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Properties of Protective Malarial Antibody

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Summary. Properties of protective malarial antibody have been studied in cultures of *P. knowlesi* giving average parasite multiplication rates of sixfold in 24 hours. Parasite growth was assessed by incorporation of [^{3}H]-leucine into protein.

Immune serum has little effect upon the growth of intracellular parasites, but prevents reinvasion of red cells and inhibits the succeeding cycle of parasite development. Protective antibody is present in relatively low titre in immune sera even after long immunization and this may explain certain characteristic features of malarial immunity.

Protective antibody in the sera studied is associated with IgG and IgM; its action is not complement dependent, but requires at least two combining sites per molecule. Anti-malarial antibody has several features in common with viral neutralizing antibody.

INTRODUCTION

Malarial infection leads to the production of specific antibodies detectable by several immunological techniques (reviewed in Sadun, 1966). Although these antibodies may cross-react with various plasmodial species, immunity to malarial infection is largely species specific. In addition, there is poor correlation between the immune status of an individual and observed levels of malarial antibody. These observations indicate that much anti-malarial immunoglobulin has no protective effect against the plasmodial infection (Targett and Voller, 1965). Nevertheless, protective antibody has been demonstrated by passive transfer tests in monkey (Coggeshall and Kumm, 1937) and human infections (Cohen, McGregor and Carrington, 1961) and, to a lesser degree, in rodent malaria (Fabiani and Fulchiron, 1953; Bruce-Chwatt and Gibson, 1955; Isfan, 1966; Briggs, Wellde and Sadun, 1966; Diggs and Osler, 1969). Such tests have not, however, provided a suitable basis for detailed studies on the mechanism of malarial immunity. Recently, serum from monkeys immune to *Plasmodium knowlesi* has been shown to inhibit specifically the cyclical proliferation of this simian parasite maintained *in vitro* (Cohen, Butcher and Crandall, 1969). The present paper describes the application of this technique to give information about levels of protective antibody in immune sera, the distribution of such antibody among the major immunoglobulin classes and its mode of action on the parasite; this work has been the subject of a preliminary report (Cohen and Butcher, 1970).

MATERIALS AND METHODS

Host–parasite system

Rhesus monkeys (*Macaca mulatta*) of either sex, weighing 2–3 kg were maintained in separate cages in a room artifically illuminated between 7 a.m. and 7 p.m. *P. knowlesi*

parasites (anonymous Malayan strain isolated in 1964 from *Macaca irus*) were obtained initially from Professor P. C. C. Garnham at the London School of Hygiene and Tropical Medicine. Parasites were stored as whole blood at −70° in an equal volume of 15 per cent glycerol in 0·9 per cent NaCl. During a series of experiments parasites were maintained by weekly passage between previously uninfected monkeys. When parasitaemia became asynchronous after repeated passage, infections were again initiated from the frozen material.

Parasite counts

The first appearance of parasitaemia was assessed on thick blood films haemolysed in water and stained with Giemsa. Parasite density was determined on the basis of erythrocyte counts and the percentage of red cells parasitized determined by counting up to 10^4 cells on thin blood films. Differential parasite counts were based upon the classification of 100 parasitized cells observed on thin blood films stained with Giemsa.

Immunization

P. knowlesi infections are almost invariably fatal in previously uninfected rhesus monkeys. Newly infected animals were therefore protected with mepacrine hydrochloride (repeated 20 mg doses) given by intramuscular injection for 3–4 weeks. Animals were subsequently challenged with up to 10^8 parasites ten to fourteen times over a period of 17–23 months; all animals were shown to be completely resistant to 10^8 parasites before bleeding. Blood was collected by cardiac exsanguination into heparin (about 25 U/ml) and the plasma stored in two separate pools (Table 1) at −15° for up to 6 months. Plasma was dialysed

TABLE 1

DATA CONCERNING IMMUNE RHESUS SERUM POOLS ANALYSED

Pool	No. of donors	Duration of immunization	No. of challenges	Final challenge
I (27.5.69)	4	17–20 months	11–12	10^8 parasites
II (17.9.69)	4	17–23 months	10–14	10^8 parasites

against the appropriate electrolyte solutions before use in culture experiments; a precipitate (mainly fibrinogen) which formed after thawing and dialysis was removed by centrifugation. Supernatant solutions are referred to below as immune sera.

Dr A. Voller kindly supplied a sample of immune serum from a rhesus monkey which had been infected 2 months previously with 10^8 *P. cynomolgi bastianelli* parasites and before bleeding was shown to be resistant to challenge with 5 ml of blood infected with the same parasite.

Absorption of immune serum

A sample of blood containing mature schizonts was centrifuged and the parasite rich layer washed twice with phosphate buffered saline (PBS). A volume of 8 ml of washed cells was mixed with 320 ml of freshly prepared 1 : 10,000 saponin in PBS. The saponin treated parasites were washed twice in PBS and added to four times their volume of immune serum; the suspension was left on a rotary mixer for 16 hours at 4°. After centrifugation to remove parasites, two further absorptions for 30 minutes at 37° and for 2 hours at 4° were carried out as above, using freshly prepared washed schizonts. The serum was finally spun at 10,000 rev/min for 1 hour at 2° in a Sorvall bench centrifuge and the supernatant solution was dialysed against PBS at 4°.

Preparation of Ig fractions

These were prepared from normal and immune (pool 1) sera using the procedures indicated in Table 2. Protein solutions were concentrated by pressure dialysis at 2° and Ig concentrations were adjusted to those of whole serum before assay.

TABLE 2

DATA FOR VARIOUS RHESUS MONKEY PREPARATIONS TESTED FOR ANTI-MALARIAL ACTIVITY; IMMUNE Ig FRACTIONS WERE ISOLATED FROM POOL 1 (TABLE 1)

Preparation	Fractionation	IgG (mg/ml)	IgM (mg/ml)
Normal serum	—	13	0·7
Immune Pool 1	—	13	0·8
Immune Pool 2	—	13·5	0·3
Whole Ig	0·4 Saturated $(NH_4)_2SO_4$ (Reppt. × 3)	13	0·4
IgG (2)	DEAE—0·015 M phosphate, pH 8·0	13	<0·1
IgG (1)	DEAE—0·02 M and 0·025 M phosphate, pH 8·0	13	<0·1
IgM	Sephadex G200—0·05 M Tris 0·1 M NaCl, pH 8	<0·1	0·8
Normal IgG	DEAE—0·04 M phosphate, pH 6·0.	11	<0·1

Determination of Ig concentration

This was carried out by ring diffusion on gel plates. Sheep antisera specific for human IgG and IgM which cross react with the corresponding monkey Igs together with standard solutions of human IgG and IgM were used (Cwynarski, 1968).

Enzymatic digestion of IgG

Normal and immune IgG preparations were digested with papain for 16 hours at room temperature according to the method of Porter (1959) and with pepsin for a similar period according to the method of Nisonoff *et al.* (1960). Undigested IgG was in both instances removed by gel filtration on Sephadex G200. Solutions of Fab+Fc (papain digest) and $F(ab_1)_2$ (pepsin digest) were concentrated by pressure dialysis.

Reaction of monkey sera with anti-IgA and anti-IgE

A concentrated Ig solution was prepared by salt precipitation (0·4 saturated $(NH_4)_2SO_4 \times 3$) of specific sheep anti-human IgA serum which cross reacted with monkey IgA. An excess of this immune Ig was added to volumes of normal and immune serum which were incubated at 37° for 30 minutes and left at 2° overnight. After centrifugation the supernatant sera no longer reacted with anti-IgA on immuno-electrophoresis.

Aliquots (3 ml) of normal and immune serum were also reacted with 0·6 ml of specific sheep anti-human IgE serum kindly provided by Dr S. G. O. Johansson. This antiserum, raised against a monoclonal human IgE, has been shown to react with rhesus monkey IgE (Ishizaka and Ishizaka, 1968).

Culture of parasites

The method used was based upon the studies of Geiman, Siddiqui and Schnell (1966) and Trigg (1967) and is described in detail elsewhere (Butcher and Cohen, 1970). The main modifications introduced were as follows: (i) infected red cells for culture were washed twice in phosphate-buffered saline and resuspended in a mixture of horse serum

and homologous normal monkey serum; (ii) a protein hydrolysate was replaced by the amino acid mixture used in Eagle's medium together with glutamine (14·6 mg/100 ml), co-enzyme A (10·2 mg/100 ml), ATP (37 mg/100 ml) and AMP (26·3 mg/100 ml); (iii) flasks were gassed with 90 per cent N_2, 5 per cent CO_2 and 5 per cent O_2. Cultures were carried out for 24 hours in conical flasks fitted with ground glass sampling ports. Each flask contained 0·25 ml of packed erythrocytes (of which 0·5–1·7 per cent were initially parasitized), 3·0 ml of culture medium, 0·15 ml horse serum and various volumes (0·05–0·6 ml) of rhesus serum or Ig fractions (normal or immune). Samples (0·2 ml) were taken at intervals while maintaining positive pressure within flasks.

In vitro *measurement of* [^3H]*leucine incorporation*

Parasite growth during *in vitro* culture was measured by incorporation of [^{3}H]-leucine from a medium containing no unlabelled leucine. Samples of culture fluid suspensions were added to 1 ml 0·08 per cent unlabelled leucine in physiological saline. After centrifugation the supernatant fluid was removed and cells washed three times in 10 per cent trichloracetic acid and then in alcohol and ether. Precipitates were dissolved in 0·5 ml concentrated formic acid at 37° and 0·5 ml H_2O was then added to each. Aliquots (0·2 ml) were added to 20 ml scintillation fluid (Buhler, 1962) for measurement of radioactivity.

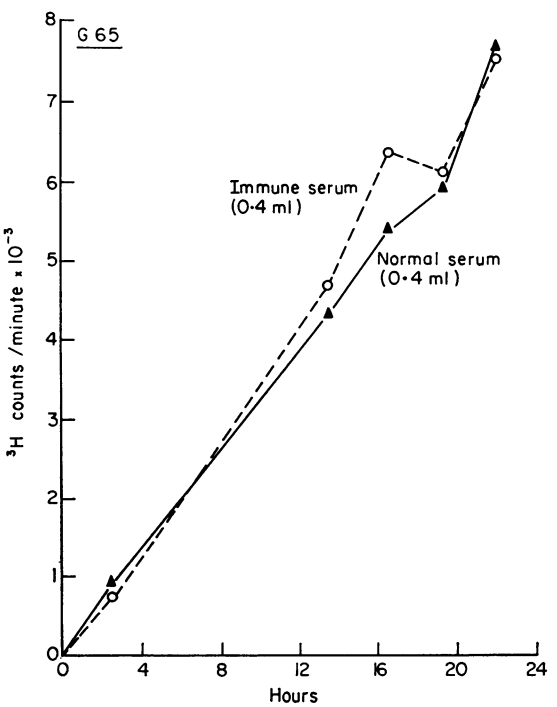

FIG. 1. *In vitro* incorporation of [^{3}H]leucine into *P. knowlesi* parasites during development from rings to mature schizonts in the presence of normal and immune rhesus sera. Parasitaemia was 2 per cent at the beginning and end of the experiment. Differential parasite counts: at 0 hours, rings 100 per cent, 17 hours, rings 2 per cent, trophozoites 80 per cent, schizonts 18 per cent, 22 hours, rings 5 per cent, trophozoites 9 per cent, schizonts 86 per cent.

TABLE 3

In vitro MULTIPLICATION RATES OF *P. knowlesi* PARASITES

Serum	Parasites		Medium†	Gas‡	No. of cultures	No. of expts. showing given multiplication rate											Mean multiplication rate
	Stage*	Washed				0	×1	×2	×3	×4	×5	×6	×7	×8	×9	×10	×11	
Rhesus (autologous)	T	–	Original	1	14	1	2	7	2	2								2·0
Rhesus (homologous)	T	×2	Modified	1	13		3	1	2	2	1	4						3·9
Horse	T	×2	Modified	1 or 2	14			1	3	1	3	2	4					5·0
Horse+rhesus (homologous)	T	×2	Modified	2	43	1	2	1	3	5	6	4	6	6	3	5	1	6·2
Horse+irus	T	×2	Modified	2	8						1	1		4		1	1	8·4
Horse or rhesus (homologous)	R	×2	Modified	2	7			1	2		1	2	1					4·6

* Stage of development at beginning of culture. T = trophozoites and some early schizonts, R = rings.
† See Methods.
‡ 1, 95 per cent air 5 per cent CO_2; 2, 90 per cent N_2, 5 per cent CO_2, 5 per cent O_2.

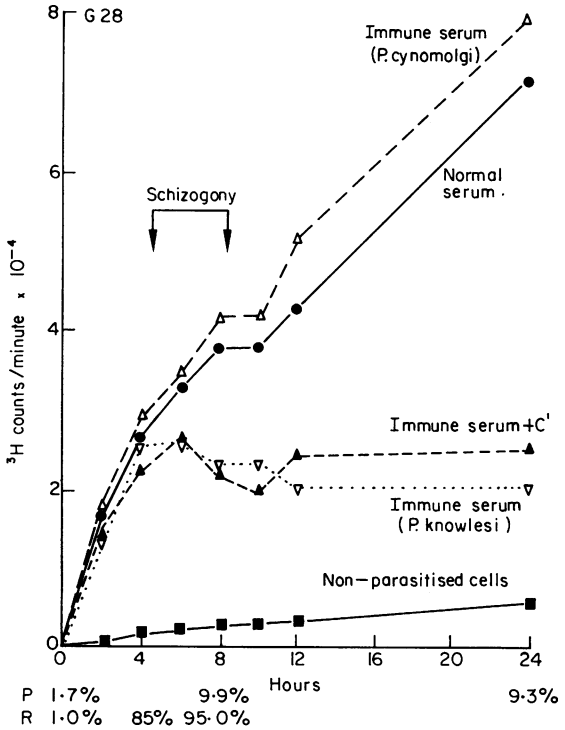

FIG. 2. *In vitro* incorporation of [^{3}H]leucine into *P. knowlesi* parasites and into non-parasitized red cells in the presence of normal and immune rhesus sera. The period of schizogony (parasite division and red cell reinvasion) is indicated by arrows. P = percentage of red cells parasitized and R = percentage of parasites which are young ring forms, in flasks containing normal serum.

RESULTS

In vitro GROWTH OF *P. knowlesi*

The object of these experiments was to replicate in the laboratory the 24-hour cycle of parasite growth, multiplication, erythrocyte invasion and renewed growth shown by *P. knowlesi* in the monkey host. In particular, because the number of merozoites produced by a mature *P. knowlesi* schizont varies from 6 to 16 (Garnham, 1966) it was necessary to achieve *in vitro* multiplication rates of at least six-fold.

Parasites for culture were obtained by heart puncture 3–10 days after inoculation of previously uninfected monkeys (*Macaca mulatta*). Starting with whole blood containing trophozoites and early schizonts and using a culture technique based on methods described by Geiman *et al.* (1966) and Trigg (1967), the average multiplication rate was 2·0 in 24 hours. Better results were achieved after altering the technique as described under Methods and the importance of each modification will be evaluated elsewhere (Butcher and Cohen, 1970). With the method finally adopted average multiplication rates were six-fold in cultures initiated with trophozoites and five-fold with ring forms of *P. knowlesi* (Table 3). In cultures initiated with trophozoites and immature schizonts, the parasites reinvaded red cells after 6–8 hours and developed to the stage of trophozoites by 24 hours.

[^{3}H]LEUCINE INCORPORATION BY *P. knowlesi* IN THE PRESENCE OF NORMAL SERUM

Parasites grown from rings through the stage of trophozoites to early schizonts in the presence of normal rhesus serum, incorporate [^{3}H]leucine into trichloracetic acid precipitable material at an approximately linear rate (Fig. 1). When parasite division (schizogony) and red cell reinvasion commence—as shown by the rising percentage of young ring forms and the increased total parasite count—the rate of [^{3}H]leucine incorporation diminishes, then becomes stationary for about 2 hours and is subsequently renewed during the succeeding cycle of parasite development (Fig. 2). Cultures of normal rhesus blood incorporate leucine at a level which is less than 10 per cent of that with parasitized blood (Fig. 2).

EFFECT OF IMMUNE SERUM

Immune serum (0·3–0·4 ml serum per flask containing 0·25 ml red blood cells) has no appreciable effect upon the rate of leucine incorporation during intracellular growth of rings, trophozoites and schizonts, e.g. Fig. 1 and the beginning of experiments shown in Fig. 2. With the onset of schizogony, however, leucine incorporation is reduced in the presence of immune serum and no further uptake occurs, indicating that the succeeding cycle of parasite development is completely suppressed (Fig. 2). This action does not appear to be complement dependent, because heating the immune serum at 56° for 1–3 hours or

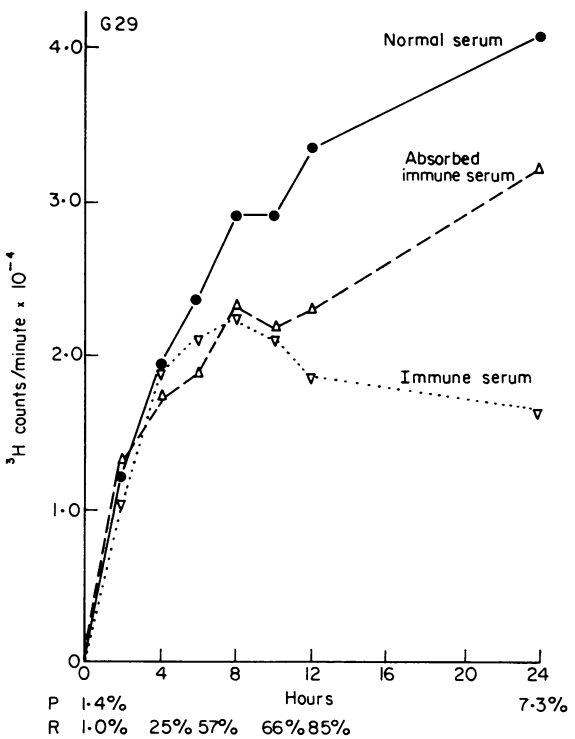

FIG. 3. *In vitro* incorporation of [^{3}H]leucine into *P. knowlesi* parasites in the presence of normal, immune and immune absorbed sera. The latter serum was absorbed with *P. knowlesi* schizonts obtained by saponin lysis of red cells (see Methods). P = percentage of red cells parasitized and R = percentage of parasites which are young ring forms, in flasks containing normal serum.

the addition of fresh monkey serum as a source of complement, does not appreciably alter the response (Fig. 2). The specificity of the reaction is shown by the fact that *P. knowlesi* incorporates leucine normally in the presence of serum (0·3 ml serum per flask containing 0·25 ml erythrocytes) from a rhesus monkey immune to *P. cynomolgi bastianelli*. *In vivo* tests show that there is no cross immunity between these two species of plasmodia (Voller and Rossan, 1969). In addition, immune serum absorbed with mature *P. knowlesi* schizonts obtained by saponin lysis of infected erythrocytes, inhibits leucine incorporation less effectively than whole serum (Fig. 3). With the immune absorbed serum, leucine uptake was reduced during schizogony, as compared with the normal, but then recommenced indicating that a proportion of parasites had entered a second cycle of development.

In vitro ASSAY OF PROTECTIVE ANTIBODY

Assay of normal and immune preparations were carried out in culture flasks containing 0·25 ml of washed red blood cells with an initial parasitaemia of 0·5–1 per cent. The dose dependent nature of parasite inhibition is illustrated in Fig. 4 for pool 1 antiserum (Table 1). It is apparent that in flasks containing less than 0·3 ml antiserum increasing proportions of cultured parasites enter a second cycle of development.

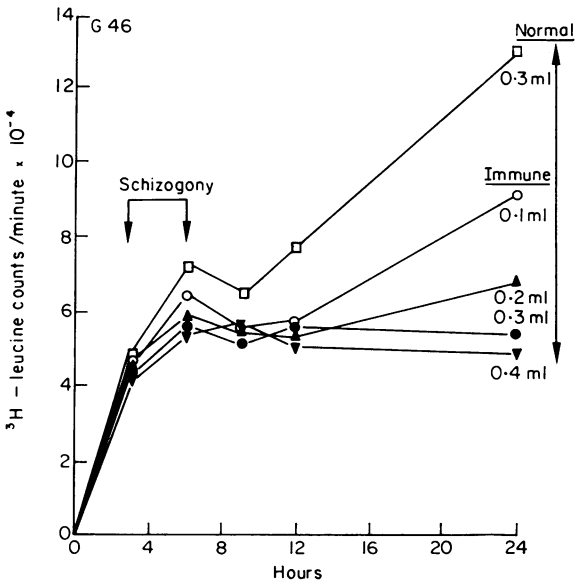

FIG. 4. Incorporation of [^{3}H]leucine by cultures of *P. knowlesi* in the presence of normal serum and various doses of immune serum (pool 1, Table 1). The vertical arrow on the right shows the normal growth increment (see text). The initial parasitaemia was 1 per cent and the multiplication rate in flasks containing normal serum was 6·5-fold. Schizogony denotes the period of parasite division.

As a basis for comparing different immune preparations, dose response data relating to inhibition of the second cycle of *in vitro* growth were derived from radioactivity measurements at 24 hours. In all experiments the standard inhibitory dose of immune serum was taken as 0·4 ml of Pool 1 (Table 1) since this consistently produced complete suppression of the second cycle of development. The normal growth increment was taken as the difference between 24 hour radioactivity values in flasks containing 0·4 ml of the standard

TABLE 4

INCORPORATION OF [^{3}H]LEUCINE INTO PARASITE PROTEIN IN THE PRESENCE OF NORMAL RHESUS SERUM AND VARIOUS VOLUMES OF IMMUNE SERUM (G46)

Serum		Packed erythrocytes (ml)	Per cent erythrocytes parasitized		[^{3}H]leu c.p.m.					Increment*	Per cent normal increment
Pool	Volume (ml)		0 hours	24 hours	3 hours	6 hours	9 hours	12 hours	24 hours		
Normal	0·3	0·25	1·0	6·5	4700	7200	6500	7700	13,100	8200	100
	0·4	0·25	1·0	—	4200	5500	57000	5100	4900	0	0
Immune	0·3	0·25	1·0	—	4300	5700	5200	5600	5400	500	6
Pool 1	0·2	0·25	1·0	—	4600	5800	5500	5100	6800	1900	23
	0·1	0·25	1·0	–	4400	6500	5500	5500	9200	4300	52

* See text and Fig. 4.

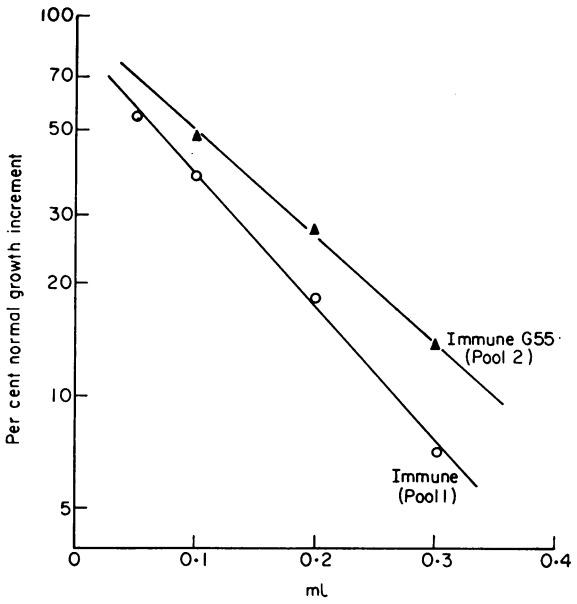

FIG. 5. Dose response data for immune sera, pools 1 and 2 (Table 1).

immune serum and those containing normal serum (Fig. 4). Radioactivity increments in other flasks were expressed as percentages of the normal (Table 4) and these values showed an approximately linear relationship to dose on a logarithmic plot (Fig. 5). The mean data from four experiments for immune pool 1 (Table 1) and one experiment for pool 2 (Table 1) indicate that these sera, which were both obtained from monkeys immunized for about 18 months, contain similar titres of protective antibody. It is apparent from Fig. 5 that when red cells (0·25 ml) were mixed with a physiological proportion of immune serum (0·3 ml) the amount of protective antibody was near the threshold required for inhibition of progessive parasite growth in cultures containing 0·5–1·0 per cent of infected red cells.

NATURE OF PROTECTIVE ANTIBODY

Various Ig fractions were prepared from immune rhesus serum (pool 1) by methods indicated in Table 2. The IgG and IgM content of these was determined by immunodiffusion and for the purpose of assay was adjusted to that of the original immune serum pool (Table 2).

A whole Ig fraction prepared by $(NH_4)_2SO_4$ precipitation simulated the inhibitory effect of the whole immune serum (Fig. 6). However, IgG fractions of fast or slow electrophoretic mobility and IgM had significantly less inhibitory activity when assayed at concentrations corresponding to those of the immune serum. The data in Fig. 6 show that the antimalarial effect of whole immune serum or salt precipitated Ig can be accounted for by the additive effects of IgG and IgM. In accordance with this finding, inactivation of either IgA or IgE by addition of an excess of specific anti-IgA or anti-IgE had little effect upon the inhibitory action of the immune serum (Fig. 7). Similarly, attempts to inactivate IgE by heating at 56° for 2–4 hours produced little change in the antimalarial titre of the immune serum.

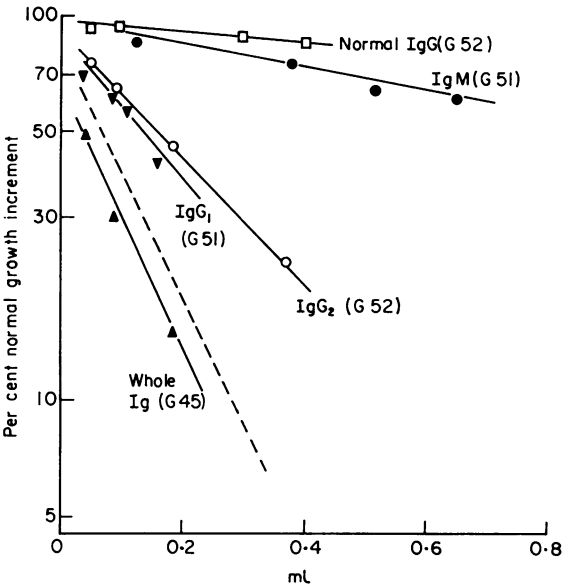

FIG. 6. Dose response data for immune preparations of whole Ig, IgG and IgM and for normal IgG. Fractions were tested at concentrations corresponding to those of the original immune serum (Table 2). The dotted line shows the response for immune serum, pool 1 (see Fig. 5).

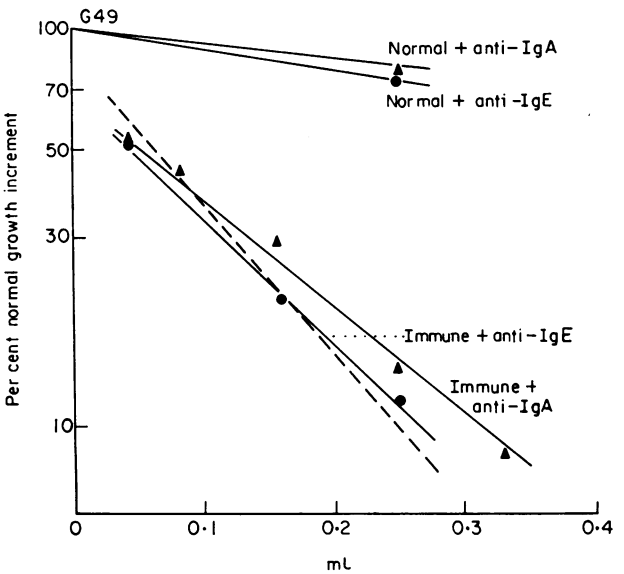

FIG. 7. Dose response data for normal and immune (pool 1) sera to which were added excess sheep anti-IgA γ-globulin and anti-IgE serum (see Methods). The dotted line shows the response for the original whole immune serum, pool 1. Recorded volumes are corrected for dilution by the anti-IgA and anti-IgE preparations.

Studies on the enzymatic splitting of γ-globulin have provided a means for investigating the molecular basis of various antibody reactions (Porter, 1959). The role of bivalence and the importance of the Fc fragment in malarial antibody were studied by comparing papain fragments (univalent Fab together with Fc) and peptic fragments (bivalent $F(ab_1)_2$) at equivalent concentrations. The immune papain digest had no demonstrable anti-malarial effect, whereas the peptic fragment simulated the anti-malarial action of whole IgG tested at equivalent concentration (Fig. 8).

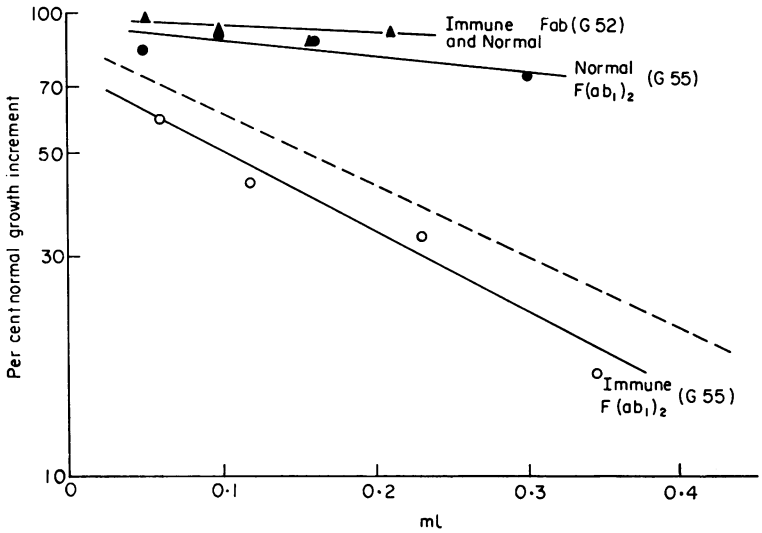

FIG. 8. Dose response data for papain (Fab+Fc) and pepsin $F(ab_1)_2$ fragments of normal and immune IgG. Concentrations of fractions tested were Fab+Fc 13 mg/ml, $F(ab_1)_2$ 8·7 mg/ml. Dotted line shows the response for the original whole IgG (13 mg/ml).

DISCUSSION

Malarial immunity in many species, including the rhesus monkey, becomes manifest very slowly during the course of the disease and is associated with persistence of the infecting organism as shown by the occurrence of periodic low-grade parasitaemia in clinically immune subjects, and the induction of malarial relapse by splenectomy in immune animals. This form of non-sterilizing, relative immunity occurs in many protozoan infections and has been described by such terms as 'premunition' and 'tolerance' (Sergent and Sergent, 1956). This characteristic response could be a consequence of the fact that the plasmodium traverses the plasma only briefly during passage of merozoites between erythrocytes; this must limit its access to immunologically competent cells so that levels of protective antibody may remain inadequate for complete suppression of parasite multiplication even after long infection. This concept is supported by the results of the present study. In rhesus monkeys repeatedly immunized for about 2 years and clinically resistant to challenge with 10^8 parasitized red cells (equivalent to about 0·01 per cent of circulating erythrocytes), the titre of protective antibody was near the threshold required to inhibit progressive parasite growth in cultures containing 0·5–1·0 per cent of infected red cells. It seems likely that titres of protective antibody are also low in human adults exposed to lifelong malarial infection, since the passive immunization of African infants required doses

of immune adult γ-globulin equivalent to about half the total circulating Ig pool of the recipient (Cohen, McGregor and Carrington, 1961).

In the present study protective antibody was demonstrated in IgG of fast and slow mobilities and in IgM. The antimalarial action of whole immune serum was quantitatively accounted for by summation of the inhibitory effects of IgG and IgM. Although IgM is less active than IgG when assayed at their relative serum concentrations, IgM has significantly greater antimalarial potency per unit weight of protein and this difference would be further enhanced by expressing the results on a molar basis. The association of protective antibody with IgM was not observed in hyperimmune rat serum (Stechschulte, Briggs and Wellde, 1969) and in the case of chronically immunized monkeys could be attributed to the appearance of antigenic variants of *P. knowlesi*. Such variants of the simian parasite distinguishable by a schizont agglutination test (Eaton, 1938; Brown and Brown, 1965), appear during the course of chronic infections and each might induce a primary immune response associated with IgM antibody (Brown and Brown, 1965). However, the present investigation provides no evidence for serological diversity among the distinct classes of protective malarial antibody. Thus, the *in vitro* response to both IgG and IgM was linear over the range investigated and at the highest doses tested IgG produced 80 per cent and IgM 40 per cent inhibition of normal parasite growth. These results suggest that, in regard to the parasite population analysed, IgG and IgM contained protective antibodies of comparable specificities. Persistence of IgM antibody after prolonged immunization has been observed with other particulate antigens (Torrigiani and Roitt, 1965).

The antimalarial activity of immune IgG has been demonstrated previously in human *P. falciparum* and *P. malariae* infections by passive transfer tests in young African children (Cohen, McGregor and Carrington, 1961) and also was inferred from the occurrence of neonatal immunity in the offspring of immune mothers and the presence of protective antibody in such newborn infants' sera (Edozien, Gilles and Udeozo, 1962). The protective activity of serum from rats immune to *P. berghei* was associated mainly with 7S immunoglobulin as shown by *in vivo* protection tests (Diggs and Osler, 1969). In other passive transfer tests using the same rodent parasite the most potent fractions contained electrophoretically fast IgG and IgA and this suggested the possibility that IgA may have antiparasitic activity (Stechschulte, Briggs and Wellde, 1969). In the present experiments there was no evidence that monkey IgA contained significant titres of protective malarial antibody. The failure to demonstrate activity in monkey IgE is of interest because levels of IgE are high in human (Johansson, Mellbin and Vahlquist, 1968) or monkey (Ishizaka, Ishizaka and Tada, 1969) parasitic infections and this, together with earlier observations on the common occurrence of immediate type sensitivity (Catty, 1969), had suggested a possible protective role for IgE in parasitic diseases.

Immune serum has little effect upon the growth of parasites within cells, but suppresses the cycle of development which follows schizogony. This suggests that protective malarial antibody prevents reinvasion of red cells by combining with free merozoites, although the possibility of an effect on mature schizonts cannot be excluded. This action is not complement dependent and peptic fragments of IgG which cannot fix complement, retain the antiparasitic action of intact antibody. Since peptic fragments lack Fc it is evident that this portion of the molecule has no role in parasite inactivation. However, bivalence appears to be an essential feature of protective malarial antibody since univalent Fab fragments are inactive.

It is evident from these findings that protective malarial antibody has many features in

common with some viral neutralizing antibodies (Burnet, 1960). Both appear to inhibit cell invasion, act independently of complement, require at least two combining sites to exert their protective action and do not affect the behaviour of organisms already within cells. In the well-studied bacteriophage neutralization system the effectiveness of bivalent as compared with monovalent antibodies (Stemke and Lennox, 1967; Klinman, Long and Karush, 1967; Stemke, 1969) is not attributable to differences in size (Klinman, Long and Karush, 1967; Stemke, 1969), but apparently reflects the considerably greater affinity of molecules carrying two combining sites (Greenbury, Moore and Nunn, 1965). In malaria, the agglutinating capacity of bivalent antibody may also be significant since microscopical observations have shown that merozoites liberated into normal serum during schizogony remain discrete before reinvading red cells, but in the presence of immune serum the free parasites tend to agglutinate and fail to penetrate erythrocytes (Butcher, unpublished observations).

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