Chapter Six

IMMUNOLOGY

One of the objectives of the project was to study the immune response of the population, by means of a number of serological tests, over the period before, during and after the application of control measures aiming at the temporary removal of as much of the specific antigenic stimulation as feasible (see Chapter 1). Most of the results presented in the following chapter have already been published (40, 117).

Material and Methods

The study design, the serological sampling scheme, and the control operations applied have been described in Chapters 2 and 3 (see in particular pp. 30,32 and 43-49, Fig. 1 and 5, and Table 1). The timing of the 8 serological surveys in relation to the 3 phases of the project (baseline; intervention; post-intervention) and to the 23 parasitological surveys is shown pictorially in Fig. 43. The degree of parasitological control achieved was described in Chapter 5. In summary: (1) the seroimmunological study was conducted from 1971 to 1975 in 2 village clusters treated in 1972 and 1973 with propoxur and high-frequency distribution of sulfalene-pyrimethamine (about 1800 persons, see Table 1) plus 1 untreated control village (about 1150 persons); in late 1972 and in 1973 infants were excluded from MDA unless and until found positive; in the wet season of 1974, in the previously treated population, 4 rounds of chloroquine were administered at 5-week intervals to those aged less than 10 years (see pp. 48-49); (2) during the intervention period (1972-1973), the prevalence of malaria was reduced to 1-5% ; transmission was

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a The work described in this chapter was done by or under the supervision of Dr R.L. Cornille-Bragger, Mr T.S. Ashkar and Dr H.M. Mathews, with the advice and assistance of Dr I.A. McGregor, Dr A. Voller, Dr I.G. Kagan and Dr D.S. Rowe, and with the assistance in the field of Mr J. Storey.

brought to a very low level, but not interrupted; (3) during the post-intervention phase (1974-1975), there was a rapid resurgence of malaria, and the parasitological findings demonstrated a moderate loss of immunity in the previously treated population.

The serological methods selected were listed in Chapter 2 (see p. 33). Some details regarding their utilization in the project are given below; further information is available elsewhere (38, 39, 173, 174).

The levels of immunoglobulins G (IgG) and M (IgM) were determined by a modification of the method described by Mancini et al. (101). Working standards (IR 0172 Standards) were prepared especially for the investigation so as to take into account the very high levels of serum IgG and IgM in the study population. The IgG standard contained 520 international units per millilitre (IU/ml) as defined by Rowe et al. (141). The IgM standard contained 915 IU/ml. The results are expressed in IU/ml or as a percentage of the IR 0172 Standards. The international reference preparation WHO 67/97 (see 141) was included on each plate and processed in the same manner as the specimens. The precision and accuracy of the method as used in Garki are discussed in a Technical Note (12).

The precipitating antibodies against ***P. falciparum*** antigen (Ouchterlony test) were detected by a modification of the method of McGregor et al. (110). The number of bands of precipitation was recorded. Antigen 672, used for serological surveys 1-5, was procured from the heavily infected placental blood of a Nigerian (Ibadan) woman; antigen P1 14, used for serological surveys 6-8 was procured from similarly infected placental blood of a Gambian woman. Methodological aspects have been discussed in Technical Notes (34, 36). Occasionally bands of precipitation were observed between adjacent sera on the plates, indicating the presence of soluble antigens; such "S" antigens were first reported from the Gambia (111); the cases detected in Garki are reported in a Technical Note (3 7).

The indirect fluorescent antibody (IFA) titres were determined by the method of Voller & O'Neill(157). Antigen films of ***P. falciparum*** (Lagos strain), ***P. malariae*** (Ward strain USA), and ***P. brasilianum*** were prepared by Dr Voller from the blood of experimentally infected Aotus monkeys. The ***P. brasilianum*** antigen replaced the ***P. malariae*** antigen for serological surveys 6-8. A monospecific sheep anti-human IgG conjugate was used. In the older age-groups, the IFA test was performed only on a subsample of the population. A limited amount of work was also performed with a monospecific anti-human IgM conjugate; titres were much lower, even after separation of the IgM fraction of the serum (33).

The passive (or indirect) haemagglutination antibody (PHA or IHA) test was performed at the Center for Disease Control, Atlanta, GA,

Table 21

Age-specific prevalence of *P. falciparum* and *P. malariae* before intervention; comparison of parasitological and serological findings

Parasite species	Test		Definition of "positive"	Prevalence by age-group (in years)[a]					
				1-4	5-8	9-18	19-28	29-43	244
P. falciparum (*P.f.*)	Parasitological surveys No. 1-8		≥1 film positive for *P.f.*	0.98 (272)	0.99 (359)	0.95 (395)	0.76 (392)	0.78 (731)	0.78 (458)
	Serological survey No. 2	Precipitin	>/1 band	0.92 (265)	0.99 (358)	1.00 (395)	0.99 (391)	1.00 (731)	1.00 (454)
		IFA	≥20	1.00 (198)	1.00 (186)	1.00 (123)	1.00 (53)	1.00 (77)	1.00 (51)
		IHA	≥16	0.91 (209)	0.94 (309)	0.96 (369)	0.98 (338)	0.98 (628)	0.99 (367)
		IHA	≥32	0.87	0.91	0.93	0.97	0.98	0.99
P. malariae (*P.m.*)	Parasitological surveys No. 1-8		≥1 film positive for *P.m.*	0.53	0.81	0.61	0.26	0.30	0.30
	Serological survey No. 2	IFA	≥20	0.95	1.00	1.00	1.00	1.00	1.00

a The figures in parentheses represent the number of persons examined at all 8 baseline parasitological surveys or by each serological test at the end of the baseline period (second serological survey, coinciding with the 8th baseline parasitological survey); the same number was examined by the IFA-Pm as by the IFA-Pf tests.

USA. The specimens from the first serological survey were tested against a P. ***knowlesi*** antigen according to the method of Rogers et al. (139). The specimens from serological surveys 2-8 were tested against a ***P. falciparum*** antigen according to the method of Mathews et al. (102); antigen was prepared from ***Aotus*** monkeys infected with blood-passaged ***P. falciparum.*** About 250 sera were retested blindly in Kano by the method of Meuwissen (113); there was relatively good correlation ($r = +0.711$) between the results with the 2 methods (35).

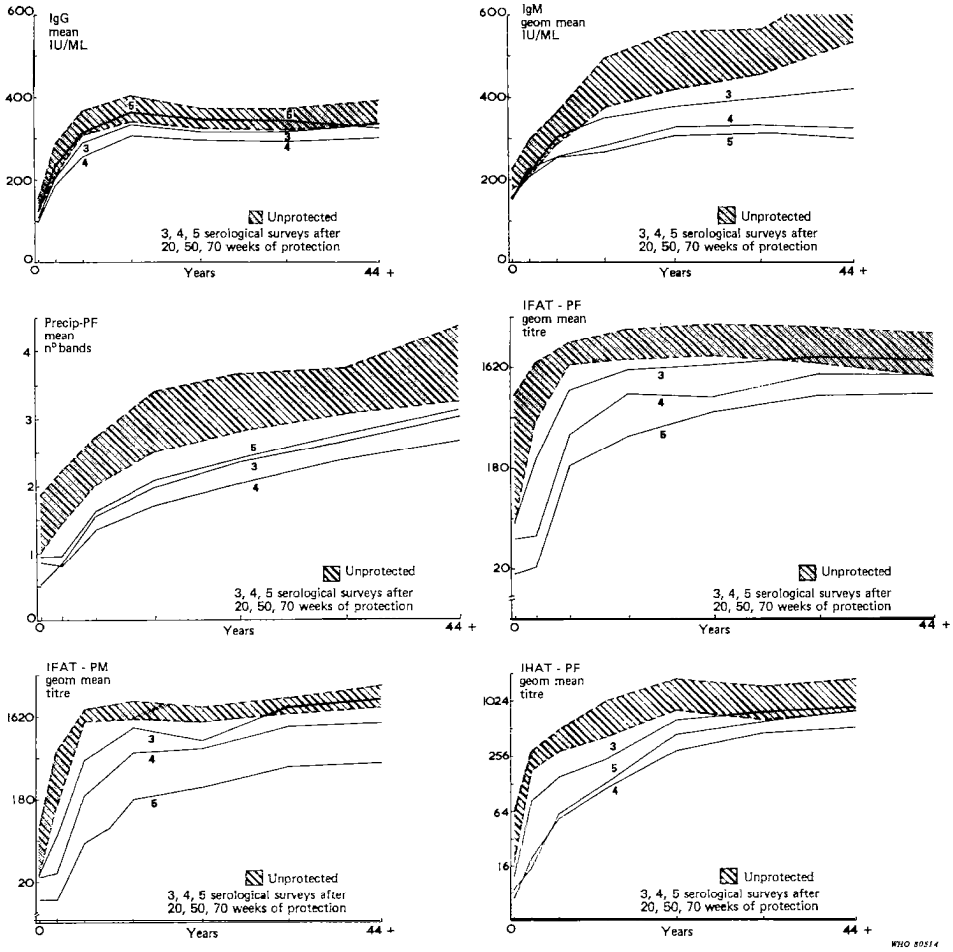
Measurement precision within a survey was monitored by repeated testing either of specimens collected or of standards; comparability between surveys was monitored by the re-examination of specimens from a given survey at subsequent surveys. Some moderate changes in sensitivity were observed, which should be taken into account in the interpretation of the findings: for IgG and IgM, an increase between serological surveys 7 and 8; for the precipitin test, an increase between serological surveys 3 and 6 and a decrease between serological surveys 6 and 8; for the IFA-Pf test, an increase between serological surveys 4 and 5 and a decrease between serological surveys 7 and 8; for the IFA-Pm or IFA-Pb test, a decrease between serological surveys 5 and 8.

Serological Results by Age, Survey and Treatment

Within each survey, differences between villages treated in the same way were neither systematic nor significant; therefore the results have been analysed by age and serological survey in both the unprotected population (all villages during the baseline period; 1 village cluster during and after the intervention period) and the protected population (2 village clusters during and after the intervention period).

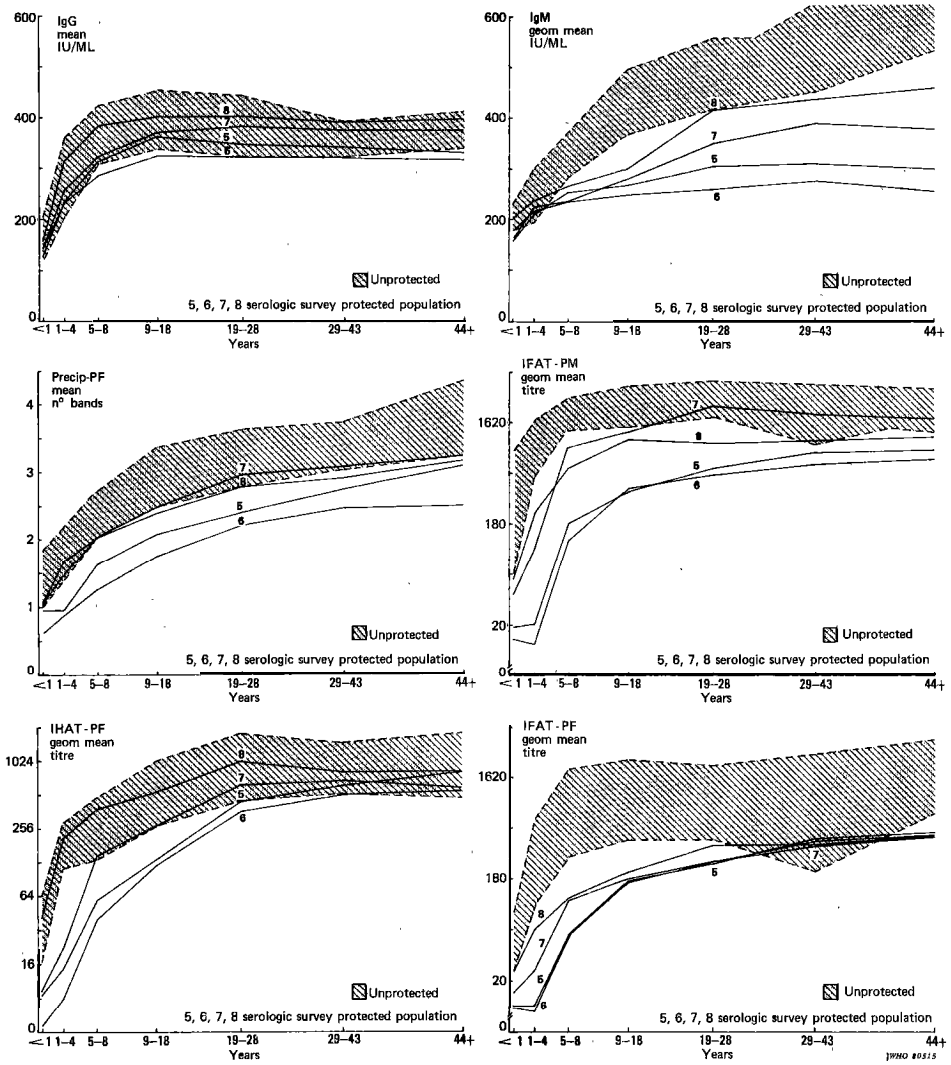
The results for each test are presented in this section and are illustrated in a series of figures and 1 table. Table 21 shows the proportion of the population aged 1 year or older that was positive by each specific serological test before intervention. The numbers examined given in the table are typical of the numbers examined serologically per survey and age-group. Figure 48 shows the average results of each test by age, as the range of 5 surveys in the unprotected population, and after 20,50 and 70 weeks of control in the protected population (i.e., at serological surveys 3,4 and 5. Figure 49 shows the average results as the range of 7 surveys in the unprotected population, and at the end of the intervention phase (and 5, 10 and 22 months later) in the protected population (i.e., at serological surveys 5, 6, 7 and 8). Figures 50 and 51 show the detailed distribution of the results of 4 tests, in selected age-groups of the 2 popu-

Fig. 48. Levels of IgG, IgM and malaria antibodies, by age, treatment and survey, in the unprotected population (range of 5 surveys) and in the treated population at serological surveys 3, 4 and 5



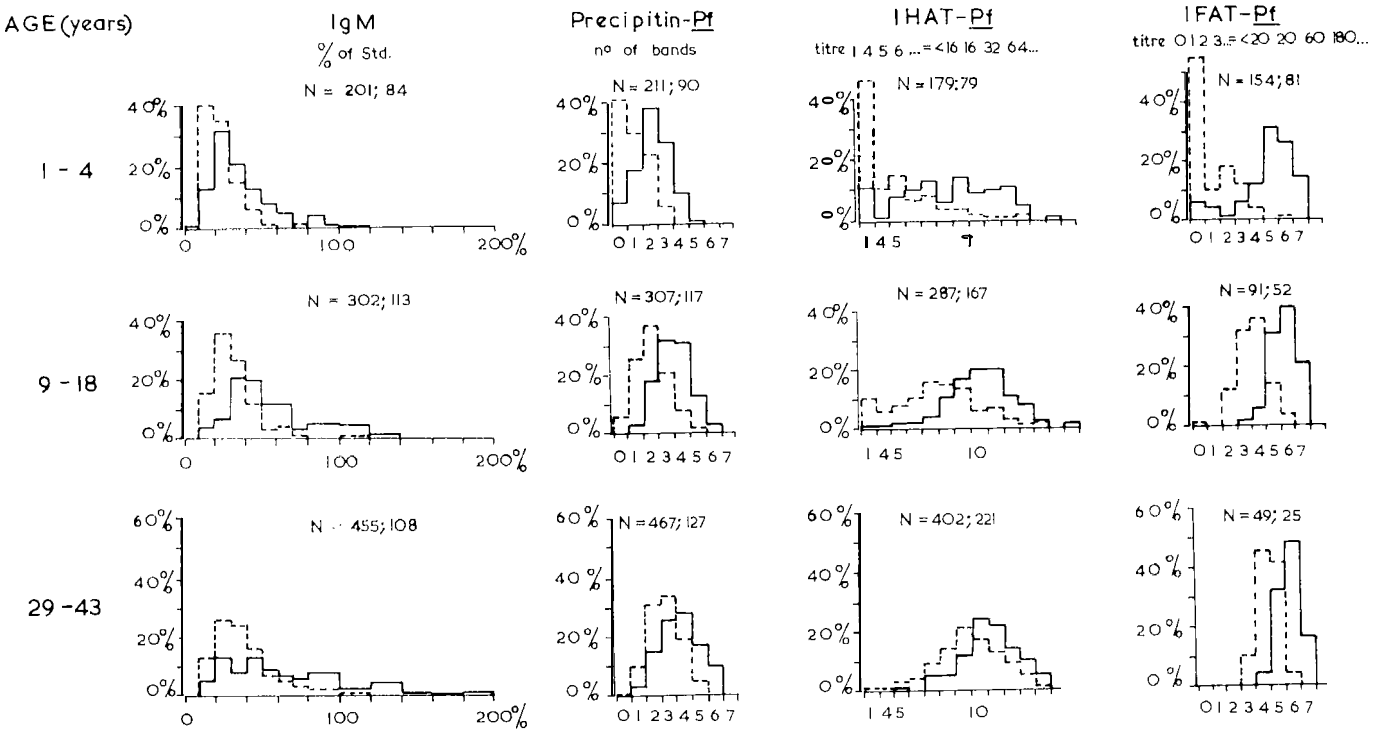
lations, at the end of the intervention period (i.e., at serological survey 5, in the wet season of 1973) and at the end of the follow-up period (i.e., serological survey 8, in the wet season of 1975); the 2 remaining tests-JgG and IFA-Pm or IFA-Pb-are excluded because the first showed very little difference between the 2 populations and because the

Fig. 49 Levels of IgG, IgM and malaria antibodies, by age, treatment and survey, in the unprotected population (range of 7 surveys) and in the treated population at serological surveys 5,6,7 and 8, i.e., after 16 months of protection and 5,10 and 22 months after the end of the intervention phase



second behaved nearly like the IFA-Pf. Figure 52 shows, by survey, the age-adjusted average and 2 selected age-specific averages of each test in the protected population, expressed as a fraction of the concurrent result in the unprotected population.

Fig. 50. Distribution of IgM levels and results of the precipitin-*P. falciparum*, IHA-*P. falciparum* and IFA-*P. falciparum* tests in the protected (broken lines) and unprotected (solid lines) populations in 3 age-groups, after 1½ years of protection (serological survey 5)[a]



[a] N = No. protected; No. unprotected. The 2 populations are significantly different in all cases: $p < 0.001$ by the χ^2 test.

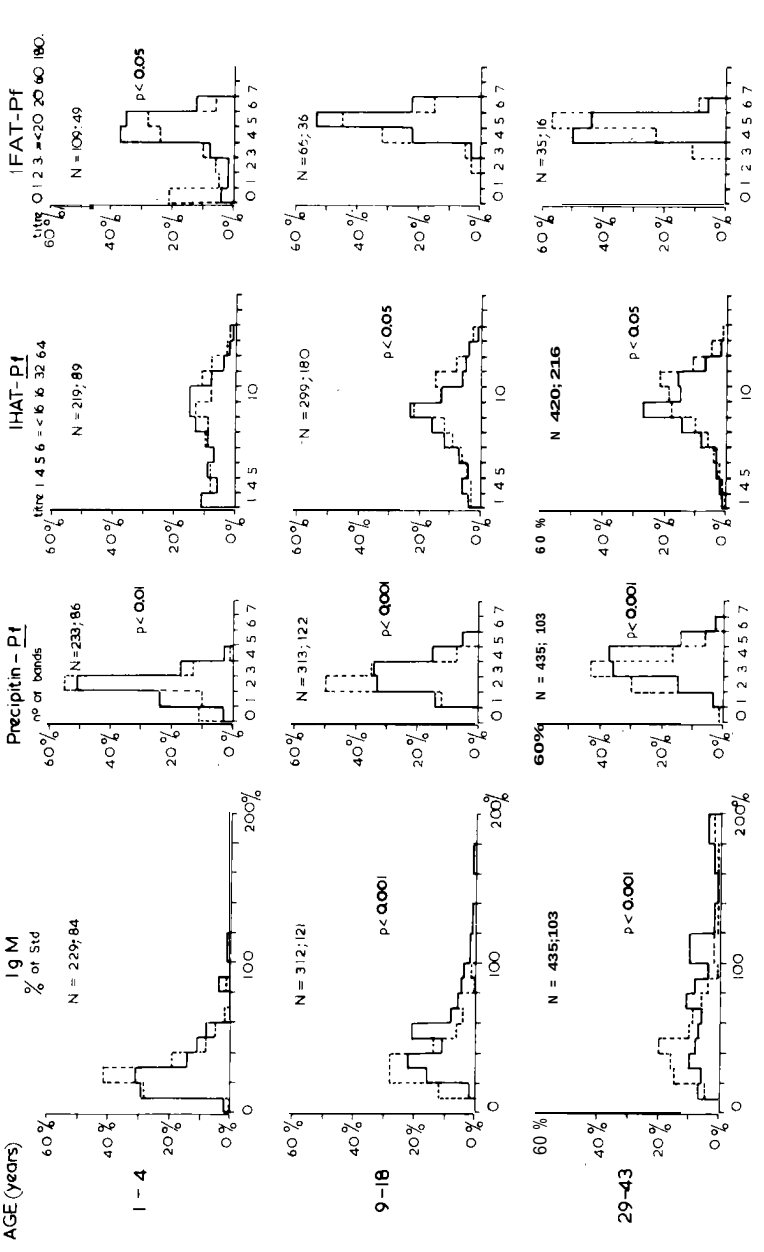


Fig. 51. Distribution of IgM levels and results of the precipitin-P. *falciparum*, IHA-P. *falciparum* and IFA-P. *falciparum* tests in the protected (broken lines) and unprotected (solidines) populations in 3 age-groups at serological survey 8, i.e. 2 years after the end of the intervention phase a

a N = No. protected; No. unprotected. The values of p were determined by the χ^2 test.

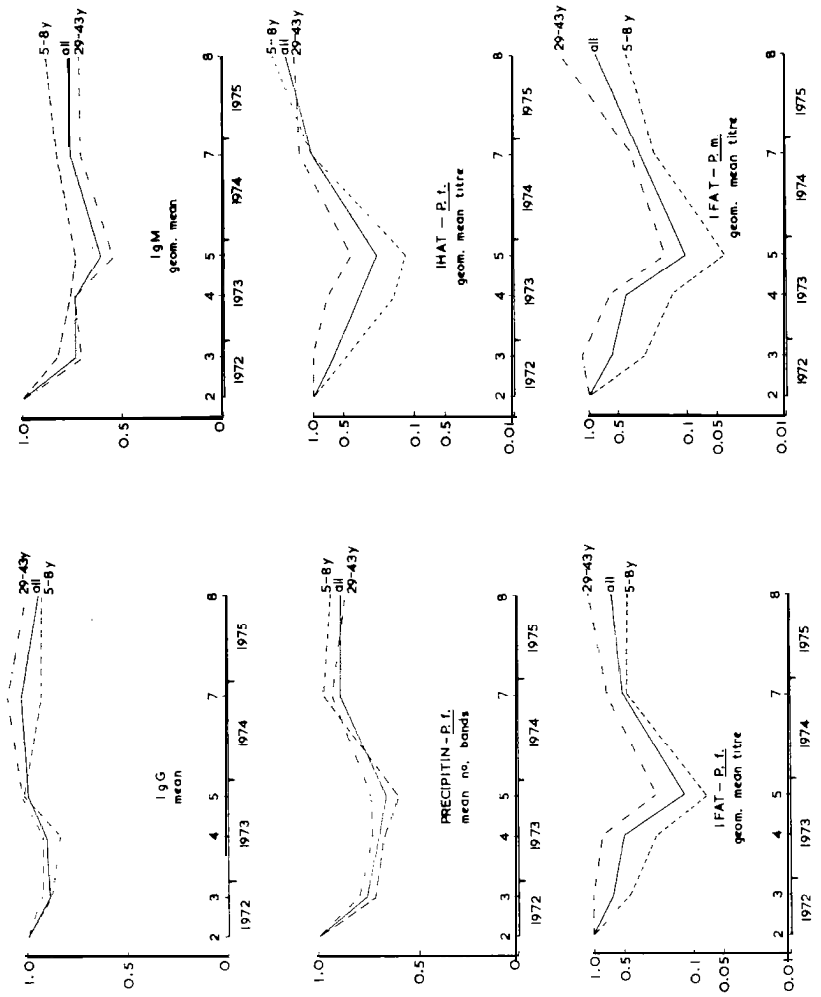


Fig. 52. Serological results during the intervention phase (1972–1973) and 1 and 2 years after the end of the intervention phase, expressed as a fraction of the concurrent results in the untreated control population

Immunoglobulin G

In the unprotected population, the mean serum concentration of IgG was about 150 IU/ml in the infants, rose to about 250 IU/ml in the 1-4 year age-group, then to 300-400 IU/ml in the 5-8 year age-group and stayed at that level in the older age-groups (Fig. 48). In the protected population, a slight decrease was observed after 20 weeks of control in the 1-28 year age-groups, and after 50 weeks of control a further slight decrease was found in the age-groups above 1 year of age. After 70 weeks of control, however, an increase over the results of the previous survey was observed in all age-groups, and mean levels of IgG observed being the same as in the unprotected population in the 5-43 year age-groups and slightly lower in the other age-groups. During the follow-up period, there was no great difference between the protected and unprotected populations (Fig. 49).

Immunoglobulin M

In the unprotected population, the mean level of serum IgM was about 200 IU/ml in the infants and rose gradually with age up to 500-600IU/ml in the older age-groups. After 20 weeks of control, there was a decrease in all age-groups that was more marked in those over 19 years of age. After 50 weeks of control, an additional decrease was observed in the older age-groups (Fig. 48), and during the same dry-season period a decrease was also noted in the unprotected population. After 70 weeks of control, the level of IgM seemed to remain stable in the age-groups < 1, 1-4 and 5-8 years and a further decrease was observed in the older age-groups (Fig. 48). At the same time, an increase was noted in the unprotected population SO that the difference between protected and unprotected population became the largest observed in the course of the project (see Fig. 52). This difference, very significant in all age-groups, was especially large in the older age-groups (Fig. 50 and 52). The distribution of the results of that survey also showed a greater percentage of persons with a low level of IgM in the protected than in the unprotected population and this was particularly noticeable in the ≥9-years age-groups (Fig. 50).

In the previously protected population, the average IgM level decreased from surveys 5 to 6, increased rapidly from surveys 6 to 7 and somewhat more slowly from surveys 7 to 8 (Fig. 49). At the last survey, the IgM level was still clearly lower than in the untreated control population, in particular in the older age-groups (Fig. 51 and 52). In comparison with other tests and results, the IgM level is the one which remained the furthest below its original level.

Precipitin (Ouchterlony) test-P. *falciparum*

In the unprotected population nearly 100% were positive by or before the age of 5 years (Table 21). After infancy the number of bands increased with age throughout life (Fig. 48). At the last survey, results in all age-groups were higher than ever before. In the protected population, a marked decrease in the number of precipitin bands were observed after 20 weeks of control in all age-groups. After 50 weeks of control, a further decrease in the number of bands was found in the ≥ 5 years age-groups. After 70 weeks of control, the number of precipitin bands observed in the protected population had again increased, the results being very similar to those of the third serological survey (Fig. 48). Since a similar or even larger increase from the fourth to the fifth survey was also observed in the unprotected population, the results of the last 2 surveys should be examined in the light of the drift in test sensitivity described above (see p. 176).

At the end of the intervention phase, the difference between protected and unprotected, the largest one observed in the course of the project, was very significant in all age-groups, and was especially large in the younger age-groups (Fig. 50 and 52); the number of negatives (i.e., individuals showing no bands) was much higher in the protected than in the unprotected population in the age-groups < 1, l-4 and 5-8. There were also a few negative results in the older age-groups (Fig. 50).

After the end of the intervention phase, the average number of bands of precipitation in the previously protected population showed a time-trend similar to the one shown by the level of IgM, except that it did not increase between surveys 7 and 8 (Fig. 49 and 52). As already mentioned, there was a decrease in the sensitivity (number of bands detected in a given serum) of the test between surveys 7 and 8. At the last survey, the average number of bands was still less than in the unprotected population in all age-groups except the 5-8 years (Fig. 5 1). In terms of the proportion positive (i.e., having at least 1 band of precipitation) there was still a significant difference in the 3 youngest age-groups: 0.603 versus 0.875 in infants ($p<0.001$)0.837 versus 0.965 in the l-4 years old ($p<0.001$); and 0.977 versus 1.000 in the 5-8 years old ($p<0.05$).

Indirect fluorescent antibody (IFA) test-P. *falciparum*

In the unprotected population 100% were positive by the age of 1 year (Table 21); there was a rapid increase in titre with age up to a plateau that was already reached in the 5-8-year age-group. After 20 weeks of control the titre decreased in the 0-28-year age-groups. After 50 weeks of control there was a further decrease in antibody titres in the 0-28-year age-groups and a smaller decrease in the ≥ 29-years age-groups. After 70

weeks of control there was again a decrease in antibody titres affecting all age-groups (Fig. 48). At the end of the intervention phase, the IFA-Pf antibody titres in the protected population had decreased to the lowest mean value observed, whereas in the unprotected population, after a slight decrease at the end of the dry season (fourth serological survey), the titres had reached their highest value at the fifth serological survey. The difference between protected and unprotected was the largest one observed in the course of the project; very significant in all age-groups, it was especially large in the younger age-groups (Fig. 50 and 52). In the protected population the proportion of sera with "negative" results had markedly increased in the age-groups < 1 and l-4 years, and more sera with lower titres were observed in the older age-groups (Fig. 50).

During the post-intervention phase, the average IFA-Pf titre showed, in the previously protected population, the same time-trend as the result of the precipitin test-i.e., it decreased between surveys 5 and 6, increased rapidly between surveys 6 and 7, and did not increase between the last 2 surveys (Fig. 49). In this last interval, it decreased in absolute terms (Fig. 49) but increased in relation to the control population (Fig. 52), and there was also some of the decrease in test sensitivity (titre of a standard positive serum) already mentioned. At the last survey, the average titre was still lower than in the control population and the difference was significant below 9 years of age (Fig. 51).

Indirect fluorescent antibody (IFA) test-P. *malariae or P. brasilianum*

In the unprotected population 100% were positive at or before the age of 5 years (Table 21); the IFA-Pm antibody titres rose with age more slowly than for P.falciparum. During the intervention phase, the titres decreased progressively in all age-groups following a pattern similar to that of the IFA-Pf antibody titres (Fig. 48), and the number of "negative" sera increased in the O-43-year age-groups. After 70 weeks of intervention, almost all sera were negative in the age-groups < 1 and l-4 years and more sera with lower titres were observed in the 5-8-year age-group.

During the post-intervention phase, in the previously protected population, there was no decline in average titre between surveys 5 and 6 (Fig. 49), although there was a decline in test sensitivity (titre of a standard positive serum). There was a very slow increase from surveys 6 to 7 to 8 (Fig. 49 and 52). During the same period, there was a further apparent decrease in test sensitivity and in the titres of the untreated control population. At the last survey, average titres were still lower than in the untreated control population, and the difference was significant at ages l-18 years.

Passive indirect haemagglutination (IHA) test-P. *falciparum*

A different antigen (P. *knowlesi)* was used in the first serological survey, the results of which were very different from those observed at the subsequent surveys using a specific antigen ***P.*** *falciparum,* thereby suggesting the greater sensitivity of the specific antigen. The results of this first survey Will not be discussed further in the present section.

In the unprotected population, the proportion positive in all age-groups was slightly lower by the IHA than by the precipitin and IFA tests (Table 21); after infancy, the titres for IHA-Pf rose with age up to a plateau reached by the age of 19-28 years. After 20 weeks of control, there was a decrease in titres in the l-28-year age-groups. After 50 weeks of control, the titres were further decreased in all age-groups. After 70 weeks of control there was a slight rise in the titres in all the ≥ 1-year age-groups (Fig. 48). This last change observed in the protected population corresponds to an even greater increase in titres in the unprotected population and this difference between protected and unprotected was the largest one observed; it was very significant in all age-groups after the age of 1 year, being most significant in the younger age-groups (Fig. 50 and 52).

At the end of the intervention phase, a comparison between the protected and the unprotected populations showed a greater number of "negatives" in the age-groups < 1 and l-4 years, lower titres in the 5-18-year age-groups and a small decrease in the age-groups ≥ 29 years (Fig. 50).

After the end of the intervention phase, the average IHA titre in the protected population showed the same time-trend as that of the level of IgM (Fig. 50 and 52); by the last survey, the protected population had higher titres than the control population, in all age-groups, a difference that was significant in all age-groups except the 1-4-years old (Fig. 51).

The Longitudinal Study of Infants

Definition of 2 populations of infants

The serological results among infants born after parasitological survey 1 were used to establish their average serological history. Because the surveys were conducted at lO-week intervals, an infant was assumed to be 5, 15, 25, 35 . . . weeks old, at the first, second, third, fourth . . . parasitological surveys after birth. The following groups were compared:

(1) unprotected infants, including all those born in the untreated villages (follow-up cluster No. 2) after parasitological survey 1 and those

born in the treated villages (follow-up clusters No. 5 and 7) prior to treatment, i.e., up to parasitological survey 8; the oldest unprotected "infants" included were 135 weeks old;

(2) infants protected from birth, including only those born after parasitological survey 8 in follow-up clusters No. 5 and 7; the oldest protected "infants" included were 65 weeks old.

Among the unprotected infants, the subgroup of those who were born after parasitological survey 8, and who were therefore the exact contemporaries of the protected infants, was compared with the total: differences were neither significant nor systematic, with the possible exception of the IFA-P. ***malariae*** test for which the 2 sets of results will be presented. Starting with parasitological survey 8, the protection in follow-up clusters No. 5 and 7 (area Al) had consisted of spraying with propoxur and mass drug administration; infants were excluded from drug treatment until found positive, which happened in a very small number of infants, mostly at low densities and without apparent effect on the serological averages. The parasitological conversion rates for ***P. falciparum*** and ***P. malariae*** were estimated for the 2 infant populations, and the corresponding cumulative prevalence curves were calculated by the formula

$$1-\{\exp(-h(t-n))\}$$

where h = the daily infant conversion rate (ICR), t = age in days, and ***n*** = 10 for ***P. falciparum, 20*** for ***P. malariae.*** The observed cumulative prevalences were found to be very similar to the calculated ones, which are presented for comparison with the serological results.

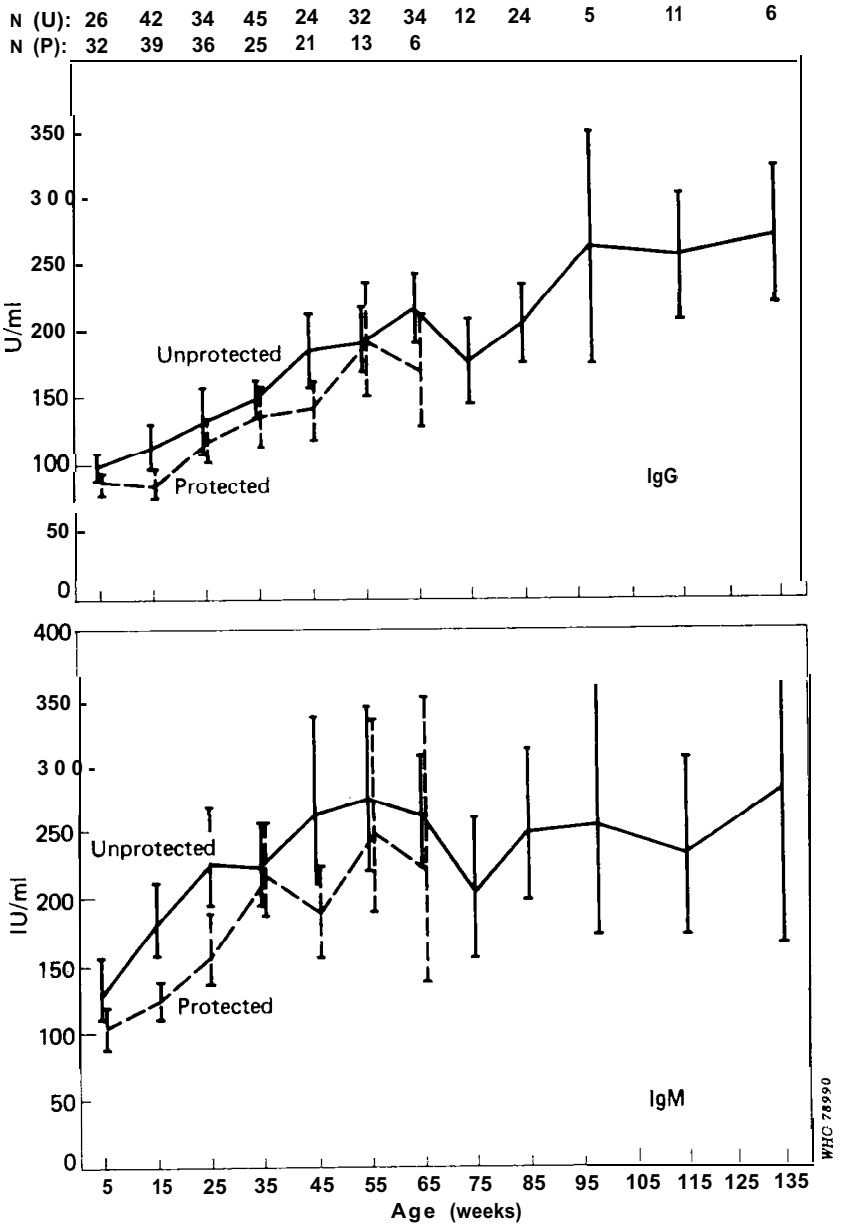
Immunoglobulin levels

In the unprotected infants, the IgG level rose throughout the first 135 weeks of life, from 100 to 275 IU/ml, while the IgM level rose from 125 to 250 IU/ml during the first 35 weeks of life, after which it was relatively stable up to 135 weeks. In the infants protected from birth the levels were slightly but systematically lower (Fig. 53).

Malaria antibody levels

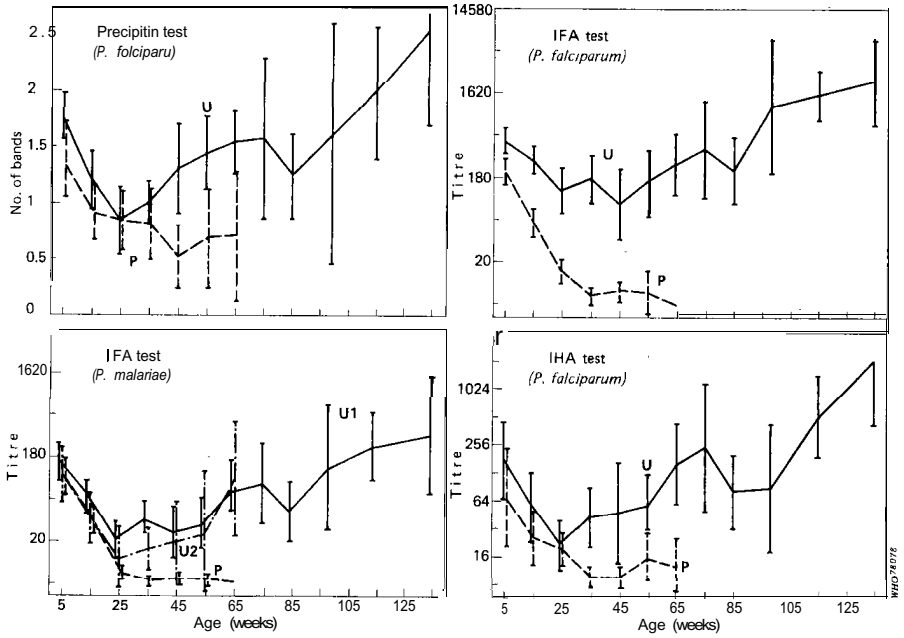
Figure 54 shows the mean antibody levels, by age, in the 2 populations of infants; for computing the geometric means shown for the IFA and IHA titres, an IFA titre of <20 was converted to 6.7 and an IHA titre of < 16 was converted to 8. In the unprotected infants the levels of malaria antibodies fell after birth to a minimum, after which they again rose. The minimum was reached at 25 weeks for the precipitin ***(P.falciparum),*** IFA ***(P. malariae)*** and IHA ***(P.falciparum)*** tests and at only 45 weeks for the IFA ***(P.falciparum)*** test. ,

Fig. 53. Levels of IgG and IgM (mean and 95% confidence limits) in the infants of the unprotected (solid line) and protected (broken line) populations, by age a



a N(U) = numbersexamined in the unprotected population; N(P) = numbersexaminedin the protected population.

Fig. 54. Levels (mean and 95% confidence limits) of precipitating, IF and IHA antibodies against *P. falciparum* and of IF antibodies against *P. malariae* in infants, by age, in the unprotected (U) and protected (P) populations a



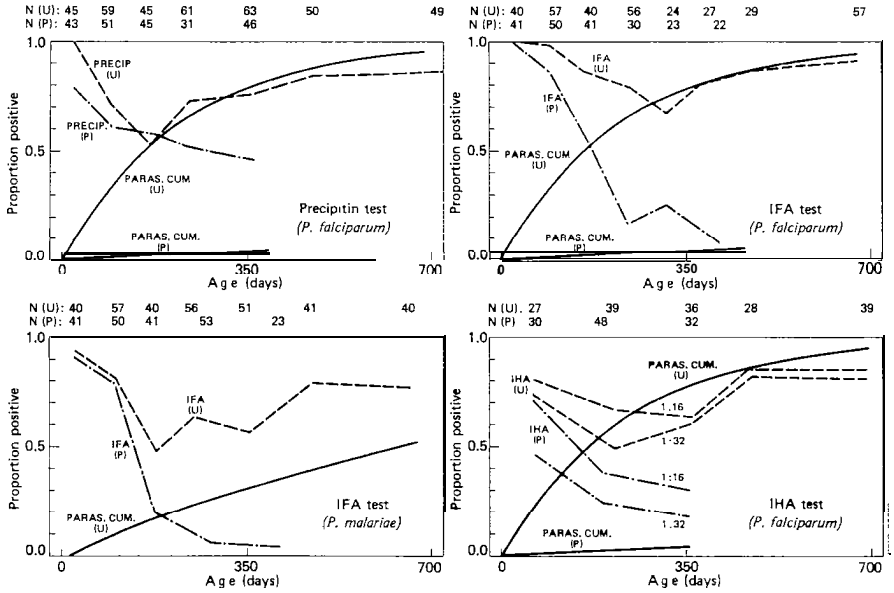
a **UI = total unprotected; U2 = unprotected born after onset of intervention phase. See Fig. 55 for the numbers examined in both populations.**

In the infants born into the protected population, the levels of ***P. falciparum*** antibodies at the first survey after birth, i.e., at about 5 weeks, were lower than in the unprotected infants, and this difference was significant for the number of bands of precipitation and the IFA results. The IFA-Pf, IFA-Pm and IHA-Pf titres fell rapidly to very low "negative" levels, reaching a minimum at the age of 35 weeks for the IFA-Pf and IHA-Pf and at the age of 25 weeks for the IFA-Pm; after that, the titres remained low in contrast to the rise observed in the unprotected population. In the case of the precipitin test ***(P. falciparum),*** the level, expressed as the number of precipitation bands, fell to a point lower than that observed in the unprotected population but still clearly above 0; a minimum was reached by the age of 45 weeks.

Proportion positive by the serological tests

Figure 55 shows the proportion positive in the 2 populations of infants, by age and as detected by the 4 specific serological tests. In the

Fig. 55. Proportion of infants positive for malaria antibodies, by age, compared with the cumulative proportion positive, calculated from the parasitological conversion rate, in the unprotected (U) and protected (P) populations (positive = 1/20 by the IFA test, 1/16 or 1/32 by the IHA test) a



a N(U) = numbersexamined in theunprotected population; N(P) = numbersexamined in the protected population; paras.cum. = cumulative parasite prevalence.

unprotected infants the proportion positive decreased after birth to a minimum, after which it increased steadily; in the infants protected from birth it decreased faster and to much lower levels.

In the unprotected infants, the proportion positive for *P. falciparum* by the precipitin test decreased from 1 .OO at the age of 5 weeks, to about 0.50 at the age of 25 weeks (175 days), after which it increased; between the ages of 175 and 700 days, the proportion positive was very close to the cumulative prevalence expected from the parasitological infant conversion rate estimated in the same population. In the infants protected from birth, the proportion positive was significantly lower in the youngestgroup ($\chi^2 = 8.34$) and continued to decrease beyond the age of 175 days.

The proportion positive for *P. falciparum* antibodies by the IFA test decreased in the unprotected infants from 1 .00 at the age of 5 weeks to 0.67 at the age of 45 weeks (315 days), after which it increased and was very close to the calculated cumulative prevalence of the infection in the same population. In the infants protected from birth, it was also 1 JO at

Table 22

Serological results, at survey 19 or22 (i.e., in thefirst or second wet season after the end of the intervention period) of infants born between surveys 18 and 19, **21** and 22, respective@: comparison between the previously protected and control populations

Village clusters		IgGa	IgM[a]	Precipitin-Pf[b]	IFA-PfC	IFA-Pm[c]	IHA-Pfd
2 (control)	$\bar{x}$	25.2	26.2	1.89	3.44	2.44	6.11
	$S_{\bar{x}}$	5.1	9.1	0.26	0.29	0.18	0.81
	N	9	9	9	9	9	9
5 8 7 (previously protected)	$\bar{x}$	18.1	12.3	1.68	2.78	1.50	6,29
	$S_{\bar{x}}$	1.8	1.3	0.23	0.33	0.20	0.58
	N	16	16	19	18	18	17
		ns.	n.s.	n.s.	ns.	p <O.Ol	n.s.

a Percentage of standard IR 0172.
b Number of bands of precipitation.
c Coded titre, where 0, 1, 2, 3.... = <20, 20, 60, 180
d Coded titre, where 3,4, 5, 6 = <16, 16, 32, 64....

the age of 5 weeks, after which it decreased faster and for a longer time than in the unprotected to about 10% at the ages of 55 and 65 weeks, i.e., not much higher than the cumulative prevalence of infection expected in the same population from the low infant conversion rate.

The proportion of the unprotected infants positive for ***P. malariae*** antibodies by the IFA test decreased from 0.93 at the age of 5 weeks to 0.48 at the age of 25 weeks, after which it increased slowly; in the infants protected from birth, it was nearly the same (0.90) at 5 weeks of age, after which it decreased to much lower levels (about 0.05 at 1 year) than in the unprotected population.

For the IHA test, 2 definitions of positive titres-namely, 16 or higher and 32 or higher-were applied. In the unprotected infants, the proportion positive for ***P.falciparum*** antibodies decreased after birth to a minimum, after which it increased. Its relationship to the calculated cumulative prevalence of the infection is less striking than for the precipitin and IFA tests, but the proportion of infants examined by the IHA was lower. At the age of 25 weeks or over, the proportion with a titre of 16 or higher was a slightly better indication of cumulative prevalence than the proportion with a titre of 32 or higher. In the infants protected from birth, the proportion positive decreased to about 30% or 20%, according to the definition used, by the age of 1 year.

Serological results in the newborn in the post-intervention f ollow-up period

Among the infants examined, a small number were born between parasitological surveys 18 and 19, or between surveys 21 and 22, i.e., they were about 5 weeks old at one of the last 2 serological surveys. The results (Table 22) showed that such newborn of the previously protected population had lower test figures than the controls (the exception was the IHA-Pf), but the difference was significant for the IFA-Pm alone.

Variation by Sex

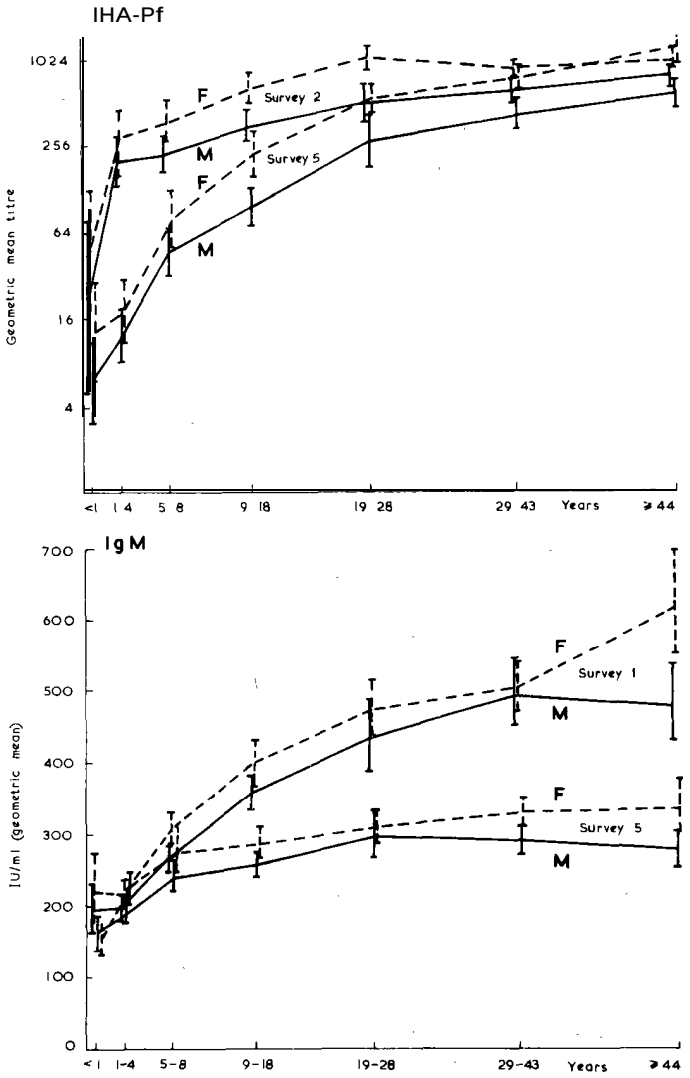
The systematic comparison between the serological results for males and females showed a consistent difference for 2 of the 6 tests: the females had on the average a higher level of IgM and a higher titre of IHA antibodies against ***P,falciparum,*** both before and after intervention with propoxur and drugs. The intervention reduced the average results of both sexes to about the same extent, SO that the difference remained nearly the same (Fig. 56). During the post-intervention period, females continued to have, on the average, more IgM and higher IHA-Pf titres than males. The magnitude of the difference was not apparently affected by the increase in both the female and male averages during the resurgence of malaria.

Relationship between the Results of the Same Serological Test in the Same Personat Different Surveys

Correlation coefficients were calculated for each test and for each pair of serological surveys, in the protected and unprotected populations. The actual variables used were % IgG, log % IgM, number of precipitation bands for the Ouchterlony test, and log titre for the IFA and IHA tests; all these variables had an approximately normal distribution.

Table 23 gives the correlation coefficients between the test results for the 2 populations ***(a)*** at survey 2, just before the onset of intervention with insecticide and mass drug administration, and at survey 5, carried out 70 weeks later, and (b) between the second and last surveys. Except for IgG, the correlation coefficients are all significantly different from 0.

Fig. 56. Levels of IgM and the IIHA-P. *falciparum* titres (means and 95% confidence limits) of males and females, before (survey 1 or 2) and after 70 weeks of protection (survey 5)



They are strong only for IgM and IHA. There was no great difference between the correlations observed in the unprotected and protected populations, and also no great difference between the correlations between surveys 2 and 5 and those between surveys 2 and 8.

Table 23

Correlation coefficients (r) between the results of the same immunological test at serological surveys 2 (dry season, 1972) and 5 (wet season, 1973) or 8 (wet season, 1975) in the unprotected and protected (or previously protected) populations, after adjustment for age a

Test	Surveys2and5				Surveys2and8			
	Population				Population			
	Unprotected		Protected		Unprotected		Protected	
	r	N^b	r	N^b	r	N^b	r	N^b
IgG	0.224	460	0.017'	1218	0.052[c]	366	0.041[c]	1 028
IgG	0.676	458	0.630	1 217	0.666	365	0.661	1 027
Precipitin-Pf	0.287	512	0.330	1285	0.328	371	0.354	1 068
IFA-Pfn	0.207	218	0.356	361	0.263	116	0.186	206
IHA-Pf	0.667	623	0.686	962	0.618	557	0.514	659

a Adjustment for age consisted in transformation of each result into standard deviations above or below the mean for the corresponding age-group

b N = number examined

c Not significant; all the other coefficients in the table are significant at the 1% level.

In summary, the position of a person with respect to the average for his age-group was relatively stable over a period of at least $3\frac{1}{2}$ years for IgM and IHA-Pf, and to a lesser extent also for the precipitin-Pf, IFA-Pf and IFA-Pm. This relative stability was little affected by the intervening period of marked and relatively rapid decrease of the average results during the intervention phase, nor by the subsequent period of increase during the post-intervention phase. For IgM, the strength of the correlation between surveys increased with increasing age, suggesting that the variation within persons decreased with increasing age, while the variation between persons increased (see Fig. 50).

Relationship between the Results of Different Serological Tests in the Same Personat the Same Survey

Correlation coefficients were calculated for each pair of tests, at each survey, in the protected and unprotected populations; the variables actually used were the same as in the preceding section. At the fifth serological survey (Table 24) many of the age-adjusted correlation coefficients were found to be significantly positive in both the populations, and the findings were similar at the other serological surveys. Although

Table 24

Correlation coefficients (r) between the results of different pairs of immunological tests, at serological survey 5 (wet season, 1973), in the unprotected and protected populations, after adjustment for age a

Pair of tests	Population			
	Unprotected		Protected	
	r	*N*	*r*	*N*
IgG-IgM	0.131	561	0.113	1 710
IgG – Precipitin-Pf	0.141	560	-0.040	1 712
IgG – IFA-Pf	0.223b	285	-0.105	527
IgG – IFA-Pm	0.256'	285	-0.116	527
IgG – IHA-Pf	0.235[b]	504	0.005	1 526
IgM – Precipitin-Pf	0.278[b]	558	0.170	1 710
IgM – IFA-Pf	0.091	284	0.006	527
IgM – IFA-Pm	0.077	284	-0.044	527
IgM – IHA-Pf	0.270[b]	502	0.175	1 524
Precipitin-Pf – IFA-Pf	0.3376	298	0.346[b]	551
Precipitin-Pf – IFA-Pm	0.319[b]	298	0.261[b]	551
Precipitin-Pf – IHA-Pf	0.411[b]	544	0.411[b]	1 537
IFA-Pf – IFA-Pm	0.541[b]	302	0.621[b]	551
IFA-Pf – IHA-Pf	0.445[b]	266	0.539[b]	461
IFA-Pm – IHA-Pf	0.265[b]	266	0.3536	461

a Adjustment for age consisted in transformation of each result into standard deviations above or below the mean for the corresponding age-group;

b Significant at the 1% level.

when the correlations were significant, they were not very strong. In both the protected and unprotected populations, the most strongly related pairs were: IFA-Pf and IFA-Pm; IFA-Pf and IHA-Pf; precipitin-Pf and IHA-Pf.

Relationship between Parasitology and Serology

Method of analysis of the baseline data

At each of the 2 baseline serological surveys, for each of the 6 serological tests, and within each age-group, persons were grouped according to their serological results. For example, for the precipitin test 5 groups were defined by the number of bands of precipitation, namely, 0, 1, 2, 3 and >4; while for the IHA-Pf 4 groups were defined by titre, namely, <16, 16-32, 64-128 and $\geq$256. The definitions were the same for all age-groups, and were selected SO as to produce relatively large numbers of different serological results in as many age-groups as poss-

Table 25

Distribution, by test result and age, of persons included in the study of the relationship between serology and parasitology

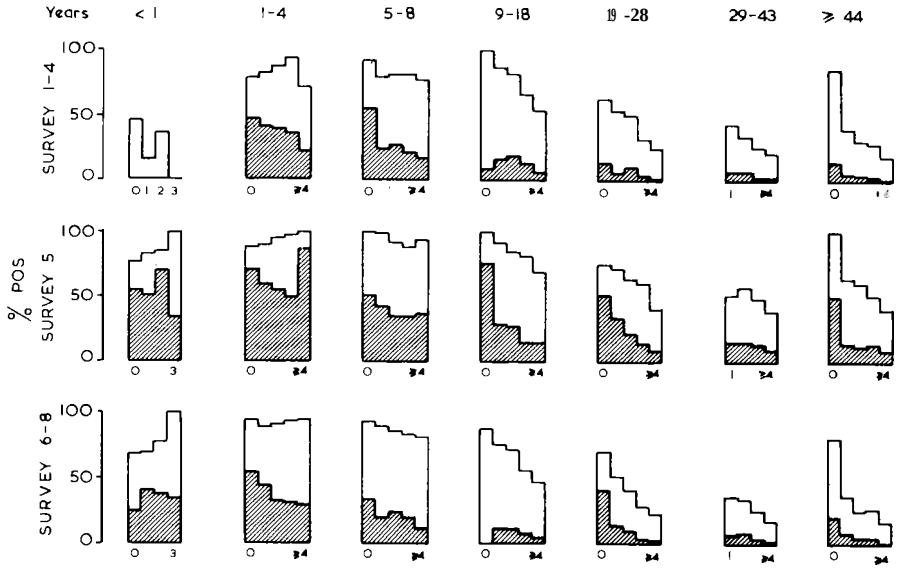
Survey No.	Test	Result	<1 year	1-4 years	5-8 years	9-18 years	19-28 years	29-43 years	≥44 years
5	Precipitin-*P. falciparum* test (No. of bands)	0	13	16	6	4	4		2
		1	42	76	71	39	18	2:	14
		2	13	109	166	117	71	130	51
		3	3	65	120	163	171	286	156
		≥4	0	7	36	73	133	318	236
5	IHAT-P. *knowlesi* (titre)	<16	4	21	70	43	28	51	26
		16-32	7	65	110	128	92	136	90
		64-128	8	36	57	64	80	151	66
		2256	5	25	41	66	108	247	154
8	IH AT- *P. falciparum* (titre)	*<16*	11	19	*19*	14	6	10	3
		16-128	12	65	78	74	41	49	29
				84	159	200	173	362	199
		2≥2 0484	2	41	53	81	118	207	136
8	IFAT-P. *malariae* (titre)	<20	23	9	0	0	0	0	0
		20-180	48	58	16	2	1	0	0
		540		29	17	17	10	7	3
		1 620	39	56	81	54	21	29	20
		≥4 860	0	46	72	50	21	41	28
5	IGM, %IR0172Standard	1 = I-19%	18	86	67	22	22	54	27
		2 = 20-39%	23	110	209	168	120	211	133
		3 = 40-69%	3	17	81	134	146	222	109
		4= ≥70%	2	3	28	39	98	243	164

ible (Table 25). Once the persons were thus grouped according to their serological results, the groups were compared in terms of the parasitological results of the same individual persons throughout the baseline period, i.e., at parasitological surveys 1-8. This allows the comparison of the groups, defined by their serological result at parasitological survey 5 (serological survey 1), in terms of their parasitology before, during, and after the serological survey-namely, at parasitological surveys 1-4, 5, and 6-8 respectively. The parasitological variables examined included the parasite rates (the proportions of persons positive) and the parasite density indices (the proportions of fields of thick blood films positive).

The precipitin test *(P. falciparum)* and *P. falciparum* parasitaemia

Figure 57 shows a strong negative association between the test results and the ***P. falciparum*** parasite rates in the age-groups ≥ 9 years; this association is practically the same whether one considers the parasitology before, during or after the serological survey. In the < 1-year age-group, the association with parasitaemia is negative before the serological sur-

Fig. 57. ***P. falciparum*** parasite rate and ***P. falciparum*** gametocyte rate (shaded) at parasitological surveys 1-4, 5 and 6-8, according to age and to the number of bands (0 to 4+) in the Ouchterlony precipitin-P. ***falciparum*** test at parasitological survey 5



vey and positive during and after the survey. The association probably changes from a positive to a negative one around the age of 5. A negative association is also observable between the test results and the *P. falciparum* gametocyte rates at the age of ≥1 year before, during and after the serological survey; it appears for much earlier ages than the negative association between precipitins and the parasite rate. Variation in the *P.falciparum* trophozoite density, as a function of the test result, was very similar to that in the *P. falciparum* parasite rate. The association with density may become negative earlier in life than the association with the parasite rate. The density of *P.falciparum* gametocytes shows a strong negative association with the test result earlier in life than is the case with trophozoite density.

The IHA-P. *knowlesi* or IHA-P. *falciparum* test and *P. falciparum* parasitaemia

For the IHA test a *P. knowlesi* antigen was used at the first serological survey only. A strong positive association was found between the IHA-Pk titre and the *P. falciparum* parasite rate in the < 1-year age-group, and a strong negative association at ages ≥9 years (Fig. 58, upper half); while this figure illustrates what happens to the parasitology after the serological survey; the same relationships were observed before and during the serological survey. There is a positive association between the IHA-

Fig. 58. *P. falciparum* parasite rate and *P. falciparum* gametocyte rate (shaded) at parasitological surveys 6-8 according to age and IHA titre (<16, 16-32, 64-128, ≥256) or IaM level(1,2,3,4 = I-19%, 20-39%,40-69%, ≥70% of standard) at parasitological survey 5

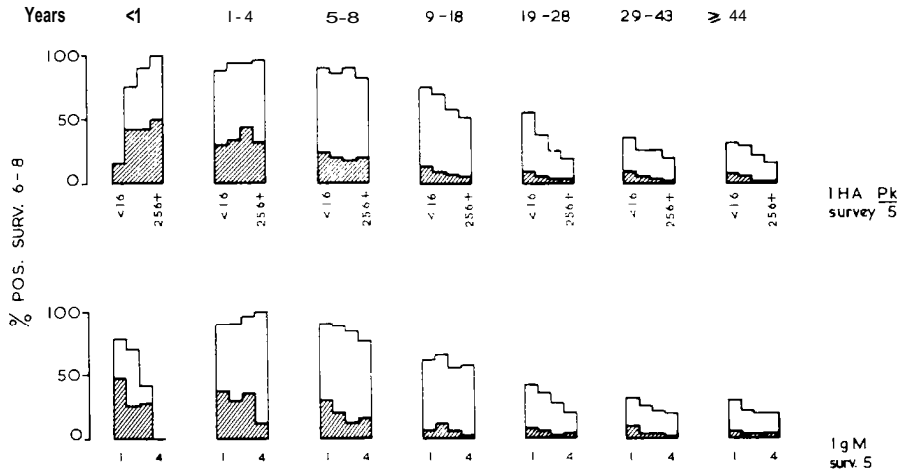
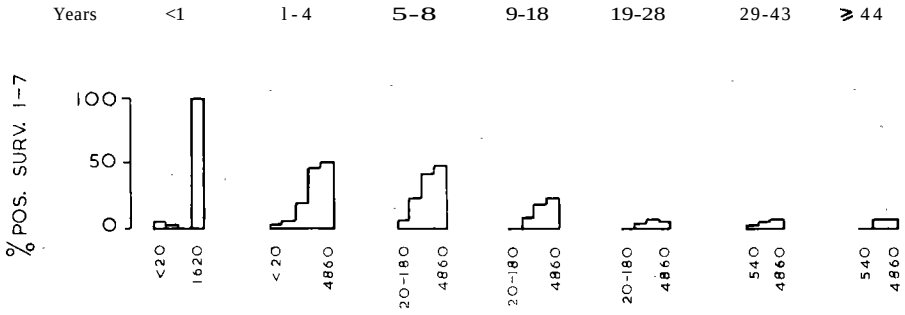


Fig. 59. *P. malariae* parasite rate at parasitological surveys l-7, according to age and titre (<20, 20-180, 540,1620, ≥4860) of the IFA-P. *malariae* test at parasitological survey 8



Pk titre and the ***P. falciparum*** gametocyte rate, in the <l-year age-group and a negative association at ages ≥ 5 years. The same relationships were again observed before and during the serological survey. A high titre is associated with decreased gametocytaemia, and this association may precede the one with decreased parasitaemia. The association of the IHA-Pk titre with ***P. falciparum*** trophozoite and gametocyte densities shows the same trend with somewhat more irregularities. The associations between the IHA-Pf titre at the second serological survey and ***P. falciparum*** parasitaemia are very similar to those just described for the IHA-Pk test at the first survey.

The IFA test and homologous parasitaemia

The associations in this test are less obvious than above. The numbers examined were smaller and several strata were empty or nearly SO. In the case of the IFA-Pf test there was a positive association between the test result and concurrent ***P. falciparum*** parasitaemia up to the age of 5 years and no association for the older ages. In the case of the IFA-Pm test there was a positive association between the test result and ***P. malariae*** parasitaemia in all age-groups; strong up to the age of 19 years, it was weaker thereafter. This association is very clear between the IFA-Pm titre at parasitological survey 8 and the ***P. malariae*** parasite rate at surveys l-7 (Fig. 59), and also between the IFA-Pm titre at survey 8 and the ***P. malariae*** parasite density at surveys l-7.

Immunoglobulin levels and parasitaemia

Figure 58 (lower half) shows the relationship between the IgM level at survey 5 and the ***P. falciparum*** parasite and gametocyte rates at para-

sitological surveys 6-8. The association is negative in most age-groups, including infants. At survey 8, there was a positive association in infants between the IgM level and the ***P. falciparum*** parasite rate; otherwise the trend shown in Fig. 58 was also seen in the remainder of the results concerning IgM.

For the IgG level, no clear-tut association, either positive or negative, was found with parasitaemia by the approach adopted.

Relationship between parasitological and serological findings during and after the intervention phase

The foregoing has shown that during the baseline period 3 test results-namely, the IgM level, the number of bands of precipitation, and the IHA titre-were negatively associated with ***P.falciparum*** parasitaemia except in the youngest age-groups, i.e., parasitological positives had lower serological results (Fig. 57 and 58). By contrast, during the intervention phase, while the average results were decreasing, the association became positive, i.e., parasitological positives had higher serological results, and this positive association extended progressively into older age-groups. The greatest serological difference in that direction between positives and negatives was reached at serological survey 6. After that, while the prevalence of malaria returned towards its baseline level and the serological results were again increasing, the difference between positives and negatives decreased, and then changed signs, starting with the older age-groups. Thus the association between parasitology and serology again became negative, returning to what it was during the baseline phase (Fig. 60).

Discussion

Age and the development of the active immune response in the unprotected population

The levels of immunoglobulins and, after early infancy, the prevalence and number of bands in the precipitin-Pf (Ouchterlony) test and the titres of antibodies detected by the IFA-Pf, IFA-Pm and IHA-Pm (or IHAT-Pk) tests all increased rapidly with age.

This undoubtedly reflects the development of an active immune response to malaria and possibly also, in the case of the immunoglobulin levels, to other infections. The results of the different tests followed a

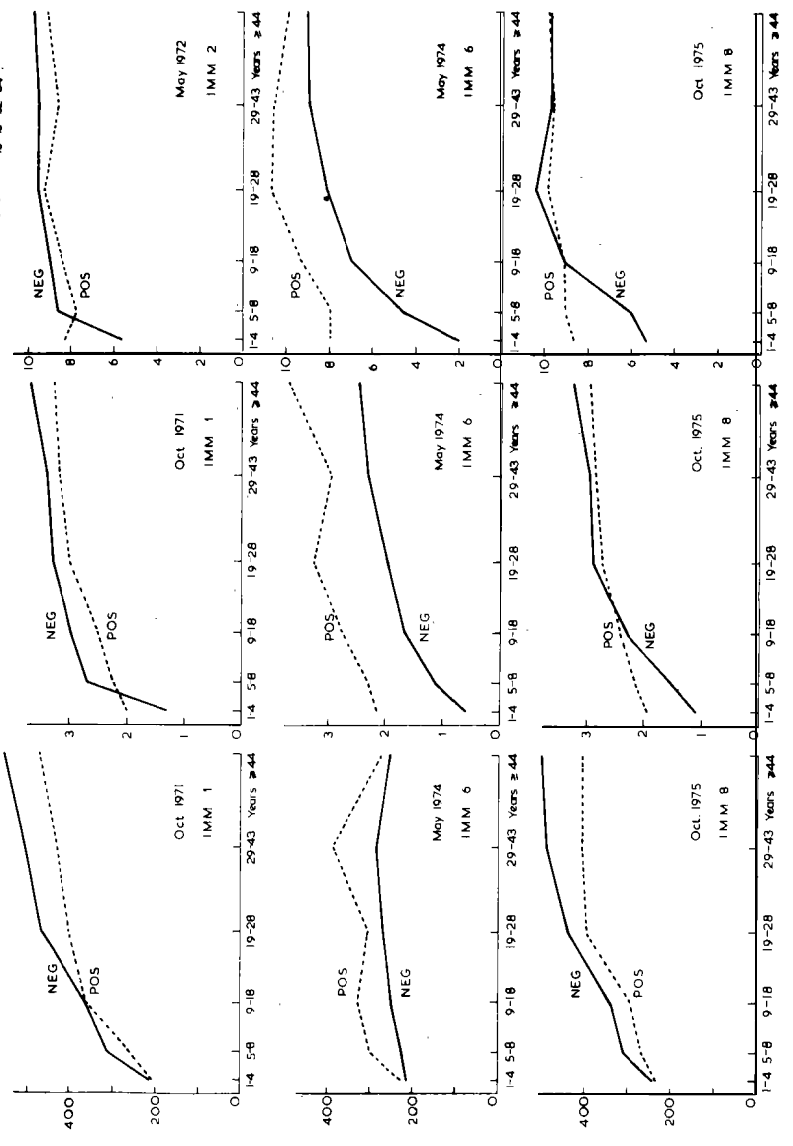


Fig. 60. Serology of parasitological positives and negatives (*P. falciparum*), before intervention (IMM I-2), after $1\frac{1}{2}$ years of intervention plus one dry season (IMM 6), and in the second wet season after termination of the intervention (IMM 8)

somewhat different pattern. Four test results reached a plateau: IFA-Pf by the age of l-4 years; IgG and IFA-Pm by the age of 5-8 years; and IHA-Pf or IHA-Pk by 19-28 years. Two test results increased throughout life: the level of IgM and the number of bands in the precipitin-Pf test. It should be noted that if the results of the precipitin test are expressed as the percentage positive (i.e., having at least 1 band), then the 100% ceiling is practically reached by the age of 5-8 years. This different behaviour of different tests according to age is important for the interpretation of serological results, although it may be an artefact; in particular, it is possible that the IFA and IHA titres level off to a plateau if the number of molecules of immunoglobulins available at high dilutions of an initially small volume of plasma or serum falls beiow a critical threshold.

The research design of the serological component of the Garki project was influenced by the studies carried out in the Keneba area of the Gambia (108, 109, 112, 140), and a comparison between the 2 sets of results is therefore of particular interest. In both cases the serological tests included IgG, IgM, the Ouchterlony precipitin-Pf test and the IFA-Pf test; both areas are well documented demographically and epidemiologically, and they represent nearly unmodified highly endemic situations of the tropical savanna of West Africa, with a predominance of ***P. falciparum*** .

The age-specific parasite rate rose more slowly and to a lower and probably later peak in the Keneba area than in Garki. This suggests that Keneba had either a lower inoculation rate or a higher recovery rate, possibly reflecting a higher consumption of antimalarial drugs.

The average level of IgG reached its adult plateau approximately by the age of 5 years in both areas (140). It seems, however, that the increase, as a function of age, was steeper in Garki and that the plateau was in fact reached sooner. The average level of the adult plateau in Keneba oscillated between 50% and 60% of the working standard (concentration: 4750mg IgG/lOOml), which corresponded to 295-354 IU/ml and was nearly identical to the range of 322-353IU/ml observed for the $\geq$ 5-years age-groups in Garki at the first serological survey (the conversion factor used was 1IU = 80.4pg IgG). For comparison, sera from normal British adults, studied concurrently with the Gambian sera, had an average IgG level of 12OOmg/1OOml or 149 IU/ml, i.e., about half the Keneba or Garki level.

In Keneba, there was little or no systematic change in the average level of IgM between the ages of about 6 months and 6-7 years; in Garki, such an early plateau was not observed. With this difference, the increase continued throughout life in both areas, and in both areas it was largely due

to an increased dispersion into high levels. The actual average level was probably higher in Garki: in Keneba the (arithmetic) average level of IgM at the age of 15 years was about 35% of the standard, or 260 IU/ml (1IU = 8.47pg IgM), while in Garki the (geometric) average level for the 9-18-year age-group was 372 IU/m1at the first survey.. If the same kind of average (arithmetic or geometric) were used for both areas, the difference would be even larger. For comparison, sera from normal British adults, studied concurrently with the Gambian sera, had an average IgM level of 89mg/lOOml, 'or 105 IU/ml, i.e. less than one-third of the Garki level .

The proportion found positive by the precipitin-Pf test rose faster, as a function of age, and reached a plateau of nearly 100% earlier in Garki than in Keneba. In both areas, the average number of precipitation bands increased with age.

In the IFA-Pf test the geometric mean titre rose sooner and more steeply in Garki, where it reached a plateau by the age of 5 years, than in Keneba, where it was still rising at the age of 10 years. The geometric mean titres actually recorded were higher in Garki. However, the IFA methods were different: in Keneba a trophozoite antigen was used, in Garki a schizont; in Keneba an anti-human globulin was used as conjugate, in Garki a specific anti-human IgG.

Significant differences were detected between villages in the Gambia but not in Garki; this could be explained by the greater parasitological differences actually observed between villages in the Gambian area. In summary, the serological differences between Garki and Keneba cari probably be explained by the corresponding parasitological differences.

Serological changes in the protected population

The main objective of the serological study was to measure the changes in the serological results after a reduction of the antigenic stimulation during the intervention phase. Interpretation of the results depends on the specificity and effectiveness of the control methods applied. These methods were very effective in reducing the antigenic stimulus, with respect to both ***P. falciparum*** and ***P. malariae,*** as demonstrated by the parasitological data (see Chapter 5). Propoxur, however, may have reduced antigenic stimuli other than malaria parasites, carried by the same or other vectors, while sulfalene and pyrimethamine were probably more specific at the doses used. For these reasons, the changes observed in the nonspecific tests (levels of IgG and IgM) may not have been entirely due to a reduction in malaria1 antigenic stimulation.

A specific objective of the serological study was to determine which

test results changed under protection by propoxur and sulfalene-pyrimethamine, and at what rate. Change under protection may be measured against baseline values (for which in this study there was no significant difference between the 2 populations) or against concurrent values in the unprotected controls. In Fig. 52 the second approach is taken. It is assumed that the controls are stable, SO that changes in their results are attributed to changes in the sensitivity of the test systems; the analysis is presented in a series of cross-sections with updated age-groupings, SO that change in the results of an age-group are due both to variations in the individual results and to movement of persons between age-groups through aging; the second effect becomes negligible with increasing age, because groups become wider and the results less variable.

It may be assumed that everybody in the protected population, except infants, had undergone antigenic stimulation by ***P. falciparum*** and that the great majority had undergone antigenic stimulation by ***P. malariae.*** Again excepting negative infants, the population received sulfalene-pyrimethamine, with a mean coverage of 85%.

Five of the 6 tests gave clearly decreasing results under protection; the 5 included the nonspecific IgM test, which in the baseline period also showed an association with parasitological immunity, and the exception was IgG. This suggests that malaria was an important factor in the high levels of IgM but not in those of IgG or that malaria caused an increase in IgG which persisted even after the removal of the antigenic stimulus.

The estimated rate of change of a test result will obviously vary with the way the result is expressed. For example the proportion "positive" decreased more slowly than the number of precipitation bands (precipitin-Pf test) or than the antibody titres (IFA-Pf, IFA-Pm and IHA-Pf tests). The level of IgM and the number of precipitation bands in the precipitin test decreased more slowly than the IFA and IHA titres.

The changes observed in the serological results of the population under protection may depend not only on the initial value of the serological results and the effectiveness of protection, but possibly also on the prior history of antigenic stimulation. Under the particularly intense stimulation observed in the study area, the specific malaria1 antibodies (IFA-Pf) reached a high level at an early age and remained at about the same level throughout life; however, the introduction of treatment revealed differences between age-groups which were not apparent in the unprotected population (Fig. 48). Either these differences could have been present before treatment but masked by the existence of an Upper limit of test sensitivity according to the hypothesis presented on p. 201, or they could reflect basic differences such as increasing involvement of the immune system with increasing age and prolonged antigenic stimulation.

Serological changes during the post-intervention phase

After the end of the intervention phase (end of 1973), malaria remained at a very low level during the dry season of 1974; this explains why the serological results continued to decline. After that, in the wet seasons of 1974 and 1975, although the entomological factors of transmission and the infant conversion rate were still below their baseline levels, the prevalence of ***P. falciparum*** increased above the baseline levels in those age-groups without systematic protection. This higher level of parasitaemia must probably be attributed to a lower level of immunity (see p. 165). The prevalence of ***P. malariae*** increased more slowly and was, at the last survey, still clearly below its baseline or control level (see Chapter 5).

The 5 serological results which had decreased clearly during intervention increased in 1974-1975. One of them, the IHA-Pf titre, became significantly higher than the control, reflecting the temporary increase in prevalence of parasitaemia above its baseline or control level. This reflection was delayed: although the prevalence of parasitaemia was more clearly excessive in 1974 than in 1975, it was only in 1975 that an excessive titre of IHA-Pf was demonstrated. The 4 other results had not yet reached, in 1975, their baseline or control levels. For the IFA-Pm titre, this is easily explained by the parasitological findings. For the IgM level, and the results of the precipitin-Pf and IFA-Pf tests, it must mean that they increase more slowly than the prevalence of ***P. falciparum.*** If one considers the increase of a serological result in 1974-1975 in relation to its decrease in 1972-1974, it is the increase of the IgM level which was the most delayed.

The longitudinal study of infants

The results of the 4 specific tests (precipitin-Pf, IFA-Pf, IFA-Pm, IHA-Pf) show that levels of malarial antibodies decrease in early life, both in the unprotected and protected populations; these tests apparently detect maternal antibody. The results suggest either that in an unprotected population the antibodies detected by the precipitin-Pf and IHA-Pf tests persist longer than those detected by the IFA-Pf and IFA-Pm tests, or that the first 2 tests as actually used were less specific. Protection of the mothers (including drug administration) may have decreased the amount of maternal antibodies detectable by the precipitin-Pf, IFA-Pf and IFA-Pm tests.

In the unprotected population, the proportions positive by the 3 P. falciparum tests (precipitin, IFA, IHA) rose after a certain age and became very similar to the cumulative prevalence of ***P. falciparum*** estimated

parasitologically. The tests therefore seem to be good indicators (and possible estimators) of the effective inoculation rate. In this context the definitions used for "positive" appear satisfactory from the point of view of sensitivity; for the IHA test, the Center for Disease Control (CDC), Atlanta, GA, USA, considers a titre of ≥ 32 rather than one of 16 as diagnostic with a satisfactory specificity; this makes the test somewhat less sensitive than the others. The data of this study indicate a better fit for the IHA test at titres of 16 or higher, while for the IFA test the rapid decline to nearly 0 in the infants of the protected population suggests that a titre of 20 is quite specific.

In the unprotected population, the proportion positive by the IFA-Pm test remained higher than the cumulative prevalence of P. malariae estimated parasitologically, while in the protected population it dropped rapidly to nearly 0. These findings could be explained in part by cross-reactions between ***P. falciparum*** and ***P. malariae*** in the IFA tests; there was a positive correlation between the results of the two IFA tests (see p. 193). The findings would also be compatible with a high sensitivity and specificity of the IFA-Pm, combined with a relative insensitivity of the method by which the infant conversion rate for ***P. malariae*** was estimated.

With respect to the IgG and IgM levels, there was relatively little difference between the infants of the protected and unprotected populations, suggesting that only a small part of the early rise in IgG and IgM is attributable to malaria.

The levels of IgG and IgM and the results of the precipitin-Pf and IFA-Pf tests in the unprotected infants were compared with the corresponding results from Keneba, Gambia (109,112,140). The findings of the two studies were on the whole similar but showed the following differences: (1) the decline in IgG observed in the first few weeks of life in the Keneba study was not observed in Garki where the 2 youngest age-groups were, on the average, 5 and 15 weeks old; (2) the proportions positive by the precipitin-Pf and IFA-Pf tests decreased after birth to clearly lower minima in Keneba than in Garki, before rising again. This last difference could be a result of either a higher test sensitivity or a lower test specificity in Garki, or of a higher level of transmission in Garki; the parasitological findings from the 2 areas suggest the latter explanation.

Comparison between males and females

Females had, on the average, a higher level of IgM and a higher titre of antibodies detected by the IHA test (both before and after control), i.e., they had higher results in 2 of the 3 tests which were associated with the individual's parasitological immunity (see p. 194). Below 5 years of age,

males and females had the same parasite rate; after the age of 5, females had slightly lower ***P. falciparum*** and ***P. malariae*** parasite rates than males (see p. 125). Since it is unlikely that-differences in the misclassification of age between thesexes- could explain the parasitological and serological differences between males and females, these may be interpreted as reflecting a higher average level of immunity in the females. Moreover, since below 5 years of age no parasitological difference was detected, this higher level of immunity in females is probably due to a stronger immune response rather than to a stronger antigenic stimulus.

In 1974-1975, during the resurgence of malaria in the previously treated population, the parasitological difference between males and females was greater than in either baseline or control populations (see p. 156). There was, however, no increase in the serological difference. This suggests that females have not only a stronger humoral response, but also either a stronger natural immunity or a stronger cellular response.

The correlation between different surveys in the same person

There was a positive correlation between the results of the same test in the same person at different surveys. This correlation was relatively strong only for the IgM level and the IHA-Pf titre. In addition, the variation of IgM within each person decreased with increasing age, while the variation of IgM between persons increased with increasing age. This could be attributed to a stable differential exposure between persons or, more likely, to a differential response (probably genetically determined) for a given antigenic stimulation; it may be that, under the prevailing heavy antigenic stimulation, varying relatively little between persons, each person tended towards his or her characteristic maximum level of IgM.

The position within a person's age-group is relatively stable for more than 3 years, including the periods of decrease and increase in the average results during and after the intervention phase. The correlation between pre- and post-intervention serology was as strong in the protected as in the unprotected population, while the correlation between pre- and post-intervention parasitology was weaker in the protected than in the unprotected population (see p. 156). The findings suggest that the level of immune responsiveness and its parasitological and serological effects are rather stable and determined early in life, possibly by the genome; and that in later life, in a situation of high endemicity, variations between persons in the frequency of parasitaemia have less effect on variations in the level of immunity than the reverse. In the Keneba study (112), the correlation between immunoglobulin levels of the same

person at different surveys was also positive, being stronger for IgM than for IgG; and it was stronger in Keneba than in Garki, particularly in the case of IgG.

The correlation between different serological tests in the same person

The positive correlation between the IFA-Pf and the IFA-Pm tests may result, at least in part, from cross-reactivity and from the fact that the random variation in sensitivity of the IFA test system from day to day must affect the results of both tests (in the same person) in the same direction (24 sera were processed for both tests on the same day).

Among the specific tests, the IFA tests in this study detected antibodies within the IgG fraction only, and the precipitin and IHA tests detected antibodies in both the IgG and IgM fractions. As the antibodies detected by the specific tests represented only a small part of the immunoglobulins, the fact that the specific tests did not show a strong correlation with total immunoglobulin levels was not too surprising.

The correlation between different serological tests had also been studied in Keneba, and there as in Garki a significant but relatively weak correlation existed between the IgG and IgM levels in the same person and between the immunoglobulin (IgG or IgM) levels and the precipitin-Pf test results.

The relationship between serology and parasitology in the individual

At the total population level, it is likely that all 6 tests used here are indicators both of contact with malaria and of partial immunity to it, that is, there is more malaria and a higher level of immunity in populations showing higher test results. The present study analyses these relationships within a population with a high endemic level.

What relationship should be expected between serology and parasitology at the individual level in population surveys? If the serological test is specific, then in early life, after the loss of passive immunity, the association should be positive. If the test result is associated with protection, and is persistent, then in later life the association should become negative. The higher the incidence rate, the earlier the expected transition from a positive to a negative association. If the serological status of a person is more stable than the parasitological one, then the association: (a) should be clearer if the findings of several parasitological surveys are combined, and (b) may keep the same sign over relatively long periods (e.g., the 18 months of baseline) whether the parasitology is examined before, during, or after the serological survey.

The negative associations observed are tentatively interpreted as an association between serological tests and relative protection, in terms of parasitaemia and gametocytaemia. This applies to 3 tests which are, in order of strength of the association, as follows: precipitin (*P.falciparum* antigen), IHA (*P. knowlesi* or *P. falciparum* antigen), and IgM concentration. Not surprisingly, these tests are a better (more stable) indicator of "protection status" than a single parasitological examination. None of the 6 tests, however, is a Perfect indicator of parasitological protection, even on a population basis; the degree of protection associated with a given level of the test increases with age, if we rule out (as we probably can) decreasing exposure with increasing age. This does not per se negate the hypothesis that the test is measuring protecting antibody; it suggests that one or more other factors (humoral or cellular) of immunity develop more slowly than what the test measures.

An alternative explanation of the negative association may be considered. The binding of antibody by parasites might, by itself, tend to produce a negative association. However, if that were the whole explanation of the negative associations actually observed, they would exist at all ages and would probably be much more obvious with the simultaneous parasitaemia than with the past and future parasitaemia; moreover, the negative association with gametocytaemia would not clearly precede the one with parasitaemia, and drugs would be expected to exaggerate the negative association rather than reverse it as they did. In the case of IgM (not all of it antibody), this alternative explanation would seem untenable quantitatively.

It has been suggested by Greenwood (75) that the large amounts of IgM produced by persons living in endemic malarious areas may be beneficial to the parasite. The finding, within specific age-groups, of a negative association between parasitaemia and the IgM level does not support that hypothesis.

The IFA titres were not, within specific age-groups, associated with protection. The persistently positive association between the IFA-Pm titre and P. *malariae* parasitaemia may be interpreted as follows: each episode of parasitaemia produces a relatively quick and short-lived increase in titre, SO that the periods with an increased titre overlap to a large extent with the episodes of detectable parasitaemia. In the case of *P.falciparum,* episodes of parasitaemia are more frequent than with *P. malariae:* even if each episode of parasitaemia produces a transitory increase in titre, the more rapid succession of episodes is sufficient to blur the relationship.

The term "parasitaemia", as it is used here, obviously means parasitaemia microscopically detected by the method employed (i.e., examination of 200 fields of thick blood films); even though the method is.

relatively insensitive, it is sufficient, for the analysis and interpretation presented in this study, that it be well standardized.

Among the 6 tests used (IgG, IgM, precipitin, IFA-Pf, IFA-Pm, IHA), the IgG test was the only one for which no definite relationship with parasitaemia was demonstrated, and the only one which showed little change under the impact of malaria control. In Keneba, the Gambia, however, a relationship between IgG and parasitaemia was demonstrated (see below).

The changing pattern of association between serology and parasitology during and after the intervention period confirms the interpretation given above to the relationship between parasitology and serology: in endemic malaria, immunity builds up gradually with age, and if a test is specific and is also an indicator of the level of immunity, one expects in the Young a positive association with parasitaemia, in the older a negative association. In other words, among the young the test result is an indicator of the degree of parasitaemia, in the present or the recent past; among the old, the test result is an indicator of the degree of immunity; the higher the level of transmission, the sooner one gets "old" with respect to malaria. The effect of drugs has been to make the old temporarily lose part of their immunity, i.e. to make them, in terms of malaria, temporarily younger.

With respect to the IHA-Pf test at the last survey, there is an apparent paradox: between the 2 populations (previously protected and unprotected), the one with the higher titre is probably the less immune, but within each population the persons who have a higher titre are probably more immune than the others.

The correlation between serology and parasitology has also been studied in Keneba (108, 109, 112, 140). In both the Garki and Keneba areas the 4 serological variables increased with age, while the parasite rates and densities decreased. The relationship was also studied within specific age-groups: the data from Keneba were analysed by comparing the serological results in persons found positive and negative at the concurrent parasitological examination; the data from Garki were analysed in the same manner and also by stratifying persons by their serological results and comparing the strata with respect to the preceding, concurrent or subsequent parasitological findings. It is the latter analysis which has been presented in this chapter. In Keneba, a positive correlation was found between IgG and the concurrent parasitaemia at O-20 years, and between IgM and the concurrent parasitaemia at O-2 years; while in Garki there was no correlation between IgG and parasitaemia and a negative correlation between IgM and concurrent or subsequent parasitaemia. The difference between the two areas with respect to IgG is difficult to explain. The difference with respect to IgM in the young

children could be explained by a higher rate of antigenic stimulation and development of parasitological immunity in Garki, but the difference in adults (no correlation in Keneba, a negative one in Garki) is puzzling; it is possible that in Keneba the situation was modified by the use of drugs to a larger extent than in Garki. With respect to the IFA-Pf test, both areas show a positive association between titre and parasitaemia at O-4 years and none thereafter. With respect to gametocytes, in Keneba there was no correlation with the IFA-Pf titre at O-4 years. In the same age-group in Garki, there was also no correlation between gametocytaemia and the IFAT-P.falciparum titre, but there was a negative correlation between gametocytaemia and the number of bands in the precipitin-Pf test.

Sensitivity and specificity of the serological tests

The sensitivity of a test is defined by the proportion of true positives it detects. To apply this to malaria serology, one needs operational definitions of "true positive" and "serological positive". If the definitions used in Table 21 are tentatively adopted, it can be seen that in the l-4 and 5-8-year age-groups everybody either had, or had had in the preceding 70 weeks, a detectable ***P. falciparum*** parasitaemia; the proportion found positive by a serological test in those age-groups was thus a measure of the sensitivity of the test. The IFA test was more sensitive than the precipitin test, which in turn was more sensitive than the IHA test; this ranking was also usual in the other surveys of the unprotected population; it differs from the one implied in a report by a WHO Scientific Group (182; in particular its Table 4 and Fig. 1). which was, however, comparing surveys made at different times and places. A high sensitivity is sometimes obtained at the cost of a low specificity. The specificity of a test is defined by the proportion of true negatives it detects. Negative controls from nonendemic areas were consistently negative in the test systems used in this study, but they were few in number. In the study population, specificity was suggested (although not quantified) by the effect of specific treatment on-the serology and by the results among infants born into the protected population.

With respect to ***P. malariae,*** everybody in the 5-8-year age-group was positive by the IFA test, but only 82% had or had had a patent parasitaemia in the preceding $1\frac{1}{2}$ years, according to the 8 baseline parasitological surveys. This means either that the serological test was relatively nonspecific or that the parasitological test was relatively insensitive; the latter seems likely since parasitaemia could easily have been missed by eight surveys at intervals of 10 weeks, and since ***P. malariae*** parasitaemia

may have been reduced by the simultaneous presence of ***P. falciparum*** in the same persons (see p. 167).

Summary

A longitudinal study on the epidemiology and control of malaria in rural West African savanna included serological surveys conducted twice a year from 1971 to 1975 in a control area and in a comparable area where control measures, including spraying with residual insecticide and mass drug administration, were applied for 2 years. The parasitological situation evaluated by microscopic examination of thick blood films indicated a maximum parasite rate of ***65%*** for ***P. falciparum*** and 20% for ***P. malariae,*** falling to 6% and 1% respectively after the control measures. In the post-intervention phase the prevalence of ***P. falciparum*** rose rapidly. The resurgence of ***P. malariae*** was slower. The serological specimens were tested for IgG and IgM, precipitin bands (Ouchterlony test), immunofluorescent antibodies and indirect haemagglutination antibodies for ***P. falciparum,*** and immunofluorescent antibodies for ***P. malariae.*** The rapid increase with age, reflecting the development of the active immune response, was detected by all tests. For IgM and precipitin the increase continued throughout life, whereas for the 4 other tests a plateau was reached at an early age. The introduction of control measures resulted in progressive decreases in all parameters except in IgG level. For the 5 tests showing changes the decrease varied with age; it was more marked in the older age-group for IgM and in younger age-groups for the 4 other tests.

Two infant populations, the one exposed to intense malaria transmission and the other protected, were compared by the 6 serological tests. The IgG and IgM levels increased with the age of the infant and were consistently, though only slightly, lower in the protected infants. The results of ***3 P.falciparum*** tests (precipitin, IFA and IHA) and one ***P. malariae*** test (IFA) were high at birth and decreased rapidly afterwards, in both populations; in the unprotected population this decrease was followed by an increase closely associated with the parasitological findings, while in the protected population the decrease continued to very low levels. Infants of protected mothers were probably born with lower levels of maternal antibody.

Females had slightly higher levels of IgM and slightly higher titres of IHA-P. ***falciparum*** antibodies. They also had slightly lower parasitaemias, and the most likely explanation is that they develop a stronger immune response.

The position of an individual person's serological result with respect to the average results of his age-group was relatively stable over the period of the study for IgM and ***IHA-P.falciparum,*** and to a lesser extent for precipitin-P.falciparum, IFA-P. ***falciparum*** and IFA-P. ***malariae.*** This relative stability was little affected by the decrease, followed by an increase, resulting from the control measures and their interruption. This finding suggested that the level of individual immune responsiveness and its parasitological and serological effect were stable and determined early in life, possibly under genetic control. A positive correlation was also usually observed between the results of different tests in the same person at the same survey, but it was generally not very strong.

The study of the relationship between a person's parasitological and serological results showed that there was no relationship between IgG and parasitology. For the 5 other serological tests , including the non-specific IgM level, there was a positive association between the test result and parasitaemia in early life. For 3 tests (IgM, precipitin and IHA) the relationship became negative in older children and adults, indicating that the test results were associated with protection. During the intervention phase the positive association extended progressively into the older age-groups. In the post-intervention phase these 3 serological results increased and the difference between parasitologically positive and negative decreased, then changed sign starting with the older age-groups, and the association became negative again. The changing patterns of association with parasitology confirmed that among the young these 3 test results were an indicator of the degree of parasitaemia in the present or the recent past, while among older persons the test result was an indicator of the degree of immunity. For the IFA-Pf test, there was no association between a person's titre and parasitology after the first few years of life; for the IFA-Pm test, the positive association persisted throughout life.

In the post-intervention phase the rate of increase for the 5 changing parameters was different, and the temporary increase in prevalence of parasitaemia above its baseline or control level was best reflected in the IHA titres. Of the results of the 4 other tests which by 1975 had not yet reached their baseline levels, those of the were the most delayed.