Chapter Seven

ABNORMAL HAEMOGLOBINS AND ABO BLOOD GROUPS

These two human polymorphisms were studied in the population included in the seroimmunological surveys, i.e., in the 2 village clusters (No. 5 and No. 7) treated in 1972-1973 with propoxur and high-frequency MDA, and the village cluster (No. 2) untreated throughout (see Chapter 6). The relationship of these polymorphisms to parasitological, serological and demographic variables was investigated. More detailed findings and discussions are available elsewhere (4, 41, 62, 120, 153).

Material and Methods

Blood was collected by finger-prick into heparinized tubes twice a year (in May, at the end of the dry season, and in October, at the end of the rains) from about 3000 persons distributed between the protected and the control villages, and 534 infants born to the same population during the study; this provided plasma for immunological and other studies, while the red cells were stored at -20°C preceding haemoglobin electrophoresis.

The haemolysates obtained from the frozen erythrocytes were diluted with distilled water to give a solution of 100 g Hb per litre and applied to cellulose acetate for electrophoresis without any further preparation (91). Separation of haemoglobins was satisfactory for the identification of major components, but it was not possible to quantitate Hb A_2 (and so to diagnose β-thalassaemia minor) because of background staining, presumably derived from stroma.Hb A and Hb S were eluted and quantitated in all strips showing Hb AS pattern,

For ABO typing, the erythrocytes from the seventh serological survey (wet season of 1974) were used. Antisera were obtained from the Schering Corporation, USA.

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a The work described in this chapter was done by or under the supervision of Professor A.F. Fleming, with the assistance in the field of Mr J. Storey and Dr R.L. Cornille-Brøgger.

Relatively large groups, e.g., the Hb AA and Hb AS, or the ABO blood groups, were compared by the χ^2 test for distributions into positive and negative, or into density classes, and by the t-test for serological results, after normalizing IgM levels and antibody titres by logarithmic transformation. Small groups, i.e., the Hb SS and the Hb AC, and the single Hb SC, were compared with the others as follows: parasitological and immunological results from persons with Hb SS (Hb AC; Hb SC) electrophoretic pattern were compared to results from appropriate reference groups, defined as subjects from the same village or group of villages, from the same age-group, receiving the same treatment, and examined at the same survey. The comparisons were made as follows: (1) the number of blood films found positive was compared to the number expected, i.e., the sum of the proportions positive in the appropriate reference groups; (2) parasite densities and serological results were compared to the reference median (or its approximation by the arith-

Table 26

Haemoglobin electrophoresis patterns in a whole population sample in Garki and in infants born into that population subsequently: comparison with the distribution expected from the Hardy-Weinberg law

Haemoglobin electrophoresis	Initial whole population					Newborn[a]				
	Observed		Expected[b]		χ^2[c]	Observed		Expected[d]		χ^2[c]
	No.	%	No.	%		No.	%	No.	%	
AA	1 925	70.2	1 989.8	72.6	2.11	394	73.8	387.7	72.6	0.10
AS	793	28.9	675.1	24.6	20.59	126	23.6	131.5	24.6	0.23
AC	19	0.7	15.1	0.6		3	0.6	2.8	0.5	
SC	1	0.0	2.8	0.1	0.25	0	0.0	0.5	0.1	0.04
CC	0	0.0	0.0	0.0		0	0.0	0.0	0.0	
SS	4	0.1	59.1	2.2	51.37	11	2.1	11.5	2.2	0.02
Total	2 742	99.9	2 741.9	100.1	74.32 df=3 p<0.001	534	100.1	534.0	100.0	0.39 df=3 p>0.9

a Born after the commencement of the project; the haemoglobin type was determined at an age varying between a few days and several months.

b Calculated by the Hardy-Weinberg law from the Hausa and Fulani gene frequencies and from the relative numbers of the 2 ethnic groups.

c Test of goodness of fit, comparing observed and expected.

d As in note *b*, and assuming equal birth rates,

metric or geometric mean, as appropriate); (3) if there was no significant difference between the numbers observed and expected, or between the numbers above and below the reference median, it was concluded that the Hb SS (Hb AC) did not differ from the others; (4) if there was a significant difference, it could be due to the (moderate) dependence between surveys, and the histories of Hb SS (Hb AC) individuals were compared with those of others.

Haemoglobin Electrophoresis

The prevalence of sickle-cell trait (Hb AS) in the sample of the whole population of Garki before antimalarial intervention was 28.9%, which was significantly greater than that reported in Ibadan in the forest area of the south west of Nigeria. Hb AC was present in only 0.7% of the Garki population. The distribution of genotypes (especially of Hb AS and Hb SS) observed in Garki was very different from that expected under the Hardy-Weinberg law (Table 26, left half). Both sickle-cell trait and Hb AC were more common amongst the Hausa than the Fulani; the frequency of the Hb S gene was highly significantly greater in the Hausa.

The prevalence of sickle-cell trait was 24.2% in those under the age of 1 year and over 28% in all other age-groups (Table 27). Only 1 subject

Table 27

Haemoglobin electrophoretic patterns in a whole population sample in Garki, classified by age

Haemoglobin electrophoresis	Age (years)									
	<1		1-4		5-9		10-14		≥15	
	No.	%	No.	%	No.	%	No.	%	No.	%
AA	47	71.2	184	71.0	302	68.8	142	71.7	1 178	70.2
AS	16	24.2	73	28.2	133	30.3	56	28.3	486	29.0
SS	2	3.0	1	0.4	1[a]	0.2	0		0	
AC	1	1.5	1	0.4	3	0.7	0		13	0.8
CC	0		0		0		0		0	
SC	0		0		0		0		1	
Total	66	99.9	259	100.0	439	100.0	198	100.0	1 678	100.0

a Later reclassified as being 10-14 years when interviewed and found to have retarded growth.

(0.05%) was seen with sickle-cell anaemia over the age of 9 years.

The mean proportion of Hb S in 750 sickle-cell trait subjects was 33% ± 5% (SD), and there was no variation with age. The distribution showed a normal curve. Hb S never exceeded 45% of the total.

Haemoglobin electrophoresis was performed on 534 infants born between 1971 and 1974. They were from a few days to several months old when first examined. The Hb genotype distribution observed was close to the expected as calculated from the gene frequency (Table 26, right half).

Haemoglobins and Genetic Fitness

Let the Hb AA, Hb AS, and Hb SS have selection coefficients s, 0 and ţ respectively; the corresponding fitnesses are W(AA) = (l-s), W(AS) = 1, and W(SS) = (l-t). The oldest Hb SS person was in the 10-14-years age-group, and he had retarded development (Table 27). If t = 1, we have:

$$q = \frac{s}{1+s},$$

where q is the S gene frequency (25) and we can estimate s from q:

$$s = \frac{q}{1-q} = \frac{0.146}{1-0.146} = 0.171$$

and the fitness ratio $\frac{W(AS)}{W(AA)} = \frac{1}{1-0.171} = 1.206$

The heterozygote (Hb AS) had an advantage of approximately 21% over the normal homozygote (Hb AA) in this population. This may have resulted from either greater survival or greater fertility. Following Ruck-nagel & Neel (142), differential survival was estimated by the formula:

$$\frac{\text{proportion AS in adults/prop. AS in newborn}}{\text{proportion AA in adults/prop. AA in newborn}}$$

In Garki (Tables 26, 27) this gave

$$\frac{(486/1678)/(126/534)}{(1178/1678)/(394/534)} = \frac{486/126}{1178/394} = 1.20,$$

which was significantly greater than 1 ($p<0.05$, by χ^2), similar to the

fitness ratio and sufficient to explain it.

The fertility of Hb AA and Hb AS women was estimated by the following formula:

$$\left(\frac{1000}{3}\right)\left(\frac{\text{No. live births to AA women, between surveys 1 and 16 (3-year period)}}{\text{No. AA women aged 15-44 alive at survey 9 (middle of period)}}\right)$$

and similarly for AS women; this gave fertility rate per 1000 per year.

The result was:

$$\left(\frac{1000}{3}\right)\left(\frac{201}{437}\right) = 153.3 \text{ per 1000 per year for the Hb AA}$$

and $$\left(\frac{1000}{3}\right)\left(\frac{90}{203}\right) = 147.8 \text{ per 1000 per year for the Hb AS}$$

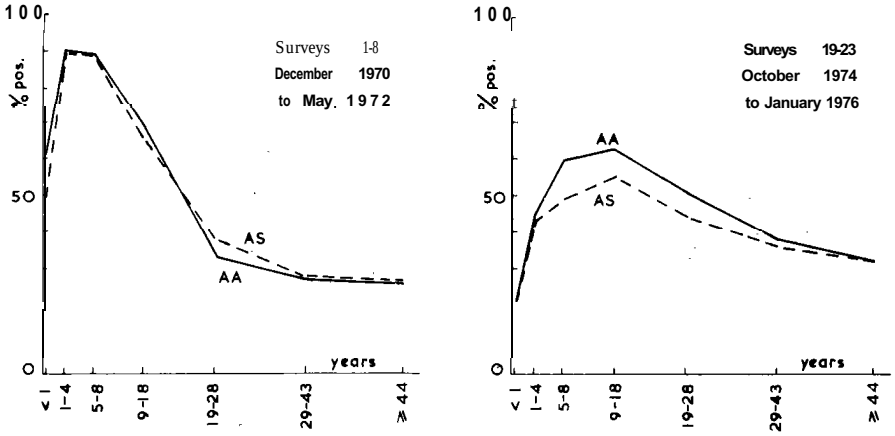
The two fertility rates were remarkably similar ($\chi^2 = 0.03$; $p>0.8$). In order to maintain a prevalence of 29% of sickle-cell trait in adults solely through differential female fertility, the female with sickle-cell trait would have had to contribute to the next generation at a rate that was 1.54 times greater than that of the normal female. In that case, the expected distribution of genotypes at birth (336.5 AA, 149.7 AS, and 14.8 SS) would have been different from that observed (Table 26; $\chi^2 = 6.79$ for 2 degrees of freedom, giving $p<0.05$), and from that expected under the Hardy-Weinberg law.

Sickle-cell Trait and Malaria

Normal homozygotes (Hb AA) were compared with those with sickle-cell trait (Hb AS), with respect to prevalence and density (percentage of fields positive) of *P. **falciparum*** asexual stages and gametocytes, *P. **malariae,*** and *P. **ovale.*** The comparison was made by age, within each of the 8 baseline surveys. In addition, the results of those born after the first survey were used in a second way, to compare Hb AA and Hb AS at 5, 15, 25...65 weeks, i.e., at the first, second, third...seventh parasitological surveys after birth.

The only systematic or significant differences concerned the *P. **falciparum*** asexual stages in infants and young children. The analysis of the surveys by year of age leads to the probable conclusion that, in the dry season, between the ages of 1 and 3 years, the Hb AS had less *P. **falciparum*** asexual stages than the Hb AA; and that the difference was one of density rather than prevalence; there was no significant difference in

Fig. 61. Age-specific prevalence of *P. falciparum*, before intervention (mean of 8 surveys, all villages) and during resurgence of malaria after intervention (mean of 5 surveys, villages previously treated with propoxur and MDA)



the wet season. The analysis of the results in those born after survey 1 leads to the probable conclusion that between the ages of 30 and 59 weeks the Hb AS had a lower prevalence and density of ***P. falciparum*** asexual stages than the Hb AA. Incidence and recovery rates of ***P. falciparum*** parasitaemia were estimated by the method of Bekessy et al. (7). They were similar in the Hb AS and Hb AA at all ages, with one exception which may have been due to chance (62).

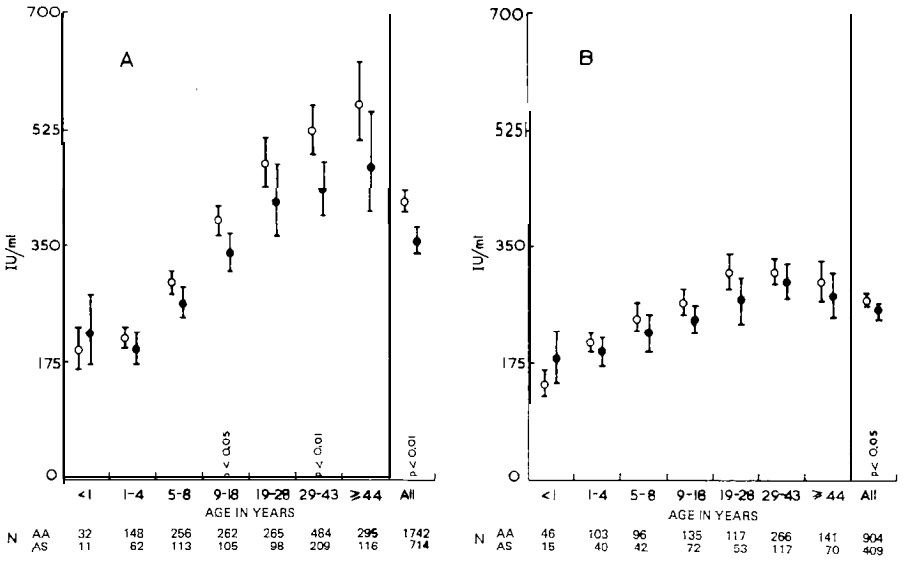
In 1974- 1975, after the intervention with propoxur and mass administration of sulfalene-pyrimethamine was terminated, malaria progressively returned to its baseline level. During this period of resurgence, in the previously treated villages, the prevalence of ***P. falciparum*** tended to remain somewhat lower among the Hb AS than among the Hb AA in the age-groups between 5 and 29 years (Figure 61). Only one difference was significant: in October 1974, in the age-group 5-8 years, 93% (68/73) of the Hb AA were positive, as compared with 77% (24/31) of the Hb AS ($\chi^2 = 3.85$).

Sickle-cell Trait, Immunoglobulins and Malaria Antibodies (see 41)

Immunoglobulins

There was no striking difference between the Hb AA and Hb AS with respect to IgG. In the unprotected population, in 7 surveys, the only

Fig. 52 Immunoglobulin M: age-specific geometric mean level of IgM (and 95% confidence limits) in persons with haemoglobin AA (o) and haemoglobin AS (•) genotypes (A) in the wet season of 1971 before intervention, and (B) in the wet season of 1973 after 70 weeks of antimalarial intervention



significant differences were the following: the Hb AS had more IgG at under 1 year of age on 2 occasions, while they had less IgG in the age-group 5-8 years on 1 occasion, in the age-group 29-43 years and for all ages combined on 2 occasions. None of the differences was large. In the protected population, there was no significant difference.

With respect to IgM, the results were rather more remarkable. The differences were not large, but fairly consistent and significant in several cases. At the first survey, the mean was slightly but not significantly higher in Hb AS under the age of 1 year. After one year of life, the IgM was on the average higher in Hb AA subjects; this difference increased with age and was significant in the age-groups 9-18 and 29-43 years and in all ages combined (Fig. 62, graph A). At the second survey (May, the end of the dry season 1972), all differences were in the same directions as at the first survey and were significant in the age-groups 9-18 ($p<0.001$), 19-28 and 29-43 years ($p<0.05$) and for all age-groups combined ($p<0.001$). Protection against malaria was followed by a progressive decline of IgM in both groups and a decrease of the difference between Hb AA and Hb AS (Fig. 62, graph B).

When the data from unprotected infants born after the onset of the project are regrouped by age (± 5 weeks), there is very little difference between the AA and AS with respect to either IgG or IgM.

Ouchterlony (precipitin) test with *P. falciparum* antigen

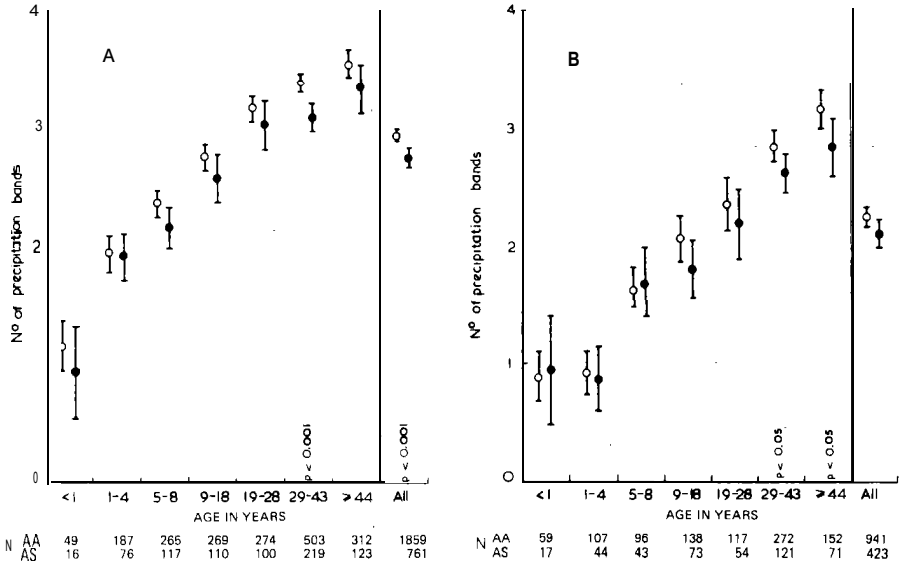
There was a rather clear and consistent difference between the Hb AA and Hb AS. At the first survey there were on the average more precipitin bands in Hb AA subjects in all age-groups, the difference being significant in age-group 29-43 years and for all ages combined (Fig. 63, graph A). At the second survey, the differences between Hb AA and Hb AS were in the same direction (except in the age-group l-4 years), and were significant in the age-groups 9-18 ($p<0.05$), 19-28 and 29-43 years ($p<0.01$) and for all ages combined ($p<0.001$).

Protection was followed by a fall in the number of bands seen in both groups and a decreasing difference between them; at the end of the second wet season under protection (October 1973), there was almost no difference below 9 years, but in older age-groups there were still more bands on the average in Hb AA subjects, the difference being significant in the age-groups 29-43 and >44 years (Fig. 63, graph B).

Indirect fluorescent antibody test

There were some differences between Hb AA and Hb AS, but they

Fig. 63. Precipitin test with *P .falciparum* antigen: age-specific mean number of bands of precipitation (and 95% confidence limits) in persons with haemoglobin AA (o) and haemoglobin AS (•) genotypes (A) in the wet season of 1971 before intervention, and (B) in the wet season of 1973 after 70 weeks of antimalarial intervention



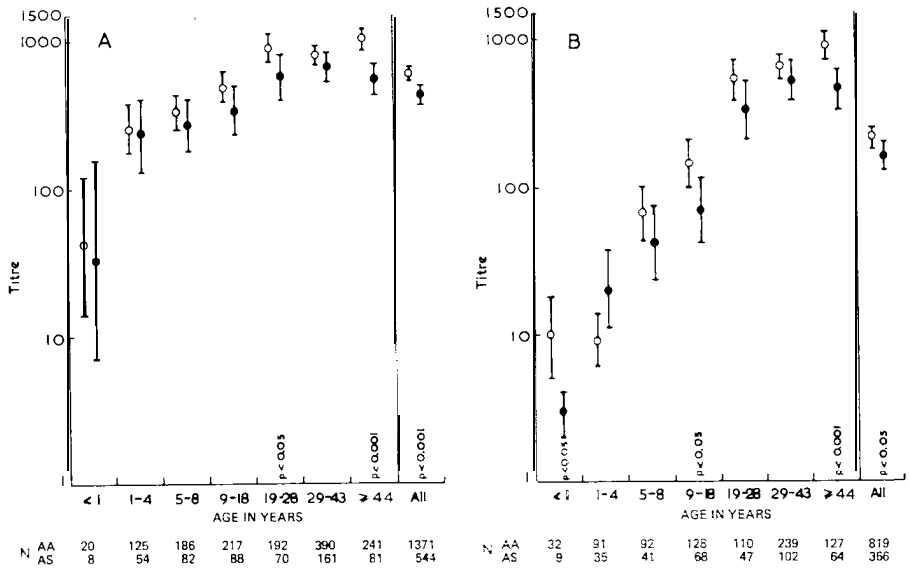
were not very consistent. With a ***P. falciparum*** antigen, at the first survey, the mean titre was higher in Hb AA subjects except in the age-groups 19-28 and ≥44 years, but none of the differences was significant. However, in the second survey (dry season 1972), the mean titre was higher in Hb AA in the age-groups 5-8 ($p<0.01$) and 9-18 years ($p<0.05$) and for all ages combined ($p<0.05$). Protection was followed by a sharp decline of titre in both groups, especially in the younger age-groups and a decrease of the difference between the two groups.

With a ***P. malariae*** antigen, titres tended to be lower in Hb AS subjects but differences were less regular than with the other antibody tests, and none was significant at either of the first 2 surveys. Protection against malaria was followed by a sharp decline of titre in both groups, especially in the younger age-groups, and there was no consistent pattern of difference between Hb AA and Hb AS.

Indirect haemagglutination test with *P. falciparum* antigen

The second survey was the first occasion on which this test was performed using ***P.falciparum*** antigen. The mean titre was higher in Hb AA subjects in all age-groups, the difference increased with age

Fig. 64. IHA test with *P. falciparum* antigen: age-specific mean titre (and 95% confidence limits) in persons with haemoglobin AA (o) and haemoglobin AS (•) genotypes (A) in the dry season of 1972 before intervention, and (B) in the wet season of 1973 after 70 weeks of antimalarial intervention



throughout life, and was significant in the age-groups 19-28 and ≥44 years and for all ages combined (Fig. 64, graph A). The titres decreased in the protected population but remained nearly systematically higher in the Hb AA than Hb AS subjects at the end of the second wet season under protection, the IHA titre was still significantly lower in the AS below 1 year of age, in the age-groups 9-18 and ≥44 years and for all ages combined; in the age-group l-4 years the difference was reversed (Fig. 64, graph B).

Immunology by sex, tribe and haemoglobin

Serological differences between Hb AA and Hb AS subjects were similar in males and females, and in both the Hausa and the Fulani.

Immunology, malaria parasitology and haemoglobin

A negative correlation was observed in this population between the IgM, Ouchterlony precipitation bands and IHA titre against ***P.falciparum*** antigen on the one hand and ***P. falciparum*** frequency and density on the other (see pp. 194-199 and 207-210). This pattern was found in both Hb AA and Hb AS subjects, without any significant difference between the two groups.

Findings in the Mb SS Persons (see 120)

There were 147 blood examinations performed on a total of 14 Hb SS persons. The number of blood films positive for ***P. falciparum*** trophozoites, ***P. falciparum*** gametocytes and ***P. ovale*** was less than expected, but not significantly so. The Hb SS persons were remarkably less frequently positive for ***P. malariae*** than expected. The difference was very significant ($p<0.01$) but may have been exaggerated by dependence between surveys, and individual histories should be considered. All Hb SS individuals contributed to the results, and in particular 9 persons in the age-group l-4 years contributed from 1 to 16 examinations; 2 Hb SS persons aged l-4 years were examined at all 8 baseline surveys and both were always negative for ***P. malariae*** ($p = 0.036$, or 0.19^2, where 0.19 is the frequency of the event (8 negative results) in the reference population).

When Hb SS subjects were positive for ***P. falciparum*** below the age of 5 years, the density of trophozoites was more frequently below the median of the corresponding population than above ($p<0.05$). When the

results were analysed by person rather than by film, it was found that among the 8 persons concerned 6 were more frequently below the median, 1 more frequently above ($p = <0.055$ in a one-tailed sign test).

The 14 subjects with Hb SS were examined at 1 or more of the 8 serological surveys by 1 or more of the 6 serological tests.

IgM concentrations were significantly more frequently above than below the average of the reference group after the age of 1 year. There was no significant pattern in the distribution of IgG levels.

Antimalarial antibody titres were more frequently below than above average in the indirect fluorescent antibody (IFA) test against *P. falciparum* and *P. malariae* and in the indirect haemagglutinating antibody (IHA) test against *P. knowlesi* (2 tests only, both at above 1 year and both below average) or *P. falciparum.* The differences were weaker (not significant) below 1 year of age. The number of precipitation bands against *P. falciparum* in the Ouchterlony test were also more often below average than above, but not significantly.

Results obtained from the same person were not independent, so individual histories were analysed. Hb SS individuals over 1 year of age were significantly more often below average than above average as regards their IFA titres against *P. falciparum* and *P. malariae.* They were more frequently below than above average as regards the IHA titres against *P.falciparum* (or *P. knowlesi)* and more frequently above than below average for IgM concentration, but these differences were not significant.

Findings in the Hb SC Person

There was 1 male, of about 45 years, who had Hb SC. He was examined in 10 parasitological surveys without antimaiarial protection; he was found to have all forms of parasitaemia slightly more frequently than the average for his reference group (for example, *P.falciparum* trophozoites were observed 7 times, the expected number being 3.2). However, none of the differences was significant. The same person was examined in the 8 serological surveys for IgG, IgM, Ouchterlony precipitin and IHA; his IgG and IgM levels were more frequently above than below average, but no result was exceptional.

Findings in the Hb AC Persons (see 153)

Altogether 269 observations were made on 21 Hb AC subjects and compared with their appropriate reference group. The differences be-

tween the observed number of positive blood films and the expected number were mostly small and unsystematic. Parasite densities also showed no systematic or significant difference between Hb AC persons and the general population.

The 21 Hb AC subjects were examined at 1 or more of the 8 serological surveys by 1 or more of the 6 tests. No significant differences were found between Hb AC subjects and their appropriate reference groups for IgM concentration or any of the antimalarial antibody tests. However, IgG concentrations in Hb AC were more frequently above average than below ($p<0.05$), and this tendency was most obvious in the protected population during the period of antimalarial intervention, which lasted over 2 transmission seasons and the intervening dry season. Of 22 observations on 7 individuals at 3 surveys during intervention, 19 were above average ($p<0.01$).

ABO Blood Groups and Malaria

The distribution of the study population according to ABO blood groups is discussed in a Technical Note (4). The overall frequencies were 45.5% 0,23.4% A, 26.1 % B and 5% AB. The Fulani had more A and less B than the Hausa.

The ABO blood groups were compared in detail with respect to their parasitological and serological results. No significant or systematic difference was detected. In particular, there was no systematic or significant difference between the ABO blood groups regarding the number of times individual persons were positive during the baseline period (parasitological surveys l-8). There was also no systematic or significant difference between the ABO blood groups during the resurgence of malaria in the post-intervention phase.

Discussion

Haemoglobin genotype frequencies, fitness, and parasitology

The 28.9% of Hb AS and 0.7% of Hb AC observed in Garki confirm what is known about the geographical distribution of abnormal haemoglobins, a subject which has been reviewed by Livingstone (92). In northern Nigeria, among studies using electrophoresis, the frequency of

Hb AS ranged between 19.0% and 32.6% and the frequency of Hb AC between 0.7% and 1.3%. Studies using the sickling test yielded a lower range of frequencies for the sickle-cell trait.

Given the limited viability of the Hb SS, some factor is required to maintain an appreciable frequency of Hb S in a population; even though the mutation rate to this allele is quite high (1.7×10^{-3}) it is inadequate without some additional factor of favourable selection (1.55). There is quite conclusive clinical and epidemiological evidence that Hb S is maintained at high frequency by the partial protection of Hb AS persons against ***P. jiilciparum*** (I, 93). The only previous study of malaria and sickling on a sizeable northern Nigerian population was conducted in the even drier climate of Sokoto (160); the sickling test was positive in 11% and the prevalence of malaria was significantly lower in sickle-cell children than in normal children, a difference which was pointed out by Allison (2), but not by the authors. Partial protection against ***P. falciparum*** in Hb AS subjects was also clearly demonstrated in the present study. Our findings thus confirm those of most earlier workers elsewhere and are in complete contradiction with the more frequent and dense parasitaemia described in Hb AS children in Accra (138).

The most probable mechanism by which Hb AS persons are protected against ***P. falciparum*** is that oxygen consumption by the parasite in the red cell causes sickling, followed by phagocytosis, thus breaking the malaria cycle (94). In addition, with the recently developed continuous culture systems, it has been shown that at a lowered oxygen tension Hb AS and Hb SS cells, when compared with normal cells, exhibit increased resistance to invasion and maturation of ***P. falciparum*** parasites (68a, 131a). The partial protection is translated into a lower mortality. When transmission is intense, as in Garki, immunity to malaria is acquired early and the advantage of the Hb AS is concentrated in the very young age-groups and consists entirely in a better chance of survival, reflected in the age-specific prevalence of the trait. In areas of low transmission, sickle-cell trait still protects against the complications of malaria in pregnancy (3) and so increases female fertility, for example, amongst the Black Caribs (60). In Garki, there was no difference in fertility between Hb AA and Hb AS women. It is probable that women of child-bearing age living in areas of high transmission have attained levels of immunity at which sickle-cell trait confers no advantage, or only protection against the development of gross splenomegaly and anaemia in the occasional pregnant subject (61). Raper has speculated that, as transmission increases, the frequency of the sickling trait increases up to a maximum frequency beyond which a further increase in transmission would so shorten the period of low immunity, in which the Hb AS have an advantage, that the frequency of the trait would decrease (137).

The small parasitological difference observed in Garki between the Hb AA and Hb AS appears insufficient to explain the large difference in survival. As an additional paradox, the parasitological difference was observed in the dry season, whereas the incidence, prevalence and density of ***P. falciparum*** and mortality from malaria are all much higher in the wet season. It should, however, be remembered that malignant ***P. falciparum*** infections last only a few days so that most episodes would be missed by surveys conducted every 70 days; moreover, some ill children may have been relatively inaccessible for study. It is interesting to note that in Zambia, in the first observations to suggest the protection offered by the sickle-cell trait, Beet (6) found a lower prevalence of malaria in sicklers in the dry season but not during the rains, when transmission was highest. The definitive evidence of protection comes from the study of sick children and post-mortems (93).

Serology of the Hb AA and Hb AS

Most of the seroimmunological findings are new. Edozien et al. (52) found that among unprotected children aged 12-26 months in southern Nigeria the Hb AS had on average slightly more gammaglobulins, and interpreted their finding as indicating that the relative protection of Hb AS individuals against malaria resulted from their ability to mount a stronger immune response. In Garki, Hb AS infants had sometimes more IgG and IgM than the Hb AA, but the difference was not very consistent and only rarely significant. After the first year of life, however, the Hb AS had on the average less IgM and less precipitating and haemagglutinating antibody against ***P. falciparum*** than the Hb AA; they also tended, albeit somewhat less consistently, to have less IgG and fluorescent antibodies against both ***P. falciparum*** and ***P. malariae.*** Even when they were significant, these serological differences were not large, and there was considerable overlap between individual results. It is remarkable that the 3 tests for which the Hb AS have clearly lower results are precisely the 3 tests whose results were found to be associated with parasitological protection (see Chapter 6 and Ref. 40); it is also remarkable that these serological differences become detectable at the age at which the parasitological differences cease to be detectable. The following interpretation is proposed. The Hb AS cells' resistance to parasitic invasion and growth, and their sickling and phagocytosis ***(64a, 94, 131a),*** reduce the antigenic stimulation and hence the immune response in the Hb AS. To dispose of parasites, however, their immune response is assisted by sickling itself, so that there is no difference in parasitaemia. In infancy, however, when neither the Hb AA nor the Hb AS have much immunity, the sickling trait gives a clear parasitological advantage to the Hb AS; it is also possible that the very beginning of their immune

response is facilitated by an effect of sickling on the processing of antigen, which could explain a transitory rise of their immunoglobulin levels above those of the Hb AA.

The above interpretation would also be congruent with 3 more observations, 2 made in Garki, the other from the literature:

(1) the serological differences between Hb AA and Hb AS tend to disappear when antigenic stimulus and immune status are reduced by mass drug administration;

(2) during resurgence of malaria in a population whose immunity status has been depressed by drugs (see Chapter 5), the parasitological advantage of the Hb AS over the Hb AA extends to older children and adults;

(3) the sickle-cell trait gives almost complete protection against the tropical splenomegaly syndrome, characterized by high IgM and antimalarial antibody levels (10, 61, 76).

The Hb SS

Hb SS persons tended to have fewer ***P. falciparum*** parasites and lower IFA and IHA titres against ***P. falciparum*** than the general population. This may be explained in the same way as the similar findings in the Hb AS. The Hb SS had fewer ***P. malariae*** parasites and lower IFA titres against ***P. malariae*** than the general population, while the Hb AS had the same amount of ***P. malariae*** parasites and questionably lower IFA titres against ***P. malariae*** than the Hb AA. It is possible that ***P. malariae*** causes a moderate degree of intracellular hypoxia sufficient to produce a sickling in Hb SS red blood cells, but not in sickle-cell trait cells. Hb F may offer some protection against ***P. malariae (21),*** but it is unlikely to explain the relative rarity of ***P. malariae*** in Hb SS persons in Garki, because this relative rarity was observed mainly in the age-group 1-4 years when Hb F concentrations in cases of sickle-cell anaemia in Africa are generally not more than 10% of total Hb (83).

The Hb AC

Previous workers have failed to demonstrate any resistance of the Hb AC to malaria (93). In Garki also, the Hb AC had the same level of ***P.falciparum*** parasites and the same antibody titres against ***P.falciparum*** as the general population; they may even have had somewhat more ***P. malariae,*** which would not support the suggestion of Livingstone (92), based on geographical distribution, that Hb AC might protect against this species of malaria.

IgG concentrations tended to be higher than average in Hb AC individuals, and this was more noticeable during protection against malaria;

this suggested that Hb AC subjects were better able to produce (or had a long-lasting) IgG against some antigen or antigens other than malaria. This could confer advantage to the heterozygotes in certain environments, sufficient to balance the moderate disadvantage experienced by Hb CC subjects, and so maintain the gene at high frequency. The present study was conducted in an area of West Africa where Hb C has low frequency, and this hypothesis is most likely to be refuted or confirmed in areas of high frequency of Hb AC such as northern Ghana or Upper Volta.

The ABO blood groups and malaria

There is a relatively extensive literature on the ABO blood groups and malaria, which has been reviewed by Livingstone (93). Certain authors found significant differences (not always the same ones), while others found no difference. Wood et al. (166) and Wood (16.5) studied the human host selection of A. ***gambiae*** S.S according to ABO blood-group status; they found that 0 was more attractive than B, which was more attractive than A or AB, in the following ratios: 5:4.3:3.3.

In the present project, no significant difference, either parasitological or serological, was detected between ABO blood groups. The inclusion of all members of a geographically defined population in the present study probably ensured that the different ABO groups were exposed to the same environmental factors, i.e., that the comparison between the ABO groups was unbiased. It is possible that some differences exist at lower levels of transmission; for example, a moderate differential attraction of ***A.gambiae*** s.l. towards the various ABO blood groups could produce a parasitological difference when the average man-biting rate is low, but not when the average man-biting rate is very high, as it was here. However, even during the resurgence of malaria, no significant or systematic difference was detected, in contrast to the concurrent enhancement of the differences between males and females (see p. 156 and Fig. 45) and between Hb AA and Hb AS persons (see p. 218 and Fig. 61).

Summary

Haemoglobin electrophoresis was performed in the population included in the serological study, i.e., in 2 village clusters treated for 1½ years with propoxur and high-frequency MDA of sulfalene-pyrimethamine (every 2 weeks in the wet season, every 10 weeks in the dry season) and in 1 untreated comparison village cluster. Two abnormal haemo-

globins, S and C, were found, at the frequencies expected from the literature. In the whole population (N = 2742) the genotype frequencies were: 70.2% AA, 28.9% AS, 0.7% AC, 0.1% SS, plus a single SC person; these frequencies are very different from those expected under the Hardy-Weinberg law, and reflect the well-known low fitness of the Hb SS genotype, and the increased fitness of the Hb AS genotype. Among 534 newborn, the genotype frequencies were: 73.8% AA, 23.6% AS, 0.6% AC, 2.1% SS; these frequencies fit the Hardy-Weinberg law very closely. This suggests that the advantage of the Hb AS is one of survival, not of fertility, and was confirmed by the study of the age-specific genotype frequencies and by the direct estimation of the fertility of Hb AS and Hb AA women. Differential fertility could, however, be a significant factor at lower levels of transmission, and consequently lower levels of immunity in women of child-bearing age.

In infancy and early childhood, the Hb AS had a somewhat lower prevalence and density of *P falciparum* parasitaemia than the Hb AA, in agreement with most of the literature. During the resurgence of malaria after its near removal for 1½ years, the parasitological advantage of the Hb AS extended into older age-groups.

In later childhood and beyond, the Hb AS had somewhat lower levels of IgM, fewer bands of precipitin against a *P. falciparum* antigen, and lower titres of antibody in the IHA (PHA) *P. falciparum* test. These differences tended to disappear when malaria was reduced to a very low level by propoxur and drugs.

Fourteen Hb SS persons were compared to their reference groups of the same age and from the same time and place, with respect to parasitological and serological results. The most striking finding was the low prevalence of *P. malariae* among the Hb SS.

Twenty-one Hb AC persons were compared to their reference groups. The most striking finding was a somewhat higher level of IgG in the Hb AC. The difference was enhanced when malaria was controlled.

The single Hb SC person was not remarkable either parasitologically or serologically.

No significant parasitological or serological difference was detected between the ABO blood groups.