

tion than that resulting from the continuous system. Further, all γ -globulin bands obtained by the discontinuous system were located closer to the albumin zone as noted previously¹³. Nevertheless, certain pathological γ -globulins (1b, c, d) still migrated towards the cathode.

Paper electrophoresis at pH 8.6 in citrate barbiturate buffer of the sera investigated demonstrated in each case a significant increase of the proteins which migrated with mobilities corresponding to those of the γ -globulins. The increase over the normal value was 23–34 relative per cent. As judged by ultracentrifugal analysis the 7S-globulins of the same sera varied between 30 and 40 per cent. The 19S-component appeared approximately normal in concentration. Abnormal plasma proteins with sedimentation coefficients of 10 and 14S, respectively, were observed in one case (1e) only.

The present starch-gel electrophoretic investigation on pathological γ -globulins demonstrates that this procedure offers an advantage over paper electrophoresis in that certain abnormal γ -globulins can be resolved into several bands. This resolution seems to indicate that these pathological γ -globulins display a discontinuous spectrum with respect to apparent mobility in contrast to the continuous spectrum of the normal γ -globulins. If the mobility of the 7S- γ -globulins may be related to the net charge of these molecules, then this observation

can be interpreted to mean that certain pathological γ -globulins are synthesized in such a way that they carry relatively unlike electrostatic net charges.

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Note added in proof. After this communication had been submitted, an article by Owen, Got and Silberman (*Clin. Chim. Acta*, 3, 605; 1958) came to our attention, in which the heterogeneity of the γ -globulins of patients with myeloma is described.

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GAMMA-GLOBULIN AND ACQUIRED IMMUNITY TO HUMAN MALARIA

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MALARIAL infection is followed by the development of immunity which is specific for the homologous parasite, but ineffective against heterologous strains and species¹⁻⁴. The epidemiological effects of acquired immunity in man are seen among the inhabitants of hyperendemic malarious regions who show a consistent pattern of susceptibility to infection. Infants born in such regions are relatively resistant during the first three months of life and thereafter all children suffer severe and recurrent attacks of the disease. Clinical malaria becomes comparatively infrequent in later childhood and among adults is rarely seen in acute form^{5,6}.

The response of phagocytic cells in malarial infection was shown in early histological work⁷, and for many years resistance to the disease was considered to be exclusively cellular in nature. In 1937, Coggeshall and Kumm⁸ demonstrated the passive transfer of malarial immunity in rhesus monkeys and so established the presence of protective antibodies. However, the therapeutic effect of large doses of immune serum was evident only against minimal numbers of parasites and has not been demonstrated convincingly in man. The belief is therefore still prevalent that circulating antibodies play a relatively unimportant part in acquired malarial immunity⁹.

Passive Transfer of Human Malarial Immunity

In this investigation the role of circulating antibody in malarial immunity has been studied in subjects living in The Gambia which is a hyperendemic area of West Africa. The therapeutic effect of γ -globulin prepared from the serum of apparently immune adults has been tested in young African children suffering from severe clinical malaria with dense parasitaemia. The γ -globulin prepared by chromatography on columns of diethylaminoethyl cellulose¹⁰ was homogeneous on electrophoresis and in the ultracentrifuge appeared as a main peak with a sedimentation coefficient ($S_{20,W}^0$) = 6.78; an additional heavier component (10S) comprised less than 5 per cent of the total protein. The remainder of the protein eluted from the columns (referred to here as γ -free serum) was shown by serological tests to contain about 70 mgm. 7S γ -globulin and 20 mgm. 19S γ -globulin/gm. total protein.

Twelve children aged 4 months–2½ years (weight 5.4–12.6 kgm.) admitted with a clinical diagnosis of malignant tertian malaria and an initial parasitaemia of 10,000–230,000/c.mm. received γ -globulin by intramuscular injection at intervals of 8–24 hr. for 3 days. The total dose was 1.2–2.5 gm./child which

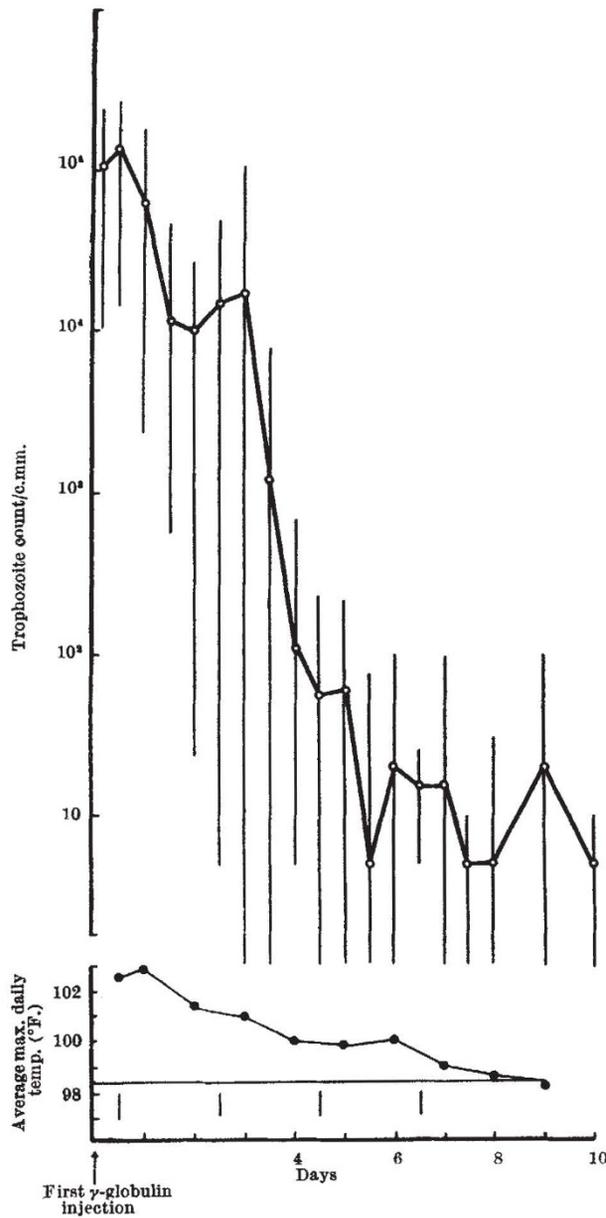


Fig. 1. Trophozoite counts (mean and range shown on a logarithmic scale) and average maximum daily temperatures in 12 children with acute *Plasmodium falciparum* malaria treated with immune γ -globulin. The time-scale refers to days after the first γ -globulin injection. Vertical lines below indicate approximate times of expected schizogony.

was equivalent to 10–20 per cent of the recipient's own γ -globulin.

The infections were predominantly synchronous and γ -globulin therapy was usually started when the peripheral blood contained a high density of young ring forms. Blood examinations were made at 12-hourly intervals and the course of parasitaemia is shown in Fig. 1. By the fourth day after the inception of treatment parasite counts were always less than 1 per cent of the initial values; by the ninth day trophozoites were not detectable in 8 out of 12 cases and the maximum count was 80/c.mm. (Fig. 1). The fall in parasitaemia was accompanied by progressive alleviation of clinical illness although temperatures did not usually return to normal before the seventh

day (Fig. 1). No alterations in parasite morphology have been observed during treatment; however, in malignant tertian infections only immature parasites are detectable in peripheral blood. The immune γ -globulin appears to be effective both against *Plasmodium falciparum* and *P. malariae* (Fig. 2), but probably has no action against gametocytes (Fig. 3).

Two children aged 2–3 years failed to respond to the injection of 1.2 gm. γ -globulin. They had been admitted with pneumonia and measles respectively,

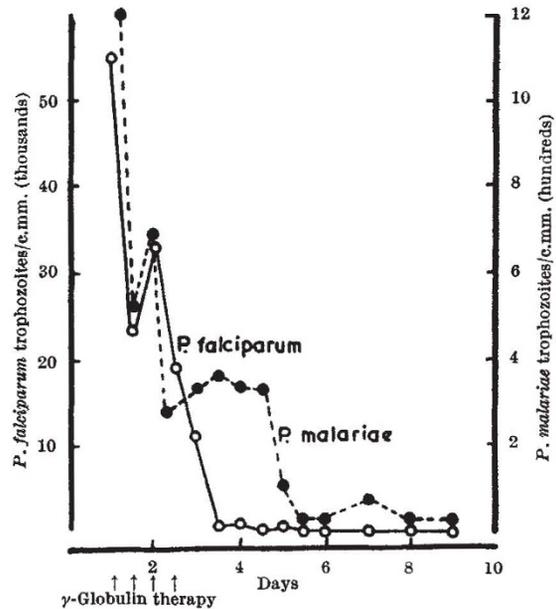


Fig. 2. Trophozoite counts of *Plasmodium falciparum* and *P. malariae* in a child with a mixed infection treated with immune γ -globulin; arrows show times of administration of γ -globulin by intra-muscular injection.

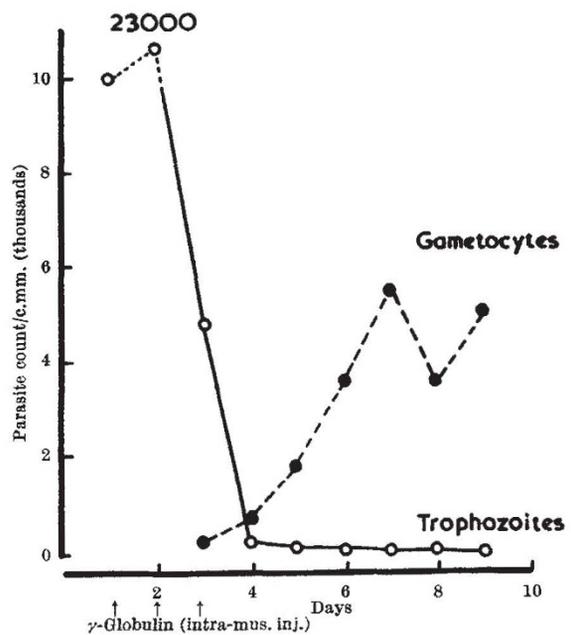


Fig. 3. Trophozoite and gametocyte counts in a child with acute *Plasmodium falciparum* malaria treated with immune γ -globulin. Arrows show time of administration of γ -globulin by intra-muscular injection.

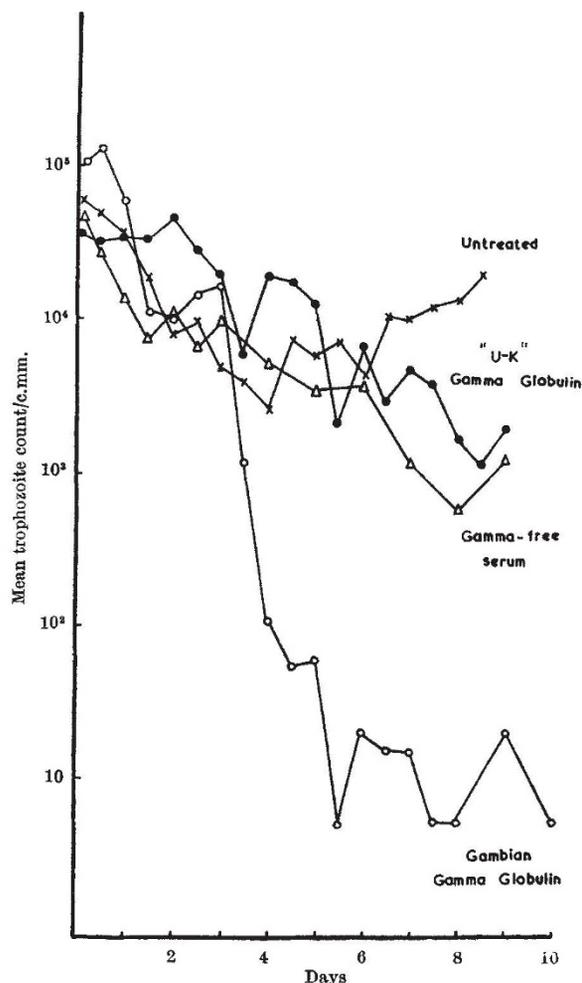


Fig. 4. Mean trophozoite counts (logarithmic scale) in four groups of children with acute *Plasmodium falciparum* malaria. The treated children received either (i) γ -globulin prepared from blood donors in Great Britain, or (ii) serum prepared from Gambian adults and containing 7 per cent 7S γ -globulin and 2 per cent 19S γ -globulin, or (iii) γ -globulin prepared from Gambian adults

and in both *P. falciparum* parasitaemia of relatively low density (8,000 and 16,000/c.mm.) was an incidental finding. Failure in these cases was probably due to inadequate dosage since 3 children of

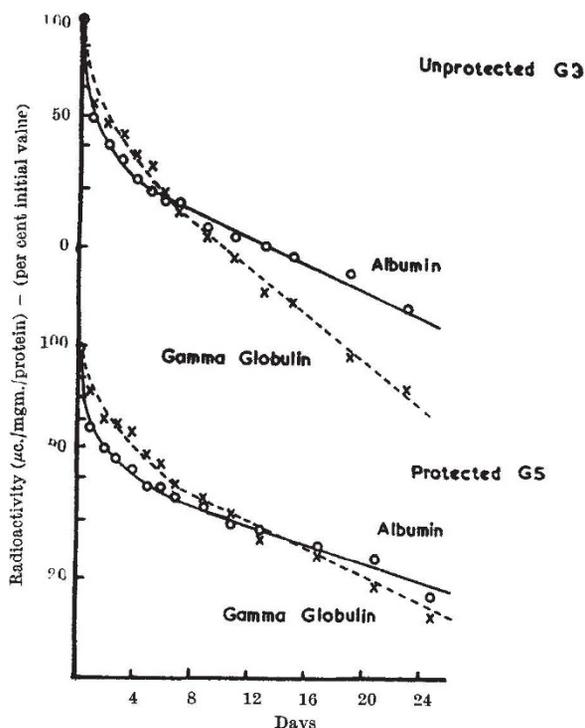


Fig. 5. Plasma elimination of ^{125}I -albumin and ^{125}I - γ -globulin in an unprotected adult Gambian and in a similar subject who had received 'Pyrimethamine' (25 mgm./week) for 6 years

comparable weight (10-12 kgm.) responded to the injection of 1.8-2.5 gm. γ -globulin.

In further experiments, 7 children with acute *P. falciparum* malaria received the γ -free fraction of Gambian serum (0.8-1.4 gm.). A single batch was used in all experiments and no child has developed signs of serum hepatitis. In addition, 4 children received γ -globulin prepared from the sera of blood donors in Great Britain (Lister Institute, Elstree, Herts.) and 2 were kept under observation without anti-malarial therapy. Trophozoite counts usually decreased in all these children. However, the response was irregular, and between the fourth and tenth days the mean trophozoite counts were considerably greater than those observed after treatment with adult African γ -globulin (Fig. 4).

Table 1. TURNOVER DATA (MEAN AND RANGE) IN ADULT SUBJECTS INJECTED WITH LABELLED ALBUMIN AND γ -GLOBULIN

Subjects	No.	Total protein	gm. (per cent)	Intra-vascular pool (gm.)	Distribution ratio	Turnover-rate		
						Per cent I-V pool/d.	gm./d.	mgm./kgm./d.
Albumin								
Unprotected Gambians	4	8.1 (7.6-8.7)	3.6 (3.1-4.2)	86 (61-110)	1.3 (1.2-1.4)	10.8 (8.6-12.3)	9.1 (6.8-12.0)	170 (150-222)
Protected Gambians	5	8.3 (7.8-8.6)	4.1 (3.8-4.3)	91 (86-102)	1.2 (1.1-1.5)	10.5 (7.1-14.3)	9.4 (6.7-12.3)	176 (124-241)
W. Africans in Great Britain	4	8.6 (8.4-8.7)	4.6 (4.2-4.9)	113 (108-115)	1.3 (1.1-1.5)	10.4 (9.9-11.0)	11.7 (11.4-12.2)	159 (149-177)
Europeans in Great Britain (ref. 12)	11	7.8 (7.2-8.4)	4.5 (4.1-5.8)	123 (95-145)	1.4 (1.1-1.2)	10.4 (9.1-12.1)	12.7 (10.5-17.1)	185 (136-257)
γ -Globulin								
Unprotected Gambians	5	8.6 (8.3-9.2)	2.7 (2.2-2.9)	66 (57-82)	0.8 (0.7-1.0)	13.5 (11.8-15.7)	8.8 (7.9-9.7)	169 (139-203)
Pregnant unprotected Gambians	1	7.6	1.8	50	0.8	9.6	4.8	84
Protected Gambians	5	8.3 (7.8-8.6)	2.1 (1.5-2.4)	47 (30-67)	0.7 (0.6-0.8)	11.5 (8.5-15.4)	5.2 (4.6-5.7)	98 (80-113)
W. Africans in Great Britain	4	8.6 (8.4-8.7)	1.8 (1.6-2.0)	45 (38-55)	0.8 (0.7-0.9)	8.2 (7.8-9.3)	3.7 (2.7-4.3)	59 (42-57)
Europeans in Great Britain (ref. 11)	5	7.8 (7.2-8.4)	1.1 (1.0-1.1)	37 (28-44)	0.8 (0.6-0.9)	5.1 (4.0-5.7)	2.1 (1.5-2.4)	23 (18-28)

Distribution ratio = ratio extra- to intra-vascular protein mass

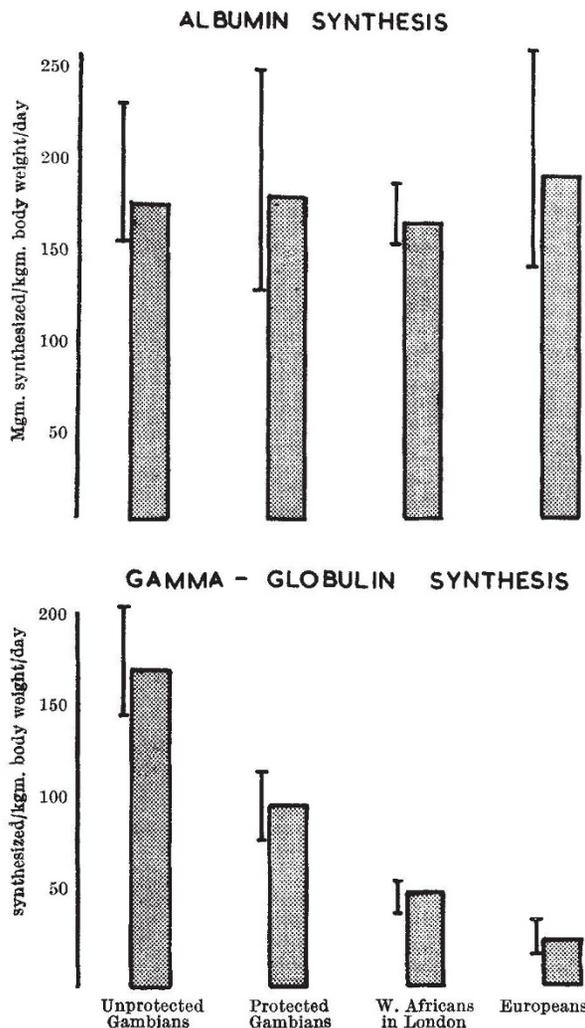


Fig. 6. Comparison of the average rates (and range) of albumin and γ -globulin synthesis in malarious and non-infected Africans and in healthy Europeans living in Great Britain

γ -Globulin Synthesis in Relation to Malarial Infection

The distribution and turnover of separate plasma protein fractions can be measured simultaneously by using two isotopes of iodine (iodine-131 and iodine-125) having different γ -emission spectra¹¹. Using this method, rates of albumin and γ -globulin synthesis have been measured in apparently healthy Gambian adults exposed to malarial infection but not showing detectable parasitemia. Similar studies were carried out on 5 Gambian adults who had received an anti-malarial prophylactic weekly for 4-5 years (Fig. 5) and also on 4 West African medical students who had suffered from malaria in childhood, but had been resident in England for 3-10 years. The experimental procedure has previously been described^{11,12}; results were analysed according to the method of Matthews¹³.

The rate of albumin synthesis was similar in the three groups of Africans and within the range for healthy Europeans. On the other hand, the rate of γ -globulin synthesis in unprotected Gambians was about 7 times that observed in Europeans; protection by anti-malarial therapy considerably reduced the rate of γ -globulin synthesis (Table 1 and Fig. 6). The rapid turnover of γ -globulin in unprotected adults

cannot be attributed to the selective catabolism of isotopically labelled malarial antibody since γ -globulins prepared from European and adult Gambian donors have identical elimination-rates (Fig. 7). Considerably less γ -globulin was synthesized by an unprotected subject who was three months pregnant at the time of study (Table 1); this finding is of interest in relation to the increased susceptibility to malarial infection described in pregnant women living in hyperendemic areas^{14,15}.

West Africans resident for some years in the United Kingdom synthesized less γ -globulin than Gambian subjects maintained on anti-malarial therapy (Fig. 6); this difference must be attributed to environmental factors other than malarial infection. After several years in Great Britain, West Africans continue to synthesize γ -globulin at almost twice the rate observed in healthy Europeans; this may be due to environmental influences of early life or to genetically determined differences in γ -globulin metabolism.

Mechanism of Acquired Malarial Immunity

The preparation of γ -globulin from pooled sera of apparently hyperimmune donors and the use of susceptible children as recipients likely to have been exposed to homologous parasite strains, have provided optimum conditions for demonstrating the potency of naturally occurring malarial antibodies active against *Plasmodium falciparum* and probably also against *P. malariae*. The results show that the mechanism of acquired malarial immunity is basically similar to that observed with many other infections, being primarily dependent on the presence of protective antibodies which are associated with 7S γ -globulin. The response to immune γ -globulin

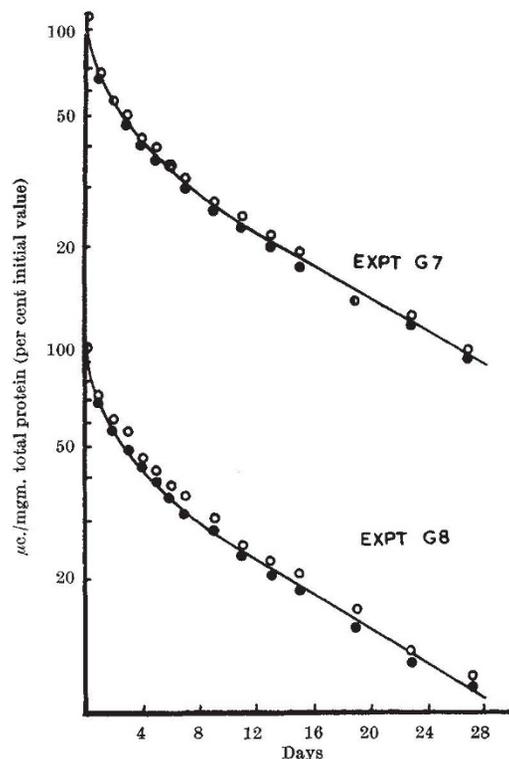


Fig. 7. Comparison in two unprotected African adults of the plasma elimination of γ -globulin prepared from European and Gambian donors and labelled with iodine-125 and iodine-131 respectively. ●, European γ -globulin; ○, Gambian γ -globulin

considered in relationship to the cycle of parasite development as well as the absence of morphological changes in the parasites of peripheral blood, suggest that antibody acts either on mature intracellular forms or on merozoites liberated from red blood cells. Protection is of limited duration and a treated subject was again susceptible three months after receiving γ -globulin. Other fractions of immune serum including 19S γ -globulin did not appear to have a protective effect nor did the γ -globulin of European blood donors.

These findings support the hypothesis that the relative resistance of infants born in hyperendemic areas is due to passive transfer of protective antibodies from the mother. This process may, however, be rendered relatively ineffective by partial loss of malarial immunity during pregnancy^{14,15}, which appears to be associated with a reduction in the rate of γ -globulin synthesis. Other factors, such as selective vector biting¹⁶, deficiency of *p*-aminobenzoic acid in milk-fed infants^{17,18} and the presence of a high proportion of foetal haemoglobin in circulating red cells¹⁹ may contribute to the suppression of malarial infection during early life.

In areas of hyperendemic malaria, immunity is established only after long exposure to intense infection. This slow response is probably attributable both to the inherently poor antigenicity of the parasite and to the serological diversity of naturally occurring plasmodia. Acquired immunity is associated with hypergammaglobulinæmia^{20,21} and with a striking increase in the rate of γ -globulin synthesis. Protection of Gambian adults against malaria over a period of several years reduced the average daily synthesis of γ -globulin by 3.5 gm., which is more than the total daily production in healthy Europeans. Since plasmodial infection leads to the production of complement-fixing antibodies²² and precipitins²³,

neither of which is species specific, it is likely that a considerable part of the γ -globulin synthesized in response to malaria is not protective antibody.

We wish to thank Sir Charles Harington, whose interest led to the initiation of this work, and Prof. R. R. Porter for much helpful discussion. We are grateful to Dr. D. Harling for clinical supervision of patients, Dr. W. d'A. Maycock for supplies of U.K. γ -globulin and Miss Margaret Polley for serological estimations of 19S γ -globulin. One of us (S. C.) is in receipt of a Medical Research Council grant.

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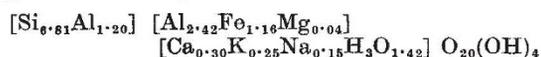
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MOISTURE EXPANSION RELATIONSHIPS FOR A FIRED KAOLINITE-HYDROUS MICA-QUARTZ CLAY

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IN carrying out a detailed study of the moisture expansion characteristics of a clay containing 30 per cent kaolinite (poorly organized), 30 per cent hydrous mica (poorly organized) of the following approximate composition:



35 per cent quartz, 2 per cent haematite, and 1 per cent anatase, I have established relationships that might be found useful in interpreting the results from other clays and clay products.

The curves of moisture expansion versus firing temperature for specimens exposed to air for 90 days, and for specimens autoclaved at 220 lb./sq. in. for 2, 8, 30 and 200 hr. (measurements beginning from the same datum; specimens removed from the muffle at 300° C. and cooled for 2 hr. in a dry desiccator before initial measurement) show that the autoclave expansion curves parallel the natural expansion curve and that at a firing temperature of

about 1,000° C. all have a maximum in expansion. If, however, autoclave expansion is plotted directly against natural expansion (Fig. 1), non-linear relationships exist over the firing range 950–1,200° C. for all periods of autoclaving, and the points below and above 1,000° C. separate into two curves for 30 and 200 hr. of autoclaving. (Linear relationships exist if autoclave expansion is plotted against the log of natural expansion.) The non-linear relationship expresses the fact that for this clay the ratio of autoclave expansion to natural expansion varies widely with firing temperature, and implies that there must be several reactive components produced over the range of firing temperature covered by the specimens. Mineralogical analysis has established that these reactive components are amorphous matter, glass, and anhydrous clay minerals. Amorphous matter, which is responsible for the coincident moisture expansion peak at about 1,000° C. in autoclaved and naturally exposed specimens, is equally reactive to water vapour in the air or to steam under pressure,