Chapter Five

PARASITOLOGY

This chapter includes the study of the malaria parasites in the blood of the selected study population under natural conditions (i.e., during the pre-intervention phase, and in the comparison villages left untreated during the intervention phase), under the effect of intervention measures (residual insecticides with or without the administration of drugs, as indicated in Chapter 2), and for 2 years after the end of the intervention phase.

The study design and the control operations are described in Chapters 2 and 3. For the timing of the 23 parasitological surveys see Fig. 43. The parasitological findings have been the object of several reports and documents (116, 152, *171, 172, 185).*

Methods

Method of hlood examination

For the kind of epidemiological investigation planned in Garki, a method was needed which was simple, reproducible and sensitive, which measured not only prevalence but also density (preferably simultaneously), and in which the probability of diagnosis of a given species was, as far as possible, independent of the presence of other species.

A review was made of available methods (17,48,49,56,57,168), and those requiring the measurement of an exact volume of blood, or an additional erythrocyte or leukocyte count, or involving the mixture, in fixed proportions, of the blood with a standard suspension of fowl erythrocytes, were all discarded for the sake of simplicity. Counting the proportion of parasitized erythrocytes would be the preferred method only

a The work described in this chapter was done by or under rhe supervision of Mr J. Storey and Mr Hussaini Yacim.

for high-density infections, which are a minority in an area of high endemicity; in addition, in the absence of an erythrocyte count, the method would lose much of its value. Counting parasites against leukocytes, without a leukocyte count, was not discarded without preliminary trial. Reproducibility and sensitivity are considered below (p. 111). TO make the diagnosis of a given species independent, as far as possible, of the presence of other species, it was decided to pursue the examination either for a predetermined time or for a predetermined number of microscopie fields.

After this review, the following methods were selected for a preliminary tria1 (149):

Method	*Blood film*	*Lens system*	*Examination time*	*Method of counting*
1	thick	standard	10 min	Number of fields examined and number of fields positive for ***P. falciparuh*** asexual forms, ***P.f.*** gametocytes, ***P. malariae,*** ***P. ovale,*** respectively.
2	thick	standard	20 min	
3	thick	wide-angle	5 min	
4	thin	wide-angle	10 min	
5	thick	standard	10 min	Number of leukocytes, ***P.f.*** gametocytes, other malaria parasites, respectively.

Fields examined and positive were counted with the help of a 5-key tally counter .

Method 2 was only slightly more sensitive than method 1, which in turn was more sensitive than the others. There was a strong correlation between the density measured against leukocytes, following Bruce-Chwatt (17), and the proportion of fields positive on the thick film. The latter is expected to give a poor discrimination among the highest densities, but this was not considered essential for the purpose of an epidemiological investigation.

Method 1 was adopted, except that the 10 minutes examination time was replaced by an examination area of 200 fields, which requires about 10 minutes to caver.

The “standard lens system” consisted of paired x 7 oculars (field 18.5) and an x 100 oil-immersion objective; the diameter of a microscope field is therefore 18.5/100 = 0.185 mm. Only the “best part” of the film was examined, the thickness of which Dowling & Shute (49) estimated to be 0.09 mm. The senior parasitologist of the Garki project had worked with Dowling and Shute, and the thickness of the films was, in principle, the

same. The volume of blood corresponding to 200 fields was therefore $200\,\pi\left(\frac{0.185}{2}\right)^2 0.09 = 0.48$ or about 0.5 mm^3.

Collection and processing of blood films

The surveys were aimed to achieve total coverage of the populations of the villages selected. Collections were made by house-to-house visit, with a second visit to the homes of absentees. Collections usually took place between early morning and midday, except during the period of high agricultural activity (July and August), when collection took place from early afternoon till dusk. Identical preprinted identity numbers were stuck to the person's record form, the blood examination form and the slide; linkage between the result and the person was made by computer.

Slides were desiccated for at least 24 hours, and stained for 30 minutes in a 3% Giemsa solution. The standard of staining was consistently high. Schüffner's dots stained clearly as a pink halo around the more mature trophozoites and later stages of P. *ovale,* making differentiation between this species and *P. malariae* reasonably straightforward.

Parasitology staff

The national staff consisted of: 1 senior technician; 3 laboratory technicians (supervisors) with 3 or more years of training and experience in a WHO malaria research laboratory; 8 laboratory assistants (microscopists) trained for 3-6 months in blood collection, staining and malaria microscopy techniques; 3 laboratory attendants. General supervision was ensured by the WHO parasitologist.

The laboratory assistants (microscopists) performed the routine blood examinations used in the analysis, each microscopist examining 25 slides per day on the average; all specific diagnoses were confirmed by a more senior person.

Sensitivity and reproducibility of the blood examination method

Effect of doubling the volume of blood examined

In 1970-1973, a systematic sample of one-fifth of the slides was examined for 400 fields instead of 200. The sample was representative with respect to age, sex, time and place. In the two groups of blood films obtained for the whole of the baseline phase (Table 15), it was found that doubling the standard volume of blood examined (200 fields) produces

Table 15

Percentage of positives among thick films examined for 200 fields and among thick films examined for 400 fields: all villages and surveys I-8 combined

Parasite	Parasite rates (%)		Increase (%)	
	in 38 258 films examined for 200 fields	in 8 821 films examined for 400 fields	absolute	relative
	(a)	*(b)*	*(c) = (b) – (a)*	*(d) = (c)/(a)*
P. falciparum				
asexual stages	48.9	53.7	3.7	9.8
gametocytes	12.7	16.4	5.3	29.1
any stage	50.9	56.2		10.4
P. malariae	15.3	18.9	3.6	23.5
P. 0 vala	2.4	2.9	0.5	20.8

relative increases in prevalence of 10% for ***P.falciparum, 24%*** for ***P. malariae,*** 21% for ***P. ovale.*** The present analysis of the project's results is based on the examination of 200 fields (see p. 114).

Independent re-examination of slides

Throughout the duration of the project, a systematic random sample of the blood films examined by the microscopist was re-examined by a supervisor. The microscopist did not know which of his films would be re-examined, and the supervisor did not know the result of the first examination. During the baseline phase, 10% of the films were thus re-examined; when the prevalence decreased during intervention, this percentage was increased.

Table 16 gives the results, for the 23 surveys combined, in terms of prevalence. The microscopists were, on the average, somewhat less sensitive than their supervisors. The microscopists found overall prevalences of 30.0%, 8.5%, 9.2% and ***0.9%*** for ***P. falciparum, P.f.*** gametocytes, ***P. malariae*** and ***P. ovale,*** respectively, versus the 31.6%, 10.0%, 10.8% and 1.2% of the supervisors (Table 16). The microscopists also found somewhat fewer positive fields than the supervisors: the ratio of the total number of fields found positive by the microscopists to the number found positive by the supervisors was ***0.96*** for ***P. falciparum*** asexual stages, ***0.88*** for ***P. falciparum*** gametocytes, ***0.89*** for ***P. malariae, 0.74*** for ***P. ovale;*** these ratios are very similar to the corresponding ratios for prevalence (see column g in Table 16). More striking than the difference in sensitivity between microscopists and supervisors was the increase in pre-

Table 16

Results of the independent double examination for 200 fields of 12 382 slides: parasite rates, all villages and surveys (I-23) combined

Parasite	Slides (%) found positive by:			Parasite rates (%) according to:			Ratios between parasite rates		
	micro-scopists only	super-visors only	both	microscopists	supervisors	both combined	microscopist / supervisor	microscopist / both combined	supervisor / both combined
	(a)	*(b)*	(c)	(d) = (a) + (c)	*(e) = (b) + (c)*	*(f) = (a) + (b) + (c)*	*(g) = (d) / (e)*	*(h) = (d) / (f)*	*(i) = (e) / (f)*
P. falciparom, any stage	3.8	5.3	26.3	30.0	31.6	35.4	0.95	0.85	0.89
P. falciparum, gametocytes	2.4	3.9	6.1	8.5	10.0	12.4	0.85	0.69	0.81
P. malariae	2.3	3.9	6.9	9.2	10.8	13.2	0.85	0.70	0.82
P. ovale	0.2	0.5	0.6	0.9	1.2	1.4	0.73	0.61	0.84

valence resulting from combining the 2 examinations-up to 35.4%, 12.4%, 13.2% and 1.4% for *P. faiciparum, P. falciparurn* gametocytes, *P. malariae* and *P. ovale,* respectively (Table 16). Microscopists and supervisors were also compared within each individual survey: there was some variation, but no time trend (185). The present analysis of the project's results is based on the results obtained by the microscopists.

The distribution of positive films by the number of positive fields

This distribution was studied in order to see whether it fitted any known distribution, whose properties could then be used to estimate a likely proportion of false negatives (latent positives). The approach failed, in particular because there were more films with 2 fields positive than with 1 field positive, both in the films examined for 200 fields and in those examined for 400 fields. A plausible explanation is that, once a positive field is found, the remaining fields are examined more carefully (see also 15).

The parasitological indices used

Theparasite rate (PR): defined as the proportion (or percentage) of persons positive for *P. falciparum* (any form), *P. falciparum* gametocytes, *P. malariae* and *P. ovale,* respectively, by the examination of 200 fields of thick film. Films examined for 400 fields and found positive were allocated to positive or negative by a random experiment based on the number of fields found positive: e.g., if, out of 400 fields, 2 were positive for *P. falciparum* asexual stages and 1 for *P. falciparum* gametocytes, the probability that the film would have been positive after 200 fields was $1 - (\frac{1}{2})^2 = 0.75$ for *P. falciparum* asexual stages, $1 - \frac{1}{2} = 0.5$ for *P. falciparum* gametocytes, $1 - (\frac{1}{2})^3 = 0.875$ for *P.falciparum* any stage. In fact, very few of the "positive 400" were classified as "negative 200'', because, as stated above, there were very few films with a single positive field.

The parasite density index (PDI): here defined as the proportion of fields positive for *P. falciparum* asexual stages, *P. falciparum* gametocytes, *P. malariae* and *P. ovale,* respectively, out of the fields examined in a given population. It is an estimate of the parasite load in that population. For this index, the films examined for 200 and 400 fields were simply pooled. It will be noted that this PDI is different from the PDI commonly used in the malariological literature (e.g., 17)

The positive parasite density index (PPDI): here defined as the proportion of fields positive for *P. falciparum* asexual stages, *P. falciparum*

gametocytes, *P. malariae* and *P. ovale,* respectively, in pensons found positive for the species considered (i.e., in the case of *P. faiciparum,* persons positive for *P. falciparum,* any form) in a given population. It is an estimate of the density of infections in that population. For this index, as for the PDI, the films examined for 200 and 400 fields were simply pooled.

The 3 indices are related as follows: PDI = PR x PPDI; this relationship is, however, exact only if all persons are examined for the same number of fields.

Parasitological Findings in the Absence of Intervention

In this section are presented the parasitological observations made during the baseline phase in the 8 village clusters selected for follow-up, as well as some of the observations made in the untreated control village clusters in the intervention phase (clusters No. 1 and 2) and in the post-intervention phase (cluster No. 2).

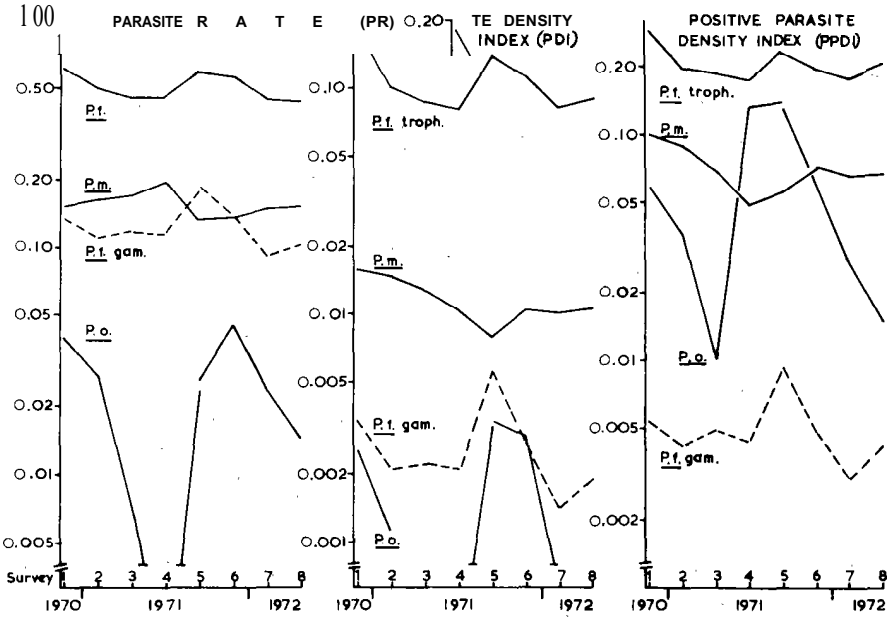
The situation described is really unaffected by antimalarial measures. No insecticides were used in the area in the past. The amount of antimalarials used was negligible: (1) there are only 2 dispensaries in the district, in Garki and in Gwarzo, the latter outside the study area, and an analysis of their utilization of antimalarials and of the origin of the attending patients led to the conclusion that their influence on the study was negligible (148); (2) the ambulant drug-Sellers in the markets carried nonspecific antipyretics but no antimalarials.

Prevalence and density of parasites

Prevalence and density by age, season and year

Figure 20 shows, at each of the 8 baseline surveys, the parasite rate (PR, proportion of persons positive) the parasite density index (PDI) and the positive parasite density index (PPDI). The small discontinuity in the curves, at survey 5, corresponds to the addition of 6 villages to the clusters of villages studied (see Chapter 2). Surveys 5 and 6 correspond to the wet season of 1971. The prevalence and density of *P. falciparum, P. falciparum* gametocytes and *P. ovale* clearly increase in the wet season and decrease in the dry season (the high PPDI for *P. ovale* at survey 4, which seems to contradict the above statement, corresponds to only 8 positive persons). The *P. falciparum* microscope picture in the early part of the transmission season was reminiscent of an epidemic situation with

Fig. 20. The crude parasite rate and density, a by species b and survey; baseline phase, ail villages combined



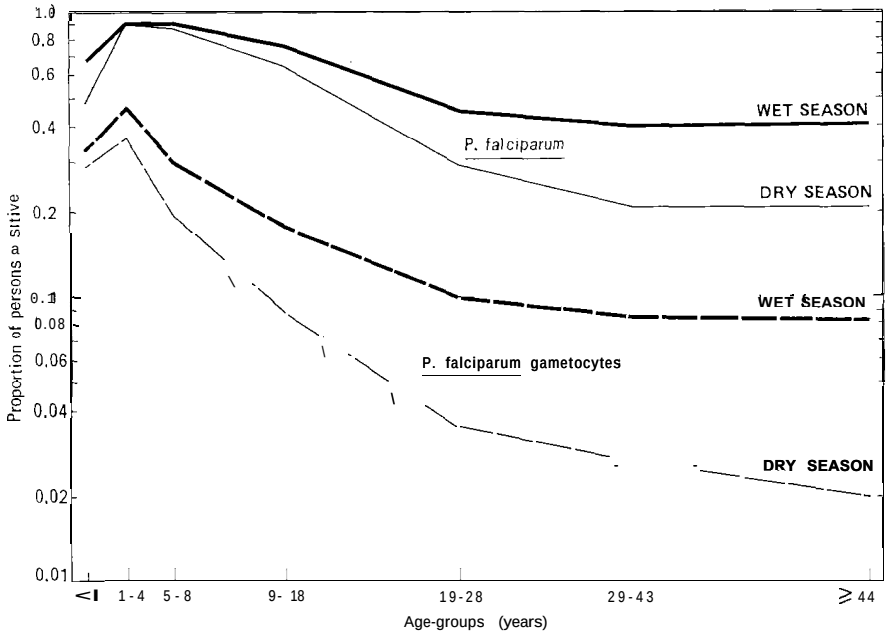
a **PR** = proportion of persons positive; **PDI** = proportion **of fields positive; PPDI = proportion of fields positive in persons positive for the species.**

b ***P.f. = P. falciparum; P.f. gam. = P. falciparum*** **gametocytes;** ***P.m. = P.malariae; P.0. = P.ovale.***

very high parasite densities and a noticeable increase in circulating schizonts. ***P. malariae*** behaved differently: its prevalence reached its seasonal maximum at the end of the dry season (survey 4), its minimum early in the wet season (survey 5); the density of patent infections (PPDI) reached its seasonal minimum before the wet season (survey 4, when prevalence is highest), and its maximum late in the wet season (survey 6).

Figures 21 and 22 show the combined effect of age and season on the prevalence and density of ***P. falciparum*** and its gametocytes. The combined data from the 16 villages (about 5000 persons), surveyed 5 times in 1971 at regular intervals of 10 weeks, were used; the surveys with the highest and lowest crude prevalence of ***P. falciparum*** (any form) and gametocytes, respectively, were chosen. Fig. 21 covers the parasite rates (PR), i.e., proportions of persons found positive by the examination of 200 microscope fields of thick film; Fig. 22 covers the density of trophozoites and gametocytes in persons found positive, where the density of trophozoites is defined as follows: { (number of fields positive for

Fig. 21. Prevalence (parasite rate) of *P. falciparum* (trophozoites and/or gametocytes) and of *P. falciparum* gametocytes only, by age and season; baseline phase, all villages combined



trophozoites)/(number of fields examined)} /(proportion of persons positive for ***P. falciparum*** trophozoites and/or gametocytes); the density of gametocytes is defined similarly (positive parasite density index, PPDI). The parasite rate decreases by age; this is due to increasing immunity, expressing itself as increasing recovery and/or decreasing detectability and/or decreasing susceptibility. The amplitude of the seasonal variation in parasite rate increases with age. The net proportion of positives becoming negative in the dry season increases with age by a factor of about 10, while the net proportion of negatives becoming positive in the wet season decreases with age, but only by a factor of about 2. This suggests that the main effect of immunity is to increase recovery and/or to decrease detectability, rather than to decrease susceptibility. The trophozoite density decreases faster by age than the parasite rate, which suggests that part of the latter's decrease is due to decreasing detectability. Both gametocyte rate and density decrease faster by age than parasite rate and trophozoite density, respectively; this suggests that immunity decreases infectivity before increasing recovery and/or decreasing detectability.

Fig. 22. Density of trophozoites and gametocytes in persons positive for *P. falciparum* (positive parasite density index) by age and season; baseline phase, all villages combined

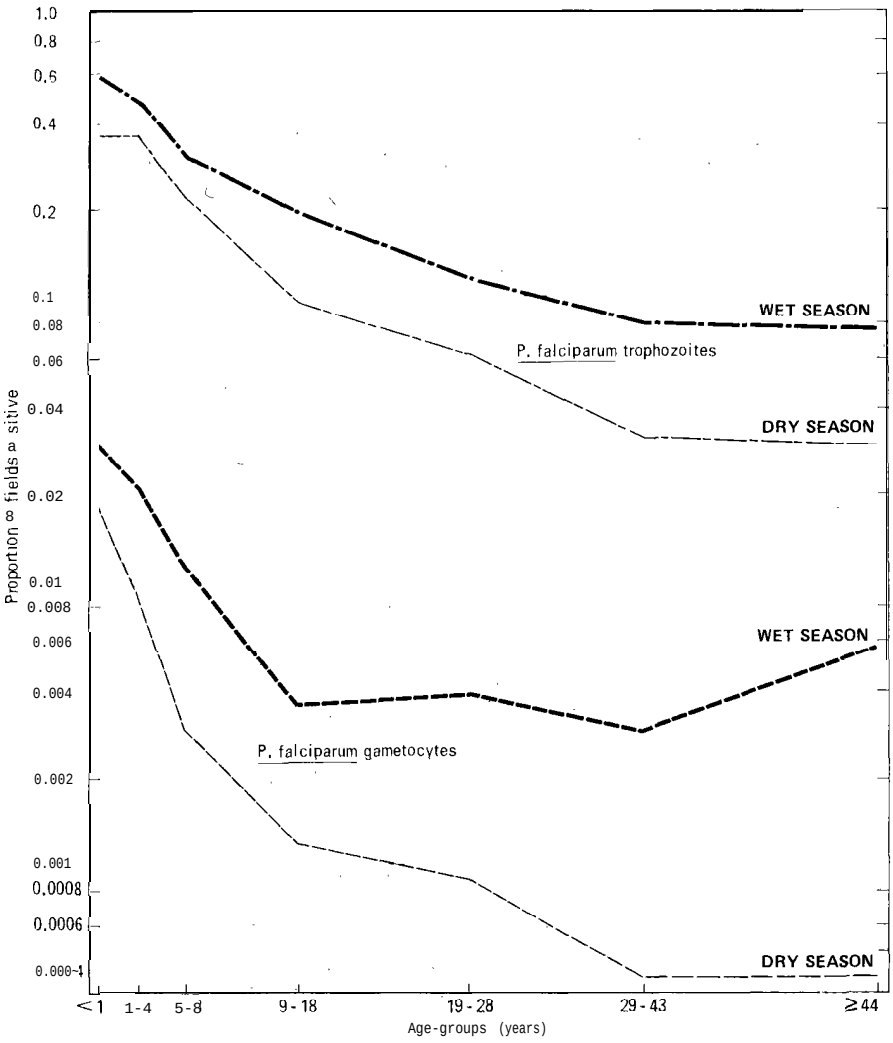
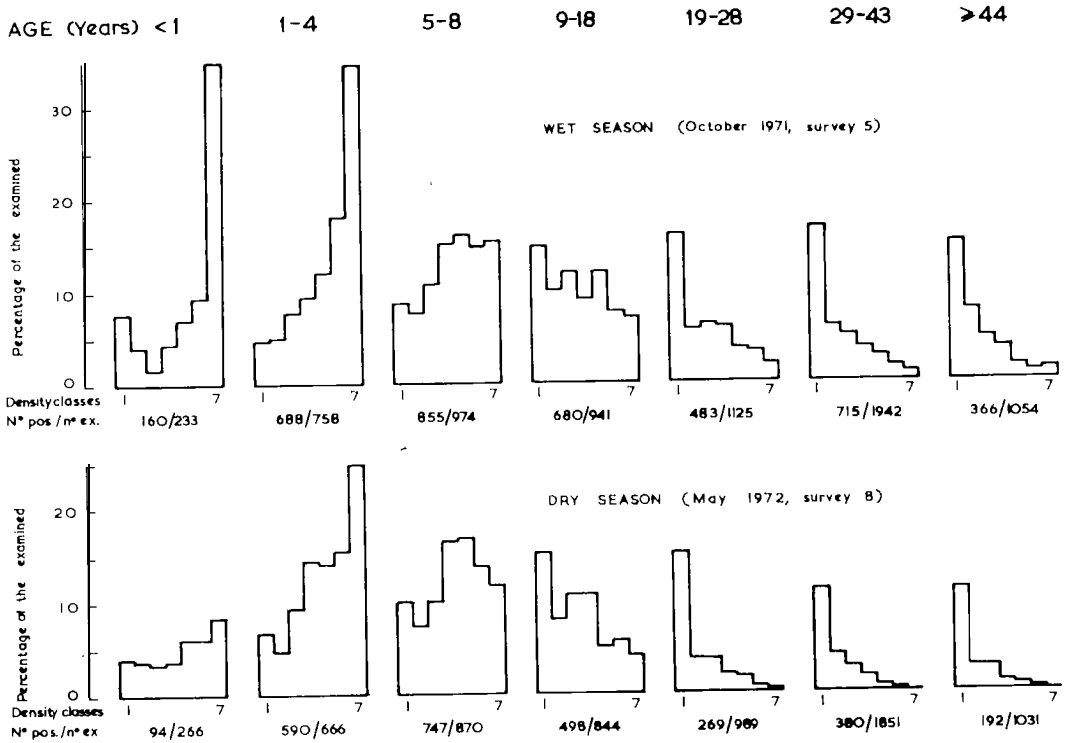


Figure 22 shows only the average densities (PPDI) without telling us anything about the distribution of the population according to density. Therefore Fig. 23 displays the actual frequency distribution of persons into density classes defined in terms of proportions of microscope fields positive. The figure shows a shift from a large predominance of relatively

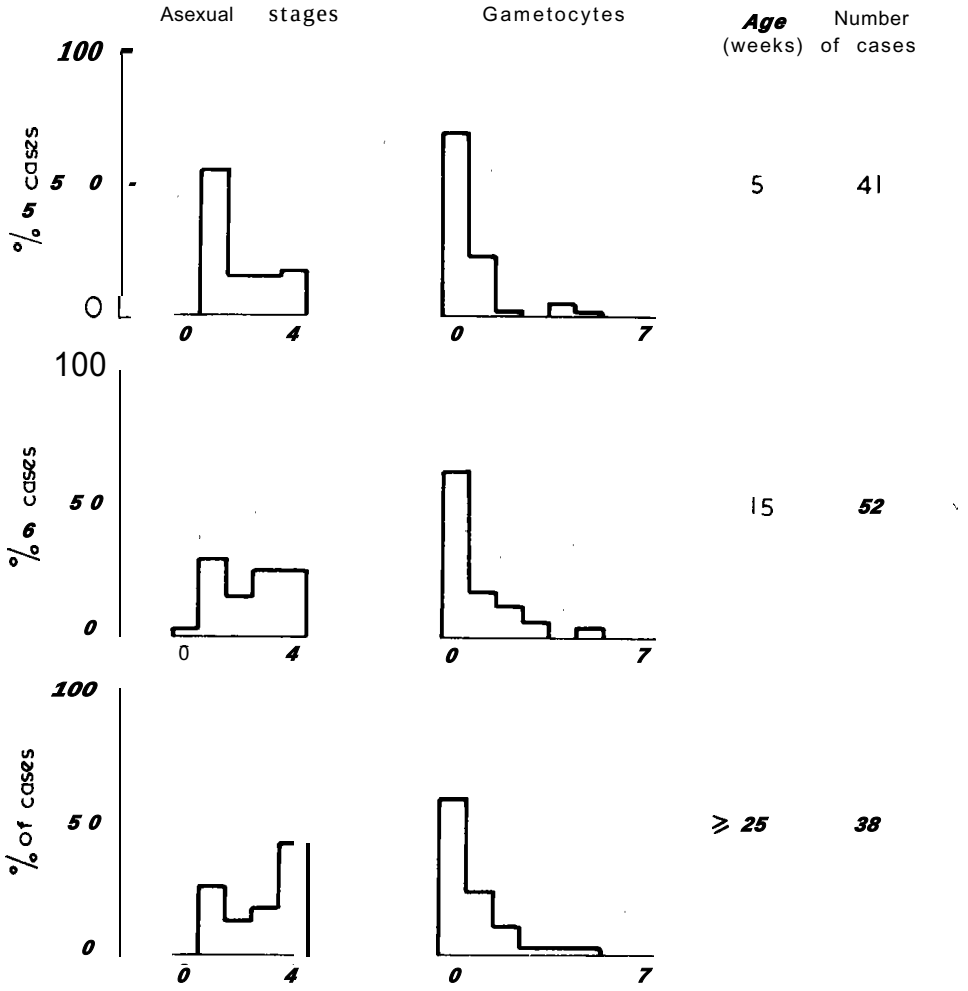
Fig. 23. Age-specific density distribution of *P. falciparum* asexual stages, by season[a]



[a] Each histogram shows the distribution of the positives (as percentage of the age-group) into 7 density classes, defined by the proportion of thick film fields positive; the upper limits of the classes are 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.0). Histograms of data collected in all clusters of villages during the pre-intervention period.

high densities in young children to a large predominance of low-density infections in adults. In most age-groups, there is also a moderate shift tow,ards lower densities in the dry season. (For the progressive increase in density of ***P. falciparum*** infections in the course of the first year of life, see Fig. 40, upper half.)

Fig. 24. Distribution of 131 first infections with ***P. falciparum*** according to the density of asexual stages and gametocytes, by age of the infant[a]

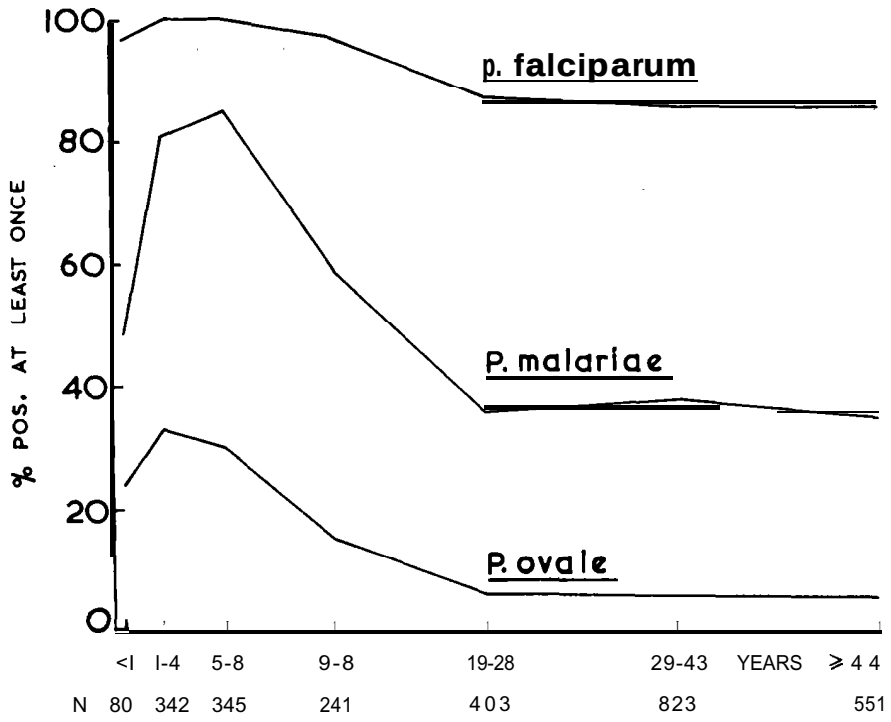


a Density of asexual forms: 0 = negative; 1,2,3,4 = up to 4%, 16%. 64%, 100% of fields positive; density of gametocytes: 0 = negative; 1,2,3,4,5,6,7 = up to 2%, 4%, 8%) 16%, 32%, 64%, 100% of fields positive.

In infants regularly followed from birth, i.e., examined every 10 weeks, the "first infections" with ***P. falciparum*** were classified according to density of asexual stages and gametocytes, by age of the infant at his "first infection" (Fig. 24). It is seen that the density of asexual stages increases with age of the infant at the time of his first infection, while the density of gametocytes is little affected.

Of the 131 "first infections", 41 were observed at the first survey after birth; there were 5 infections in infants "aged" 2-l 1 days (4 out of the 5 had a precise record of the day of birth), the remainder being 21 days old or more. The earliest "first infections" all had densities of asexual stages of less than 0.04 (proportion of fields positive) and none had gametocytes, while of the 36 others, 19 had densities of asexual stages greater than 0.04 and 13 had gametocytes. Some of the earliest infections may be

Fig. 25. Cumulative prevalence of *P. falciparum, P. malariae* and *P. ovale* over 8 surveys conducted at intervals of 10 weeks from late 1970 to mid 1972, before intervention=



a N = number of persons examined at evety one of the 8 surveys; age defined at survey 1; 16 villages combined.

congenital, but the small numbers and the accepted limitations of the data (age) do not allow of a firm conclusion.

There was some spontaneous variation between the years, as may be seen below in Fig. 42, upper half, and in Fig. 43. In particular, there was a spontaneous decline in prevalence from 1971 to 1972, followed by a spontaneous increase from 1972 to 1973.

Cumulative prevalence of patent parasitaemia, and the number of positive results per person

Figure 25 shows the age-specific cumulative prevalence, i.e., the proportion of persons found positive at least once among those examined at all of the 8 baseline surveys. For all 3 parasite species, the cumulative prevalence is much higher than the prevalence at any given survey, and a high maximum is reached in the age-groups l-4 and 5-8 years, when it was 100% for *P. falciparum,* more than *80%* for *P. malariae* and more than 30% for *P. ovale.*

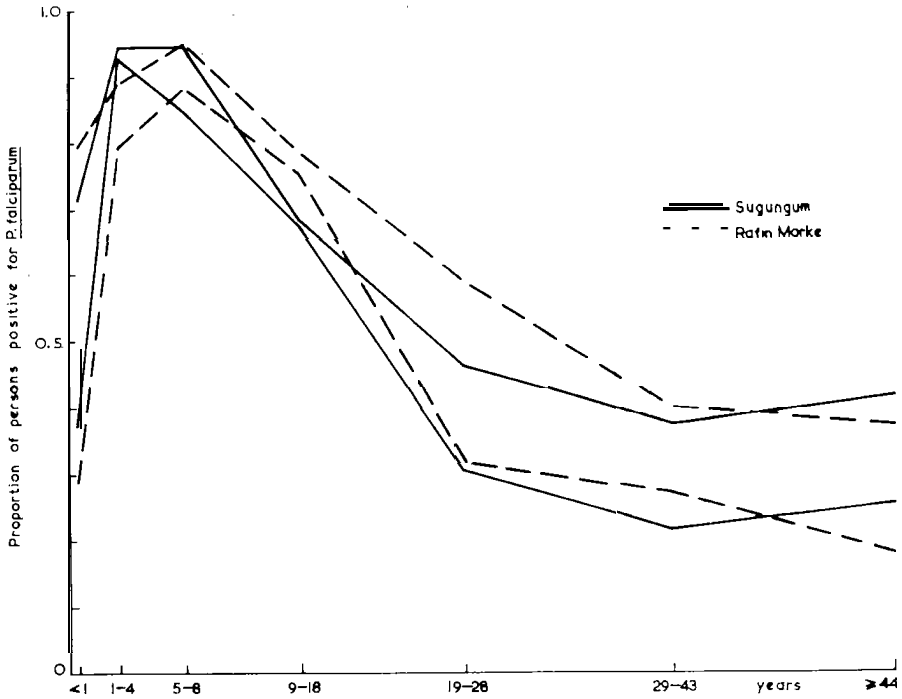
Persons examined at all 8 baseline surveys were classified within specific age-groups by the number of times (O-8) they were found positive. The resulting distribution was compared to the binomial distribution with the same average. This binomial distribution would be expected if results were randomly distributed between persons and surveys. The observed distribution is consistently more dispersed, i.e., there is an excess of persons persistently positive and of persons persistently negative. If the actually observed seasonal variation is taken into account and if the results were randomly distributed between persons but not between surveys, the expected distribution would be even less dispersed than the simple binomial distribution, and the above conclusion regarding parasitological heterogeneity within age-groups would be reinforced.

Geographical variation

For each of the 22 villages covered by surveys 5-8, age-adjusted average proportions positive were computed. This average prevalence varied among the villages between 40.6% and 59.1% for *P. falciparum,* between 9.9% and 21.9% for *P. malariae,* and between 1.1 % and 6.9% for *P. ovale.* The 3 species, including *P. ovale,* were thus present in every one of the 22 villages. For *P. falciparum* the variation between villages was significant ($p<0.001$). For each species, the villages were ranked by prevalence; the rank correlation coefficients were + 0.491 between *P. falciparum* and *P. malariae* ($p<0.05$), + 0.411 between *P. malariae* and *P. ovale* (n.s.) and + 0.108 between *P. falciparum* and *P. ovale* (n.s.).

There was also some variation between villages with respect to the age-specific prevalence of *P. falciparum,* as shown in the case of Sugungum and Rafin Marke (Fig. 26). These are the compact villages having res-

Fig. 26. Age-specific prevalence of *P. falciparum,* before intervention, in the villages with the highest (Sugungum) and lowest (Rafin Marke) vectorial capacity a



a For each **village** the upper curve **represents the wet season (average** of surveys 5 and 6) and the lower curve represents the dry season (average of surveys 3 and 4).

pectively the highest and lowest vectorial capacities (see Chapter 4 and pp. 270-273). In Rafin Marke, the peak of parasitaemia is reached later than in Sugungum (in the 5-8-years age-group rather than in the 1-4-years age-group), and the subsequent decline as a function of age is slower (see the 19-28-years age-group).

Prevalence % was analysed by type of village, with the following results:

Type of village	*Number of* villages	*P. falciparum* Average	*P. falciparum* Range	*P. molariae* Average	*P. ovale* Average
Compact	15	53.5	(48.8-59.1)	14.6	2.8
Mixed	5	41.5	(45.5-48.5)	13.0	2.1
Scattered	2	44.1	(40.6-47.0)	13.2	3.0

P. ***falciparum*** is more common in the compact villages than in the scattered ones, and mixed villages occupy an intermediate position; the

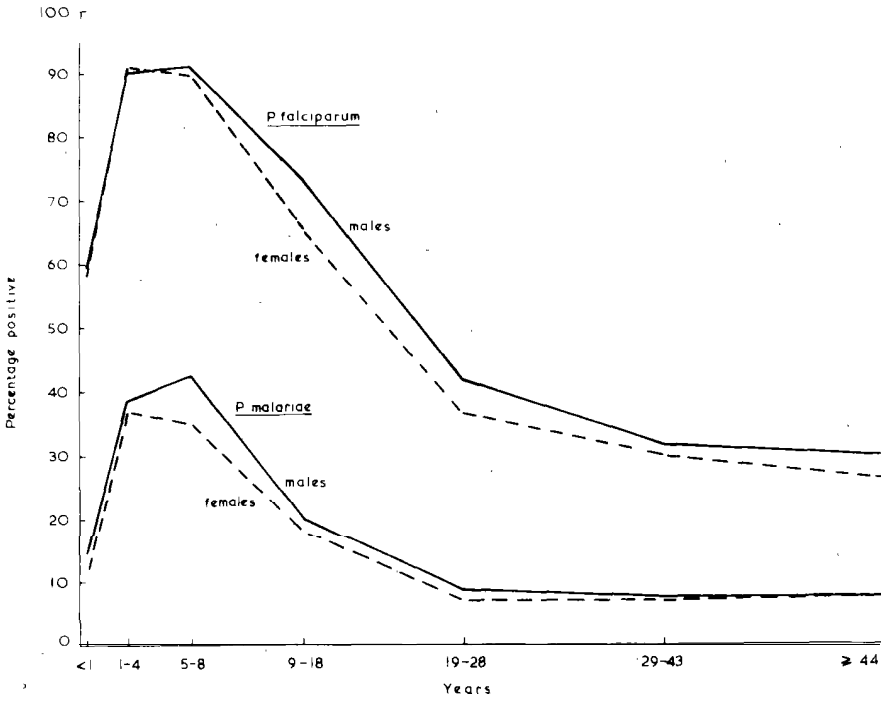
variation between the different types of village is very significant ($p < 0.001$).

The prevalence *(%)* of *P. falciparum* in compact and scattered settlements was also analysed by age, with the following results.

Age-group (years)	*Type of settlement*	
	compact	*Scattered*
O-8	**84.0**	**85.7**
9-28	**53.1**	**42.7**
>29	**30.7**	**20.7**

It is thus only in the older age-groups that *P. falciparum* is less common in the scattered settlements. These parasitological differences are also visible when one compares the compact and scattered sections of individual village clusters.

Fig. 27. Age-specific prevalence of *P. falciparum* and *P. malariae,* by sex; 16 villages combined, average of surveys 1 to 5



Comparison between males and females

The comparison between the sexes for the prevalence of *P. falciparum* and *P. malariae* was made with the year-long data for surveys l-5 combined (Fig. 27). Evidently after 5 years of age, males have rather consistently higher average parasite rates (and parasite densities) than females; several of the differences are statistically very significant (e.g., for *P. falciparum,* the crude prevalence or the prevalence in the age-groups 9-l 8 and 19-28 years, and for *P. malariae,* the crude prevalence or the prevalente in the age-group 5-8 years).

Effects of population movements

The study population is relatively mobile (see Chapter 8). This may have an effect on the understanding of the local epidemiology and on the result of control measures. At each parasitological survey, those who had been present at the preceding survey were compared with those who had been absent, and those who were present at the next survey were compared with those who were absent at the next survey. This was done by individual villages, by village clusters and for the whole population. Only small and unsystematic differences in prevalence were detected, both before and after age-adjustment. One can conclude that there was no difference with respect to malaria, either between those who were leaving and those who were staying or between those who were coming in and those who were already there, and that in the absence of control measures the local epidemiological situation is not significantly affected by the mobility of part of the population studied.

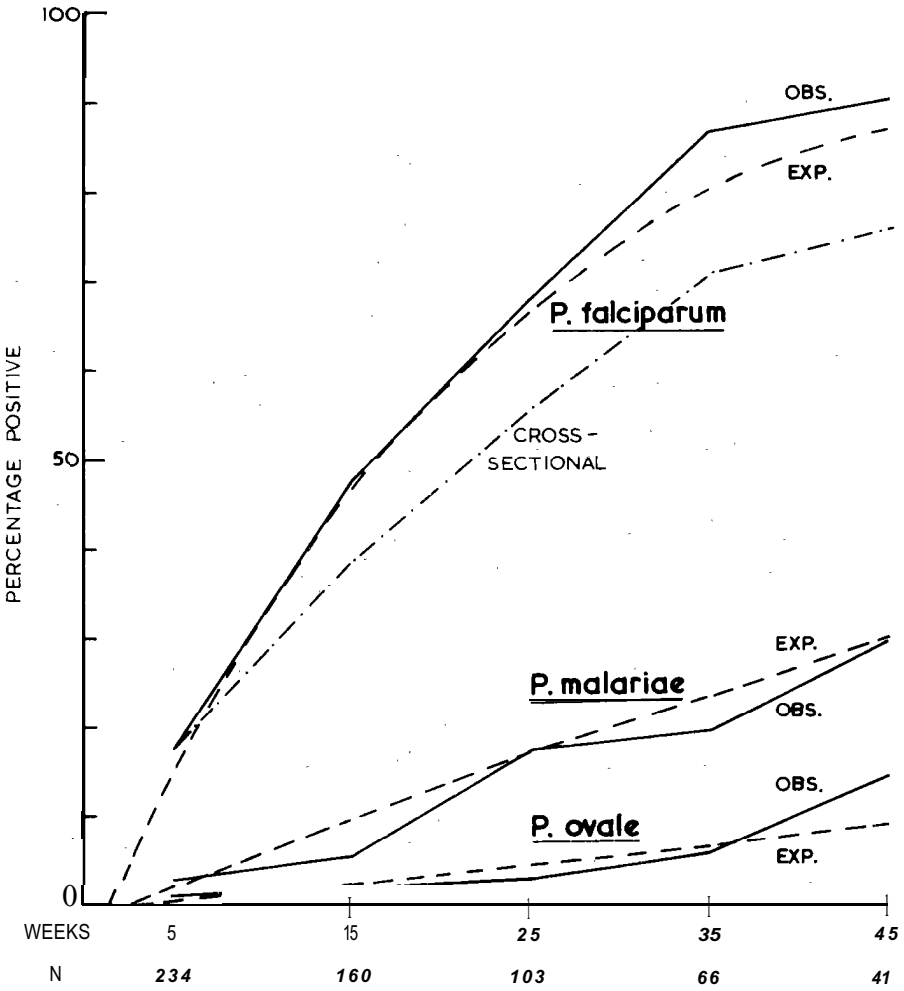
Incidence and recovery rates (conversion and clearance rates)

Infant conversion rates

The daily infant conversion rate (ICR) was estimated by the formula $ICR = -1n\ (1-P)/\ t$, where P is the proportion found positive for the first time for a specified parasite after a given interval, and t is the length of the interval in days. The average ICR over several intervals was calculated by using a weighted average for *P.* Given the daily conversion rate, it is possible to calculate the expected cumulative prevalence by age, i.e., the expected proportion of infants that are, or have been, positive by a given age (Fig. 28); this may be compared to the cumulative prevalence actually observed, for each of the 3 species. All infants born between surveys 1 and 8 in the 16 villages studied throughout were included up to survey 8 or up to the first missed survey. The calculated ICR is the average for the baseline period, and somewhat smaller than the yearly average,

because the baseline period includes 2 dry seasons but only 1 wet season. The infants found positive at the first survey after birth, included in the observed cumulative prevalence, were not included in the estimation of

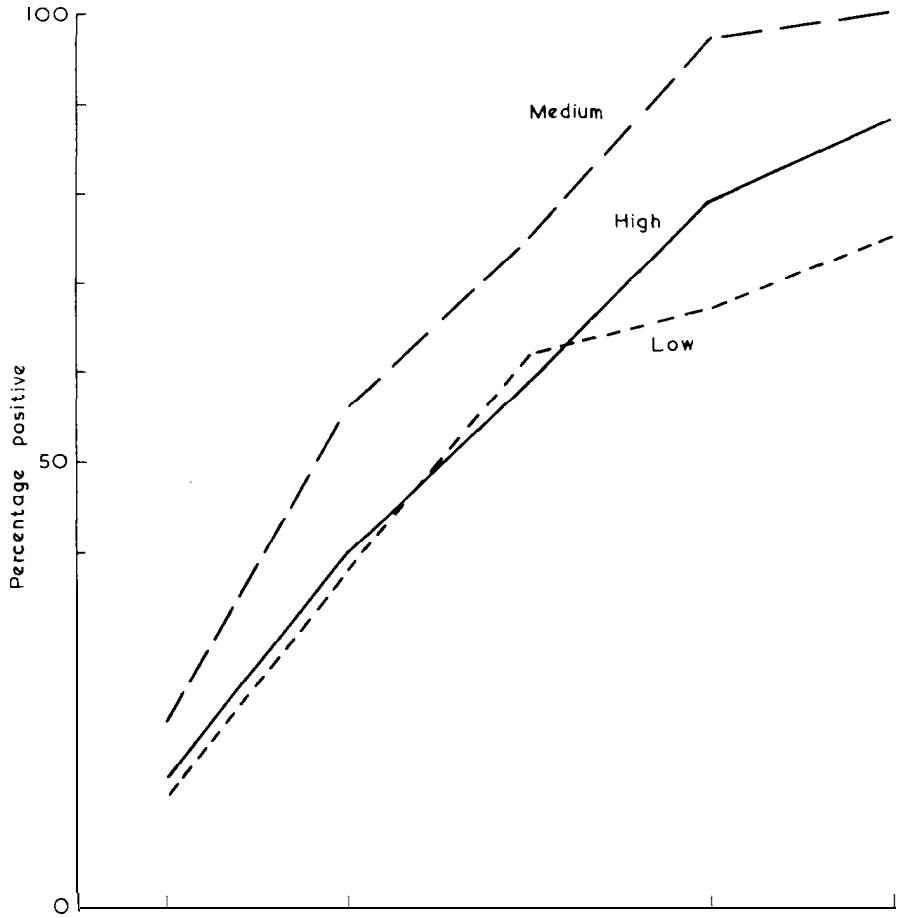
Fig. 28. Observed cumulative prevalence of *P. falciparum, P. malariae* and *P. ovale,* by age of infants regularly followed after birth at IO-week intervals compared with the cumulative prevalence expected from the average infant conversion rate ICR; a baseline phase, all villages combined



a Calculatedfrom: $x(t) = 1-\exp\{-h(t-n)\}$, whereh = *ICR* = 0.0066 for *P. faiciparum,* 0.0012 for *P. mslariae,* and 0.00031 for P. ovale; n, the incubation period (in days) = 10 for *P. falciparum, 20* for *P. malariae* and *P. ovale.* The graph also shows the cross-sectional prevalence of *P. falciparum* in the same infants.

the infant conversion rate. The estimated daily infant conversion rates were 0.0066, 0.0012 and 0.00031 for ***P. falciparum, P. malariae*** and ***P. ovale*** respectively, i.e., the rate was $5\frac{1}{2}$ times greater for ***P. falciparum*** than for ***P. malariae,*** and 4 times greater for ***P. malariae*** than for ***P. ovale.*** In the case of ***P. falciparum*** the observed cumulative prevalence is compatible with the hypothesis that the conversion rate is constant

Fig. 29. Cumulative prevalence of *P. falciparum* in infantsfollowed longitudinally, byage and by the relative level (low, medium, high) of the vectorial capacity of the village; baseline phase, all villages combined



Weeks	5	15	25	35	45
N High	66	42	27	19	16
N Medium	114	81	55	35	17
N Low	54	37	21	12	8

during the first year of life, after allowing for an initial incubation period, i.e., there is no effect of maternal antibody on incidence (see Discussion). Fig. 28 also shows the age-specific cross-sectional prevalence of falciparum parasitaemia in the same infants (the percentage of infants actually positive at a given age). The cross-sectional prevalence is clearly lower than the cumulative prevalence, i.e., infants recover (from patent parasitaemia) at an appreciable rate (see next section).

Figure 29 shows the cumulative prevalence of ***P. falciparum*** in infants, as a function of age, in 3 groups of villages defined by their vectorial capacities. The villages with the "medium" vectorial capacity had the highest observed cumulative prevalence of ***P. falciparum*** in infants, but the differences were not significant (see also p. 148 and Table 17). Note that, in relation to the critical vectorial capacity (see Chapter lO), even the "low" vectorial capacity is in fact very high.

The infant conversion rate showed a marked seasonal variation, both for ***P.falciparum*** (see Fig. 35, 67 and 68) and for ***P. malariae (see*** Fig. ***35).*** For ***P. falciparum,*** there was also a spontaneous decrease from 1971 to 1972, and an increase from 1972 to 1973 (see Fig. 68 and Table 19; see also pp. 59 and 60).

Incidence of and recovery from patent parasitaemia in the general population

For each species, transition frequencies between consecutive surveys were determined, i.e., the numbers N + + , N + -, N--, N- + , where N + + is the number of persans positive at both surveys, N+ - the number positive at the first survey and negative at the second, etc. From these transition frequencies, daily rates of transition between the negative and positive states can be derived as shown by Bekessy et a1.(7). Fig. 30 and 31 show the age-specific transition rates from negative to positive (daily conversion rate $\hat{h}$) and from positive to negative (daily clearance rate $\hat{r}$), in the 16 villages studied throughout. Yearly averages were obtained by summing the numbers N + + , etc. over 5 successive lO-week intervals. ***P. falciparum*** has the highest conversion rate and the lowest clearance rate, i.e., it is with ***P. falciparum*** that episodes of patent parasitaemia are most frequent and of longest duration. ***P. ovale*** has the lowest conversion rate and the highest clearance rate, i.e., its episodes of patent parasitaemia are both the rarest and the shortest. ***P. malariae*** has intermediate conversion and clearance rates.

The conversion rates $\hat{h}$ increase in early life, then decrease; the mean conversion rate varies between age-groups by factors of about 2, 5 and 3 for ***P. falciparum, P. malariae,*** and ***P. ovale*** respectively (Fig. 30). In early life, the clearance rates $\hat{r}$ may decrease in the case of ***P. falciparum,***

Table 17

Entomological inoculation rate and Infant conversion rate for *P. falciparum,* in the wet season of 1971, before intervention, in villages grouped according to their vectorial capacities

Vectorial capacity	Village clusters a	Daily entomological inoculation rate (EIR)[b]	Daily infant conversion rate for *P. falciparum* (ICR)[c, d]	Ratio ICR/EIR
Lowest	4, 5 (3 villages)	0.229	0.0128 ± 0.0033 (16/25)	0.056
Intermediate	1,2, 6, 8 (8 villages)	0.252	0.0163 ± 0.0027 (43/59)	0.065
Highest	3, 7 (5 villages)	0.940	0.0124 ±0.0017 (17/27)	0.013

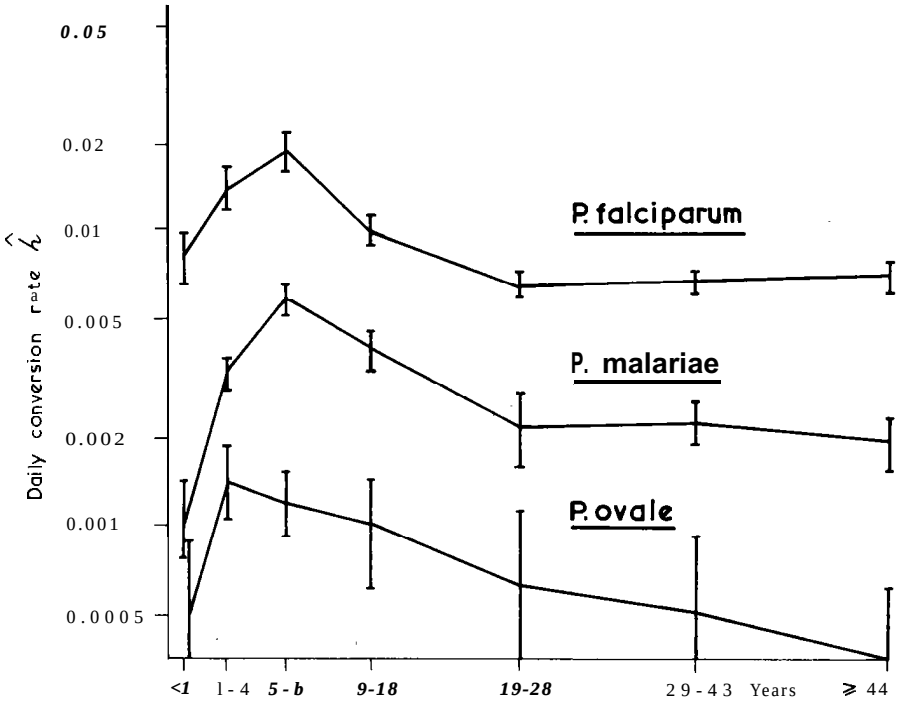
a The 6 villages added at the fifth survey are excluded.

b Weighted by the number of infant periods of risk in each village cluster.

c In parentheses: number of conversions/number of infant-periods of risk. The intervals considered are those between surveys 4 and 5 and between surveys 5 and 6, of average duration t = 80 days.
ICR = -ln *(1-p)* / f, where p is the ratio shown.

d Standard error of ICR = $S_p / t(1-p)$, where $S_p = 1/\sqrt{(N-+/p^2) + (N--/(1-p)^2)}$, where $N-+$ and $N--$ = numbers converting and remaining negative respectively in the interval.

Fig. 30. Daily incidence rate of patent parasitaemia (conversion rate) for 3 species of parasite, by age; baseline period, 16 villages, 1-year averages and 95% confidence limits

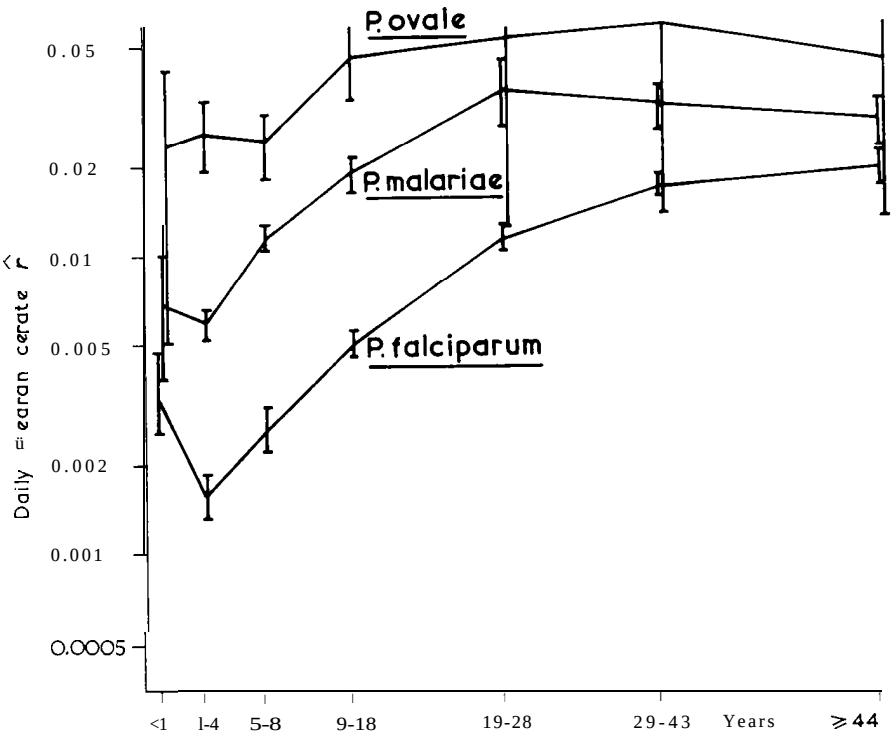


or show no systematic variation as with ***P. malariae*** and P. ***ovale;*** later in life, the clearance rate increases towards an adult plateau; the mean clearance rate varies, between age-groups, by factors of about 10,5 and 2 for ***P. falciparum, P. malariae*** and ***P. ovale*** respectively (Fig. 31).

The expected equilibrium parasite rate $\hat{h}/(\hat{h} + \hat{r})$ was very close to the one actually observed, except in infants, who had not had time to reach equilibrium.

Figure 32 shows the variation of the conversion rate $\hat{h}$ by age and season. Since there was no significant difference between th,e three oldest age-groups, they have been combined. The curves can probably be interpreted as follows: the difference between the wet season level and the dry season level represents new infections; the dry season level represents mainly relapses of old infections. According to that interpretation, infant conversions represent mainly new infections; the infants' curve in Fig. 32 is indeed very similar to the infant conversion rate calculated on the basis of "first infections" only (see Fig. 67); after infancy, an increasing proportion of "conversions" are relapses, up to a maximum

Fig. 31. Daily recovery rate from patent parasitaemia (clearance rate) for 3 species of parasite, by age; baseline period, 16 villages, 1-year averages and 95% confidence limits



in the group of 5-8 years; after the age of about 5 years, the rate of new infections remains about the same, while the rate of patent relapses decreases.

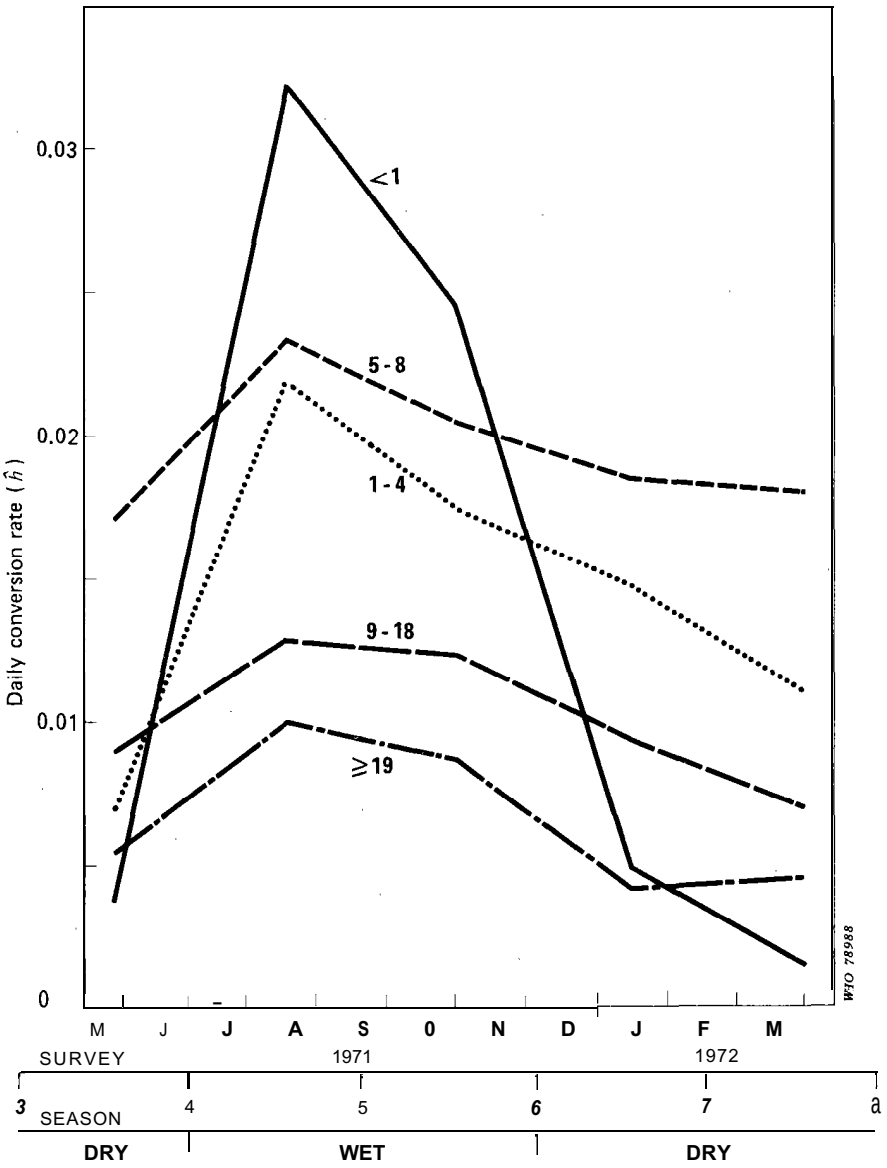
By comparison between villages, it appears that $\hat{r}$ tends to decrease (episodes of parasitaemia tend to be longer) as the vectorial capacity increases; this may be an effect of superinfection.

Relationship between parasitological and entomological findings

This section looks into the relationship between the parasitological variation by season, year and place (see pp. 115 and 122) and the entomological findings (see Chapter 4).

The seasonal variation in prevalence, density and incidence of ***P. falciparum*** and ***P. ovale*** parasitaemia appears directly related to the seasonal variation in the density of vectors, the entomological inoculation rate

Fig. 32. Estimated daily incidence rate of patent *P. falciparum* parasitaemia (conversion rate), by age and season; baseline period



and the vectorial capacity. The relationship between the seasonal variation of ***P. malariae*** and the entomological findings is more complex (see below).

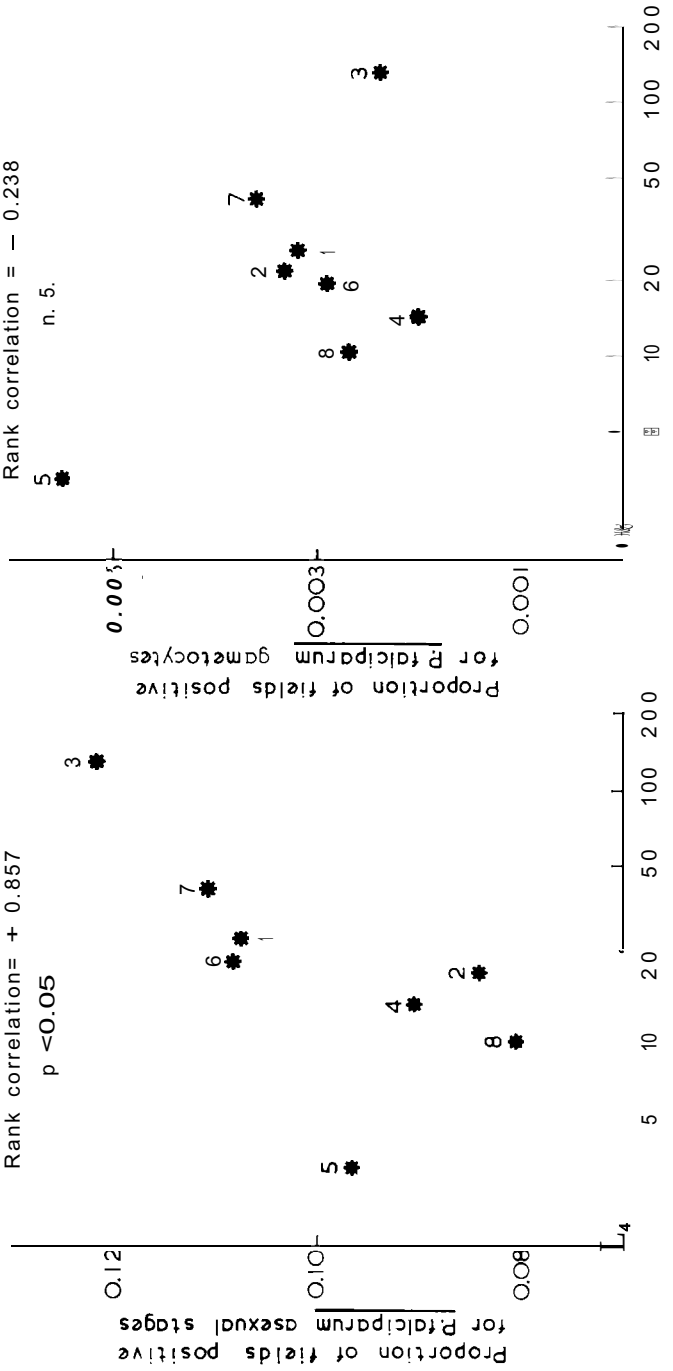


Fig. 33. Correlation between density of vectors and density of ***P. falciparum*** asexual stages and gametocytes in 8 villages (1-8) a

a Density of vectors = No. of A. gambiae Sl. and A. funestus females by PSC, average for 1971 wet season. Density of parasites = proportion of fields positive among all fields examined, average of surveys 3-7, covering 1 year and including 1971 wet season.

The spontaneous decrease in prevalence and incidence of ***P. falciparum*** from 1971 to 1972, and their spontaneous increase from 1972 to 1973, in the untreated control villages (Fig. 42, upper half; Fig. 68) corresponded to the contemporary spontaneous changes in vector density and vectorial capacity (Fig. 7, 8 and 42; Tables 7 and 32).

The relationship between parasitological and entomological variation between villages was investigated in the 8 compact settlements in which both the indoor-resting density and the man-biting rate were estimated. The average age-adjusted parasite rates and densities for surveys 3-7, covering one whole year, were compared with the average vector densities in the wet season of 1971, corresponding to parasitological surveys 4-6. There was a positive correlation. It was strongest between the indoor-resting density of vectors (number of females/hut) and density of asexual stages of ***P.falciparum*** in persons positive for the species (Fig. 33, left); the correlation was significant. On the other hand, between the same index of vector density and the density of gametocytes in persons positive for ***P. falciparum*** the correlation was negative although not significant (Fig. 33, right).

In the scattered settlements, adults have, on the average, a lower prevalence of ***P.falciparum*** than in the compact settlements (see p. 123). Only limited entomological observations are available from the scattered settlements, but they suggest on the other hand that the man-biting rate is usually higher in the scattered than in the compact settlements (see p. 94). The parasitological and entomological findings could be reconciled by the hypothesis that the level of transmission is higher in the scattered settlements and that this produces a higher level of immunity, hence a lower level of parasitaemia, in the adults. The data, however, do not allow the hypothesis to be tested.

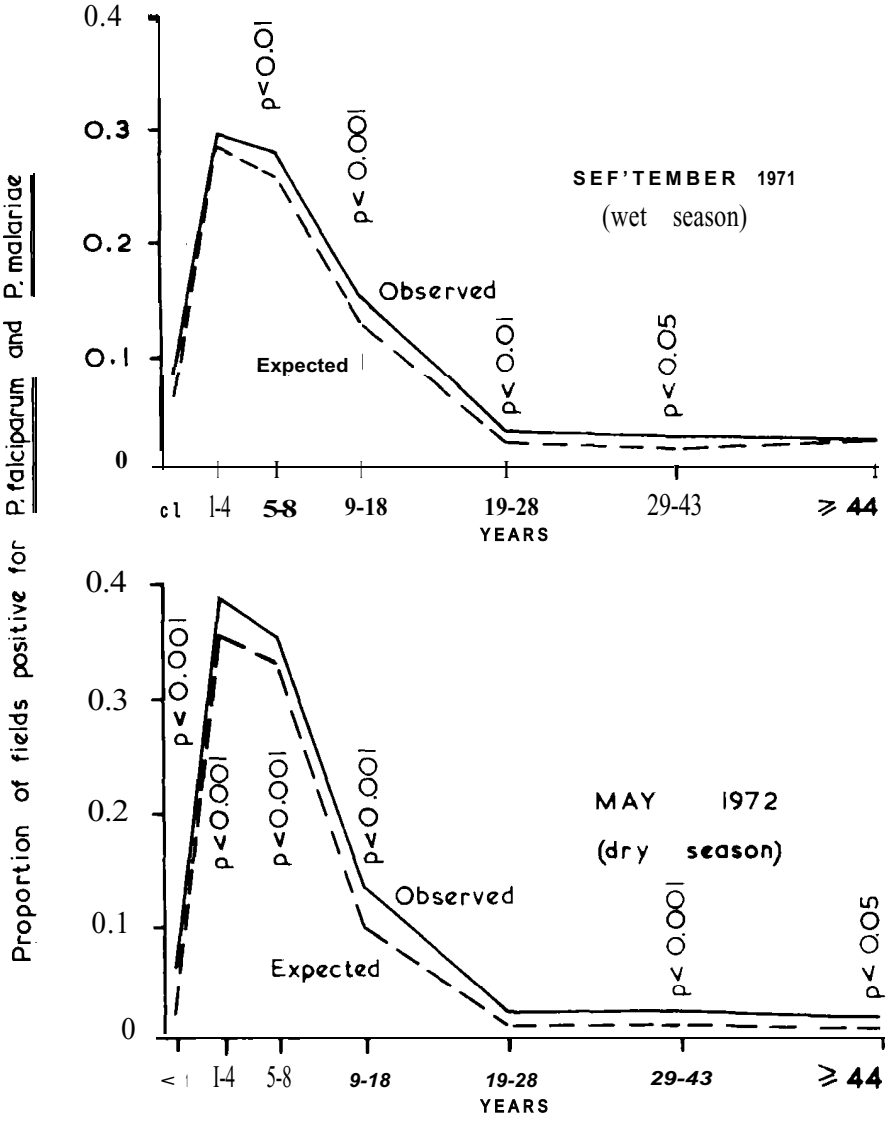
Between the study villages, i.e., between villages with high to very high levels of transmission, an increase in vector densities and vectorial capacities produced an increase in entomological inoculation rates (see Chapter 4), but no increase in infant conversion rates (Table 17, Fig. 29). As a consequence, at the highest vectorial capacity, there is a decrease in the ratio between the entomological inoculation rate and the infant conversion rate, i.e., a decrease in the proportion of inoculation resulting in a patent infection.

Mixed infections and the relationship between *P. falciparum* and *P. malariae*

This topic has been the subject of an unpublished report (116), which may be applied for by the reader who desires more details.

At each of the 8 baseline surveys, within specific age-groups, the pre-

Fig. 34. Observed orevalence of mixed infections with *P. falciparum* and *P. malariae* (in the wet and dry seasons in 22 untreated villages) compared with prevalence expected under the hypothesis of independence between the species



valence of double and triple infections was regularly greater than that expected under the hypothesis of independence between the species. The excess of multiple infections was often statistically significant, although it was usually not very large. An example was the excess of mixed parasit-

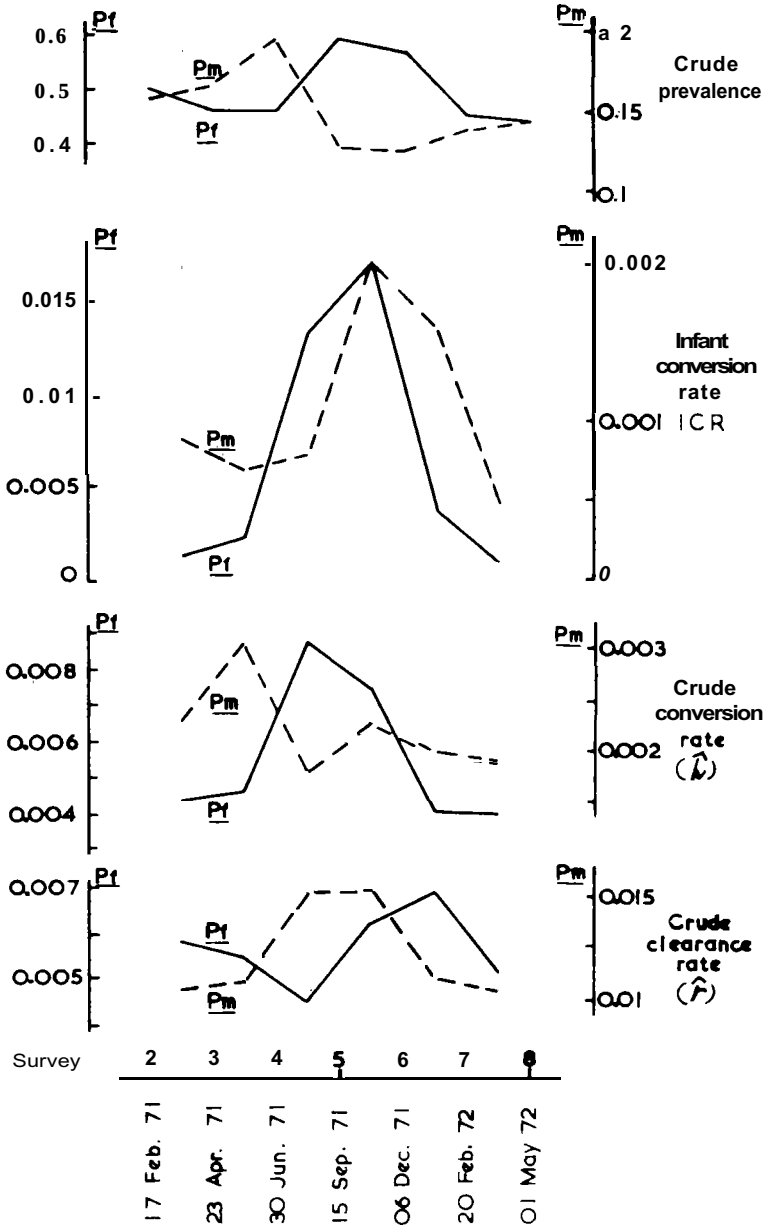
aemias of ***P. falciparum*** and ***P. malariae*** in both the wet and the dry seasons (Fig. 34).

Among persons examined at all 8 baseline surveys, within specific age-groups, there was a significant correlation between the number of times a person had been found positive for a given species and the number of times the same person had been found positive for either of the 2 other species. The correlation was strongest between ***P. falciparum*** and ***P. malariae,*** between which it remained significant even when only odd-numbered surveys were considered for one species and only even-numbered surveys for the other species. In addition, if a person was positive a given number of times for ***P. falciparum*** and a given number of times for ***P. malariae,*** the two tended to be found simultaneously, although the opposite would have been expected from the contrast between the 2 species with respect to seasonal variation (see Fig. 20, and the text below).

The relationship between ***P.falciparum*** and ***P. malariae*** was also analysed with respect to the changes observed between consecutive surveys. At a given survey, a person is either positive for ***P. falciparum*** and for ***P. malariae,*** or positive for ***P. falciparum*** only, or positive for ***P. malariae*** only, or negative for both. At the next survey, he may be in the same state, or may have moved to any of the other 3 states. The fate of a group of persons, between consecutive surveys, with respect to ***P. falciparum*** and ***P. malariae*** can be represented by a 4 x 4 transition matrix. This was done for each of the 7 age-groups and for each of the 7 intervals between consecutive baseline surveys. The observed distribution in the 4 x 4 matrices was compared to the one expected under the hypothesis that transitions with respect to one species are independent of transitions with respect to the other species. There was a great difference between observed and expected: the probability of acquiring or keeping either species was larger if the other species was initially present. The results so far presented in this section thus point to a positive association between ***P. falciparum*** and ***P. malariae.***

On the other hand, their seasonal variations are dissociated. The contrast in the seasonal variations of ***P. falciparum*** and ***P. malariae,*** noted above (see p. 116 and Fig. 20), was explored further. The crude prevalente, infant conversion, crude conversion and crude clearance rates in 16 untreated villages were plotted to compare directly one ***Plasmodium*** species with the other (Fig. 35). The prevalence was based on the examination of about 5200 persons per survey. For the estimation of the infant conversion rate, the number of infants available for first parasitological conversion ranged, by species and interval, between 34 and 111. The crude transition rates, $\hat{h}$ and $\hat{r}$ calculated according to the method of Bekessy et al. (7), are based on the examination of about 4700 persons

Fig. 35. Prevalence of *P. falciparum* (Pf) and *P. malariae (Pm),* by survey, and transition rates between consecutive surveys, in 16 untreated villages a



a All curves scaled so that their seasonal peaks are at the same level.

per pair of consecutive surveys. A strong negative correlation was shown between the seasonal variations of the prevalences of ***P. falciparum*** and ***P. malariae,*** respectively (Fig. 35, top); in particular, in the early wet season a rapid decrease in the prevalence of ***P. malariae*** coincided with the rapid increase in the prevalence of ***P. falciparum.*** Both the negative seasonal association and the rapid change in opposite directions in the early wet season were also found regularly in individual villages, and in different years in the untreated villages. Also shown in Fig. 35, in these cases by interval between consecutive surveys, are the infant conversion rate (the rate at which infants become positive for the first time) and the crude conversion and clearance rates (the rates at which the average person becomes parasitologically positive or negative, respectively). In the case of ***P. falciparum,*** the seasonal variations of the infant and crude conversion rates are very similar and changes in either of the rates are closely followed by concordant changes in the prevalence. In the case of ***P. malariae,*** the infant and crude conversion rates behave very differently: the crude conversion rate is associated with the prevalence, as in the case of ***P. falciparum,*** but the infant conversion rate is clearly dissociated: its seasonal variation follows, with a small delay, that of the ***P.falciparum*** infant conversion rate, and both follow clearly the seasonal variation in vector density; in the case of ***P. malariae,*** this means that the seasonal peak of the crude prevalence occurs about 35 weeks after the seasonal peak of the infant conversion rate.

The relationship between transition rates and prevalence was explored as follows: Ross's mode1 was used to predict the prevalence of ***P. falciparum*** and ***P. malariae*** at surveys 3-8 given the prevalence at survey 2 and the transition rates in the intervals between consecutive surveys; a the infant conversion rate was adjusted by multiplying it by the ratio between the yearly average crude conversion rate (surveys 3-8) and the yearly average infant conversion rate; this ratio was equal to 0.88 for ***P. falciparum*** and to 1.8 for ***P. malariae.*** As expected in both species the crude conversion and clearance rates predicted the seasonal variation of the prevalence very well; if the crude conversion rate was replaced by the adjusted infant conversion rate, the prediction is still qualitatively of the right shape for ***P.falciparum,*** while for ***P. malariae*** the predicted seasonal variation is the inverse of the one actually observed.

Given the above relationship between infant and crude conversion rates, it is of interest to study the variation of the apparent incidence

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a The following formula was used: $x_t = \frac{h}{h+r} - \left(\frac{h}{h+r} - x_o\right) e^{-(h+r)t}$ where x_o, x_t = the prevalence (proportion positive) at times o and ***t;*** t = the interval in days; and ***h,r*** = the daily incidence and recovery rates (58).

rate, or conversion rate $\hat{h}$ by season and age. For ***P.falciparum*** the seasonal variation in apparent incidence rate is very similar in all age-groups ***(see*** Fig. ***32).*** For ***P. malariae,*** up to 5 years of age the pattern is about the same as that of the infant conversion rate: apparent incidence is maximal in the season of high vector density; at ages $\geq$19 years it is the reverse: the apparent incidence is lowest in the wet season, highest in the dry; the age-group 5-l 8 years shows an intermediate pattern.

The Intervention Phase: Parasitological Effect of the 3 Control Strategies

The phases of the project, the 3 control strategies applied, and the allocation of the 8 follow-up village clusters to the 3 strategies and to the untreated control group, are described in Chapter 2. The control operations are described in Chapter 3 (see in particular Fig. 5, showing the timing of mass drug administrations, as well as parasitological surveys).

The present section describes the parasitological effects of the intervention. The observations are presented in about the same order as those made in the absence of intervention (see p. 115), i.e., essentially by method of parasitological assessment (e.g., prevalence, infant conversion rate, etc.). The overall effect of each of the 3 control strategies is considered in the discussion.

Prevalence and density of parasites

Prevalence and density by survey (or season) and treatment

Figures 36 and 37 show the crude prevalence of ***P.falciparum*** and ***P. malariae,*** respectively, by survey and treatment, through the baseline and intervention phases. Before intervention, the 4 groups (pairs of village clusters) were very similar. Propoxur alone (B) had a small but definite effect on the prevalence of ***P.falciparum,*** no effect on the prevalence of ***P. malariae.*** Propoxur plus Iow-frequency MDA (A2) brought the prevalence of ***P. falciparum*** down to about 10% in the wet season of 1972, further down to about 2% in the dry season of 1973, but did not prevent it from rising to 28% at the end of the wet season of 1973.

In that wet season, there was no decrease in spraying or MDA coverage (see Chapter 3, in particular Fig. 5) nor any decrease in the relative impact of propoxur (see p. 80), but there was an increase in the underlying vector density, also noted in the untreated control villages (see pp. 60 and 77). Propoxur plus high-frequency MDA (Al) produced almost

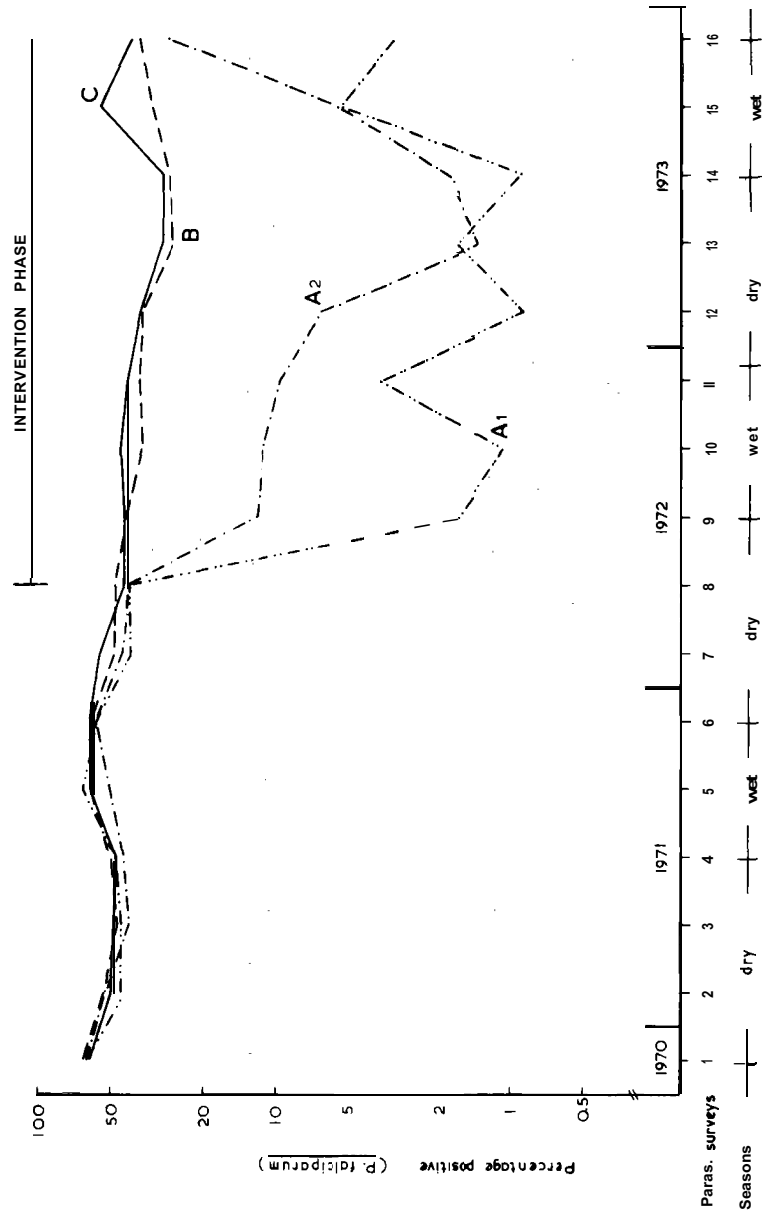


Fig. 36. Crude percentage positive for *P. falciparum*, by survey and treatment throughout the baseline and intervention periods a

a Interventions: propoxur in Al, A2, B; mass administration of sulfalene-pyrimethamine in A2 every 10 weeks, and in Al every 2 weeks in the wet season and every 10 in the dry season.

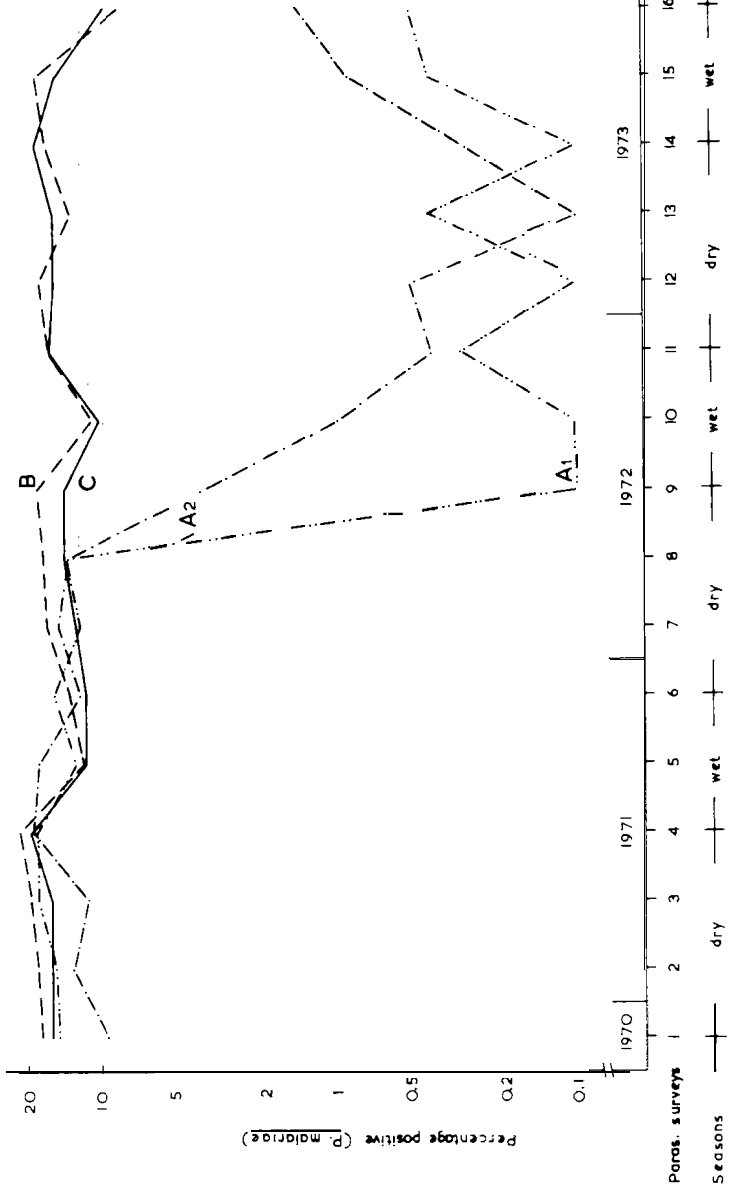


Fig. 37. Crude percentage positive for *P. malariae*, by survey and treatment, throughout the baseline and intervention periods [a]

[a] Interventions: propoxur in AI, A2, B; mass administration of sulfalene-pyrimethamine in A2 every 10 weeks, and in AI every 2 weeks in the wet season and every 10 in the dry season. No result was zero; all results smaller than 0.15% are drawn as 0.1%.

immediately, i.e., by the first intervention survey, a reduction of the prevalence of ***P. falciparum*** to about 2 % after which the prevalence oscillated between 1% and 5% for the rest of the intervention phase (recorded seasonal peaks: 3.7% and 5.3% in 1972 and 1973, respectively). The effect of propoxur and MDA (A2 and Al) on the prevalence of ***P. malariae*** was qualitatively similar to their effect on the prevalence of ***P. falciparum,*** but quantitatively greater (in relation to the baseline prevalence).

The diagnosed prevalence of ***P. ovale,*** which in the wet season of 1971 (baseline) reached 3-6% according to the (future) treatment group, decreased to very low levels in 1972 and even lower levels in 1973 in all groups and in the untreated controls. In surveys 9-16, only 20, 60, 10 and 2 films were classified as positive for ***P. ovale,*** in groups C, B, A2 and Al, respectively (in B, 46 of the 60 were diagnosed at survey 9).

Table 18 shows the seasonal averages of the crude prevalence of ***P. falciparum, P. falciparum*** gametocytes and ***P. malariae,*** by treatment, through the baseline and intervention phases; it also expresses the intervention results as a proportion of the results obtained in the same season in 1971. In the untreated control villages (C) there was a spontaneous decrease in the prevalence of ***P. falciparum*** (from 60.4% in the wet season of 1971 to 43.3% and 47.5% in 1972 and 1973), without decrease in ***P. malariae.*** With propoxur alone (B) there was a somewhat larger decrease in the prevalence of ***P. falciparum*** (from 60.1% in the wet season of 1971 to 36.8% and 35% in 1972 and 1973), with little or no decrease in ***P. malariae.*** With propoxur and MDA (A2 and Al), the prevalence of ***P.falciparum*** decreased to much lower levels (10.4% and 2.4% in the wet season of 1972; 16.5% and 4.2% in 1973) and the prevalente of ***P. malariae*** decreased even more in relation to the baseline values. The changes in the prevalence of ***P. falciparum*** gametocytes in the various treatment groups were sometimes larger, sometimes smaller than the changes in the prevalence of ***P. falciparum*** without clear-tut pattern.

Certain findings regarding the age-specific prevalence and density of parasitaemia are presented in Fig. 38, 39 and 40. The age-specific prevalente of ***P. falciparum*** in each of the 4 treatment groups in the wet season of 1971 (i.e. before intervention) and in the second wet season of the intervention phase (1973) is shown in Fig. 38, upper half. Before intervention there was little difference between the groups; propoxur alone (B) reduced prevalence in all age-groups, more so below 5 years and above 19 years; the addition of MDA (A2, Al) reduced prevalence to a low level in all age-groups.

The age-specific prevalence of ***P. malariae*** in the untreated controls (C) and in the villages treated with propoxur alone (B) is shown in

Table 18

Crude parasite rate *(%)* **of *P. falciparum, P. falciparum*** gametocytes, and ***P. malariae,*** by season a and treatment [b]

Parasite		1971 Baseline period Dry season Surveys 2-4	1971 Baseline period Wet season Surveys 5-6	1972 Dry season Surveys 7-8	1972 Wet season Surveys 10-11	1973 Intervention period Dry season Surveys 12-14	1973 Intervention period Wet season Survey 15--16
P. falciparum	C	47.2	60.4	48.7	43.3 (0.72) [a]	32.1 (0.68)	47.5 (0.79)
	B	48.7	60.1	47.5	36.8 (0.61)	30.3 (0.62)	35.0 (0.58)
	A2	46.1	52.7	42.9	10.4 (0.20)	3.2 (0.07)	16.5 (0.31)
	A I	44.7	59.7	41.9	2.4 (0.04)	1.2 (0.03)	4.2 (0.07)
P. falciparum gametocytes	C	11.5	18.8	10.0	12.0 (0.64)	7.0 (0.61)	16.9 (0.90)
	B	12.2	16.1	9.9	8.9 (0.55)	6.4 (0.52)	11.0 (0.68)
	A2	8.6	14.3	9.4	3.7 (0.26)	0.1 (0.01)	8.9 (0.62)
	A I	11.4	18.0	10.2	0.1 (0.01)	0.2 (0.02)	1.2 (0.07)
P. malariae	C	17.3	11.3	13.3	13.0 (1.15)	16.3 (0.94)	11.9 (1.05)
	B	19.5	12.7	16.9	13.3 (1.05)	15.7 (0.81)	13.3 (1.05)
	A2	14.4	15.1	14.2	0.7 (0.05)	0.3 (0.02)	1.2 (0.08)
	A I	17.1	14.1	13.1	0.2 (0.01)	0.2 (0.01)	0.5 (0.04)

a Dry season = average of first 3 surveys of the year (except survey 9, omitted because it fell in the intervention phase); wet season = average of last 2 surveys of the year.

b Treatment (starting aftersurvey8): propoxur in B, A2, Al; MDA in A2(every 1Oweeks) andAI (every 2weeks in thewetseason, every 10 weeks in the dry season).

c The observed parasite rate (PR) divided by the PR of the same treatment group in the corresponding season in 1971 (e.g., 43.3/60.4 = 0.721.

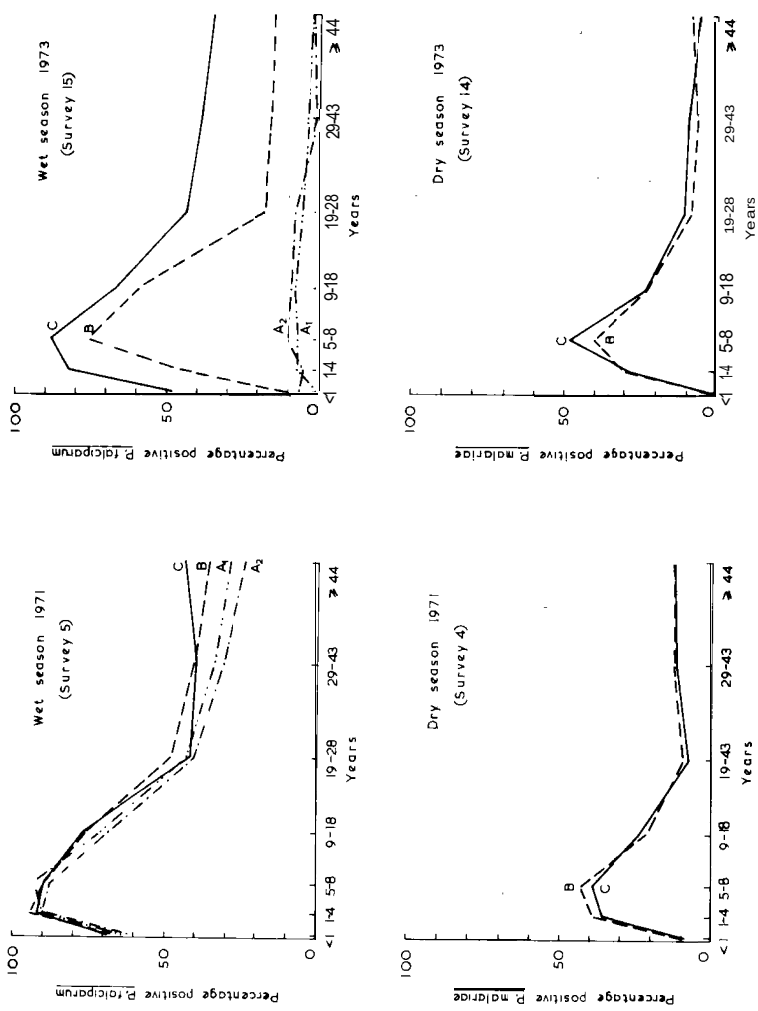
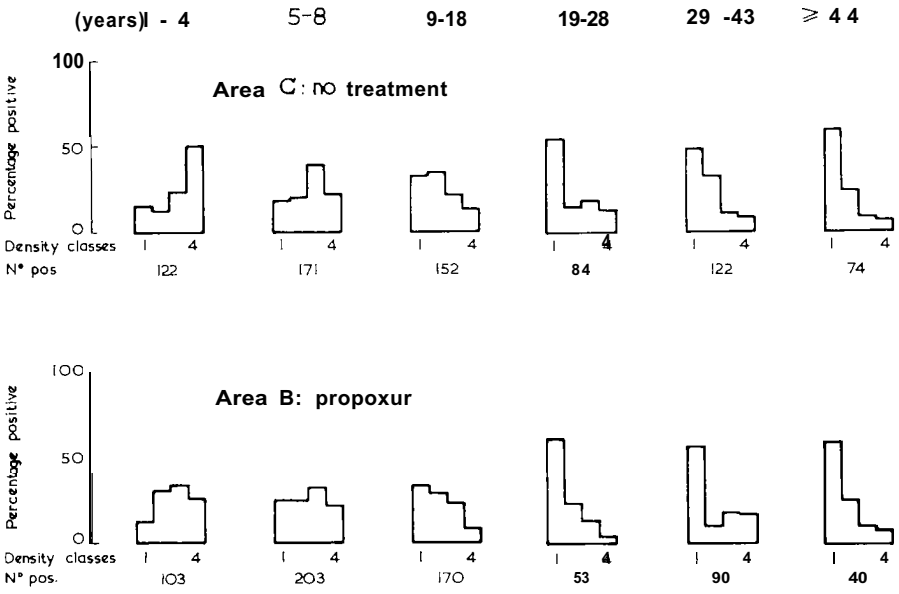


Fig. 38. Age-specific parasite rates for *P. falciparum* at surveys 5 (wet season, baseline period) and 15 (second wet season of intervention period) by treatment (Al, A2, B, C) and for *P. malariae* at surveys 4 (end of dry season, baseline period) and 14 (end of dry season, intervention period), by treatment (B, C)[a]

a Interventions: propoxur in Al, AZ, B; MDA in A2 every 10 weeks; MDA in Al every 2 weeks in the wet season and every 10 in the dry season.

Fig. 39. Age-specific distribution of persons positive for *P, falciparum* asexual stages, according to the density of the infection, in untreated villages and in villages sprayed with propoxur alone in the second wet season of intervention (survey 15)[a]



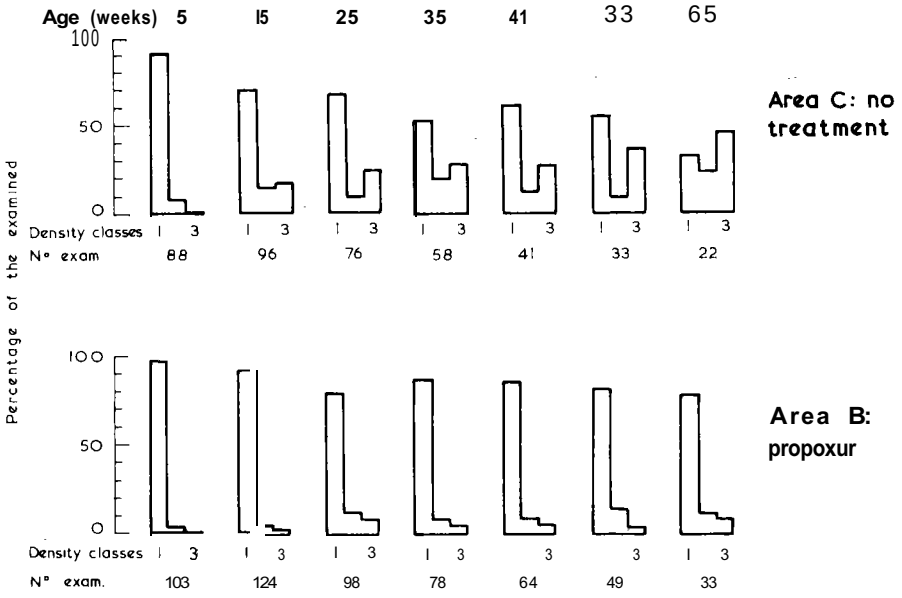
a Positivesaredistributed intoclasses 1-4defined bythefollowing upperlimits: 4%, 16%. 64% and 100% of microscope fields positive on the thick film.

Fig. 38, lower half: there was no difference either before or during intervention; the prevalence of ***P. malariae*** in A1 and A2 (not shown) was similar to the others before intervention and very low during intervention.

Figures 39 and 40 compare the untreated control villages (C) and the villages treated with propoxur alone (B) in terms of density of ***P. falciparum*** infections. Fig. 39 shows the distribution of positives into 4 density classes in the wet season of 1973 in age-groups from l-4 years to ≥44 years. In the age-group l-4 years, ***P. falciparum*** parasitaemias are less dense in the sprayed villages than in the controls. After 5 years of age, there is no significant difference.

Figure 40 compares the infants born into the 2 groups of villages in terms of parasitaemia at 5, 15, 25 etc. weeks of age, i.e., at the lst, 2nd, 3rd, etc. survey after birth: with propoxur, prevalence increases more slowly, and infections have on the average a lower density of asexual stages. There was no significant difference between sprayed and unsprayed villages with respect to prevalence and density of gametocytes.

Fig. 40. Density distribution of *P. falciparum* asexual stages in infants born in the intervention period, in untreated villages and in villages treated with propoxur alone[a]



a 3 classes: negative, those with up to 16% positive fields, and those with more than 16% positive fields.

With MDA (Al and A2) the density of the relatively small number of positive was quite variable.

Number of positive results per person

In the absence of treatment (C) or with propoxur alone (B), the distribution of persons by the number of times they were positive for *P. falciparum* in surveys 9-16 continued to be very different from random, even within specific age-groups, with large excesses of persistent negatives and persistent positives, as in the baseline period (see p. 122). With high-frequency MDA (Al), the distribution of positive results did not differ from a random distribution, even when all age-groups are combined. With low-frequency MDA, the distribution was non-random, but the difference was not large.

Variation between villages

There was some variation in the parasitological picture between similarly treated villages; the variation was unrelated to the small recorded variations in coverage by either spraying or MDA.

In the absence of drug administration-i.e., in the untreated controls (C) and in the villages treated with propoxur alone (B)-the parasitological differences between villages was related to the corresponding entomological differences (see p. 151).

The MDA record of positive and negative individuals

In the villages in which drugs were administered (Al and A2), the MDA record of the positives (nearly all *P. falciparum)* was compared to the average; since positives were a small minority, the average MDA record is practically the same as that of the negatives.

With low-frequency MDA (A2), 67% of the positives found at surveys 9-16 had been treated at the previous MDA round, i.e., 10 weeks earlier, versus 85% in the general population. The difference was largest at survey 9, when only 33 of the 185 positives had been included in the first MDA 10 weeks earlier. The difference was present at all surveys except survey 16, when the relatively large number of new positives had received the average treatment 10 weeks earlier.

With high-frequency MDA (Al), 61% of the positives had been treated at the last previous round (the time interval between drug administration and blood examination was variable, see Fig. 5). The earliest infections detected after treatment were 47 cases from surveys 9, 10 and 14; they were detected 10-15 days after a recorded treatment; this represents 1% of all the persons treated in the 3 relevant MDA rounds.

Positives had thus been treated significantly less frequently than average. Nevertheless, at both MDA frequencies, the majority of positives had been treated, which suggests that a significant proportion of them represent new infections caused by local transmission.

Effect of population movements

The type of analysis used to explore the parasitological effect of popu-

During the intervention period, in the untreated control villages, there continued to be no difference at a given survey between those who had been present at the preceding survey and those who had been absent, nor between those who were present and those who were absent at the next survey. This was also the case in the villages treated with propoxur alone, while in the villages treated with MDA those leaving and entering had almost consistently higher parasite rates than the others, both with and without age-adjustment. The contribution of imported cases may be estimated in the following way. At survey 14, i.e., at the onset of the transmission season of 1973, there were 26 positives for *P.falcipantm* out of 1268 persons examined in the follow-up villages of area A2 and 19 positives out of

1820 persons examined in the follow-up villages of area A1. If those who were absent at the previous survey (13) had sustained at survey 14 the same parasite rate as the others, there would have been only 10 positives in the follow-up villages of area A2 and 17 positives in the follow-up villages of area A1.

Incidence and recovery rates (conversion and clearance rates)

Infant conversion rates

Figures 68-70 in Chapter 8 show the infant conversion rates for P. ***falciparum*** between consecutive surveys, i.e., for each lO-week period in 1971-1973 during both the baseline and intervention phases in the untreated control villages (C; Fig. 68), in the villages treated with propoxur alone (B; Fig. 69) and in the villages treated with propoxur and MDA (A2 and A1; Fig. 70). The natural variation of the infant conversion rate, already noted (see pp. 128 and 134), is shown in Fig. 68. Propoxur alone clearly reduces the infant conversion rate, and this to a larger degree than the crude prevalence (Fig. 69; see also Figs. 36 and 38). The addition of MDA reduces the infant conversion rate to a very low level, but not to 0 (Fig. 70).

Table 19 shows the infant conversion rates (ICR) for ***P. falciparum*** in the wet seasons from 1971 until 1975 in the follow-up village clusters grouped by treatment, along with the corresponding entomological inoculation rates (EIR), and the ratio ICR/EIR (see next section). The 4 groups in 1971 had a very similar baseline ICR (0.012 to 0.016); during the intervention phase (1972, 1973) there was some spontaneous variation of the ICR (Group C; see pp. 128 and 134). All interventions clearly reduced the ICR beyond what could be attributed to spontaneous variation, while none of them interrupted transmission for any length of time. Propoxur plus MDA (A2 or A1) caused a greater reduction than propoxur alone (B), and high-frequency MDA (A1) caused a greater reduction than low-frequency MDA (A2), but the differences between the 3 strategies in their impact on the ICR are mostly not significant.

Incidence of and recovery from patent parasitaemia in the general population

In the villages sprayed with propoxur (B), there was a clear decrease in the conversion rate for ***P. falciparum*** (Fig. 41). The wet-season conversion rate after spraying was about equal to the dry-season conversion rate before spraying, while the dry-season rate after spraying was lower still. At the same time, the clearance rate increased above the prespraying values. These changes are best illustrated for the ages ≥ 19 years.

Table 19

Daily entomological inoculation rate (EIR) and the daily infant conversion rate (ICR) for *P. falciparum*, in the wet seasons of 1971 through 1975, in villages grouped according to treatment

Group; Village clusters	Treatment (in 1972-73)	Variable	Year (wet season only)				
			1971 (t = 79.5)[b]	1972 (t = 70)	1973 (t = 77.5)	1974 (t = 14)	1975 (t = 14)
C; 1,2	None	EIR[a]	0.17	0.14	0.18		
		ICR[c]	0.016 (31/43)	0.005 (20/67)	0.009 (34169)	–	–
		ICR/EIR	0.09	0.04	0.05		
O; 3,4	Propoxur	EIR	0.71	0.006	0.06		
		ICR	0.012 (22/36)	0.002 (16/105)	0.002 (19/118)	–	–
		ICR/EIR	0.02	0.33	0.03		
A_2; 6, 8	Propoxur + low-frequency MDA	EIR	0.47	0.020	0.013		
		ICR	0.014 (13/19)	<0.0008 (0/52)[d]	0.001 (8/104)	–	–
		ICR/EIR	0.03	—	0.08		
A_1; 5, 7	Propoxur + high-frequency MDA	EIR	0.35	0.021	0.017	0.058	0.093
		ICR	0.016 (13/18)	<0.0005 (0/87)[d]	0.0002 (3/196)	0.005 (18/243)	0.009 (44/384)
		ICR/EIR	0.05	—	0.01	0.09	0.10

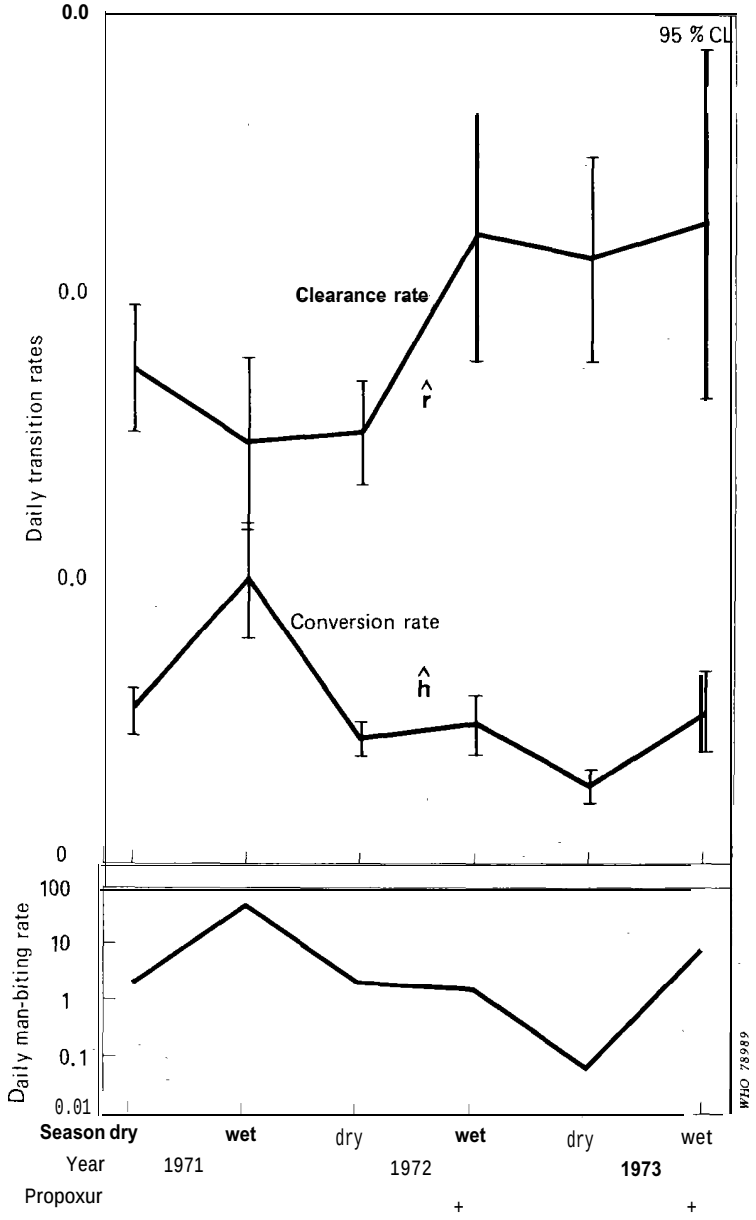
a Weighted, within each group, by the number of infant-periods of risk in each village cluster.

b Average interval between surveys.

c ICR = -ln(1-p) /t, wherep = conversion ratio (given in parentheses).

d When no infant converted, an upper limit for theICR was calculated asfollows: assuming a Poisson distribution of conversions, the probability thatn infants remain negative for t days, given an average daily ICR of h, is e^{-nht}; this probability was set at 0.05, and the expression was solved for h.

Fig. 41. Conversion and clearance rates of P. falciparum parasitaemias, at ≥ 19 years, and man-biting rate by season before and during application of propoxur; 4 villages combined



The method of estimation of the transition rates between surveys assumes constant rates in the interval (7), and is not applicable in the villages treated by MDA, because the drugs produce a sudden increase in the clearance rate.

Relationship between parasitological and entomological findings

For the untreated comparison villages (C), the relationship has already been considered above (p. 131). In the villages treated with propoxur and MDA (Al and A2), the effect of the drug administration was so great (at least when given in addition to the application of insecticide) that it obscured any straightforward relationship between entomological and parasitological variables.

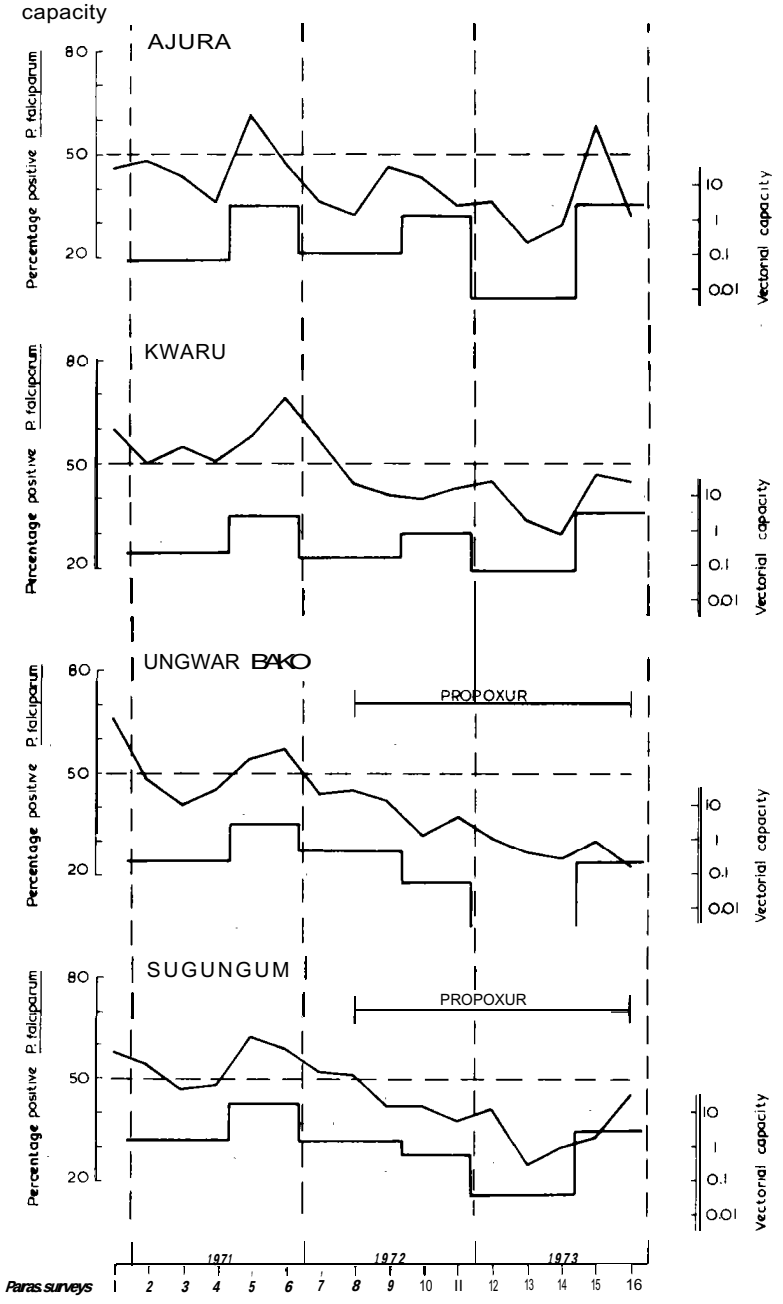
In the villages treated with propoxur alone, the relationship was clearly visible. In the 2 compact villages treated with propoxur alone and also investigated in detail, i.e., Ungwar Bako and Sugungum (see Table l), the prevalence of ***P.*** *falciparum* before, during and after spraying were closely proportional to the logarithm of the estimated vectorial capacity (Fig. 42), just as it was in the 2 untreated villages similarly investigated (see Table 32 below for the figures calculated for vectorial capacity). Ungwar Bako had a relatively low baseline vectorial capacity, and although transmission was not interrupted, the prevalence was probably still on the decline at the end of the 18 months of the intervention phase. Sugungum had a higher baseline vectorial capacity, and the prevalence of ***P.*** *falciparum* probably reached a new equilibrium (with seasonal oscillations) soon after the onset of the intervention.

For the sprayed villages as a whole, the changes in the falciparum infant conversion rate (see Table 19, Fig. 69) and in the falciparum conversion rate of the general population (Fig. 41) corresponded to the simultaneous entomological changes. The parallelism between the man-biting rate on a logarithmic scale and the falciparum conversion rate at ages $\leq$ 19 years may be noted in Fig. 41.

Mixed infections

In the untreated comparison villages, mixed infections with ***P.*** *falciparum* and ***P.*** malariae continued to be more common than expected under the hypothesis of independence between the species (see p. 134). This phenomenon was exaggerated in the villages sprayed with propoxur: at survey 13 (dry season), double infections were present in 29%, 29% and 17% respectively of age-groups 1-4, S-8 and 9-18 years, to be compared with the expected frequencies of 18%, 23 % and 8%.

Fig. 42. Crude parasite rate for ***P. falciparum*** in 2 untreated villages and in 2 villages treated with propoxur, at surveys 1-16, and the seasonal average vectorial capacity



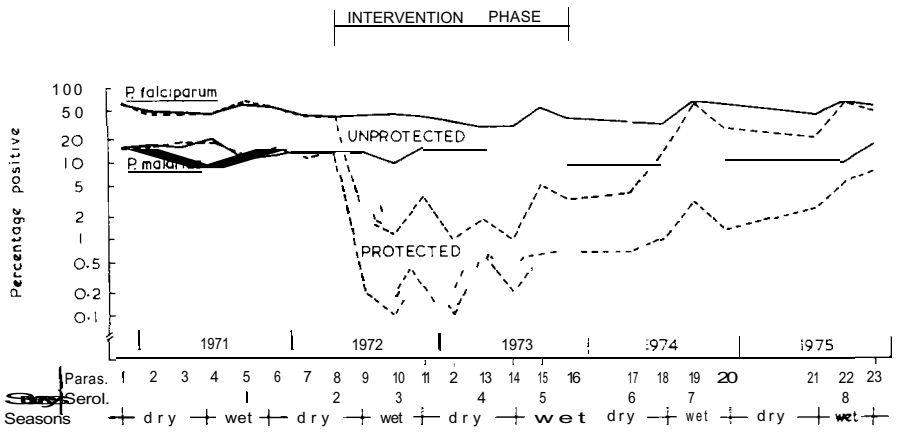
The Post-intervention Phase: The Resurgence of Malaria

As already mentioned, in the post-intervention phase (1974-1975) the investigations were limited to the villages which had received high-frequency MDA (forming clusters No. 5 and 7, or area Al), plus 1 comparison village cluster (No. 2).

Prevalence and density of parasites

The following are considered in turn: crude prevalence; age-specific prevalence (and density); prevalence by sex; the number of times the same person is found positive; first infections in infants.

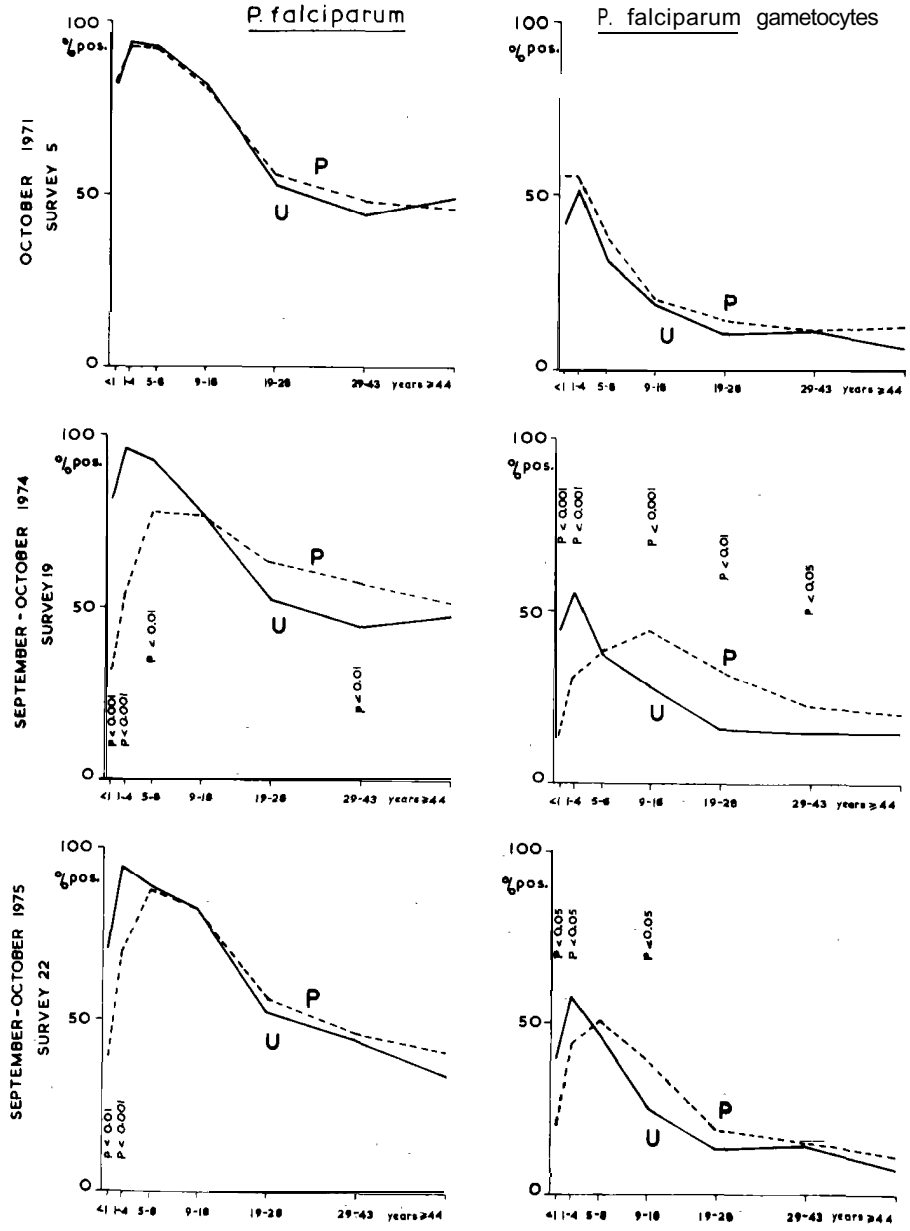
Fig. 43. Crude proportion positive for ***P. falciparum*** and ***P. maiariae*** in the unprotected population (area C) and in the population protected in 1972-73 by propoxur and high-frequency MDA (area A1)[a]



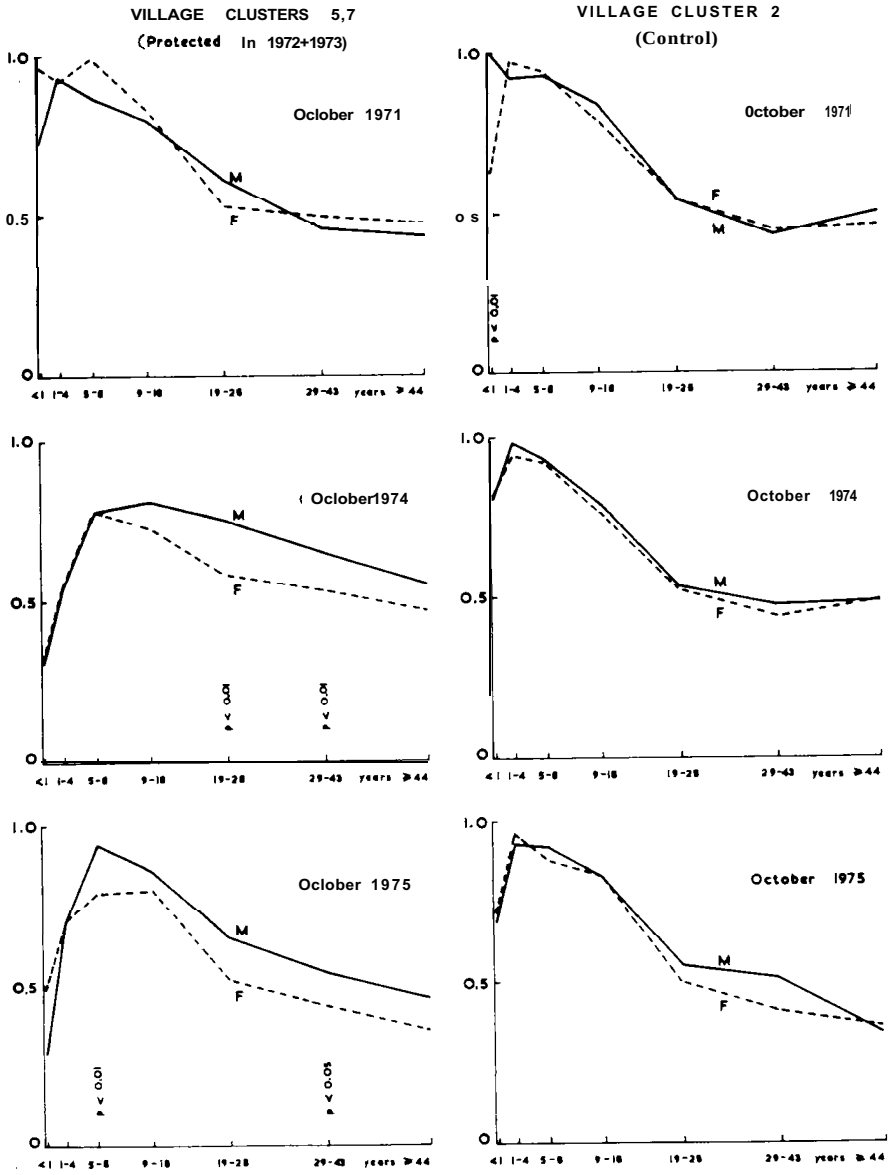
a **The intervention period lasted through** parasitological surveys 9-16.

Figure 43 shows the crude prevalence of *P. falciparum* and *P. malariae* at all surveys (l-23) in the unprotected villages (cluster No. 2) and in the villages treated in 1972-1973 with propoxur and high-frequency MDA. The similarity of the two populations before intervention and the effect of intervention have already been described (see p. 139). In the first wet season after the intervention phase (1974) the crude prevalence of *P. **falciparum*** again reached the baseline or control level; it was again below that level in the following dry season; in the wet season of 1975, it was back to the baseline or control level. The crude prevalence of *P. malariae* increased more slowly and 2 years after the end of the intervention phase it was still not back to the control or baseline level.

Fig. 44. Age-specific prevalence of ***P. falciparum*** (any form) and its gametocytes in the wet seasons of 1971,1974 and 1975 in the unprotected population (U) and in the population protected (P) in 1972-1973 by propoxur and high-frequency MDA



Fjn 45. Proportion of persons positive for *P. falciparum* by age and sex in the wet seasons of 1971,1974 and 1975 in the unprotected population and the population protected in 1972-73 by propoxur and high-frequency MDA



The age-specific prevalence of ***P. falciparum*** and of its gametocytes during the resurgence of malaria in the post-intervention phase is illustrated in Fig. 44. Above about 10 years of age, there was in the formerly protected population a temporary increase in the prevalence of ***P. falciparum*** above the control level, and even more in the prevalence of its gametocytes. The density of asexual stages and of gametocytes in positive persons (PPDI) was also somewhat higher in the previously treated villages. It should be noted that for those below 10 years of age there were systematic distributions of choloroquine in the wet season of 1974 (see p. 48).

Figure 45 compares the prevalence of ***P. falciparum*** in males and females in the October surveys of 1971, 1974 and 1975. No intersexual difference was demonstrable in the 1971 survey-i.e., in a single survey in 1 or 2 village clusters-whereas the combined results of 5 surveys in 8 clusters (see p. 125) showed the prevalence to be greater, above the age of 5, in males than in females. During the resurgence of malaria in the post-intervention years 1974 and 1975, this difference was sufficiently magnified to show clearly in the graphs for the October surveys (Fig. 45).

Table 20

Correlation coefficients between the number of positive results for ***P. falciparum*** at surveys I-8 and at surveys 18-23, in the same persons

Age at survey 1 (years)	Village cluster No. 2 fcomparison)			Village clusters No. 5 8 7 (A1)		
	N^a	*r(P.f.)*	r(*P.f.* gam.)	N^a	r(P.f.)	*r(P.f.* gam.)
<1	37	+ 0.707 0.585	+ + 0.444 0.666"“"	11 52	+0.157 0.329	+ 0.448* 0.462
1–4						
5-8	44	+ 0.515***	+ 0.536"	40	+ 0.210	+ 0.224
9-18	24	+ 0.635""	+ 0.356	47	+ 0.500***	+ 0.341
19-28	41	+ 0.663"“"	+ 0.457	114	+ 0.309**	+ 0.329
29-43	90	+ 0.553""	+ 0.602"“"		+ 0.397"	+0.158
≥44	69	+ 0.343	+ 0.077	76	+0.448	+ 0.226

a Number of persons examined at all the surveys 1-8 and 18-23.

*, **, ***: Association significant at the 5%. 1%. 0.1% levels, respectively, by the χ^2 test, applied after grouping the data in a 2 x 2 table.

The number of times a person had been found positive in the 8 baseline surveys (see p. 122) was compared with the number of times the same person was found positive in 6 post-intervention surveys (surveys 18-23). This was done for ***P. falciparum*** and for ***P. falciparum*** gametocytes in village cluster No. 2 (comparison) and in village clusters No. 5 and 7 (Al). The results obtained (Table 20) reveal that the correlation between

the pre-intervention and the post-intervention parasitological results for the same person was always positive and often significant. In village clusters No. 5 and 7, where malaria had been reduced to a very low level in the intervening period, the correlation was weaker than in the untreated comparison villages, but the positive correlation was still clearly demonstrable.

Of the 62 "first infections" observed in the wet season of 1974-1975, 59 could be classified as to age of the infant and density of the infection (proportion of fields positive). The results were compared with 84 "first infections" observed in the wet season of 1971 in the total baseline population. With respect to asexual stages, the 2 density distributions were very similar, but gametocytaemia was detected significantly less frequently in 1974-1975 than in 1971 (8/59 versus 41/84; $p<0.001$).

Incidence and recovery rates (conversion and clearance rates)

In the villages treated with high-frequency MDA in 1972-1973 (Al), the *P.falciparum* infant conversion rate in 1974-1975 was higher than during the intervention phase, but still lower than that in the baseline phase (see Table 19).

The *P. falciparum* transition rates (conversion and clearance rates) in the various age-groups of the general population could not be estimated in 1974 for comparison with 1971, but they could be estimated in 1975 (Fig. 46). It is seen that in most age-groups in the previously treated villages both the conversion rate and the clearance rate were above the 1971 baseline or the control values.

Relationship between parasitological and entomological findings

In the villages previously treated with propoxur and high-frequency MDA, the entomological inoculation rate in 1974-1975 was higher than in 1972-1973, but still lower than in 1971 before intervention. This was also the case for the *P.falciparum* infant conversion rate (Table 19), whereas the prevalence of *P.falciparum* above the age of about 10 years and the *P.falciparum* conversion rate above the age of about 5 years were greater than before intervention or in the untreated controls (Fig. 44 and 46). There was some increase in the ratio of the *P. falciparum* ICR to the entomological inoculation rate (EIR) (Table 19, last row), and a more definite increase in the ratio between the general *P. falciparum* conversion rate and the EIR (Fig. 47). Sporozoite-positive bites appeared thus more likely to succeed in establishing patent parasitaemia than before intervention.

Fig. 46. Daily transition rates from negative to positive (conversion rate h) and from positive to negative (clearance rate, *r),* for *P. falciparum* in the wet season of 1971 before intervention in the total population, and in thewet season of 1975, in the untreated population (control) and in the population previously protected by propoxur and high-frequency MDA

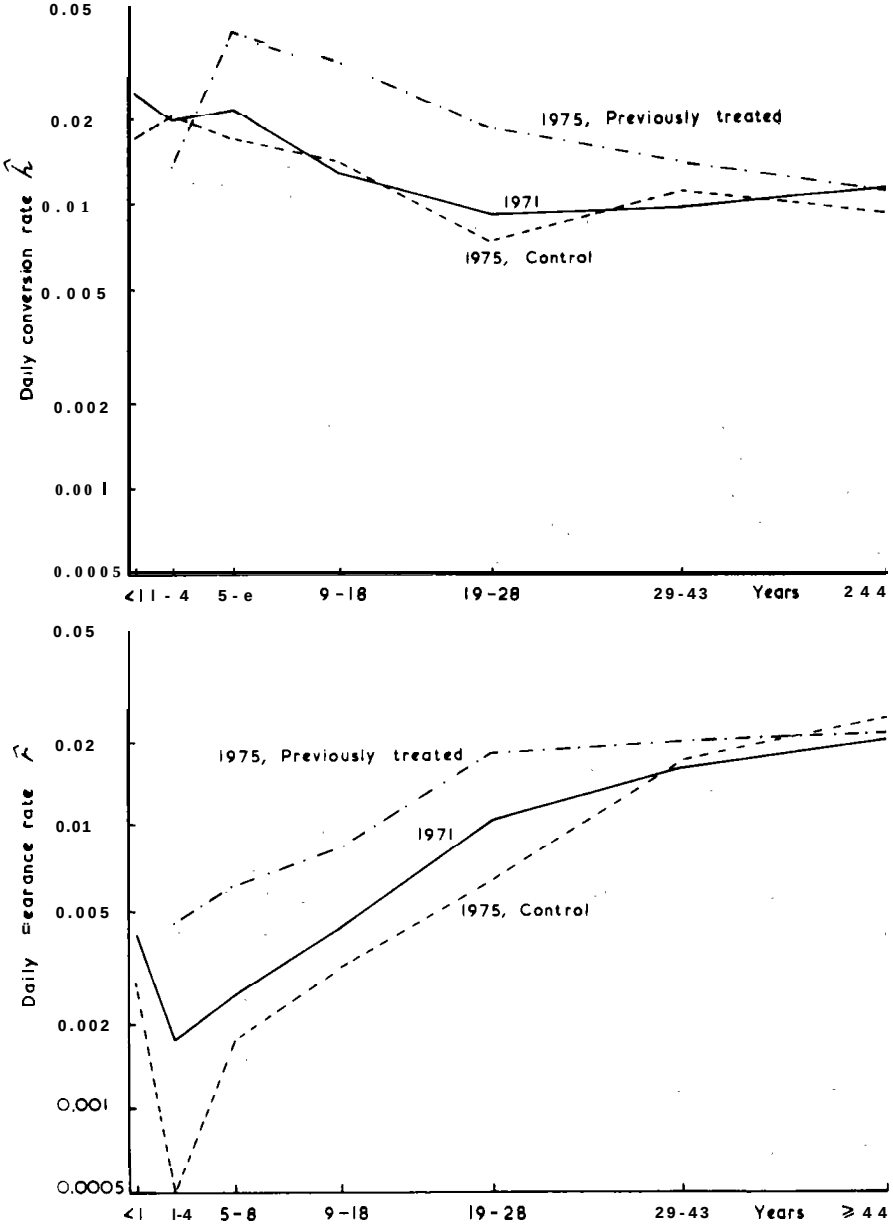
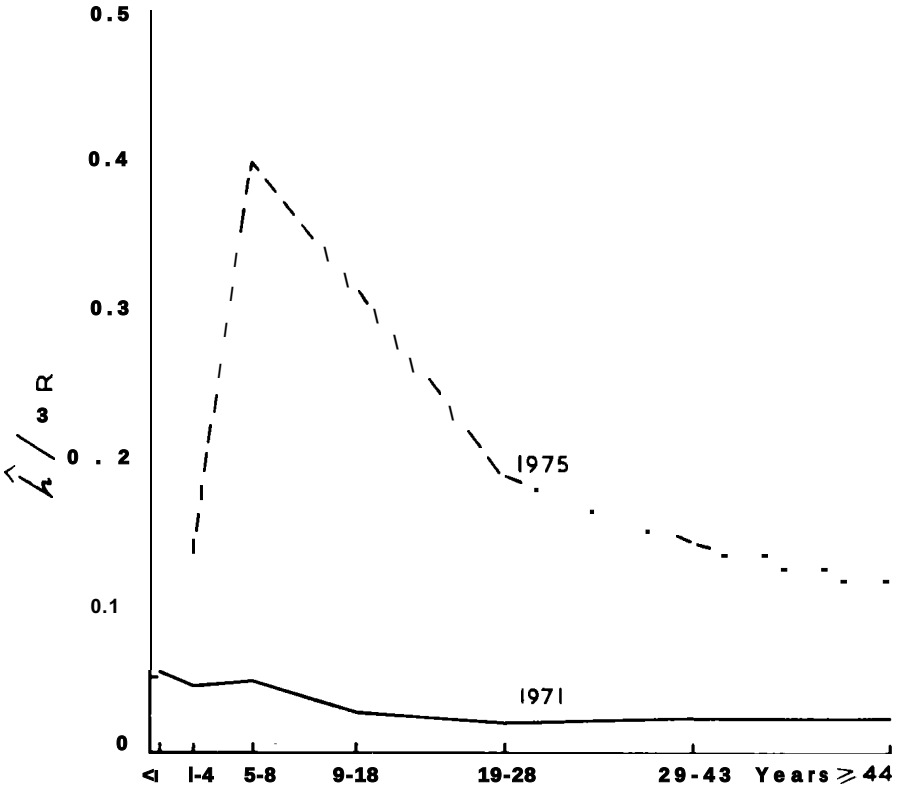


Fig. 47. Ratio between the daily conversion rate for *P. fakiparum* (h) and the entomological inoculation rate (EIR) in the wet season of 1971 in the total population, and in the wet season of 1975 in the population previously protected by propoxur and high-frequency MDA



Discussion

The parasitological effects of immunity to *P. falciparum*

The data document precisely, for this epidemiological situation, some of the known effects of immunity and add some details to the known picture.

Infants

In this situation, maternal antibody probably does not delay significantly the acquisition of the first infection: the conversion rate is

ably the same in the first few weeks of life as later (see p. 127); and the cumulative prevalence calculated from the average conversion rate of infants (estimated after excluding conversions in the first few weeks), and assuming a prepatent period of 10 days (see Fig. 28), is very close to the cumulative prevalence actually observed in the infant cohort (including conversions in the first few weeks).

The density of asexual stages of "first infections" increases with the age of the infant, while the prevalence and density of gametocytes (in infants positive for P.falciparum) show practically no change (see p. 121 and Fig. 24). The increasing density of asexual stages may be explained by the loss of passive immunity. The lack of a concurrent increase in prevalence and density of gametocytes (in the positives) may be explained by one of two hypotheses: either that passive immunity decreases the density of asexual stages without affecting the production of gametocytes (and differs in this respect from acquired immunity), or that acquired immunity with respect to gametocyte production develops to a significant extent in the first year of life. The fact that infants have a higher positive parasite density index of gametocytes than the children aged l-4 years is in favour of the second explanation.

The *apparent recovery rate* (the rate of termination of positive episodes) is significantly greater in the infants than in the next age-group (l-4 years) (see p. 128 and Fig. 31). Parasites disappear faster from the blood of infants, either because of materna1 antibody (the most likely) or because of some other difference (e.g., milk diet). Materna1 antibody would then not prevent the appearance of parasitaemia but increase the rate of removal of the parasites and thereby lower the parasite density and shorten the patent period.

The *infant conversion rate* is much smaller than the *entomological inoculation rate,* and the difference increases with vectorial capacity (see p. 134 and Table 17). Infants may be subject to a lower man-biting rate than the young adults used as baits for the night-bait collection from which the entomological inoculation rate is estimated, but this difference is probably insufficient to explain away the discrepancy and would leave unexplained the variation with vectorial capacity. So, as population immunity increases, either the susceptibility of infants must decrease (presumably through an increase in materna1 antibody), or the infectivity of sporozoite-positive mosquitos must decrease (presumably through a decrease in quantity or quality of gametocytes in the human population). If it is true that materna1 antibody has little effect on the onset of patent parasitaemia (see above), then the second explanation is the more likely one (see also the following section).

Other age-groups

In an area of high transmission such as Garki, the parasitological changes associated with age in cross-sectional surveys can be interpreted directly as effects of acquired immunity on the aging cohort because: (1) exposure to anopheline bites does not decrease with age (22); (2) mortality directly attributable to malaria (and the resulting selection) is limited to the very young (see Chapter 8); and (3) although there is some spontaneous variation from year to year (see pp. 60 and 128), it is relatively minor, and in the absence of any control measures the malaria situation may be considered to have been stable for the lifetime of the oldest inhabitants.

With increasing age (and presumably increasing immunity), the prevalence and density of patent parasitaemia both decrease. Density decreases sooner and to a greater extent than prevalence, and gametocytes decrease sooner than asexual stages (see p. 117 and Fig. 21 and 22). This was, of course, well known; the crude and simple density indices used here were, however, sufficient to show these features adequately.

The *apparent* recovery *rate* (rate of termination of episodes of patent parasitaemia) increases from the age-group l-4 years until the age-group >44 years by a factor of more than 10: this implies that the expected duration of a positive episode concurrently decreases from about 600 days to about 50 days (see p. 130 and Fig. 31). On the other hand, the tendency for the clearance rate of parasitaemia (î) to decrease with an increasing vectorial capacity (hence an increasing population immunity) (see p. 131) may be explained by superinfection.

The *apparent incidence rate* (rate of onset of episodes of patent parasitaemia) decreases, after the age-group 5-8 years, by a factor of about 2 (see Fig. 30); this may represent a decrease in the new infection rate or in the relapse rate; in the same age-groups the relative seasonal variation of the apparent incidence rate increases with age (see Fig. 32), which suggests that the relapse rate decreases faster than the new infection rate (assuming the increase in apparent incidence rate in the wet season to be due to an increase in new infection rate).

The *distribution* of persons by *the number of positive examinations* (out of 8) is nonrandom: there is, especially in the older age-groups, an excess (in comparison with a random distribution) of persons who tend to remain negative or positive (see p. 122). It is unlikely that variation in the number of inoculations can explain this, because villages with very different inoculation rates have nearly the same parasite rate. It is more likely that these differences in score reflect differences in immune status; indeed, persons who tend to remain negative have, on the average, higher levels of IgM, a greater number of bands of precipitation against a

P. falciparum antigen in the Ouchterlony test, and a higher titre in the indirect haemagglutination antibody (IHA) test with either a ***P. knowlesi*** or a ***P. falciparum*** antigen (see pp. 194 and 207).

As mentioned above, with increasing vectorial capacity there is a decrease in the ratio between the infant conversion rate and the entomological inoculation rate; it was suggested, by elimination, that this is probably due to a decreasing infectivity of sporozoite-positive mosquitos (presumably secondary to a quantitative or qualitative effect of immunity on the production of gametocytes) (see next section). Indeed, there was a negative correlation between vector density (indoor-resting density) and gametocyte density in the same village (see p. 134 and Fig. 33). There was, at the same time, a positive correlation between vector density and density of asexual stages. These findings could be interpreted as follows: at higher vectorial capacity, superinfections are more common, parasites enter the blood at a higher rate (hence the higher density of asexual stages) but the death rate of the parasites (asexual stages) is also higher, SO that fewer reach the stage of gametocyte production (assuming that the circulating parasite has to multiply once or more before going into gametocyte production).

The effect of the 3 intervention strategies on the epidemiology of *P.falciparum*

Propoxur for 1½ years (follow-up clusters No. 3 and 4, area B)

In Chapter 4 it has been shown that propoxur reduced the vectorial capacity and the entomological inoculation rate, but that the reduction was limited, probably because a large fraction of ***A.gambiae*** s.l. are exophilic, while immigration of vectors from unsprayed areas probably plays a minor role.

The prevalence and density of ***P. falciparum*** decreased with propoxur, even when the concurrent spontaneous decrease in the untreated control villages is taken into account (see p. 142 and Table 18), but the decrease was not great. The impact of propoxur (alone) on the prevalence of ***P. falciparum*** may tentatively be computed as follows, on the basis of Table 18. Let us assume that, in the absence of propoxur, area B would have undergone the same spontaneous changes as the untreated comparison area (C). Then the prevalence of ***P. falciparum*** observed under propoxur was 85% of that expected in the wet season of 1972, and 74% of that expected in the wet season of 1973."

The change in age-specific prevalence and density (see Fig. 38 and 39) suggests that propoxur produced a relatively large decrease in incidence,

a These results were obtained as follows (see Table 1.8): $36.8/\{(43.3/60.4)\ 60.1\} = 0.85$; and $35.0/\{(47.5/60.4)\ 60.1\} = 0.74$.

while the recovery rate (from parasitaemia) increases with age (i.e., with age-related immunity; see also p. 161). No change in the prevalence and density of gametocytes among positives was demonstrated.

The longitudinal study of infants revealed that propoxur had induced a marked decrease in the infant conversion rate (see p. 148, Table 19 and Fig. 69) under protection by propoxur alone; the infant conversion rate may have decreased less than the (entomological) inoculation rate; infections in infants had lower densities of asexual stages than in the control villages (see Fig. 40).

The longitudinal study of the total population shows at all ages a decrease in the incidence rate of episodes of parasitaemia, and also a decrease in their duration (see p. 148 and Fig. 41); the probable explanation of the decreased duration of positive episodes is that, with a lower inoculation rate, there is less overlap between positive episodes resulting from different inoculations or that a higher proportion of positive episodes results from old infections. The decrease in the incidence rate of episodes of parasitaemia (which confounds new infections and relapses) was probably much smaller than the decrease in the infant conversion rate (which counts, in principle, new infections only).

In the follow-up villages treated with propoxur alone, the epidemiological situation was not affected by the mobility of part of the registered population (see p. 147).

In summary, propoxur decreased the incidence rate of new infections by decreasing the vectorial capacity, and shortened the episodes of parasitaemia possibly by decreasing the risk of superinfection; consequently the prevalence decreased at a relatively slow rate, towards a new equilibrium value. In villages with a high baseline vectorial capacity (e.g., Sugungum) this new equilibrium was probably reached by the end of the intervention phase, while in villages with a (relatively) low baseline vectorial capacity (Ungwar Bako) the new equilibrium was probably not yet reached (see Fig. 42), and in the latter villages a continuation of spraying would probably have produced a further reduction in prevalente. It is possible that the probability of "success" of a sporozoite-positive bite increased; this could result from a lower level of population immunity, expressing itself by an increase in the infectivity of positives, by a decrease in resistance to parasitaemia, or by both.

Propoxur for 1½ years plus MDA (sulfalene-pyrimethamine) every 10 weeks (follow-up clusters No. 6 and 8, area A2)

The addition to propoxur of mass drug administration with sulfalene-pyrimethamine every 10 weeks (average coverage: 85 % among the registered population, see p. 46) produced a Sharp reduction in the preva-

lente and density of ***P. falciparum*** (see p. 139 and Fig. 36) and in the infant conversion rate (see p. 148 and Table 19).

Transmission was not interrupted for any length of time, witness the infant conversion rate (Table 19); indeed, a few sporozoite-positive vector mosquitos (probably not immigrants) continued to be found (see p. 84). Moreover, the appearance of positives among persons relatively well covered by the MDA suggests local transmission (see p. 147).

After a few cycles of MDA, a new average endemic level was apparently reached, with oscillations probably due mainly to variations in vectorial capacity (see Fig. 36). The large increase in prevalence and density of ***P.falciparum*** between surveys 14 and 16 is not explicable by the recorded MDA coverage, which did not drop; it coincided with a relatively large increase in vector density (see p. 60). Probably if the treatment had been continued at the recorded coverage, the prevalence of ***P.falciparum*** would have continued to oscillate within the range observed during the intervention phase (2-28%).

In the follow-up villages of this treatment group, the mobile part of the registered population almost consistently showed a higher parasite rate (see p. 147). An unregistered part of the population (e.g., temporary visitors), included in the MDA rounds as far as possible, may have had a still higher parasite rate. It is, however, unlikely that persistence of transmission was only due to human mobility, for the calculations above (see p. 148) assuming the absence of human mobility indicate that there would probably have been at least 10 positives in the follow-up clusters of area A2, and this, given the density of vectors even with propoxur treatment, was probably sufficient to give rise to the seasonal epidemic observed in late 1973.

Propoxur for 1½ years plus MDA (sulfalene-pyrimethamine) at high frequency, i.e., every 2 weeks in the wet season and every 10 weeks in the dry season (follow-up clusters No. 5 and 7, area Al)

This treatment produced a much faster decline in prevalence and density than the one obtained by propoxur plus low-frequency MDA (see Fig. 36 and 37), and probably a greater decline in infant conversion rate (see p. 148 and Table 19).

With propoxur and high-frequency MDA, transmission was not interrupted for any length of time, witness the infant conversion rates (Table 19); a few sporozoite-positive vectors, probably not immigrants, continued to be found (see p. 84); the appearance of positives among persons relatively well covered by MDA suggests local transmission (see p. 147).

Here also, after a few cycles of MDA, a new average endemic level was apparently reached, with oscillations again probably due mainly to variations in vectorial capacity; this new level was reached faster than with the low-frequency MDA, but was not much lower, except at survey 16 (see Fig. 36); it is likely that, if the treatment had been continued at the recorded coverage, the prevalence would have continued to oscillate within the low range observed during the intervention phase (1-5%).

With respect to human mobiiity, the remarks made in the case of low-frequency MDA probably apply. Here also, the mobile section of the population had somewhat higher parasite rates than the stable section, but again it is unlikely that transmission was maintained only because of population mobility (see p. 147).

The effect of mass drug administration on parasitological immunity to *P. falciparum*

When the Garki project was planned as a time-limited project, it was expected that malaria would return towards its original level after the intervention phase was finished. While offering a measure of residual protection to the population, the postintervention phase of the project allowed the study of the resurgence of malaria after its reduction to low levels (1-5%) for 2 years, and in particular the search for epidemiological evidence of a possible loss of parasitological immunity.

The epidemiological picture of ***P. falciparum*** in 1974-1975 in the previously treated population differs from the baseline and control pictures in several respects: higher proportion positive; higher proportion showing gametocytes, also among the positives; higher incidence and recovery rates from patent parasitaemia; higher ratio between either infant or general conversion rate and entomological inoculation rate. The changes are not very large, and tend to disappear over time; they are, however, quite consistent, and in several instances significant. The changes occurred while transmission, evaluated either by the entomological inoculation rate (see Chapter 4) or by the infant conversion rate, was still below the baseline or control level. The higher frequency at which infants were examined in the 1974-1975 post-intervention period could bias the estimate, but only upwards.

The above epidemiological changes could, tentatively, be explained as follows. The mass drug administrations have reduced the level of parasitological immunity, hence the rate at which merozoites are removed from the circulation. Parasitaemia originating from both new and old infections is more likely to become patent, i.e., not only do the incidence and prevalence of patent parasitaemia increase, but also the ratio between incidence rate of patent parasitaemia and entomological inocu-

lation rate. The increased likelihood that a new infection becomes patent may be described as an increase in susceptibility; the increased likelihood that an old infection becomes patent means that more episodes of patent parasitaemia are expected from 1 inoculation. In addition, more parasite lines reach the stage of gametocyte production. The increase in the recovery rate from patent parasitaemia may seem a paradox, but it could be due to a reduction in the rate of superinfection.. It would also be expected if a loss of immunity allowed, for a given inoculation, a larger number of shorter-lived relapses.

Although the infant conversion rate remained below the baseline or control level, its ratio to the entomological inoculation rate may have increased. This would suggest either that sporozoite-positive mosquitos are more infective or that infants are more susceptible. Sporozoite-positive mosquitos could be more infective because they fed on a less immune, more infective, human population, as suggested by the gametocyte data. Infants may have received less maternal antibodies; however, even in the baseline period, when infants must have been receiving a maximum of materna1 antibodies, they were apparently not protected against acquiring the infection. Materna1 antibodies probably reduce the density and duration of parasitaemia (see p. 159), but the first infections detected in infants in the previously protected population had the same density as those detected in the baseline period. The serological investigations made on the few infants born in the wet seasons of 1974 and 1975 revealed no significant difference between the previously protected and the control populations, except for the IFA-P. ***malariae*** test (see p. 191).

The fact that gametocytaemia in "first infections" was less common in the previously protected than in the baseline population may be explained as follows: infants examined every 2 weeks and treated as soon as found positive have relatively less chance to develop gametocytaemia than infants examined every 10 weeks.

There was a positive correlation, within specific age-groups, between the number of times a person was positive for ***P. falciparum*** in 8 baseline surveys (1970-1972) and the number of times the same person was positive in 6 postintervention surveys (1974-1975). This correlation existed in both the treated and untreated populations, while it was stronger in the latter. This parasitological stability could reflect either the stability of differences in exposure or the stability of differences in immunity. The second explanation is more likely, because: (1) there is a negative correlation between parasitaemia and several serological results and (2) the serological results are also quite stable, and equally stable in the two populations (see Chapter 6).

It is unlikely that the loss of parasitological immunity, discussed here, was accompanied by a loss of clinical immunity (see Chapter 9).

P. malariae, and its relationship to *P. falciparum*

The cumulative prevalence of ***P. malariae*** was very high: more than 80% of children aged l-8 years were found positive at least once among 8 examinations over 1½ years (see p. 122), and it is at least plausible that with more intensive sampling 100% would have been found positive at least once.

The findings with respect to the relationship between ***P. falciparum*** and ***P. malariae*** may be summarized as follows: (1) there is a positive association between ***P. falciparum*** and ***P. malariae*** parasitaemias within persons; having one species renders a person more likely to have, acquire or keep the other species; (2) there is seasonal alternation between the two parasites in the population; this seasonal alternation results mainly from the fact that, for ***P. malariae,*** above 5 years of age, the peak of the incidence rate of episodes of patent parasitaemia is shifted from the wet season (with high vector density and high transmission as measured by the infant conversion rate) to the dry season (with low vector density and low transmission).

The positive association between ***P. falciparum*** and ***P. malariae*** observed within persons cannot be explained away by the fact that results from different villages, age-groups and examiners were pooled. This positive association is in contradiction with the previous findings of others. According to the review of Cohen (29), most surveys showed a deficit of mixed infections, and the deficit increased with immunity.

Conflicting findings could result from differences between methods of blood examination. The examination of a fixed volume of blood (approximated in the present study by a fixed number of microscopic fields) should make the sensitivity of the examination with respect to one species independent of its sensitivity with respect to another species; on the other hand, a flexible stopping rule (such as the examination of 100 fields of all films, and an additional 100 fields of the films negative after the first 100 fields) may easily produce a spurious deficit of mixed infections. Moreover, since an increase in immunity causes a decrease in the density of infection (and the probability of diagnosis by the examination of a given volume of blood), this spurious deficit of mixed infections may increase with immunity. Among the publications reviewed by Cohen some do not state the stopping rule used, but several were using an elastic stopping rule, not fully described.

Accepting as genuine the positive association between ***P. falciparum*** and ***P. malariae*** within persons, the simplest explanation would be the following: the two infections are transmitted by the same vectors and any differences in exposure between persons apply automatically to both species. However, in Garki there is, after early childhood, a negative

association between ***P. falciparum*** parasitology and several serological indicators of the immune response (see Chapter 6). This suggests that, for that place and time, parasitological differences between persons are related to differences in their acquired immunity status, rather than to differences in current exposure. The positive association of ***P. falciparum*** and ***P. malariae*** suggests that persons who have a weaker immunity to the one also have a weaker immunity to the other and vice versa; the latter would be expected not only if immunity is partly nonspecific or heterologous but also if differences in past exposure or in immune response to a given exposure apply to both species.

In the above discussion, it is implied that heterologous immunity, if it existed, would produce an excess of mixed infections and not a deficit as expected by Cohen (29) from different premises. Obviously, the expected parasitological effect of heterologous immunity will depend on the parasitological effect of homologous immunity. With increasing immunity to malaria, episodes of patent parasitaemia become somewhat less frequent and markédly shorter; this was confirmed in Garki by the estimation of transition rates from the longitudinal observations, both for ***P. falciparum*** and ***P. malariae*** (see p. 130). If there is heterologous immunity, persons more likely to have patent parasitaemia of one species, because of a lower immunity, would also be more likely to have patent parasitaemia of the other species, and there would be an excess of mixed infections. On the other hand, if there were competition between the species, in the sense that the presence of the one would tend to prevent the presence of the other, one would expect a deficit of mixed infections, and the situation might be described as heterologous premunition.

The seasonal alternation between ***P. falciparum*** and ***P. malariae*** in the populations has been described previously but not analysed in detail. There was a time-lag of 30 weeks between transmission of ***P. malariae,*** measured by the infant conversion rate, and the crude conversion rate; the time-lag appears only after the age of 5 years, and is therefore unlikely to be a genetically determined characteristic of the parasite. Among other explanations to be considered, there are immunity and suppression of ***P. malariae*** by ***P. falciparum.*** Immunity to ***P. malariae*** increases rapidly with age but it is not known whether this would prolong the incubation period. Suppression of one species of ***Plasmodium*** by another is known from clinical observations (8) and suppression of ***P. malariae*** by ***P. falciparum*** is also suggested by the timing of observed events: the rapid increase in vector density after the onset of the wet season is rapidly followed by a marked increase in the prevalence of ***P. falciparum*** coinciding with a marked decrease in the prevalence of ***P. malariae,*** and the same sequence is observed very regularly although

the rapid increase in vector density occurs at somewhat different times in different villages and years. If ***P. falciparum*** suppresses ***P. malariae,*** it is not obvious why suppression becomes visible only at an age and immunity level at which both species become relatively scarce.

The findings could be explained by combining the concepts of competition and heterologous or nonspecific immunity. Let us suppose that either species activates "immune slots" effective against both. Parasites of both species, present in the same host, compete for these "slots". If there are more slots than parasites of either species but fewer than the total number of parasites, as in a host with a relatively high level of immunity, each species has a certain probability of disappearing (i.e., becoming undetectable in the blood film) while the other persists. The addition of parasites of one species, e.g., by inoculation, will have the following consequences: (1) there will be an increase in the number of activated "slots"; (2) if each parasite added activates one slot, there will be an increase in the ratios of the number of that species both to the number of slots and to the number of the other species, hence a decrease in the probability of disappearing; (3) for the other species, there will be a decrease in the corresponding ratios, hence an increase in the probability of disappearing. Seasonal alternation would be limited to the older, more immune, age-groups, because a species can disappear from the circulation only if there are more slots than parasites of that species.

This conceptual mode1 can be formalized by the hypergeometric probability law (116). The conceptual mode1 is also in agreement with the finding that specific antigens may stimulate nonspecific immune effector mechanisms, e.g., activated macrophages (30).

Whereas propoxur reduced the incidence and prevalence of ***P. falciparum*** and the incidence of ***P. malariae,*** it did not reduce the prevalence of ***P. malariae.*** Such an increase in the relative abundance of ***P. malariae*** could be the result of a decrease in the degree of suppression exerted by ***P. falciparum .***

If ***P.falciparum*** and ***P. malariae*** are competing with each other through some mechanism, the fact that it is ***P.falciparum*** which suppresses ***P. malariae,*** and not the reverse, is easily explained: (1) a given vector population, physiologically capable of transmitting both species, transmits ***P.falciparum*** much more rapidly, because its incubation interval between uptake of infective gametocytes from one human host and appearance of infective gametocytes in the next human host is much shorter, and because the proportion of vectors surviving the shorter extrinsic incubation period is much larger; (2) in the human host, ***P. falciparum*** multiplies faster than ***P. malariae:*** the time interval between divisions is shorter and the number of parasites produced per division is larger (65).

If suppression of ***P. malariae*** by ***P. falciparum*** is of epidemiological importance, as suggested here, then it may also be a factor in the geographical distribution of ***P. malariae,*** which has been puzzling investigators for a long time (65). In particular, the very high prevalence of ***P. malariae*** found by Sulzer et al. (154) in an isolated community of the Amazonian forest might be partly due to the remarkable absence of ***P. falciparum*** from the same community.

Observations on the relationship between ***P. falciparum*** and ***P. malariae*** were made in the past in the schoolchildren of Freetown, Sierra Leone. Between 19251926 (95) and 1931, a marked increase in the prevalence of ***P. malariae,*** "at the expense of ***P. falciparum",*** was reported (72); this was confirmed in 1932 (73) and again in 1935 (133). The absolute increase in the prevalence of ***P. malariae*** is easily explained by the use of thin films in 19251926 and of thick films in 193 1,1932 and 1935. Its increase in relation to ***P. falciparum*** is more difficult to explain, but may be due, partly to the fact that in 1925-1926 the examinations were made mostly in the wet season while the later examinations were made largely in the dry season, and partly to the implementation of a relatively effective programme of source reduction (133).

P. ovale

Authorities have commonly described ***P. ovale*** as a "rare" parasite (28, 65, 90). However, here it reaches a cumulative prevalence of more than 30% in the age-group l-4 years. The method by which this finding was obtained is relatively insensitive, on at least 3 counts: (1) 8 examinations are a small sample for a period of 70 weeks; (2) 200 microscopic fields of a thick film constitute a small blood sample; (3) the prevalence of ***P. ovale,*** determined by the junior microscopists and used as the actual result, was based on a subsample of films which covered only 73% of the prevalence determined by the senior microscopists (see column g, Table 16). It is therefore quite possible that, at this level of transmission, every member of the population is detectably positive, part of the time, for every one of the 3 species. The study of the age-specific prevalence curves leads to the same conclusion. The rapid decrease in prevalence of all 3 species after the age of 9 years can be explained neither by a decrease in exposure (22) nor by differential mortality, and must be attributed to a rapid increase in population immunity. This would imply that by that age the great majority have already been infected by all 3 species, or that at least for ***P. ovale*** a large fraction of the population is never at risk, which is unlikely.

It has been suggested that ***P. ovale*** is ***P.schwetzi*** and that the geographical distribution of the human infection depends on that of the

natural hosts of P. schwetzi, the gorilla (Gorilla ***gorilla)*** and the chimpanzee ***(Pan*** troglodytes) (27). Garki is certainly far from the nearest gorilla or chimpanzee populations, and the high prevalence of P. ***ovale*** in the human population renders the postulation of an animal reservoir superfluous.

The finding of P. ***ovale*** in every one of the 22 villages investigated is of interest; the distribution of P. ***ovale*** in West Africa has been described as focal (90); in Uganda, on the other hand, there was little or no evidence of focality (131). Within the area covered by the present investigation, focality was not apparent.

Malaria in males and females

Beyond the age of 5 years, females have somewhat lower parasite rates ***(P.falciparum*** and ***P. malariae)*** than males (see p. 125 and Fig. 27). The females have higher levels in 2 serological tests which show association with parasitological protection (see p. 191). These findings suggest that females mount a better immune response. During resurgence of ***P. falciparum,*** after its near removal for 1½ years by residual spraying and MDA, the parasitological advantage of females is enhanced (see p. 156 and Fig. 45) without a corresponding change in the serological difference (see p. 191). This suggests that females not only have a stronger humoral immune response, but also either a stronger natural immunity or a stronger and/or more rapid cellular response.

Summary

The baseline prevalence of malaria parasitaemia was very high, and the age-specific curves of prevalence and density were typical of a very high level of transmission and a high level of acquired immunity. This applies to the 3 species present: the cumulative prevalence of patent parasitaemia, after 8 surveys in 1½ years, reached its peak in the age-group 1–8 years, at 100% for ***P. falciparum,*** more than ***80%*** for ***P. malariae,*** and more than 30% for ***P. ovale.*** There was little variation between villages or between years. ***P. falciparum*** and ***P. ovale*** reached their highest frequency in the wet season, while ***P. malariae*** reached its highest frequency in the dry season, probably because of its suppression by ***P. falciparum*** in the wet season.

The infant conversion rate and the rate of onset of episodes of patent parasitaemia in the general population confirm the high level of transmission. The marked increase in the rate of clearance of patent parasitaemia with increasing age and the high ratio (up to nearly 100) between

the entomological inoculation rate and the infant conversion rate (the relative ineffectiveness of sporozoite-positive bites) confirm the high level of population immunity.

Propoxur alone had a limited effect on the incidence and prevalence of ***P.falciparum:*** the prevalence in the first and second wet seasons of spraying was respectively 85 and 74% of that expected; In the villages with the lowest baseline vector densities, some further decline would probably have resulted from a continuation of spraying, but in the villages with the highest baseline vector densities a new equilibrium had already been reached. The main reason for this mediocre result is probably the exophily of a sufficient number of vector mosquitos (see Chapter 4).

Propoxur plus mass drug administration (MDA) of sulfalene-pyrimethamine every 10 weeks reduced the prevalence of ***P. falciparum*** to 2% in the dry season, but it did not interrupt transmission for any length of time; nor did it prevent an increase in incidence and prevalence in the wet season, which ranged up to 28% in the second year of intervention (1973), when natural conditions favoured vector breeding. A new oscillating equilibrium was reached rapidly, and continuation of the intervention would probably not have modified the result. It is very unlikely that mobility of the human population, which was relatively pronounced, was the main cause of the maintenance of transmission. Here again, the main limiting factor was probably the exophily of the vectors.

Propoxur plus mass administration of sulfalene-pyrimethamine at a higher frequency (every 2 weeks in the wet season, every 10 weeks in the dry season) reduced the prevalence of ***P. falciparum*** to 1 % in the dry season and prevented its rise above 5% in the wet season. Transmission was, however, not interrupted for any length of time. As with low-frequency, MDA a new oscillating equilibrium was reached rapidly, and continuation of the intervention would probably not have modified the result. Here also, it is unlikely that population mobility was the main cause of the maintenance of transmission. Once again, the main limiting factor was probably the exophily of vectors.

The high-frequency MDA for 1½ years caused a temporary loss of parasitological immunity against ***P. falciparum*** demonstrable during the resurgence of malaria in the postintervention phase of the project.

Materna1 immunity did not reduce the incidence of new infections with ***P. falciparum*** but increased the clearance rate of parasitaemia, thus reducing the density and duration of episodes of patent parasitaemia.

Above the age of 5 years, females had a lower prevalence of ***P. falciparum*** and ***P. malariae*** than did males. During the resurgence of ***P. falciparum*** in the postintervention phase of the project, the parasitological advantage of females was enhanced.