THE GARKI PROJECT

Research on the Epidemiology and Control of Malaria in the Sudan Savanna of West Africa

by

L. MOLINEAUX and G. GRAMICCIA

Medical Officer, Research Coordination, Epidemiology and Training,

Formerly, Chief, Epidemiological Evaluation and Assessment,

Division of Malaria and Other Parasitic Diseases, World Health Organization

WORLD HEALTH ORGANIZATION

GENEVA

1980

Chapter One

INTRODUCTION

Purpose of the Publication

This publication is a comprehensive presentation of a study, carried out in an area of northern Nigeria from 1969 to 1976 by a WHO/Government of Nigeria research team, on the epidemiology and control of malaria in the African savanna. This research was planned and directed by a multidisciplinary group at WHO headquarters in liaison with the WHO Regional Office for Africa and in collaboration with the authorities and the staff of the Federal Republic of Nigeria and Kano State.

Although the work is completed with respect to the study of the epidemiology of malaria in the Sudan-type savanna, the planning and evaluation tools forged and tested there remain to be applied to the practical problems of malaria control under various ecological conditions, taking into account the various kinds of constraints. The work is continuing in particular in Bendel State, Nigeria, and its further results will eventually form the basis of a further publication.

This publication is addressed to all those interested in the problem of malaria in Africa and elsewhere. It is obviously not a complete textbook in this respect, because its contents are related to the objectives of the research project considered. Nevertheless, the scientific observations made in that project and the conclusions drawn from them should be useful to all those who either wish to know more about malaria epidemiology in its many aspects or are responsible for administrative and technical decisions for the planning, organization and evaluation of malaria control campaigns. For this second group of persons, the monograph should contain sufficient details and make, we hope, interesting and pleasant reading. For those, however, who are carrying out research on specific aspects of the epidemiology and control of malaria, this book may not contain sufficient details of the observations and results obtained. More detailed reports and analyses do, however, exist and it is from them that the summary tables, graphs and conclusions have been drawn. Some

additional information is available in separate publications, but much of it is to be found in various unpublished WHO documents that are not available in libraries or bookshops. These unpublished documents are included in the list of references, and copies may be requested by applying to the Director, Malaria Action Programme, World Health Organization, Geneva, Switzerland. In addition, most of the original data, including the longitudinal records collected during the work of the project, are stored on tape at WHO headquarters and access to these data for the purpose of further analysis can also be arranged. It is understood that any request for either documents or data will be considered on its merits and no commitment is made for the allotment of the resources that might be required.

The credit for the work described in this book goes to all those who have planned it, and even more to those who have executed it during 6 years of extremely hard field work. It would be difficult and invidious to assign the relative merit due to each one of them; however, the name and the function of all those who contributed to this work are listed at the end of the book, and a note at the beginning of each technical chapter indicates the names of those who were mainly responsible for the work described therein.

In addition to the scientific value of the observations and the practical guidelines to be derived from the conclusions, this book and the original data behind it have an important teaching value. This is especially relevant at a time when the passing of the malaria eradication period demands that the principles, methods and criteria for planning, conducting and evaluating malaria control programmes, adapted to the epidemiological, environmental and socioeconomic conditions of the areas concerned, will have to be learned again.

The book covers the 6 years' period of observations in the field. The technical chapters, dealing with each of the main disciplines applied in the study, cover for each subject the entire period—i.e., the baseline data collected before intervention, the results of the interventions and their analysis, and the observations carried out for two years after discontinuation of the control measures.

Each chapter concerned with a particular category of variables also relates them to what precedes; for instance, the chapter on parasitology cross-references the parasitological findings to the entomological findings previously described. Occasionally, the discussion of a chapter may anticipate some findings from subsequent chapters; for example, the discussion of parasitological results (Chapter 5) calls upon certain serological observations from Chapter 6. Summaries at the end of each technical chapter outline the main features therein of either scientific or practical value. In addition, the main conclusions are recapitulated in Chapter 11.

Previous Epidemiological Studies and Control Trials in Tropical Africa

This section outlines the relationship of the Garki project to previous work in tropical Africa, without, however, constituting a review of the previous work. Some further comparisons, with respect to the practical conclusions, are made in Chapter 11.

Even after the advent of residual insecticides, malaria in tropical Africa has resisted most of the attempts made for its control which were started, to a large extent, following the recommendation of the First African Malaria Conference, Kampala, 1950, that "malaria should be controlled by modern methods as soon as feasible, whatever the original degree of endemicity, and without awaiting the outcome of further experiments" (*167*). The problems of malaria control in tropical Africa have been summarized successively by Wilson (*164*), Bruce-Chwatt (*16*), Macdonald (*99*) and Hamon et al. (*80*). A number of pilot projects using a variety of control methods, singly or in association, but including indoor spraying with residual insecticides in different epidemiological conditions (Tropical forest: southern Cameroon, Liberia; Lowland savanna: northern Cameroon, northern Nigeria (Sokoto), Senegal, Upper Volta; Degraded forest: Benin, Togo (Palimé); High plateau: Uganda, Madagascar; Oceanic islands: Mauritius, Réunion; southern limits of tropical Africa: South Africa, Swaziland, Southern Rhodesia), have revealed that with the exception of the islands of Mauritius and Réunion, where malaria disappeared following the use of standardized eradication techniques, the results varied from a good response in tropical forest areas and highland savanna (with seasonal, unstable malaria) to a poor response in lowland savanna (*87*).

In these latter projects, the reasons for the unsatisfactory response to control, although assumed, were not fully investigated, nor were the individual factors fully analysed that had militated against the success of the control measures implemented, often at a cost exceeding the financial resources of the country concerned. In the dry savanna area of northern Cameroon, 2 rounds of DDT spraying at a 6-month interval in 1960-1961 produced a rather moderate reduction of the parasite rate and no interruption of transmission. It was hinted that the irritability of *Anopheles gambiae* and *A. funestus* on deposits of DDT was responsible for this lack of success, and an association of chemoprophylaxis with insecticidal spraying was therefore advocated (*23*). In Western Sokoto in the northern Nigerian savanna, 6-monthly spraying for 4 years (1954-58) with DDT, dieldrin or HCH in 3 adjoining areas produced a large reduction in indoor-resting vectors, and also in the spleen rate, parasite rate and parasite density, which were more pronounced in infants, but important

also in the age-groups 1–2 and 3–4 years. However, no indication was given on the availability and use of drugs by the population in the course of this trial-cum-control campaign (*19*). An extended spraying with DDT only (1957-1964) seemed to indicate that transmission was "interrupted through the greater part of the year, [but was] resumed during the height of the rains and through the season of maximum breeding by *A. gambiae* and *A. funestus*". In 1964, the parasite level appeared to have stabilized at levels of a sixth for infants under 1 year, of a third for children 1-4 years and a half for older children of those observed before the campaign had started. One round of mass drug administration (MDA) with chloroquine and pyrimethamine at the beginning of the rains in 1961 was surprisingly followed by higher parasite rates at the end of the wet season than those observed in the area where the MDA was not implemented. Again, no indication was given on the drugs available and used independently by the population (*47*). An intensive study was conducted (*26*) in the savanna pilot area of Bobo-Dioulasso (Upper Volta) in 1953-1958 on the effect of the use of residual insecticides (DDT and dieldrin once or twice per year) on the local vectors, and on the incidence and prevalence of malaria. Following a number of epidemiological considerations and analyses, that have been largely confirmed in Garki, the authors conclude that with the use of residual insecticides "Inhibition of malaria transmission has not been observed anywhere, restriction is limited to a slackening down, plasmodic and splenic rates remaining very much alike in the treated and in the control areas". Nevertheless, a number of questions fundamental for an epidemiological understanding of malaria or of its control in Africa, as well as its relation to the health and well-being of indigenous populations of various age-groups, and their vulnerability to malaria consequent on the decrease of immunity after various periods of control, were still awaiting fuller investigation.

After several years of largely unsuccessful attempts at interruption of transmission or effective control, a sense of defeatism arose that led to the exclusion of tropical Africa, and especially of the West African savanna, not only from the world campaign for the eradication of malaria, but also from the adoption of an extended programme of malaria control, in the absence of satisfactory answers to the fundamental questions indicated above. Nevertheless, the Fourteenth, Seventeenth and Twentieth World Health Assemblies drew attention to the need for undertaking intensive studies to solve the problem of malaria in Africa. An attempt to analyse the value of some of the factors determining the epidemiology of holo-endemic malaria in the African Sudan-type savanna through the use of a mathematical model was carried out in 1966-1969 in northern Nigeria at Kankiya (*125*). It was pointed out that the mathematical model used there presented several inadequacies and that the proposed single indices were

unable to characterize the epidemiological situation, that transmission of malaria was not controlled in spite of DDT spraying every 4 or 2½ months together with mass drug administration (MDA) to at least 80% of the population every 2 months, and that there were considerable discrepancies between the predictions of the model and the field observations. Further epidemiological studies and the design of a more realistic mathematical model of the dynamics of malaria were advocated.

Rationale of the Project

In view of the major importance of malaria as a public health problem, in particular in Africa, and considering that knowledge and understanding of the epidemiology of malaria and of its modification by control measures were insufficient, WHO proposed a multidisciplinary, longitudinal study of the epidemiology and control of malaria in an area of the Sudan savanna in northern Nigeria. A consultative group[a] was convened in Geneva in May 1969, and, after discussions with WHO staff in Geneva and a representative of the WHO Regional Office for Africa, concluded that:

"1. With respect to the problems posed by malaria in Africa, a field project is an essential complement to the present policy of improving basic health services.

"2. The project essentially should be concerned with research and fact finding. It is envisaged that the project will develop along the following lines:

(*a*) adequate study of ecological and demographic factors;
(*b*) study of epidemiological and immunological baseline data;
(*c*) elaboration of detailed planning based on a mathematical model of operations;
(*d*) implementation of operations, using the best techniques and material available at the time;
(*e*) continuous evaluation of the progress of operations and longitudinal study of the appropriate section of the population and relevant controls.

It is expected that (*a*) and (*b*) above will be completed within the first two years and that at least two years of longitudinal studies will be necessary after the first implementation of operations. Only after completing (*a*), (*b*) and (*c*) will it be possible to confirm the detailed requirements in staff, equipment and supplies. The immunological studies proposed, while considered important, should not be regarded as the main objective of the project. At the end of the project adequate provisions must be made to offset the effect of resurgence of malaria in the project area.

"3. An important aspect of the project is the construction of mathematical models that will identify and quantify factors of significance in the control of malaria. It is believed

[a] Composed of Professor P.G. Janssens, Director, Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium; Dr I.A. McGregor, National Institute for Medical Research, Medical Research Council, London, England; Mr J. Hamon, Mission Entomologique, OCCGE, Bobo-Dioulasso, Upper Volta.

that such models may eventually be applied with profit to the study of control of malaria in other parts of the world, and with modifications, to the study of other communicable diseases. Mathematical models would be expected to facilitate the forecasting of the effect of control measures.

"4. The project should be sited in an area where successful interruption of transmission of malaria remains to be proved. In this connexion, it is believed that operations which proved successful in savanna areas of Africa are likely also to be successful in other areas. For this reason, a savanna area in which details of population movements are known or can be evaluated is considered the site of choice. A second but inferior choice would be a forest area of Africa.

"5. No element of change of epidemiological factors in the study area should be considered for the first two years of the project. Thereafter the effect of operational measures should be assessed. At the termination of the project the wider applicability of the methods employed may be considered.

"6. Steps should be taken to ensure that the project is not understaffed. The quality and number of staff employed should be sufficient for the collection of adequate and reliable data for their analysis and for the proper supervision of the operational activities. In this connexion special consideration should be given to the appointment of, among others, a field statistician.

"7. Drug administration is necessary for the successful completion of the type of immunological studies that have been envisaged. However, particular consideration should be given to the mode of use of antimalarial drugs because in the past in some other projects the use of drugs has not given uniform results.

"8. Provided that planning and staffing are adequate, the project is likely to prove more successful than similar previous field projects undertaken in Africa. In this connexion, it is expected that the construction of relatively simple mathematical models based on essential elements will guide and facilitate practical intervention. These models may also help in the evaluation of new insecticides within a shorter period of time than is currently required.

"9. The project should be undertaken as soon as detailed planning permits. It is hoped that its existence and operation may stimulate governments in Africa to embark on appropriate antimalaria activities whilst intensifying their efforts to improve national health services.

"10. Consideration should be given to encouraging, possibly through WHO long-term fellowships, the attachment to the project of young scientists from different countries in Africa. The aim here would be to provide practical field experience in the different scientific disciplines involved in the project and to encourage the development of a high sense of responsibility. It is understood that the secondment of such personnel should not in any way prejudice the efficient operation of the project.

"11. WHO is the most suitable organization currently capable of conducting such a project in view of the availability of expertise in the different scientific disciplines concerned. The need for close collaboration among the units concerned is recognized and consideration should be given at an early stage as to the best methods of ensuring the necessary coordination" (*170*).

In the light of these conclusions, the Garki Project was organized and commenced its work in September 1969.

Another consultative group[a] met in February 1975 to review and analyse the results obtained and to advise on the future of the project. The group concluded that the programme had been well executed, that the information collected provided a better basis for the understanding of

malaria and for the planning of malaria control than was previously available, and that the project was an example of successful multidisciplinary research. The group stressed the teaching value of the project, and recommended the publication of the results, not only as individual scientific papers on various new specific findings, but also in the form of a comprehensive monograph (*181*).

Objectives of the Project

The specific objectives of the project, as actually implemented, may be described as follows:

1. To study the epidemiology of malaria in the lowland rural Sudan savanna. This means in particular a concentration of the study on the measurement of entomological, parasitological and seroimmunological variables and on their relationships. Also included were some meteorological, demographic and clinical variables, and the study of the prevalence of abnormal haemoglobins in the population.
2. To measure the effect of specified interventions, namely, spraying with a residual insecticide, propoxur, alone and in combination with mass drug administration, at two different frequencies, using a combination of sulfalene and pyrimethamine.
3. To construct and test a mathematical model of the transmission of malaria, and develop it into a planning tool allowing the comparison of alternative control strategies in terms of their expected effects. Specifically, the model was developed with the purpose of linking entomological and parasitological variables, in particular vectorial capacity and the prevalence of *Plasmodium falciparum*, and of calculating the expected parasitological effect of given changes in the entomological situation, natural or man-made, and of given mass drug administration schemes, taking into account the estimated role of immunity.

---

[a] The members of this group were: Professor A.F. Fleming, Department of Pathology, Ahmadu Bello University, Zaria, Nigeria; Professor P.G. Janssens, Director, Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium; Professor A.O. Lucas, Department of Preventive and Social Medicine, University of Ibadan, Nigeria; Dr I.A. McGregor, National Institute for Medical Research, Medical Research Council, London, England; Dr L.F. Delfini, WHO Regional Adviser on Communicable Diseases for the African Region, Brazzaville, Congo; Dr T. Matsushima, Team Leader, MPD-012, Kano, Nigeria.

ISBN 92 4 156061 4

© World Health Organization 1980

Publications of the World Health Organization enjoy copyright protection in accordance with the provisions of Protocol 2 of the Universal Copyright Convention. For rights of reproduction or translation of WHO publications, in part or *in toto*, application should be made to the Office of Publications, World Health Organization, Geneva, Switzerland. The World Health Organization welcomes such applications.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the Secretariat of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

The authors alone are responsible for the views expressed in this publication.

TYPESET IN ENGLAND
PRINTED IN SWITZERLAND
78/4320/1—6000—ACCENT/ATAR

CONTENTS

Page

Foreword 11

CHAPTER 1. INTRODUCTION

Purpose of the publication 15
Previous epidemiological studies and control trials in tropical Africa 17
Rationale of the project 19
Objectives of the project 21

CHAPTER 2. THE STUDY DESIGN AND STUDY AREA

Study design, variables measured, and methods used 23
Malaria control strategies 23
Project phases and calendar 28
Villages selected for follow-up and villages treated by the various strategies 28
Variables measured, frequency of measurement, and methods used 30
Recording, storage and retrieval of data 33
Services and staffing 34
Area, climate, population 35
Orohydrography 35
Climate and vegetation 35
Meteorology 37
Population 39

CHAPTER 3. CONTROL OPERATIONS

Residual spraying 41
Sprayable surfaces 41
The spraying operations 42
Operational results: the coverage achieved and the dose applied 42
Administration of drugs 43
Mass distribution of sulfalene-pyrimethamine in 1972-1973 43
Post-intervention drug distributions in 1974-1975 48
Discussion 49
Summary 50

Page

CHAPTER 4. ENTOMOLOGY

Anopheline fauna and observations regarding minor species 53
Collections as indices of density 54
Baseline findings 56
Vector density 59
Sporozoite rates and inoculation rates 64
Vector behaviour 66
Age composition and longevity 71
Vectorial capacity 73
Effect of intervention, in particular residual spraying with propoxur 75
Results of the preliminary trial of propoxur 76
Effect of propoxur on vector density 77
Sporozoite rates and inoculation rates 84
Vector behaviour 85
Age composition and longevity 86
Vectorial capacity 86
After-effect of propoxur, after discontinuation of spraying 88
Vector density 88
Sporozoite rates and inoculation rates 90
Vector behaviour 90
Age composition and longevity 92
Bioassay and chemical tests 92
Compact versus scattered settlements 94
Anopheles gambiae and *A. arabiensis* 95
Discussion 99
The estimation of the vectorial capacity 99
Transmission in Garki and its natural variation 101
The effect of propoxur 101
A. gambiae, *A. arabiensis* and intraspecific cytogenetic variation 103
Evaluation of insecticidal impact 105
Summary and conclusions 106

Page

CHAPTER 5. PARASITOLOGY

Methods 109
Method of blood examination 109
Collection and processing of blood films 111
Parasitology staff 111
Sensitivity and reproducibility of the blood examination method 111
The parasitological indices used 114
Parasitological findings in the absence of intervention 115
Prevalence and density of parasites 115
Incidence and recovery rates (conversion and clearance rates) 125
Relationship between parasitological and entomological findings 131
Mixed infections and the relationship between *P. falciparum* and *P. malariae* 134
The intervention phase: parasitological effect of the 3 control strategies 139
Prevalence and density of parasites 139
Incidence and recovery rates (conversion and clearance rates) 148
Relationship between parasitological and entomological findings 151
Mixed infections 151
The post-intervention phase: the resurgence of malaria 153
Prevalence and density of parasites 153
Incidence and recovery rates (conversion and clearance rates) 157
Relationship between parasitological and entomological findings 157
Discussion 159
The parasitological effects of immunity to *P. falciparum* 159
The effect of the 3 intervention strategies on the epidemiology of *P. falciparum* 162
The effect of mass drug administration on parasitological immunity to *P. falciparum* 165

Page

P. malariae, and its relationship to *P. falciparum* 167
P. ovale 170
Malaria in males and females 171
Summary 171

CHAPTER 6. IMMUNOLOGY
Material and methods 173
Serological results by age, survey and treatment 176
Immunoglobulin G 182
Immunoglobulin M 182
Precipitin (Ouchterlony) test—*P. falciparum* ... 183
Indirect fluorescent antibody (IFA) test—*P. falciparum* 183
Indirect fluorescent antibody (IFA) test—*P. malariae* or *P. brasilianum* 184
Passive (indirect) haemagglutination (IHA) test—*P. falciparum* 185
The longitudinal study of infants 185
Definition of 2 populations of infants 185
Immunoglobulin levels 186
Malaria antibody levels 186
Proportion positive by the serological tests 188
Serological results in the newborn in the post-intervention follow-up period 191
Variation by sex 191
Relationship between the results of the same serological test in the same person at the different surveys 191
Relationship between the results of different serological tests in the same person at the same survey 193
Relationship between parasitology and serology ... 194
Method of analysis of the baseline data 194
The precipitin test (*P. falciparum*) and *P. falciparum* parasitaemia 196
The IHA-*P. knowlesi* or IHA-*P. falciparum* test and *P. falciparum* parasitaemia 197
The IFA test and homologous parasitaemia 198
Immunoglobulin levels and parasitaemia 198

Page

Relationship between parasitological and serological findings during and after the intervention phase 199
Discussion 199
Age and the development of the active immune response in the unprotected population 199
Serological changes in the protected population 202
Serological changes in the post-intervention phase 204
The longitudinal study of infants 204
Comparison between males and females 205
The correlation between different surveys in the same person 206
The correlation between different serological tests in the same person 207
The relationship between serology and parasitology in the individual 207
Sensitivity and specificity of the serological tests 210
Summary 211

CHAPTER 7. ABNORMAL HAEMOGLOBINS AND ABO BLOOD GROUPS
Material and methods 213
Haemoglobin electrophoresis 215
Haemoglobins and genetic fitness 216
Sickle-cell trait and malaria 217
Sickle-cell trait, immunoglobulins and malaria antibodies 218
Immunoglobulins 218
Ouchterlony (precipitin) test with *P. falciparum* antigen 220
Indirect fluorescent antibody test 220
Indirect haemagglutination test with *P. falciparum* antigen 221
Immunology by sex, tribe and haemoglobin 222
Immunology, malaria parasitology and haemoglobin 222
Findings in the Hb SS persons 222
Findings in the Hb SC person 223

Page

Findings in the Hb AC persons 223
ABO blood groups and malaria 224
Discussion 224
Haemoglobin genotype frequencies, fitness, and parasitology 224
Serology of the Hb AA and Hb AS 226
The Hb SS 227
The Hb AC 227
The ABO blood groups and malaria 228
Summary 228

CHAPTER 8. DEMOGRAPHY
Methods 231
Demographic and parasitological (DP) surveys 232
Local registrars (pataucis) 232
Data-handling 232
Results 233
Distribution of the population by age and sex 233
Births 234
Deaths 236
Population movements 241
Demographic analysis of the MDA records 243
Discussion 244
Methods 244
Age and sex composition 244
Birth rate 245
Death rates, malaria and malaria control 245
Population movements 247
Summary 248

CHAPTER 9. CLINICAL SURVEYS
Nutritional anthropometry 251
Spleen surveys 253
Temperature surveys 253
Population, study design and methods 253
Results 255
Discussion 257
Nutritional anthropometric surveys 257

Page

Spleen rates 257
Temperature surveys 258
Summary 258

CHAPTER 10. THE MATHEMATICAL MODEL OF TRANSMISSION

The assumptions of the model 262
Epidemiological states and transitions 262
Superinfection 265
Dynamics of the human population 266
Vectorial capacity and inoculation rate 267
Equations of the model 268
Fitting of the model 270
Data selected for fitting 270
Estimation of model parameters 272
Testing of the model 273
Method of evaluation 273
Testing of the model against observations made in Garki before and during the application of propoxur 274
Testing of the model against observations made in Kisumu before and after the application of fenitrothion 277
Determination of the endemic level by the vectorial capacity 281
Variants of the model 283
Note on the computer programmes 283
Discussion 284
The model and reality 284
The present model and others 285
The model and the planning of malaria control 286
The model and the teaching of the epidemiology of malaria 287
Summary 287

CHAPTER 11. PRACTICAL CONCLUSIONS FOR THE FUTURE OF MALARIA CONTROL

The control of malaria in the Sudan savanna of Africa 290
Residual insecticides 290

Page

The combination of mass drug administration with residual insecticides 292
Selective chemotherapy and chemoprophylaxis 293
The use of the malaria transmission model for the planning of malaria control 294

References 297

Acknowledgements 307

Appendix 1. Staffing of the Project 308

Appendix 2. Cost of the Project 311

FOREWORD

Malaria is undoubtedly one of the worst scourges of tropical Africa. The intensity of malaria transmission, although not uniform all over the continent, has been considered the main obstacle to any type of control of the disease for some years past. In 1934 James, a well-known British malariologist, suggested that in Africa young children should not be treated for their first attack of malaria so that they could develop some immunity. Similarly, famous malariologists like Swellengrebel maintained that, in areas with holoendemic (stable) malaria, man should not interfere with the established premunition of the human population since that would increase the severity of the clinical manifestations of malaria and the mortality caused by it in older children and adults. In fact, because of the presumed intensity of transmission, in addition to the lack of health infrastructure, Africa was not included in the global malaria eradication programme which was initiated by the World Health Organization in the mid 1950s.

The excellent results obtained elsewhere with DDT house-spraying in interrupting malaria transmission encouraged the initiation of more than 20 pilot projects in various African countries during the mid 1950s and early 1960s. In some countries, particularly in forested areas, the impression was that transmission could be interrupted if total coverage with insecticides and full surveillance were carried out. However, in none of these pilot projects were quantified epidemiological data collected in the course of operations that would have permitted a proper appreciation of the intensity of endemo-epidemicity and thus facilitated the planning of malaria control, particularly for the dry savanna areas.

In the light of the experience gained from pilot projects and in view of the insufficient knowledge and understanding of the quantitative dynamics of malaria transmission and of the impact of such control measures as residual house spraying and mass drug administration, WHO decided to initiate field research to provide information on all the factors that contribute to the maintenance of intensive transmission. The northern part of Nigeria was favoured for this research, Garki District was selected, and thus the project described in these pages was born.

When the research project was drawn up, it was decided to invest rather more resources than previously in the collection of baseline data and in the evaluation of the impact of house spraying with an effective residual

insecticide, alone or in combination with mass drug administration. The development of a mathematical model of transmission and its testing against hard data were part of this effort of understanding: if one could simulate realistically the transmission of malaria, one would presumably be closer to a balanced understanding of the interplay between the factors involved and better equipped for planning future control programmes. The Garki project also provided an unique opportunity to study a battery of seroimmunological tests before, during and after the application of control measures. The research project was designed to be limited in time, rather than as a pilot project to try out on a small scale a strategy that would later be applied on a large scale and over a prolonged period of time. It was essential to know what could or could not be achieved and, as far as possible, to find out why that was so; and it was therefore considered justified to apply control measures and methods of supervision that were more expensive than would be acceptable in a control programme. Great care was taken to protect the population involved from any untoward effect of the application of more or less intensive control measures over a short time; in the event, one of the results of the project was an increased awareness and a new understanding of malaria by the population and, with that, the adoption of self-medication, which is probably one of the most immediately applicable ways of reducing morbidity and mortality from malaria in a situation of the sort encountered in Garki.

The project largely reached its objectives, as this monograph shows. While several of the more striking results have been published elsewhere in a fragmentary way, in the present work the authors have aimed at a balanced account of all aspects of the study. The wealth of data collected did not make this easy. Some of the interpretations may be even challenged. The original data, collected with exceptional and meticulous care, are stored on tape and could be made accessible upon request to allow further or different methods of analysis.

This is not the place to summarize the project, but some of the findings may be highlighted here. First of all, a very high intensity of transmission was demonstrated: the vectorial capacity, which is an expression of the likelihood of transmission of the parasite, was about a thousand times the critical value required for the maintenance of endemic malaria; the entomological inoculation rate, or number of infections offered to man per unit of time, was about a hundred times the critical value. These very high levels of transmission put malaria in tropical Africa in a category of its own. The variations observed in the intensity of transmission from year to year and from village to village were well documented. Residual spraying with propoxur did not have the expected effect on the prevalence of malaria. The care with which both the control operations and the evaluation were conducted allow some firm conclusions: coverage was as

nearly complete as possible; the insecticide was very effective against the mosquito vectors (still producing a high mortality among them at the beginning of the third wet season after the last application!), although less so in reducing malaria; immigration of vectors or humans from unsprayed villages was not a significant factor. The decisive factors were the exophily of a fraction of the *Anopheles gambiae* sensu lato and a high man-biting rate—hence the high level of transmission. The same factors are also the main reason why the addition of mass drug administration, even at high frequency and coverage, while it reduced malaria to a very low level, failed to interrupt transmission. Variations in the degree of exophily between villages and between Garki and Kisumu, Kenya (where a quite distinct field trial of fenitrothion was conducted), explain the variations between villages in the effect of propoxur and most of the difference between the effect of the insecticides used in Garki and in Kisumu. Cytogenetic investigations pointed to a genetic basis for variation in resting behaviour within each of the two species of the *A. gambiae* complex occurring in Garki, and their results correlated well with the differences between villages in the effect of propoxur. If the resting behaviour of a mosquito species is genetically determined, exophily will be a stable characteristic of individual vectors, and the usual method of interpreting the impact of residual insecticides on longevity, which tacitly assumes uniform behaviour, is overoptimistic. Turning to the parasitological observations, the longitudinal nature of the study made it possible to show that everybody was infected early in life, not only by *Plasmodium falciparum*, but very probably also by *P. malariae*, and even by *P. ovale*, commonly described as a "rare" parasite. The effect of parasitism on immunity was well demonstrated, confirming much that was known but also producing some findings that either are new or were hitherto much less well documented. The seroimmunological study also yielded significant findings regarding the relationship between the various serological tests and parasitological findings, regarding the differences in immune response between males and females, between persons with and without the sickling trait, and between individuals, and regarding the effect of a drastic reduction in antigenic stimulation on the test results. The clinical studies, although limited in scope, demonstrated interesting relationships between body temperature and parasitaemia, and a significant effect of malaria control on the frequency of fever and on anthropometric indicators of the nutritional status of children. The demographic studies demonstrated that the infant mortality rate was very high before control, that its variation between years and between seasons was strikingly associated with the corresponding variations of the infant's risk of acquiring *P. falciparum*, and that it was significantly reduced by malaria control. Last but not least, the new mathematical model, painstakingly tested

against hard facts, allows much more realistic simulations of the epidemiology of malaria, both before and after the application of control measures, than was previously possible.

The future will tell whether the volume, quality and relevance of the information produced by the project have justified the relatively high investment. There are implications for the future as regards control, teaching and research. The control of malaria in the African savanna will benefit from careful consideration of the observations made in Garki, even the negative ones; it is better to know your enemy's (malaria's) strength and resilience. Control of malaria on a broader scale will benefit from the addition to our planning tools of a new, more realistic, simulation model. The data from Garki, their interpretation (even when it is controversial), and simulation exercises based on the model will add to the materials available for teaching the epidemiology of malaria. Future field research should benefit directly from the experience, good and bad, gained in the project, and some of the findings mentioned above may also give leads for basic research.

The project was made possible by the dedication and hard work of many, in Nigeria and outside, in WHO, and in numerous national scientific institutions; they are listed in an appendix. Whatever the merit of the work, it is shared by all involved, and in particular by the members of the team in Nigeria, working hard and productively, often in very difficult conditions, and by the population of the Garki District, without whose superb and lasting cooperation none of this would have been possible. It is hoped that they and their descendants, and similarly affected populations, will reap the real benefits.

Dr T. Lepes
Director, Malaria Action Programme,
World Health Organization

Chapter Two

THE STUDY DESIGN AND STUDY AREA[a]

Study Design, Variables Measured, and Methods Used

Malaria control strategies

From April 1972 to October 1973, villages in 3 concentric areas were treated with one of 3 control strategies, as follows:

1. Residual indoor spraying with the carbamate insecticide, propoxur, for three or four rounds, at intervals of 2 months, before and during each of 2 main transmission seasons (1972, 1973) was applied in the largest area (B—see Fig. 1). The operation aimed at total coverage of buildings. Propoxur[b] was selected as being probably the most effective insecticide available, and because it had not been tried on a large scale in Africa. On account of its fumigant effect it was expected to have an impact also on exophilic vectors. A small-scale preliminary insecticide trial was conducted to test the operational methods, to select the appropriate frequency of spraying, and to obtain preliminary entomological evaluation (*103*).

2. Propoxur, as above, plus mass drug administration (MDA) of sulfalene-pyrimethamine at low frequency (every 10 weeks) was applied in area A2 (see Fig. 1). The drug distribution aimed at total coverage of the *de facto* population of the villages, excepting infants between birth and their first detected parasitaemia. These infants were used as indicators of residual transmission; they were examined more frequently than the rest of the population. The sulfalene-pyrimethamine combination was selected as being probably more acceptable to the recipient population on a usually empty stomach (food was rarely available in houses at the time

---

[a] The study was designed mainly by Dr A. Rossi-Espagnet, Dr G. Gramiccia, Dr L. Molineaux, Dr K. Dietz and Mr S. Brøgger, in consultation with many WHO staff members and consultants, listed in Annex 1.

[b] 2-(1-methylethoxy)phenyl methylcarbamate (also described as: 2-isopropoxyphenyl *N*-methylcarbamate).

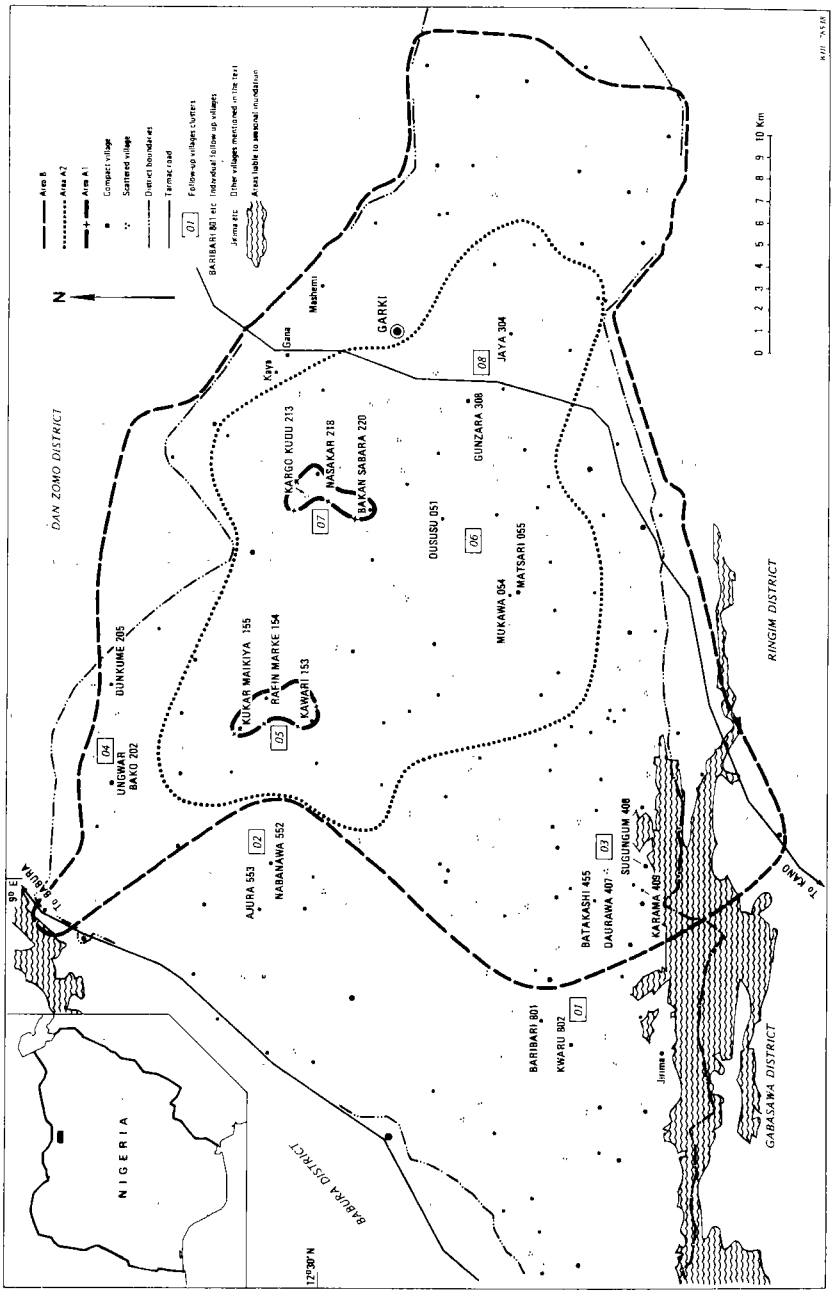


Fig. 1. The Garki project study area, showing the follow-up villages and the treatment areas

of MDA) than the standard chloroquine-pyrimethamine. A preliminary drug trial was conducted, to compare the two drug combinations in terms of clearance of *P. falciparum* trophozoites from the peripheral blood and of immediate side-effects; it was found that there was no significant difference in effectiveness, and that vomiting was less common after sulfalene-pyrimethamine, although the difference was not statistically significant (*150*). The selection of a long-acting sulfonamide (sulfalene) was made after careful consideration that took into account the limitation of the project in time, space and population affected. Two frequencies of MDA were found to be feasible on the scale envisaged, namely, either every 10 or every 5 weeks. Since computer simulations, using a preliminary version of the transmission model, indicated no great difference between the outcome from either period as long as the vectorial capacity remained well above its critical level (which was expected to be the case even after spraying with propoxur) the MDA was given at 10-week intervals.

3. Propoxur, as above, plus mass administration of sulfalene-pyrimethamine at high frequency, i.e., every 2 weeks in the wet seasons of 1972 and 1973, every 10 weeks in the intervening dry season, plus a limited amount of larviciding with temephos during the transition from the wet to the dry season in 1972 and 1973. This strategy was applied in area A1 (see Fig. 1). MDA aimed at total coverage, excepting negative infants. Temephos[a] was selected as being probably the most effective larvicide. The objective of this third, most intensive, control strategy was to reduce transmission and antigenic stimulation to the lowest feasible level, in particular for the purpose of the seroimmunological study (see p. 32). For operational reasons, this strategy could be applied only on a small scale.

While the above strategies were applied in areas B, A2 and A1, some untreated villages (area C) were studied for comparison. The project was planned as a time-limited research activity (see p. 19). After the 18-month intervention period, the following measures were taken to protect the study population against the consequences of possible malaria epidemics: in the villages treated with the third, most intensive strategy, those aged less than 10 years received 4 rounds of chloroquine, at intervals of 5 weeks, during the main transmission season of 1974; in addition in 1974 and 1975 in all villages previously treated by MDA, i.e., by the second and third strategies, chloroquine treatment was made available in each village to persons reporting with fever.

Further details regarding the control operations and the coverage actually achieved are given in Chapter 3.

---

[a] Tetramethyl-thiodi-*p*-phenylene phosphorothioate (commercial name ABATE).

Table 1
Villages included in the follow-up clusters, showing numbers of compounds, population, types and periods of observation

Area	Antimalarial measures	Village cluster No.	Village	No. of compounds (initial survey)		Population registered (survey 5)	Types of observation[a]							Period of observation
							Meteorology		Entomology			Human population		
				Compact Part	Scattered Part		Prec.	Temp. RH	PSC	NBC, ETC	ORC	DP	Imm., Hb., Clin.	
C	None	1	802 Kwaru	32	1	204	+	–	+	+	–	+	–	1970-73
			801 Baribari	27	1	179	–	–	+	–	–	+	–	
			806 Tafin Sale	0	47	330	–	–	–	–	–	+	–	
		2	553 Ajura	46	35	448	+	–	+[b]	+[b]	+	+	+	1970-76
			552 Nabanawa	89	5	700	–	–	–	–	–	+	+	
B	Propoxur in 1972-73	3	408 Sugungum	78	0	543	+	+	+	+	+	+	–	1970-73
			409 Karama	35	0	226	–	–	–	–	–	+	–	
			455 Batakashi	30	0	222	–	–	–	–	–	+	–	
			407 Daurawa[c]	13	17	182	–	–	–	–	–	+	–	
		4	202 Ungwar Bako	108	2	656	+	+	+	+	+	+	–	
			205 Dunkume[c]	85	0	481	–	–	–	–	–	+	–	
A2	(1) Propoxur + MDA (sulfalene-pyrimethamine) every 10 weeks in 1972-73.	6	55 Matsari	63	1	375	+	+	+	+	–	+	–	1970-73
			54 Mukawa	17	0	72	–	–	+	–	–	+	–	
			51 Dususu[c]	27	0	179	–	–	–	–	–	+	–	
	(2) Chloroquine to self-reporting fever cases in 1974-75.	8	304 Jaya	78	20	565	+	–	+[b]	+[b]	–	+	–	
			308 Gunzara[c]	21	18	251	–	–	–	–	–	+	–	

Area	Antimalarial measures	Village cluster No.	Village	No. of compounds (initial survey)		Population registered (survey 5)	Types of observation[a]							Period of observation
							Meteorology		Entomology			Human population		
				Compact Part	Scattered Part		Prec.	Temp. RH	PSC	NBC, ETC	ORC	DP	Imm., Hb., Clin.	
A1	(1) Propoxur + MDA (sulfalene-pyrimethamine) every 2 weeks in wet season, every 10 in dry, in 1972-73.	5	154 Rafin Marke	49	0	317	+	–	+	+	–	+	+	1970-76
			155 Kukar Maikiva	61	0	429	–	–	+	–	–	+	+	
			153 Kawari[c]	13	23	236	–	–	–	–	–	+	+	
	(2) Chloroquine below 10 years of age every 5 weeks, in wet season in 1974.	7	218 Nasakar	72[d]	0	367	+	+	+	+	–	+	+	
			213 Kargo Kudu	0	53	297	–	–	+	–	–	+	+	
			220 Bakan Sabara[c]	28	0	164	–	–	–	–	–	+	+	
	(3) Chloroquine to self-reporting fever cases in 1974-75.													

[a] Prec. = precipitation; Temp. = temperature; RH = relative humidity; PSC = pyrethrum spray collection; NBC = night bite collection; ETC = exit-trap collection; ORC = outdoor-resting collection; DP = demographic-parasitological surveys; Imm., Hb, Clin. = seroimmunological haemoglobin and clinical surveys.

[b] NBC and ETC limited to the compact part; PSC divided between the compact and scattered parts.

[c] Village added at survey 5.

[d] Including 33 new compounds built between surveys 3 and 4.

Project phases and calendar

There were four successive phases, as follows:

(*a*) *Preparatory phase:* September 1969 to September 1970: preliminary entomological and parasitological surveys; selection of study area and of the clusters of villages to be followed; drafting of study design, protocols, forms and operation manuals; testing of field and laboratory methods, including a preliminary parasitological trial comparing four methods of examining blood-films (*149*).

(*b*) *Baseline phase:* October 1970 to March 1972 (i.e., a dry season, a wet season, and a second dry season): collection of baseline data in the clusters of villages selected for follow-up; implementation of the preliminary insecticide and drug trials.

(*c*) *Intervention phase:* April 1972 to October 1973 (i.e., a wet season, a dry season, and a second wet season): application of the intervention strategies; continuation of the epidemiological study in both treated and untreated clusters of villages selected for follow-up. The duration of the intervention phase was not fixed *a priori*, but was left open between two and three consecutive main transmission seasons. In February 1972, an internal review of the results concluded that the additional information likely to be gained from a third year of intervention did not justify the expense and the expected additional loss of population immunity.

(*d*) *Post-intervention phase:* November 1973 to the termination of the project in February 1976: selective active and passive drug administration in the villages covered by MDA during the intervention phase; continuation of the epidemiological study in the villages treated with the most intensive strategy and in one cluster of untreated control villages; these were also the villages selected for seroimmunological follow-up.

Villages selected for follow-up and villages treated by the various strategies

In each of the 3 areas covered by the different control strategies (Fig. 1), 2 clusters of villages were selected for follow-up determinations. For each of these villages data are presented for the grouping of their domestic compounds (compact or scattered) and their number and population, the types of observations made, the period of observation, and the treatment applied (Table 1). The numbers of villages and their population, and the approximate area included, were highest in area B and lowest in area A1 (Table 2). The selection of villages for follow-up, their allocation to the various treatments, and the resulting subdivision of the study area were determined by a combination of principles and con-

Table 2

Numbers of villages, population and surface, in the areas treated by the 3 different control strategies

Area	Treatment	Number of villages	Population	Surface km²
B	Propoxur alone	104	32 828	550
A2	Propoxur + low-frequency MDA	54	14 129	350
A1	Propoxur + high-frequency MDA	6	1 810	12

straints. Given the available estimate of the age-structure, birth rate and expected attrition rate of the population and the expected changes in prevalence and incidence, it was considered desirable, from a statistical point of view, to follow an initial population of 2000 persons in each treatment group, or a total of 8000 persons for 3 treatments plus an untreated control group. This was also the maximum number that could be covered by parasitological survey 5 times a year. The collection of vectors from night bait was another limiting factor since it could be conducted at the required frequency (every 2 weeks in the wet season) in only 8 villages at the most and had to be limited to their compact part to allow satisfactory supervision; 8 is also the smallest number which allows independent study of 2 villages in each of the 4 treatment groups. Therefore, 8 clusters, of 2–4 villages each, including approximately 1000 persons per cluster, were selected for follow-up. They were selected throughout the district, in order to include as many as possible of the differences in intensity of transmission; they were also selected at least 5 km apart to permit, after stratification (see below), allocation of adjacent clusters to different treatments, taking into account the limited flight range of the majority of *A. gambiae, A. arabiensis* and *A. funestus*. The 3 largest villages—Garki, Gwarzo, Kargo, which were also the only villages with either a dispensary or a school—were excluded. The densities of these 3 vector species (i.e., *A. gambiae* sensu lato and *A. funestus*) were estimated during the preparatory and baseline phases as indicators of the intensity of transmission, to rank the selected follow-up village clusters. One of the 4 highest and 1 of the 4 lowest were allocated to each treatment. It was desirable to allocate contiguous areas to the same treatment, and also to reduce the effect of migrations by having similarly treated buffer zones around the evaluation villages. This led to the subdivision of the study area shown in Fig. 1 and documented in Table 2. It should be noted that the third, most intensive strategy was applied only to the 2

village clusters actually followed up. Given the available resources, the seroimmunological follow-up could cover about 3000 persons twice per year and was limited to the 2 village clusters receiving the most intensive treatment (No. 5 and 7) and to 1 untreated comparison village cluster (No. 2). These were also the villages in which the epidemiological study was continued in 1974-1975 (see p. 28).

At survey 5, 1 village was added to each of the follow-up clusters No. 3–8, in order to bring the size of the study population closer to the target of 1000 per cluster (see Table 1).

Variables measured, frequency of measurement, and methods used

The collection of data was multidisciplinary and longitudinal; i.e., it was designed in a way that would allow the study of the relationship between different variables in time or space, and make it possible to follow the history either of individual persons or of any group of persons. In each village selected for a given type of observation (Table 1), the following variables were measured:

Meteorology

Precipitation was continuously recorded by a standard automatic pluviograph (Casella); temperature and relative humidity were continuously recorded by a standard automatic thermohygrograph (Casella), placed in a standard Stevenson screen. The thermograph was regularly calibrated with the help of a mercury thermometer also placed in the screen. Additional meteorological measurements made during the nightly bite collections (temperature, relative humidity, wind velocity) have not been used in the present publication.

Entomology

The methods used were those described in the *Manual on Practical Entomology in Malaria (176)*, except as specified otherwise. The frequencies of collection were as follows:

Night-bite collection (NBC). In 1970-1973, taken every 2 weeks in the wet season, every 5 weeks in the dry season; in 1974-1975, in the wet season only, every 2 weeks in the 2 village-clusters previously treated (No. 5 and 7), twice in the season in the untreated control village cluster (No. 2); finally in 1975, in cluster No. 2, weekly for 14 weeks during the transition from dry to wet season. Each NBC was performed in 2 indoor and 2 outdoor fixed stations, with 2 human bait-collectors throughout the night in each station, i.e., for a total of 8 man-nights. Sixteen bait-collectors were employed in each NBC; at the start of each collection they

were assigned by lottery to the first or second half of the night and to the various stations; at the end of each hour, each collector's catch was stored in a refrigerated bag, and the 4 pairs of collectors were rotated between stations in a systematic way.

Stations for NBC were selected in consultation with the village headman, taking into account the expected cooperation of the occupants; the 2 stations were selected in different parts of the village, but less than 10 minutes apart on foot, for reasons of supervision. Anophelines were identified, counted, dissected for Christophers' stages (*176*); those found in stages I–IIM were examined for signs of prior oviposition by the method of Detinova (*42*); those found parous and those found in stages IIL–V were dissected for sporozoites. Either all anophelines were dissected or a representative sample with respect to hour and place (indoors, outdoors) of capture.

Pyrethrum spray collection (PSC). In 1970-1973, taken every 2 weeks, on the morning following NBC when applicable; in 1974-1975, in the wet season only, every 2 weeks in clusters No. 5 and 7, and every 4 weeks in cluster No. 2; finally in 1975, in cluster No. 2, weekly for 12 weeks during the transition from dry to wet season. Fixed PSC capture stations were selected as follows. The huts of 1 or 2 villages per village cluster (Table 1) were grouped in clusters of 2–4 huts, being whole compounds or sections of large compounds. The hut clusters were numbered in topographical sequence, and a systematic sample of 4 clusters, with a random starting-point, was drawn, so that the 4 selected clusters were dispersed within the village(s), e.g., out of 40 clusters, one would take every tenth cluster, starting with one chosen at random among the first 10. Anophelines were identified and counted, females were classified by stages of abdominal appearance, and, when feasible, dissected for sporozoites. Two further examinations were made at selected times, namely, the identification of bloodmeals by the precipitin test and the chromosomal identification of species of the *A. gambiae* complex.

Exit-trap collection (ETC). Taken on the same night as the NBC in 3 (later 5) fixed huts. ETC stations were selected according to the same principles as the NBC stations. Traps were installed before sunset, emptied after sunrise, later also between 20h30 and 21h00. The anophelines were identified and counted, the females being classified by stages of abdominal appearance.

Outdoor-resting collection (ORC). Taken in 1970-1973 only, on the same day as the PSC, in 3 villages only, in fixed artificial shelters (buried drums) within compounds and outside. The anophelines were identified and counted, the females being classified by stages of abdominal appearance. Bloodmeals were identified by the precipitin test.

The entomological observations listed above were made under the following conditions:

(*a*) the huts used for NBC, PSC, ETC were regularly inhabited; on the night of the NBC they were vacated by their normal inhabitants and occupied by the bait-collectors.

(*b*) no hut was used for more than one sampling method (NBC, PSC, ETC).

(*c*) very infrequently one hut had to be replaced by another resembling it as closely as possible.

(*d*) during the intervention period, the huts used as entomological catching stations were sprayed like any other hut, the spraymen ignoring which huts were used as catching stations.

Cytogenetic studies of the *A. gambiae* complex were also conducted in the study area during the same period. The methods and results are reported elsewhere (*31*, *145*); selected results will be presented and discussed in Chapter 4.

Demographic-parasitological (DP) surveys

The surveys covered, in principle, the total *de facto* population of selected village clusters, every 10 weeks in 1970-1973; in 1974-1975 some intervals were longer (see Fig. 43) and only 3 village clusters were followed (see Table 1). Age was estimated at registration; births, deaths, arrivals and departures were recorded; ages were updated by computer; a person absent for 4 consecutive surveys was reclassified as having emigrated. At each survey, a thick blood film was collected and examined for 200 microscopic fields; in 1970-1973 a systematic sample of a fifth of the films was examined for 400 fields. The number of fields found positive for *P. falciparum* asexual forms, *P.f.* gametocytes, *P. malariae* and *P. ovale,* respectively, was recorded.

In addition to the demographic-parasitological surveys, local residents were employed as itinerant collectors of data on births, deaths and migration. Also, in addition to the regular DP surveys, the infants excluded from mass drug administration underwent a supplementary parasitological examination in each interval between successive DP surveys; they were thus examined every 5 weeks. In 1974-1975, in the villages previously receiving MDA at high frequency, infants were examined, if present, every 5 weeks in the dry season, every 2 weeks in the wet season, as long as they were negative.

Seroimmunological surveys and haemoglobin-typing

The seroimmunological surveys covered the population of the villages selected and were carried out twice a year in conjunction with the DP sur-

veys, once in the dry season and once in the wet season (Fig. 43). Blood was collected from a fingerprick into 1 or 2 heparinized Caraway tubes and on to 1 or 2 filter papers. The following examinations were performed in Kano on plasma from the heparinized tubes: quantitative immunoglobulin determinations by single radial diffusion for IgG and IgM (*101*); indirect fluorescent antibody tests (IFA) using an IgG conjugate and *P. falciparum* and *P. malariae*, later *P. brasilianum* antigens (*157*): Ouchterlony agar gel diffusion analysis for antibodies to *P. falciparum* antigens prepared from human placentae (*110*). The passive haemagglutination test was performed in the Center for Disease Control, Atlanta, GA, USA, with the eluates from the filter paper samples for antibodies to *P. falciparum* or *P. knowlesi*, the latter for the first serological survey only (*102, 139*).

The above tests were performed regularly at each seroimmunological survey. In addition, the ELISA test, with a *P. falciparum* antigen, and also with various viral antigens, was performed in London on a sample of the sera from Garki (*158, 159*). The ELISA tests were performed at a later date and will be reported separately.

The red blood cells from the persons included in the seroimmunological surveys were sent to the Department of Haematology, University of Zaria, to determine the haemoglobin phenotypes by paper electrophoresis (*91*).

Body temperature surveys

The population covered by the seroimmunological surveys (comparison village cluster No. 2, plus clusters No. 5 and 7 in A1) was surveyed 3 times by axillary thermometry, in the middle of the wet seasons of 1973, 1974 and 1975, i.e., during the second wet season of intervention and 1 and 2 years later. The temperature surveys coincided with the 15th, 19th and 22nd demographic-parasitological surveys (see Fig. 43).

Anthropometric and spleen surveys

The above population was submitted to these surveys shortly after the wet seasons of 1973, 1974 and 1975. The following examinations were performed: height, weight, head, chest and arm circumferences, triceps skinfold (*86*) and spleen size. The head and chest circumferences were measured only in infants and children below the age of 5 years; above 10 years of age, only males were examined.

Recording, storage and retrieval of data

The data (observations and treatments) were recorded on precoded forms. The originals were sent to WHO, Geneva, and the data were trans-

ferred to tape with their precise coordinates of place, time, individual person, or mosquito, when applicable. The tapes were checked for errors and edited when appropriate. The tapes allow, in the analysis, for alternative groupings over time, space, or population, the study of the relationships between the different variables, and the extraction of the longitudinal histories of different types of units, e.g., individual persons, age-groups, villages.

The edited and documented tapes are on long-term storage in WHO, Geneva, where they can be made available for further use.[a]

Services and staffing

Services

The services required for the direction, management and implementation of the project were distributed among WHO in Geneva, the WHO Regional Office for Africa in Brazzaville, the project headquarters in Kano, and the field station in Garki.

WHO, in Geneva, was responsible for the technical direction and coordination of the project, for the processing and analysis of the data, and for international procurements.

The WHO Regional Office for Africa provided local administrative coordination and assistance, in particular through the services of the WHO Representative in Lagos.

The project headquarters in Kano was responsible for the management and implementation of the field project, and had the following laboratories and services: parasitology, serology, entomology, data recording, stores including deep-freeze facilities for sera, transport, administration, and secretariat.

In Garki the field station situated in the chief town of the district afforded a hostel for the team members, stores for equipment and supplies for field operations, simple parasitological and entomological laboratories, and repair facilities for transport and field equipment.

Staffing

The list of the staff and their period of service in the project are given in Annex 1.

Some changes occurred in the staffing both at Geneva and in the field in relation to the phasing of the project and certain reorganizations.

Frequent visits were made by staff from WHO, Geneva, to the field area, and on a few occasions the project director or team leader was called

[a] Requests should be addressed to Director, Malaria Action Programme, World Health Organization, Geneva, Switzerland.

to Geneva for meetings on planning or evaluation of the project. Field staff members were briefed in Geneva on recruitment, and some of them stopped over there for consultation or discussions during their leave.

Temporary consultants were recruited for special technical assignments, or for providing independent advice on the planning and evaluation of the project.

The field station in Garki, in addition to local watchers and keepers, was staffed during the week or sometimes longer by the staff from Kano or by temporary consultants.

Area, Climate, Population

The headquarters of the project was located in Kano, and the field station in the city of Garki, located at 100 km to the north-east of Kano.

The study area was almost coextensive with the District of Garki, Kano State, Nigeria (Fig. 1).

Orohydrography

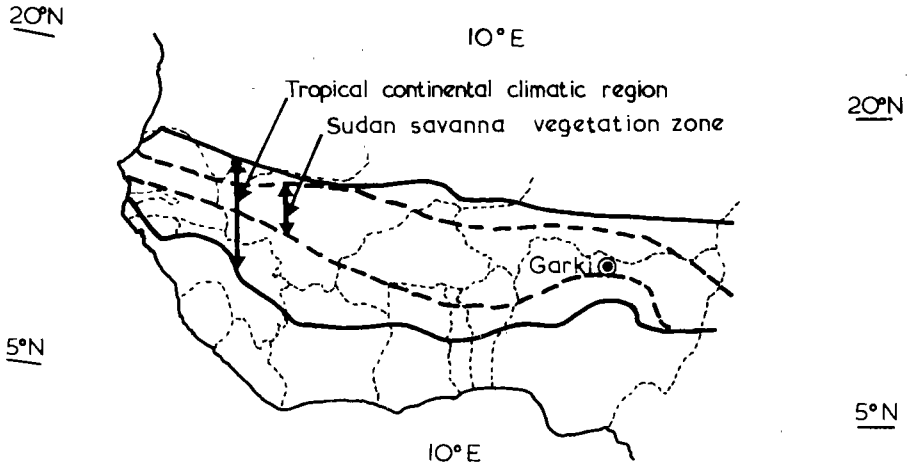
The District lies below 500 m altitude, with very little relief, being part of the Lake Chad drainage area. There is usually no permanent surface water, and the only river is the Jakiri, which is an affluent of the Hadejia and forms part of the southern boundary of the District. Marshes are formed in the wet season, but they dry up more or less completely in the latter part of the dry season. Near the Jakiri, the water table is 2–5 m deep; northwards it sharply falls to 30–50 m in the dry season over much of Garki District. Deep wells (one per village or per small group of villages) are used throughout the year. In the wet season, there are uncountable temporary collections of surface water, of various sizes and durations. Water may persist long after the rains in the borrow pits from which clay is extracted for building.

Climate and vegetation

With respect to climate, the area belongs to the tropical continental region (*84*)[a], characterized by relatively wide annual and diurnal ranges of temperature, and a restricted rainfall (250–1000 mm per annum) with

[a] Finer subdivisions of West African climates distinguish three horizontal belts within the tropical continental region; the project was located in the middle belt, called "Savanna climate" by Harrison Church and "*Climat soudanien-nord*" by Hubert (*81*). On the other hand, the project area is located near the northern limit of the "Tropical savanna" climate of Trewartha (*55*).

Fig. 2. Location of the Garki project area within the limits of the tropical continental climatic region and of the Sudan savanna vegetation zone in West Africa (*84*)



clear single maximum (Fig. 2). The yearly maximum temperature immediately precedes the rainy season, and a secondary peak of high temperature occurs at its end. Three seasons may be recognized: dry cool (November-February), dry hot (March-May), and wet (June-October). The seasons are determined by the alternating preponderance of the dry tropical continental air mass coming from the north-east (the harmattan wind) and the moist tropical maritime air mass coming from the south-west.

With respect to vegetation, the study area belongs to the Sudan savanna zone (*81, 84*). This zone according to Harrison Church (*81*, pp. 76-77),

> "is found in a belt some 120–240 miles [200–400 km] wide, from Senegal to Nigeria and beyond, north of the Guinea savanna. In the Sudan savanna, annual rainfall averages about 22–40 inches [550–1000 mm], there are seven almost rainless months, and relative humidity in early afternoons of the dry season may drop to 8%. It may be regarded as the most typical of all savannas and occurs roughly in the climatic belt of that name. It is one of the clearest climatic, vegetational and human zones, yet one of the most affected by man. It is often densely inhabited—e.g. in northern Nigeria, the Upper Volta and western Senegal—so that there is much secondary vegetation. There has also been some spreading of species from the south, and from the north—particularly of acacias. Consequently, the vegetation of this zone is difficult to define. Trees, which almost always occur singly, average 25–50 feet [8–12 m] in height and have wide-spreading crowns... There are also smaller trees, 10–20 feet [3–6 m] high, such as the acacias. Lower again is the bush or shrub of 6–20 feet [2–6 m]... Most trees lose all their leaves in the dry season... In this zone, grass shoots up only just before the rains. It is also shorter (3–5 feet [1–2 m]), less tussocky but more feathery than in the zones to the south. Consequently, it is very useful for grazing and is less deliberately burnt, so that fires are not

so violent. It is a most interesting fact that moderate grazing, by keeping the grass short, can arrest fires and so allow greater tree growth and regeneration. The original climax (xerothermic) may perhaps still be seen on isolated or inaccessible rocky slopes, or along water courses".

With respect to agriculture, the project area lies in the "millet and groundnut zone" (*81*). In the area itself, millet and different varieties of Guinea corn (giant millet, sorghum) are the main crops. They grow to heights of approximately 2 m and 4 m respectively and closely encircle most of the villages during the growing season.

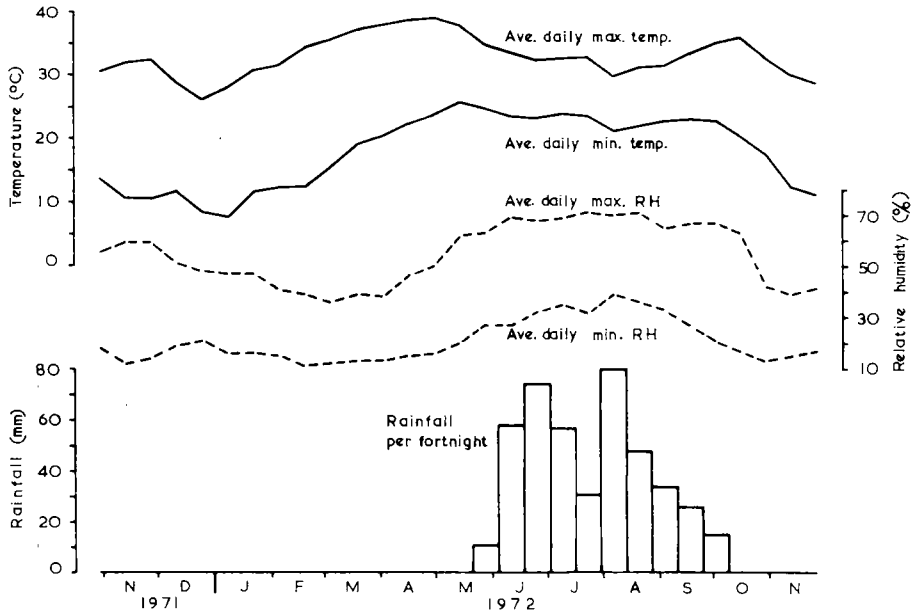
Meteorology

The meteorological observations made in the project have been the subject of an unpublished report (*178*).

Past meteorological data are available from some stations located near the study area, including Kano (*88*), but not from the study area itself. They are typical of that climatic zone. In Kano, the average yearly rainfall over a 41-year period was 886 mm, with a standard deviation of 180 mm.

The meteorological data collected by the project in the Garki district (Fig. 3), showing the rainfall and the daily maximum and minimum tem-

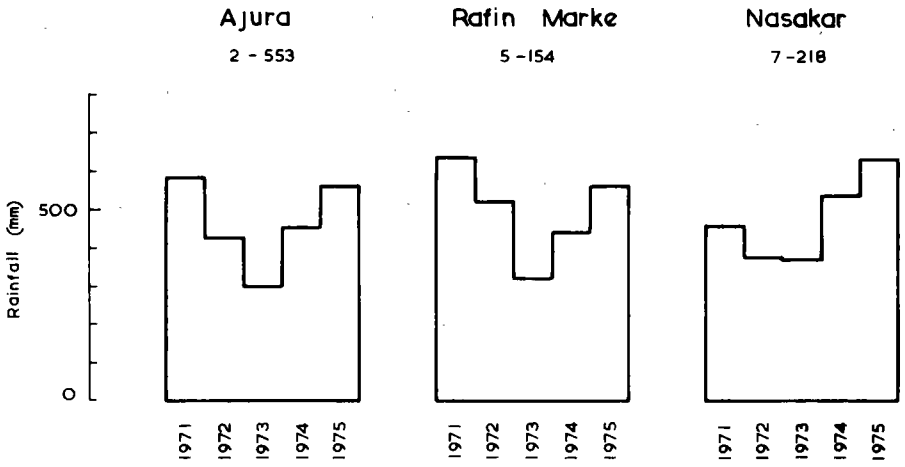
Fig. 3. Rainfall (14-day totals, average of 8 stations) and daily maximum and minimum temperature and relative humidity (14-day averages of 4 stations) in Garki district, November 1971-November 1972



perature and relative humidity in the study area over a complete calendar year (November 1971 to November 1972), allow the three seasons (dry cool, dry hot, wet) to be clearly identified.

The rainfall per annum varied, among 8 villages, from 467 mm to 691 mm in 1971, from 371 mm to 515 mm in 1972, and from 203 mm to 436 mm in 1973. There was a decrease from 1971 to 1972 and from 1972 to 1973 in every one of the 8 villages, but there was no significant correlation between the ranking by rainfall of the villages in the different years. In each of the 3 villages where observations were continued there was an increase in rainfall from 1973 to 1974 and from 1974 to 1975. Among the 3 villages, the rainfall per annum varied from 434 mm to 528 mm in 1974 and from 553 mm to 633 mm in 1975 (Fig. 4).

Fig. 4. Rainfall per year in 3 villages of the project area in 1971-1975



Rainfall in the area is discontinuous and irregular. For example, in the village of Ajura in 1975 (see Fig.9 below), the number of rainy days per month in the course of the wet season varied between 1 in April and 13 in August, while the time pattern was quite sporadic. The spatial pattern of rainy days was also quite irregular; during the years when 8 stations were in service in the study area, only about half of the rainy days involved all of them.

The distribution of the rainfall over the rainy season varies markedly from year to year, as illustrated by the figures for 1971, 1974 and 1975 in 2 of the villages (see Fig. 19 below). The changes in the time pattern of the rainy season from year to year were very similar in the different villages.

Population

The population density in the Sudan savanna zone is relatively high, and notably higher than in the belts to the north and to the south (*81*). In the project area, the population belongs almost exclusively to one of two ethnic groups, Hausa or Fulani. The Hausa form the majority: they are Negroes, live a sedentary life and are mostly farmers. Their huts are usually round, with clay walls and a thatch roof, rather closely grouped in fenced family compounds, which in turn are grouped in compact settlements. The Fulani were originally nomadic herdsmen who are believed to have come from the north as invaders. Some, the "cattle" Fulani, are still nomadic. Small groups of them with large numbers of cattle move through the project area, following the rains and the availability of grazing grounds. They live in temporary tents made of cowhides or grass. Other Fulanis have become sedentary; they are now bilingual and have intermarried to a certain extent with the Hausa. Most of the emirs and other aristocrats of northern Nigeria belong to this class. In the project area, settled Fulani practise agriculture and also keep cattle, though in smaller numbers than the "cattle" Fulani. They live in huts similar to those of the Hausa, less closely grouped in family compounds, usually unfenced; and the compounds themselves are grouped in scattered settlements, often with 100 m or more between compounds. An administratively defined village unit has a chief responsible to the district governor and may be compact, scattered or mixed. In addition to the movements of nomadic Fulani, another significant type of migration that occurs in the project area is the temporary emigration of labourers during the dry season; in the Hausa language, this seasonal migration is called "cinrani", meaning "eating away" (the dry season). Both Hausa and Fulani practise polygyny. Both ethnic groups are Moslem. The demographic data collected by the project have been indicated to a small extent above but are to be found mainly in Chapter 8.

Chapter Three

CONTROL OPERATIONS[a]

Larviciding operations were very limited (see p. 25) and only residual spraying and the administration of drugs are considered here. The epidemiological effects of the control operations are considered in subsequent chapters.

Residual Spraying

The planning, execution and operational results of residual spraying with propoxur have been described in three Technical Notes (*14, 104, 127*) and in the report on the control operations (*179*) and only the salient points are presented here.

Sprayable surfaces

The following surfaces were sprayed: the inner surface of walls and roofs of walled buildings (mostly round huts) excepting the lower 20 cm of the walls; the undersurface of furniture; the eaves of huts, the undersurface of the roofs of sheds; the undersurface of granaries. Prior to the intervention phase, a detailed geographical reconnaissance was carried out, and a survey was made of the sprayable surfaces of compounds in the area to be treated. This revealed a fair uniformity in the sprayable surface per walled hut; this surface is obtained by dividing the sprayable surface of a compound by the number of huts in the compound and includes the surface of the furniture, granaries and sheds belonging to the hut (mean 35.8 m^2; SD 6.7 m^2). On the other hand, the sprayable surface per compound varied greatly (mean 123.2 m^2; SD 89 m^2), depending chiefly on the number of huts per compound. The estimates for the spraying operations were therefore based on the number of huts, taking into

[a] The spraying operations were conducted by Mr R.V. Nambiar, Mr P.E. Lietaert, Mr E. Ramos-Camacho, and Mr V.R. Nair; the MDA operations were conducted by Dr T. Matsushima, Mr J. Storey, Mr D. Thomas and Mr S. Brøgger.

account that the sprayable surface per hut was 34 m^2 in compact villages and 38 m^2 in scattered villages.

The number of villages, the geographical area involved and the human population concerned have been given in Table 2. The number of huts to be sprayed increased from about 29 700 in the first round to about 34 100 in the sixth round. The total surface actually sprayed increased at the same time from about 1 036 000 m^2 to about 1 182 000 m^2.

The spraying operations

Propoxur 50% water-dispersible powder, suspended in 10 parts water, was sprayed with Hudson X-pert compression sprayers (3 gal US; 11.7 l) equipped with nozzle tip HSS 800 3E (G). A new swivel nozzle body (PA S.152) was introduced in 1973 to facilitate spraying eaves and the undersurface of granaries. The discharge rate was checked weekly and the pressure regulator was reset to give 760 ml per minute at a nozzle pressure of 18 psi.[a] The intended dosage was 2 g technical propoxur per m^2 of sprayable surface.

A total of 75 persons were engaged in the operation. This included 26 spraymen in 9 teams (8 regular teams of 3 and 1 mop-up team of 2). The staff was recruited locally. Spraymen received 2 weeks of training in 1972 and again in 1973. The surface sprayed per day per sprayman was about 1280 m^2 or 10–11 compounds in compact villages, and about 800 m^2 or 6 to 7 compounds in scattered villages.

In 1972, 3 rounds of spraying were applied, starting on 1 May, 5 July and 6 September, respectively. The interval between successive rounds in the same village was 61–66 days. Each round of spraying was completed about a week before the next one was started, and, in part of the area, there was some increase in vector density before the first application of insecticide. In 1973, spraying was begun earlier, and the rounds of spraying were started on 17 April, 16 June and 16 August, and a fourth round (seventh round overall) was applied to the southern half of the area, starting on 15 October. The interval between successive rounds in the same village was 56 days between rounds 4 and 5, and 60–66 days thereafter. The timing of the spraying rounds in 2 particular villages (Rafin Marke and Sugungum), is indicated in Fig. 10 and 11.

Operational results: the coverage achieved and the dose applied

Coverage was defined as the percentage of huts completely sprayed among those existing at the time of spraying. It was very high in most

[a] 18 lb/in² = 931 mm Hg = 124 kPa = 138 kN/m².

villages at most rounds. For the whole area, the coverage varied by round from 96.6% to 99%. Among all villages and rounds, it varied from 74% to 100%. In the villages selected for follow-up, coverage was 99% on the average, and varied, among villages and rounds, from 84% to 100%.

Coverage was measured immediately after spraying. The actual coverage must be somewhat lower, because between rounds some new houses are built and some old ones are repaired; this happened mainly between the first and second round of each year. Some sprayable structures may, in addition, have been missed altogether (i.e., counted in neither numerator nor denominator); the number of such structures was probably very small. One small compact village had been missed in 1972.

The average dosage of technical ingredient actually applied varied, between rounds, from 1.98 g/m² to 2.43 g/m² of sprayable surface (2.15–2.43 g/m² in 1972, 1.98–2.20 g/m² in 1973). The amount of insecticidal formulation applied per round varied, among rounds 1 to 6, from 2282 kg to 2744 kg; the grand total used in 6½ rounds was 15 845 kg.

Administration of Drugs

The spontaneous consumption of antimalarials in the study area, before or independently of the project, was negligible: the 2 dispensaries of the district, in Garki itself and in Gwarzo, are outside the study area proper and were dispensing only a negligible amount of antimalarials to the inhabitants of the study area (*148*); the ambulant drug pedlars observed in the area's markets were not dispensing specific antimalarials.

Mass distribution of sulfalene-pyrimethamine in 1972-1973

The operations

The plan for mass drug administration is outlined in Chapter 2, in terms of persons eligible and of frequency (see in particular pp. 23-25 and Table 2). The MDA operations are described in greater detail in two Technical Notes (*105, 106*) and in the report on the control operations (*179*).

A combination of sulfalene and pyrimethamine was used either as uncoated tablets or in a syrup suspension. Each tablet contained 500 mg sulfalene and 25 mg pyrimethamine. Syrup was supplied in bottles containing 10 ml fitted with a glass dropper; 1 ml of syrup contained 200 mg sulfalene and 10 mg pyrimethamine. At each filling, the dropper delivered an average of 26 drops per ml of syrup. Based on the assumption of an immunity increasing with age, and on the results of the preliminary trial, the dosage by age-group for each drug administration round was

scheduled as follows:

< 6 months	12 drops syrup	=	90 mg sulfalene + 4.5 mg pyrimethamine
6-11 months	20 drops syrup	=	150 mg sulfalene + 7.5 mg pyrimethamine
1-4 years	30 drops syrup	=	230 mg sulfalene + 12.0 mg pyrimethamine
5-9 years	½ tablet	=	250 mg sulfalene + 12.5 mg pyrimethamine
⩾ 10 years	1 tablet	=	500 mg sulfalene + 25.0 mg pyrimethamine

Whereas the tablets were given with water, the syrup was given in a solution of sugar or in fruit squash.

The people subjected to MDA outside the follow-up villages were the *de facto* population. During the first round all the occupants were registered by compound; each person was given a "person number" and at each round those present were given drugs. Persons who were absent for 2 consecutive rounds were defined as "moved", struck off the register and not counted in the population for that round. Newcomers, including births but excluding visitors (see below) were added to the register.

In the follow-up villages, rosters of occupants were prepared from those used in the demographic-parasitological (DP) surveys which had been conducted for 1½ years before MDA commenced. In this population, a person was classified as "moved" only after being absent for 4 consecutive DP surveys, i.e., for about 9 months. Persons absent for less than 4 consecutive demographic-parasitological surveys were counted as residents.

Thus the denominator used in the calculation of the coverage achieved, i.e., the registered resident population eligible for MDA, was defined differently in the 2 populations (the follow-up villages and the others) and therefore the figures for coverage are not immediately comparable.

Infants born into follow-up clusters No. 6 and 8 in area A2 were excluded from MDA as long as they were found negative by microscopic examination. An additional blood film was taken from these infants between consecutive parasitological surveys, i.e., they were examined every 5 weeks. The same rules were applied to infants born into follow-up clusters No. 5 and 7 from September 1972 onwards, i.e., after the 10th fortnightly MDA (a fortnight before the 10th parasitological survey); previously they had been included in the MDA. Infants were excluded, in order to evaluate residual transmission.

The operation was carried out by compound-to-compound visits. Every person was called by name, and the drug was given by a staff member who made sure that it was swallowed before he recorded administration. Drugs were never left for absent people to take later. Those who were absent on the first visit were, as far as possible, given the drugs on a second visit later the same day or on the next day.

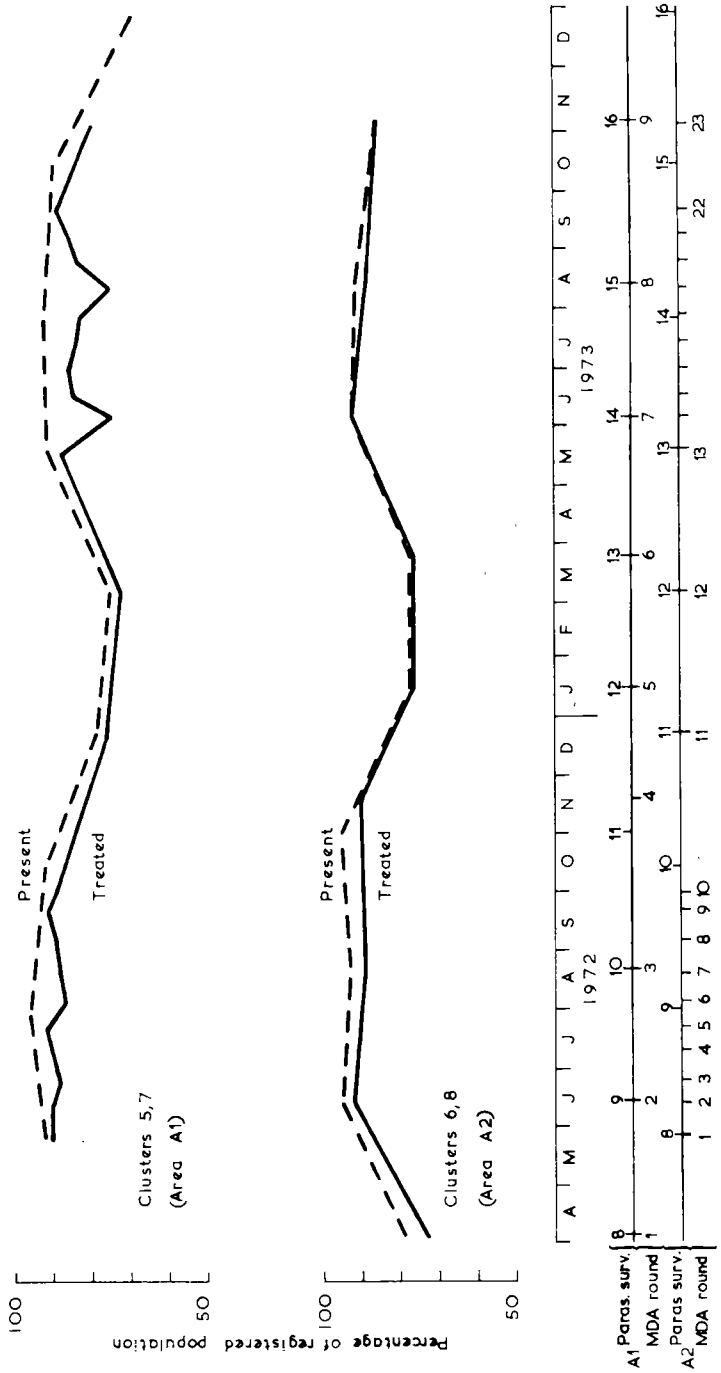


Fig. 5. Percentage of the registered population present at each parasitological survey and percentage treated at each round of mass drug administration (MDA) in 2 groups of villages where MDA was applied throughout the intervention period at 2 different frequencies (2-week and 10-week intervals)

In addition to the occupants registered by compound, visitors encountered in and around compounds at the time of administration were also given drugs, and recorded separately.

A total of 14 persons were engaged in the MDA, including 5 teams of 1 drug distributor and 1 record keeper each, mostly recruited from the district itself and trained for 1 week. Per day, each team covered 150–180 persons (25–30 compounds) in the compact villages, 90–100 persons (15–17 compounds) in the scattered villages.

The actual timing of the MDAs in the follow-up villages, as well as their relationship to the demographic-parasitological surveys, is shown in Fig. 5 in the upper line for the high-frequency MDA and on the lower line for the low-frequency MDA. The parasitological surveys were usually conducted at the end of the interval between consecutive MDAs; this means that most probably only the maxima of prevalence of parasitaemia were observed.

Operational results: the coverage achieved

Coverage was expressed as the proportion of the registered resident population treated at each MDA. In the follow-up villages, infants excluded from MDA were also excluded from the calculation of coverage. The recorded coverage of visitors was always 100%, which probably means only that, if a visitor was assessed and recorded, the person was also treated; they were excluded from the computation of coverage. The number of recorded visitors varied, by the MDA round, between 2.6% and 6.3% of the resident population, for the whole area A2 excluding the follow-up clusters No. 6 and 8; individual villages showed, as expected, more variation.

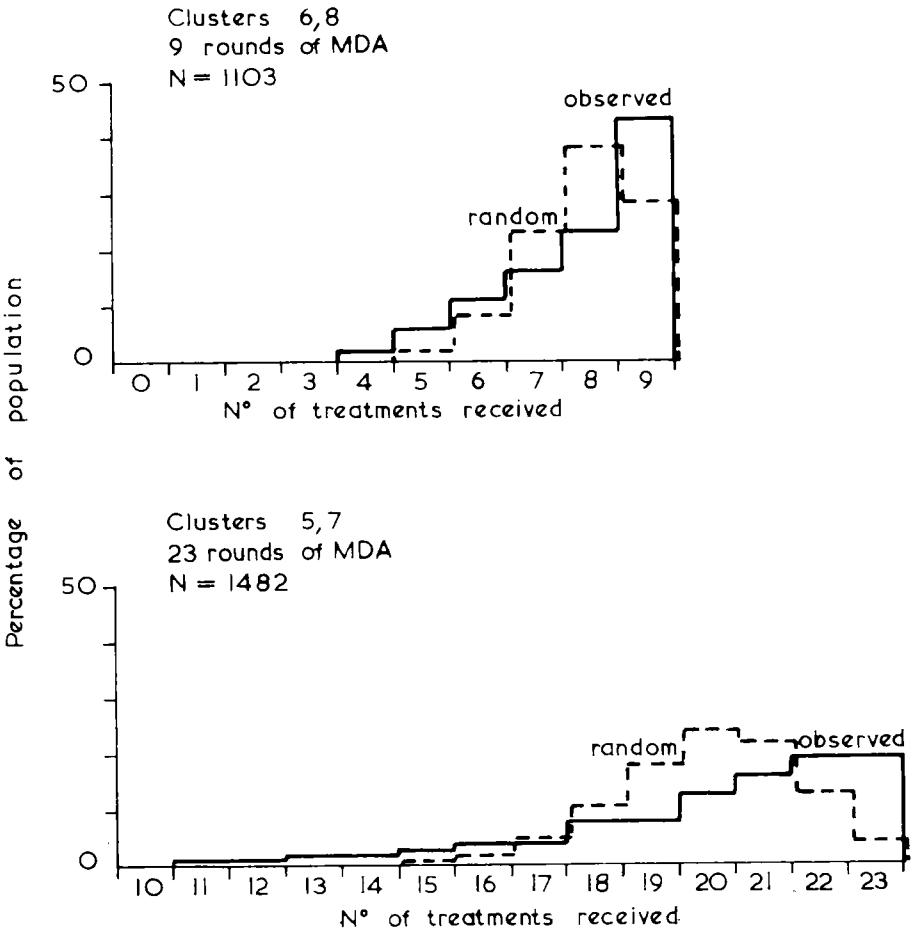
The coverage achieved at each round of MDA in the follow-up clusters is shown in the upper graph of Fig. 5 for the high-frequency MDA, and in the lower graph for the low-frequency MDA. The number of persons present at each demographic-parasitological survey and the number of persons treated at each MDA are expressed as a percentage of the registered population. In both groups of follow-up clusters, the coverage was 85% on the average, greater in the wet season, smaller in the dry season; the seasonal variation in MDA coverage followed closely the seasonal variation in absenteeism from the village (see Chapter 8), there being no long-term trend. In follow-up clusters No. 6 and 8 (Area A2) the coverage varied, by round, between 73% and 92% (by round *and* village it varied between 69% and 99%); in follow-up clusters No. 5 and 7 (area A1), coverage varied, by round, between 72% and 91%.

In the remainder of area A2, i.e., outside the follow-up clusters, the recorded coverage was 95% on the average and varied little by either round or location. Since the registered resident population was defined in

a different (narrower) way than in the follow-up clusters (see above), the figure for estimated coverage was heightened.

In follow-up clusters No. 6 and 8, where each MDA was given immediately after a demographic-parasitological survey, we can compare the resident population registered by the MDA rounds to that registered by

Fig. 6. Percentage distribution of persons according to the number of treatments received in the entire intervention period, at 2 different frequencies of MDA: comparison between the distribution actually observed and the binomial distribution with the same average[a]



[a] N = number of eligible persons, i.e., persons registered throughout the intervention period excluding a very small number of negative infants not eligible for MDA.

the demographic-parasitological surveys, and also relate the number of residents treated to the number present. The resident population, as enumerated by the MDA rounds, varied between 91% and 103% of the resident population as enumerated by the demographic-parasitological surveys. The MDA covered, on the average, 95% of the residents registered and present (excluding ineligible infants), and this proportion varied, by round, between 91% and 99%, without detectable pattern.

In each of the 2 groups of follow-up clusters receiving MDA, the persons registered throughout the intervention phase, i.e., eligible for MDA at all rounds, were classified by the number of times they had actually received the drugs, i.e., 0–9 times in clusters No. 6 and 8, 0–23 times in clusters No. 5 and 7. The resulting frequency distributions (Fig. 6) may be compared with the distribution that would have been obtained if each round of MDA had drawn an independent random sample of participators from the population, while achieving the same overall coverage. It can be seen that the actual distribution is very different from random; there is a clear excess of high and low personal MDA scores, i.e., of "good" and "bad" participators.

Post-intervention drug distributions in 1974-1975

The rationale and general plan of the post-intervention drug distributions in 1974-1975 have been outlined in Chapter 2 (see p. 25). Drug administration to persons reporting fever has been described in a Technical Note (*151*).

Starch-coated chloroquine phosphate tablets were used and the following doses of chloroquine-base were administered: below 1 year of age, 75 mg; at 1–3 years, 150 mg; at 4–9 years, 300 mg; at 10 years and above, 450 mg. In the follow-up clusters No. 5 and 7, i.e., the villages previously receiving high-frequency MDA, and in which investigations were continued, there were 4 systematic distributions of chloroquine at intervals of 5 weeks for those born (probably) after 1 January 1962, i.e., those aged less than 10 years by 1 January 1972, a date 3 months before intervention commenced. Rounds 1 and 3 followed immediately upon demographic-parasitological surveys 18 and 19; rounds 2 and 4 were given halfway between surveys 18 and 19 and surveys 19 and 20, respectively. The timing of the surveys is shown in Fig. 43.

In addition, in 1974 and 1975 in all villages previously receiving MDA, chloroquine was given to persons reporting fever. In the follow-up clusters No. 5 and 7, the drug was dispensed by project staff; 62 treatments were given to 59 persons in 1974, while 224 treatments were given to 193 persons in 1975. The percentage of the examined population that was treated varied from 0.7% to 7.3%, depending on the follow-up cluster

and period (10-week interval in the wet season). In the other villages, the distribution of chloroquine was handled by a selected villager who had been given a short instruction. Consumption was somewhat higher: in the wet season of 1974, the proportion of the population reporting fever and requesting treatment was 9.5% per fortnight on the average.

Discussion

With respect to the control operations, the following points merit some discussion: the definition and evaluation of coverage, the non-randomness of coverage, the consciousness and participation of the community in the use of antimalarials.

The definition of coverage is to some extent arbitrary, and its evaluation confronts some difficulties. This applies in particular to the coverage of the human population by a measure such as MDA, especially when no satisfactory census is available and when the population is relatively mobile. It is relatively easy to count the number of persons treated (the numerator). It is more difficult to define and to measure the number of persons that should be treated (the denominator): the resident population was probably nearly completely registered, but visitors were counted to an unknown degree of completeness, and only at points in time, and the duration of their visits is not known; in the registered population, an arbitrary rule was adopted for reclassifying long-term absentees as moved and subtracting them from the registered population, which rule for practical reasons varied between areas. When this is taken into account, it is probable that there were no real differences of coverage between the two pairs of follow-up clusters (No. 6 and 8, No. 5 and 7), or between the follow-up clusters and the surrounding area, i.e., the remainder of area A2.

The distribution of persons eligible for MDA by the number of treatments actually received was very different from random. This may be expected every time a repetitive measure is applied to a population. Ignoring this leads to an overestimation of the expected proportion of persons covered by the measure, and of the expected effectiveness of the latter; e.g., from an 80% coverage, it may be expected that, after 2 and 3 rounds, respectively 96% and 99.2% of persons will have been treated at least once, whereas in reality the proportion may be much lower. When computer simulations were used to calculate the expected effect of MDA in Kankiya, northern Nigeria (*100*), it was assumed (implicitly) that coverage was random at each round of MDA. *A posteriori,* it was shown that, as in Garki, the distribution was non-random, and this was probably one

of several reasons why the level of control achieved fell short of expectations (*126*).

Knowledge and utilization of antimalarials in the study area were practically negligible before the research project commenced. As a by-product of the project, the villagers now have an increased awareness of malaria and of the possibility of its treatment and prevention. They have acquired a modest capacity for self-help with drugs. The health services of the Kano State are expected to continue to provide the drugs.

Summary

Residual spraying with propoxur was applied, mainly to the inside of buildings, in 164 villages (including follow-up clusters No. 3–8), distributed over 900 km^2 with a total number of huts of about 30 000 and a total population of about 50 000 persons. Three rounds were applied before and during the wet season of 1972; there was some increase in vector density before the first round was completed. In 1973, spraying was started earlier, beginning in the south, and a fourth round was applied to the southern half of the area. The interval between rounds of the same year was about 60 days. In $6\frac{1}{2}$ rounds, about 16 000 kg of propoxur (50% water-dispersible powder) were applied, and the average dose was 2.15 g/m^2 (the intended dose was 2 g/m^2). Coverage, expressed as proportion of huts sprayed, was 99% on the average in the follow-up villages, and varied little between rounds or villages; since coverage was estimated immediately after each spraying round, the "true coverage" must be somewhat lower, due to building and repair activities between rounds.

Mass drug administration (MDA), using a combination of sulfalene and pyrimethamine, was applied in 60 of the sprayed villages, with a total population of about 16 000 persons, in 1972-1973, i.e., during the 2 wet seasons in which propoxur was used, plus the intervening dry season. In 6 villages (follow-up clusters No. 5 and 7), MDA was applied every 2 weeks in the wet season, every 10 weeks in the dry season, up to a total of 23 rounds; in the remaining 54 villages (including follow-up clusters No. 6 and 8) MDA was applied every 10 weeks for 9 rounds. Infants were excluded from MDA as long as they were negative; visitors were included. The proportion of the registered population covered by MDA was 85% on the average, more in the wet season, less in the dry season, owing to seasonally variable absenteeism from the villages and the study area. The distribution of eligible persons by the number of treatments actually received was not random, and showed an excess of persons receiving both more and less than the average number of treatments, i.e., an excess of

both "good" and "bad" participators; ignorance of this fact has led in the past to exaggerated expectations regarding the effect of MDA transmission.

The interventions in 1972-1973 had been planned as time-limited operations for research purposes. In 1974-1975, in the villages previously included in the MDA operations, chloroquine was used in a selective manner to minimize the effects of the expected return of the prevalence of *P. falciparum* towards its original endemic level. The operation included the administration of chloroquine to self-reported fever cases, by a responsible villager; this was continued after completion of the research project early in 1976.

Chapter Four

ENTOMOLOGY[a]

This chapter deals with the entomological observations and their interpretation, both in themselves and in their relationship with some meteorological variables and with the insecticidal control operations. The entomological sampling scheme and the methods of collection and of examination were described in Chapter 2. The relationships between the entomological variables and their epidemiological consequences are dealt with in subsequent chapters, in particular in Chapters 5 and 10. The entomological findings have been the object of WHO reports (*175, 177, 184*).

Anopheline Fauna and Observations regarding Minor Species

Eleven species were identified: *A. gambiae* sensu stricto (*A. gambiae* species A), *A. arabiensis* (*A. gambiae* species B), *A. funestus, A. rufipes, A. pharoensis, A. wellcomei, A. squamosus, A. coustani, A. maculipalpis, A. nili* and *A. pretoriensis*. Only the first 6 species were found in appreciable numbers. These numbers increased markedly during the wet season, the time lag between rainfall and mosquito density depending on the species. The 2 species of *A. gambiae* sensu lato along with *A. funestus* are the main vectors, and are the subject of the remaining sections of this chapter. The results with *A. gambiae* s.l. and *A. funestus* are presented in the next 5 sections. Findings on the relative abundance and characteristics of *A. gambiae* (s.s.) and *A. arabiensis* are presented in the section following them. Some of the findings regarding *A. rufipes, A. pharoensis* and *A. wellcomei* are given in the present section.

[a] This chapter is based on the work carried out by, or under the direct supervision of Mr G.R. Shidrawi, Mr J.L. Clarke, Mr J.R. Boulzaguet, and Mr T. S. Ashkar. The application of the Polovodova method was done by Dr N. Detinova, and the intraspecific chromosomal studies of the *A. gambiae* complex were done by Dr M. Coluzzi, both being WHO consultants in this research. Mr R.F. Fritz, Mr C. Garrett-Jones, Mr J. Hamon, and Dr K.S. Hocking assisted in the design of the entomological study.

Before intervention *A. rufipes* constituted only 0.06% of the night-bite collections (NBC), but as much as 3.5% of the pyrethrum spray collections (PSC) and 30% of the outdoor-resting collections (ORC). It was less affected by propoxur than the main vectors, and during the intervention period it formed 43.1% of the PSC (but still only 0.2% of the NBC) in the sprayed villages. In 1974-1975, in the previously sprayed villages, it still formed a somewhat larger fraction of the PSC than before intervention.

A. pharoensis was collected mainly by NBC, more commonly outdoors than indoors; it represented 4.9% of the NBC before intervention, but only 0.1% (36 individuals in 3070 collections) of the PSC. One specimen was found positive for sporozoites. The species was found in all village clusters; the highest recorded man-biting rates were 4.5 bites/man/night indoors and 25.8 bites/man/night outdoors, both in the wet season in Sugungum. This species also was less affected by propoxur than the main vectors, and during the intervention period it formed 18% of the NBC in the sprayed villages. In 1974-1975 in the previously sprayed villages, its relative abundance was back to its baseline level.

A. wellcomei was collected almost exclusively by NBC, again more commonly outdoors than indoors, and almost exclusively in Sugungum; the highest measured man-biting rates were 8.5 indoors and 17.3 outdoors. This species almost completely disappeared from the collections during the intervention period.

Collections as Indices of Density

The numbers of female mosquitos caught per collection, by means of standardized sampling methods, are used as density indices. Such indices may obviously be affected by factors other than density itself; in the case of the pyrethrum spray collection (PSC), the numbers collected might be affected by factors such as the presence of a fire, or the size of the hut, or the number of sleepers in the hut. The presence of a fire in the hut had, in fact, little or no effect on the indoor-resting density (IRD); the fires were indeed small and produced little smoke. The size of the huts in the area varied only a little (see p. 41), and could therefore not be an important factor. The relationship between the number of sleepers in a hut on a given night and the number of *A. gambiae* s.l. females caught by PSC the next morning (the indoor-resting density, or IRD, i.e., number of females per hut) was systematically investigated (Table 3). Because the IRD varies widely between times and places (see below), and as this may mask an association with the number of sleepers, the PSCs were first stratified

Table 3

Mean indoor-resting density of *A. gambiae* s.l. (No. females/hut, by PSC) as a function of the number of persons sleeping in the hut on the night preceding the PSC made in the morning; wet season of 1971 (pre-intervention), all villages combined

Density stratum[a]	Number of sleepers[b]				
	0	1	2	3	⩾4
0.1-4	0.8	1.7	1.5	2.9	2.4
	(15)	(40)	(64)	(36)	(12)
4.1-16	3.7	8.7	10.1	10.9	13.6
	(20)	(59)	(84)	(48)	(18)
16.1-64	21.6	28.1	27.2	41.1	36.6
	(18)	(52)	(77)	(56)	(20)
64.1-256	203.4	94.0	87.6	122.3	86.3
	(5)	(27)	(37)	(23)	(8)

[a] PSCs were stratified according to the mean density at the same time and place (village cluster).
[b] In parentheses: number of collections with the given number of sleepers in the given density stratum.

according to the average IRD for the given village cluster and fortnightly cycle of entomological collections. At low to medium densities (density strata 0.1–4 and 4.1–16), the IRD increased clearly between zero and 1 sleeper, but only slightly (much less than proportionally) with an increasing number of sleepers; there was a relatively large variation around the means. At high vector densities, there was no demonstrable effect of the number of sleepers. This investigation was repeated taking into account in the PSC only those females which had presumably entered the hut the night before the collection, i.e., appearing as empty, partly fed, fully fed or late fed (see pp. 66-67); this led to the same conclusions as in Table 3 where all females were included. The number of sleepers thus has a limited effect on the IRD; in addition, the number of sleepers per hut varied relatively little, either in the course of time or between village clusters, and should therefore not affect comparisons in time or space. The number of sleepers was nevertheless used to compute alternative indices of the IRD, for comparison. Three indices of indoor-resting densities were computed for *A. gambiae* s.l. and for *A. funestus:* (1) the total number of female mosquitos per hut; (2) the number of partly or fully-fed females per sleeper; (3) the number of partly, fully- or late-fed females per sleeper. The second and third indices are estimates of the number that have fed on 1 sleeper in 1 night, and are sometimes used for

estimating the man-biting rate (MBR). All 3 indices were used before intervention, to describe a time trend, i.e., the change in IRD in the course of the wet season, and to study geographical variation, i.e., to compare the IRDs of the 8 village clusters. The 3 indices behaved in parallel, i.e., they differed only by a scaling factor. This could be expected in view of what was found regarding the number of sleepers and of the fact that the proportion "fed" (by either of the definitions given above) varies relatively little in the course of the wet season or between village clusters. The 3 indices stood, on the average, in the following relationship: 1.00 : 0.21 : 0.30 for *A. gambiae* s.l., 1.00 : 0.28 : 0.31 for *A. funestus*. The first index was adopted in this study to calculate the IRD.

The number of bites per man per night, measured by NBC, was used as man-biting rate (MBR); it was computed separately for the collections made indoors and outdoors and also for the total; the latter constitutes an unweighted average MBR (see below).

The IRD, the average MBR and the exit-trap collection (ETC) (number of females per trap night) were computed, for each of the 8 villages for which the information was available, for *A. gambiae* s.l. and for *A. funestus* during the wet season of 1971 (baseline phase). The correlations between the 3 indices were quite strong and approximately linear. The simplest interpretation of this finding is that: (1) all 3 measurements are valid indices of variations in density before residual spraying; (2) the sampling was adequate; and (3) the villages are really different. The correlations were, however, not perfect and there was some variation, in both time and space, in the ratios between indices; this may result from sampling error but may also reflect differences in vector behaviour. The relation between MBR and IRD (see above) is, however, completely upset after residual indoor spraying (see pp. 77, 84).

The sensitivity of the density indices is determined by the sampling design. With 8 man-nights, 10 huts and 5 exit-traps, as normally used during the course of the present work, the lowest measurable indices are: (*a*) MBR = 0.125 bite/man/night; (*b*) IRD = 0.1 female/hut; (*c*) ETC = 0.2 female/trap/night.

Baseline Findings

In this section we shall consider not only the observations made during the baseline phase, but also some observations made during the subsequent phases in the untreated comparison villages.

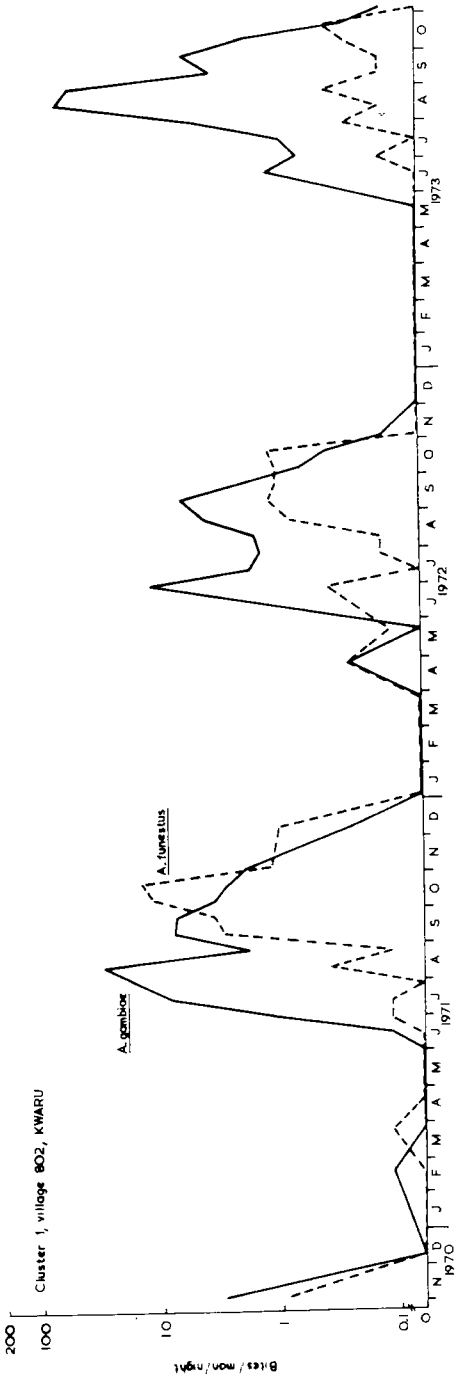


Fig. 7. Man-biting rates of *A. gambiae* s.l. and *A. funestus*, estimated from the night-bite collections in Kwaru, an untreated comparison village, in 1970-1973

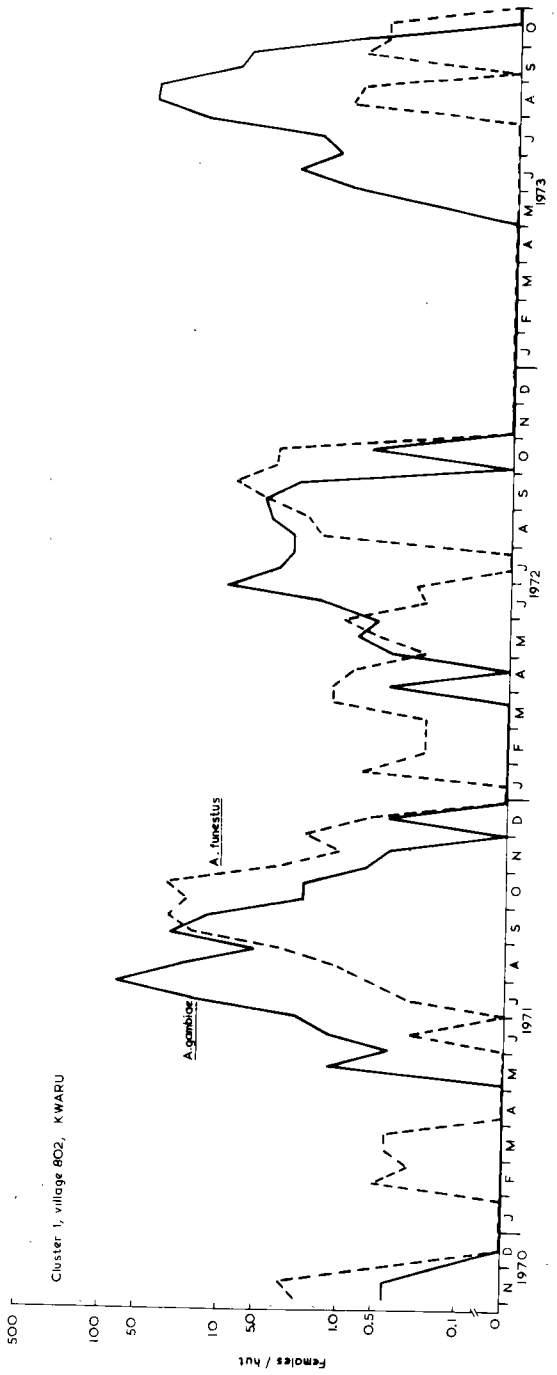


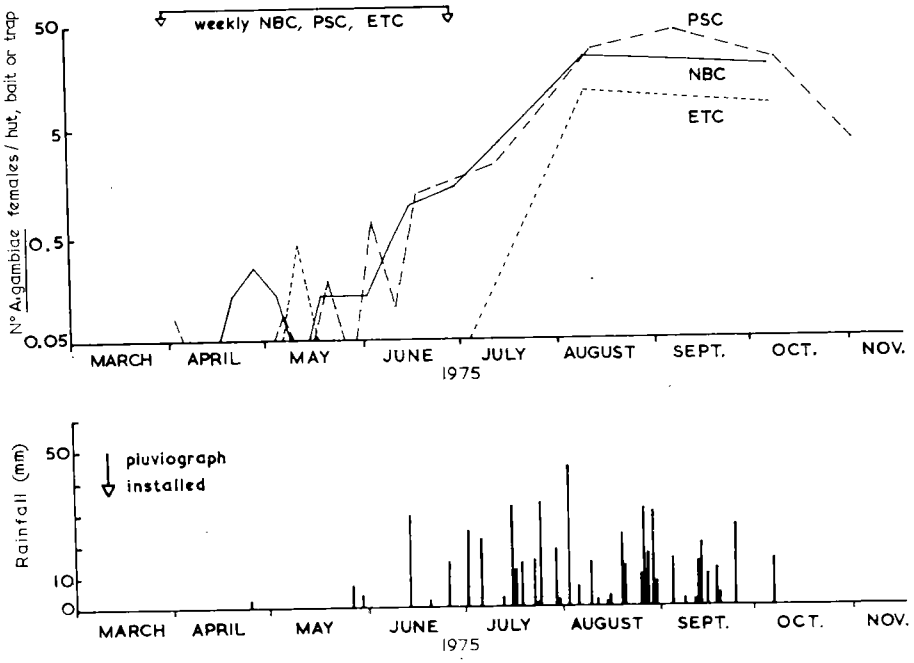
Fig. 8. Indoor-resting densities of *A. gambiae* s.l. and *A. funestus*, estimated from the pyrethrum spray collections in Kwaru, an untreated comparison village, in 1970-1973

Vector density

The average MBR (4 man-nights indoors and 4 outdoors) varied between 0 (or below the measurable threshold) and 174 and 94 bites/man/night, for *A. gambiae* s.l. and *A. funestus* respectively. Both maxima were observed in Sugungum (cluster No. 3, village 408). The IRD varied between 0 and 223 and 171 females/hut, for *A. gambiae* s.l. and *A. funestus* respectively (averages of 6 and 7 huts). Both maxima were again observed in Sugungum.

There was a very large seasonal variation in vector density, illustrated in Kwaru, an untreated comparison village, over a period of 3 years, as shown in the MBR (Fig. 7) and the IRD (Fig. 8). The yearly wave of *A. gambiae* s.l. regularly precedes that of *A. funestus*. The MBR and IRD showed the same trends. The density of *A. gambiae* s.l. decreased

Fig. 9. Rainfall and density of *A. gambiae* s.l. by night-bite collection (NBC), pyrethrum spray collection (PSC) and exit-trap collection (ETC) in Ajura, an untreated comparison village, in 1975[a]



[a] Note that the lowest measurable densities were 1/8 or 0.13, 1/12 or 0.08, and 1/5 or 0.2, by NBC, PSC and ETC respectively.

from 1971 to 1972, and increased from 1972 to 1973, while that of *A. funestus* decreased from 1971 to 1972 and decreased further from 1972 to 1973. The same trend appeared also in the ETC in Kwaru, and also in the NBC, PSC and ETC in the other untreated comparison village, Ajura. The seasonal increases in density are obviously related to rainfall (see Chapter 2). The density of *A. gambiae* s.l. rises very early in the wet season, and in 1975 a special attempt was made to follow the relationship between rainfall and densities recorded through NBC, PSC and ETC. The results obtained in Ajura (Fig. 9) illustrate this relationship, although they are somewhat ambiguous. The first rains were: 2 mm on 25 April, 7 mm on 27 May, 4 mm on 30 May, and 29 mm on 14 June. *A. gambiae* s.l. did not disappear during the dry season (see also p. 56). The first clear-cut increase in density, in particular biting density (NBC), was observed on 9 June, i.e., 13 and 10 days respectively after the rains of 27 and 30 May. At the high temperatures prevailing in that season (see Fig. 3) this is a sufficient interval to explain the increase in population by breeding following the rains (see ref. *70*), especially if some females reach the end of the dry season in the gravid state (see p. 66 and ref. *130*). We have unfortunately no direct proof of successful larval development after the small precipitations of 7 mm and 4 mm on 27 and 30 May. With respect to variation between the years, rainfall decreased from 1971 to 1972 and from 1972 to 1973 (see Chapter 2); this was reflected by the density trend of *A. funestus*, but not by that of *A. gambiae* s.l.

There was a relatively large and significant variation between villages, for all density indices, and for both *A. gambiae* s.l. and *A. funestus*. The villages with the lowest and highest vector densities were Rafin Marke (cluster No. 5, village 154) and Sugungum (cluster No. 3, village 408), respectively. Their prespraying MBR is shown in the left half of the graphs for the effect of propoxur (Fig. 10 and 11). The average MBR for the wet season of 1971 varied between villages from 6.1 to 67.2 for *A. gambiae* s.l., and from 0.1 to 24.1 for *A. funestus*. The latter species had a relatively large MBR only in Sugungum and Kwaru, both located in the south-west of the area. Surface water persists longer in that area; there is in particular a semi-perennial swamp near Sugungum. In the dry season, with the sampling methods adopted (see p. 30), the MBR and IRD dropped to 0 for some time, except in Sugungum where the MBR of *A. gambiae* s.l. and the IRD of *A. gambiae* s.l. and *A. funestus* remained low but regularly measurable. The variation between villages of the biting rate or resting density of either *A. gambiae* or of *A. funestus* was not significantly correlated with the differences in rainfall between the same villages. In addition, the variation between villages in vector density was proportionately much larger than the variation in rainfall.

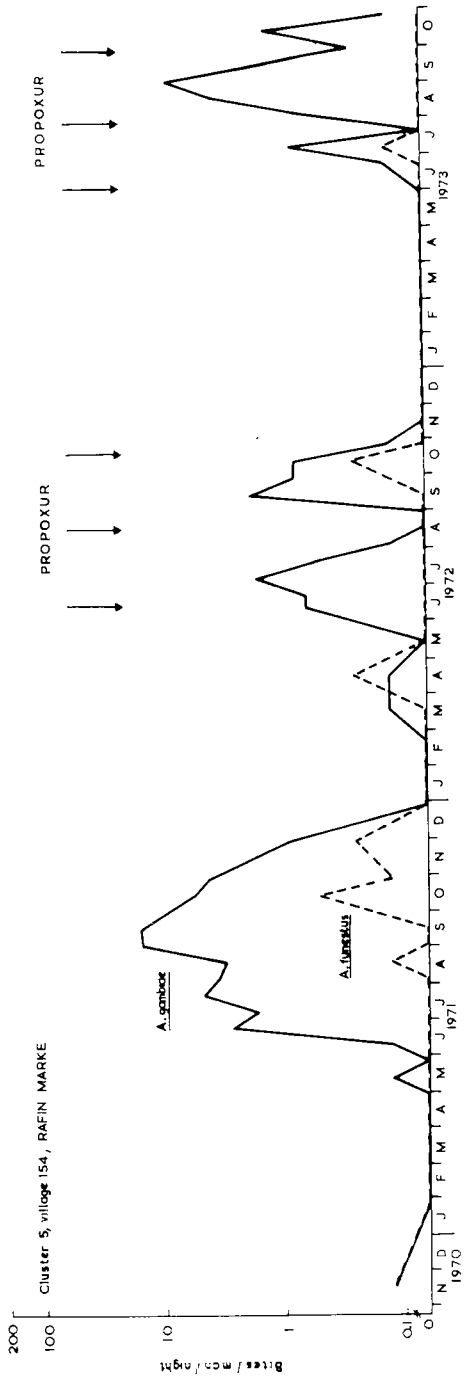


Fig. 10. Effect of propoxur on the man-biting rates of *A. gambiae* s.l. and *A. funestus*, estimated from the night-bite collections in Rafin Marke, the village with the lowest prespraying densities

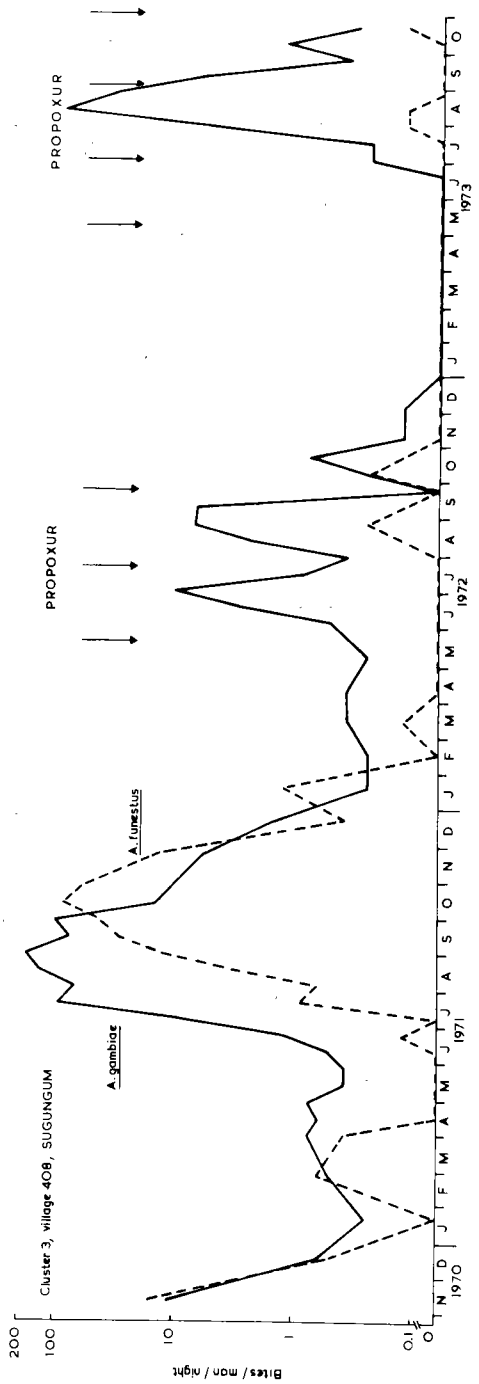


Fig. 11. Effect of propoxur on the man-biting rates of *A. gambiae* s.l. and *A. funestus*, estimated from the night-bite collections in Sugungum, the village with the highest prespraying densities

In some villages there were, not surprisingly, significant differences between different NBC or PSC stations. For the PSC a comparison was also made between the numbers collected in huts always sampled over a period of time and the numbers collected in huts missed at one or more collection cycles; since no significant difference was found, all huts sampled at a given time are included in the results presented.

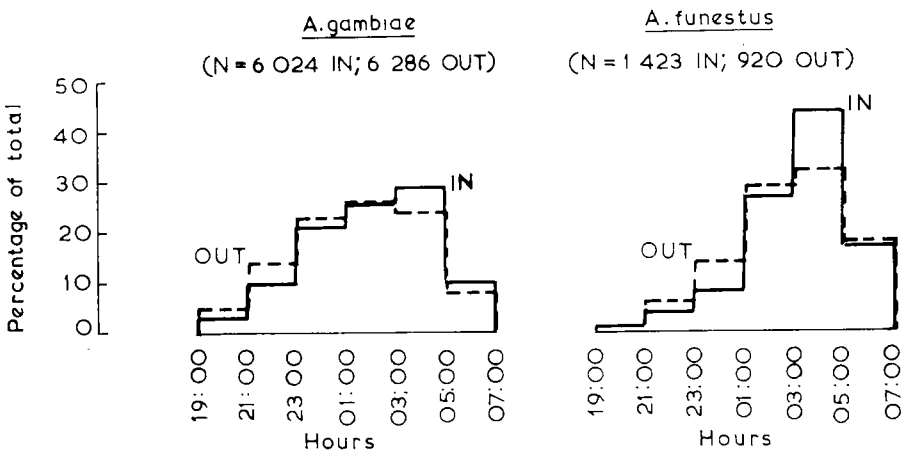
The MBR measured on baits exposed throughout the night was nearly the same indoors and outdoors in the case of *A. gambiae* s.l., but greater indoors than outdoors in the case of *A. funestus*. The ratio between the total numbers collected by NBC indoors and outdoors in the wet season of 1971 was 0.96 for *A. gambiae* s.l., 1.55 for *A. funestus*; the difference is highly significant.

The nocturnal cycle of the MBR of *A. gambiae* s.l. and *A. funestus* (Fig. 12) was determined in each successive hour of the night indoors and outdoors. It was found that both species bite mainly in the second half of the night, *A. funestus* biting later than *A. gambiae* s.l. The outdoor biting cycle, as compared to the indoor, is shifted slightly towards the beginning of the night in both species.

The exit-trap collection (ETC), as already mentioned (see pp. 56, 60), was a reflection of the MBR and IRD.

The numbers collected in the artificial outdoor shelters were very small: 199 females of *A. gambiae* s.l. and 32 of *A. funestus* collected in 3 villages (165 and 30 in Sugungum alone) during the entire baseline

Fig. 12. Biting cycle of *A. gambiae* s.l. and *A. funestus*, indoors and outdoors, in the wet season of 1971, before spraying, on human baits available throughout the night[a]



[a] Results from 300 man-nights indoors and 300 man-nights outdoors.

period. This probably shows that the sampling method is insensitive, rather than that the population resting outdoors is small.

Sporozoite rates and inoculation rates

As expected, the sporozoite rates increased in the course of the wet season. The maxima observed in 8 villages in 1971 in the *A. gambiae* s.l. collected by NBC varied between 1.9% and 11.8%. For *A. funestus* the maxima were 1.4% in Kwaru, 2.9% in Sugungum.

For both species in 1971 the sporozoite rate was greater in the vectors collected on human bait indoors than in those collected on human bait outdoors. This was very consistent in the individual villages and significant for all villages combined: 3.0% (111/3743) versus 1.9% (66/3451) for *A. gambiae* s.l. ($p<0.01$); 1.7% (12/691) versus 0 (0/234) for *A. funestus* ($p<0.05$).

The distribution of sporozoite-positive *A. gambiae* s.l. by hour of the night was very nearly the same as that of the biting density (Fig. 13). Note that the sampling for dissection, within the NBC (see p. 31), was designed to ensure equal sampling fractions by hour. For *A. funestus* the total number of positives was too small to justify an analysis by hour of the night.

The sporozoite rate was determined only for some of the PSCs and then usually on smaller numbers than for the corresponding NBC. Comparison of the NBC and PSC sporozoite rates from the same time and place reveals no systematic difference for either species.

Fig. 13. Distribution by hour of the night of the sporozoite-positive bites and of all the bites by *A. gambiae* s.l., before spraying; all villages combined

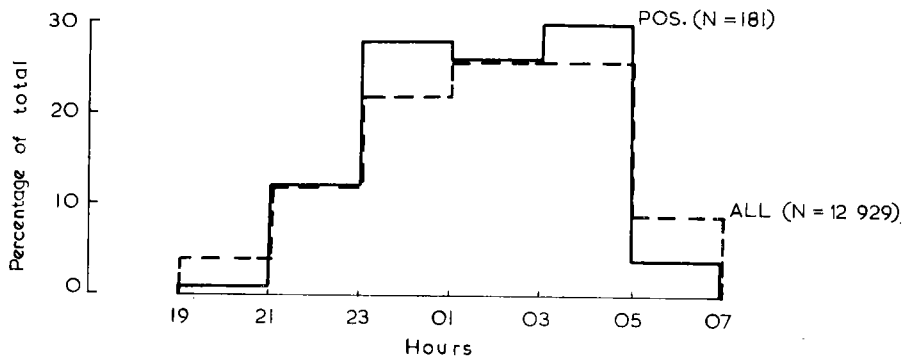


Table 4

Cumulative entomological inoculation rates (total number of sporozoite-positive bites per person) over entire wet seasons, estimated by NBC in 8 villages[a]

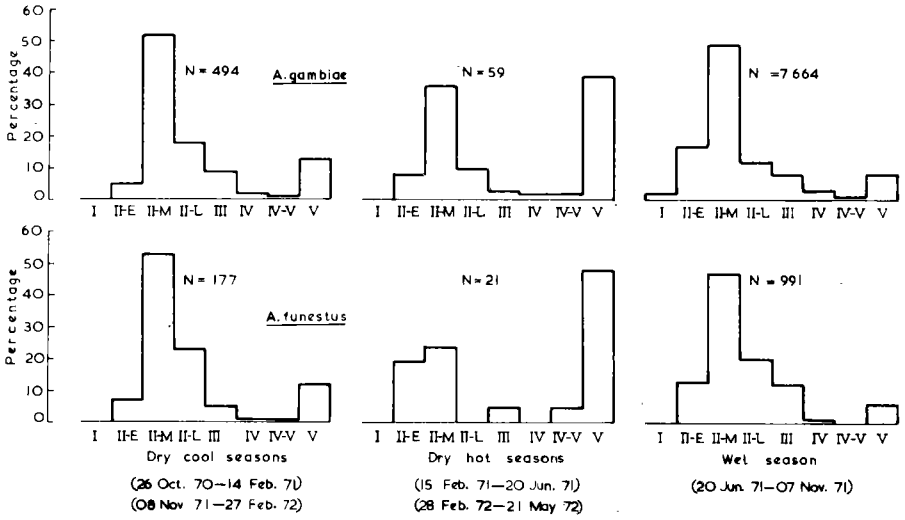
Village	Treatment in 1972-1973	1971 (21 Jun-7 Nov)	1972 (22 May-22 Oct)	1973 (18 Jun-4 Nov)	1974 (29 Jul-15 Dec)	1975 (14 Jul-30 Nov)
1. Kwaru	None	18(4)	17(2)	21(2)	—	—
2. Ajura	None	37(2)	25	28	—	—
3. Sugungum	Propoxur	132(56)	0	10	—	—
4. Ungwar Bako	Propoxur	48	3	4	—	—
6. Matsari	Propoxur and low-frequency MDA	68(2)	4	0	—	—
8. Jaya	Propoxur and low-frequency MDA	64	2	4	—	—
5. Rafin-Marke	Propoxur and high-frequency MDA	18	5	2	4	6
7. Nasakar	Propoxur and high-frequency MDA	129	0	4	16(4)	24

[a] In parentheses: the contribution of *A. funestus* to the total.

The inoculation rate (or entomological inoculation rate, EIR = MBR x SR) was computed as follows: the average MBR (IN and OUT) times the combined sporozoite rate (IN and OUT), for each species and the sum of the two. This is not the only possible method of computing the inoculation rate nor necessarily the best one.

The calculated EIR (*A. gambiae* and *A. funestus*) was found to range between 0 and a maximum of 2.2/man/night at Sugungum on 15 September 1971. The average for the wet season of 1971 varied between villages from 0.13/man/night (Rafin Marke and Kwaru) to 0.94/man/night (Sugungum). The cumulative inoculation rate in 1971 ranged from 18 in Rafin Marke and Kwaru to 132 in Sugungum (Table 4). The contribution of *A. funestus* to this total varied from 0 (4 villages) to 56 (Sugungum). In the dry season (late 1971 to mid 1972), the cumulative inoculation rate was 0, except in Sugungum where it was 13 (4 by *A. gambiae* s.l., 9 by *A. funestus*). In the untreated comparison villages the inoculation rates were relatively stable from one year to the next. The cumulative rates for *A. gambiae* s.l. and *A. funestus* combined, for the wet seasons of 1971, 1972 and 1973, were as follows: 18, 17 and 21 for Kwaru, and 37, 25 and 28 for Ajura. The contributions of *A. funestus* were 4, 2 and 2 in Kwaru, and 2, 0 and 0 in Ajura.

Fig. 14. Distribution, by Christophers' stages, of *A. gambiae* s.l. and *A. funestus* collected by night-bite collection, according to season, in 1970-1972, before spraying; all villages combined



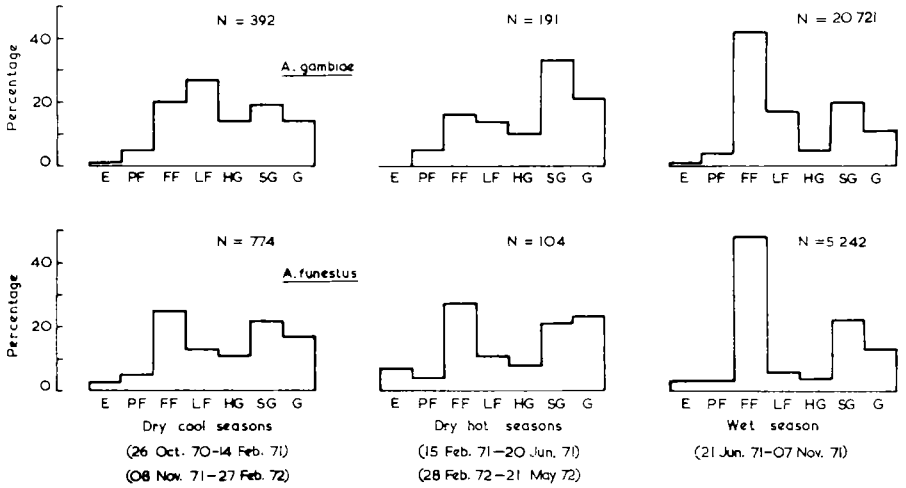
Vector behaviour

Feeding cycle

Figure 14 shows the distribution of *A. gambiae* s.l. and *A. funestus* collected by NBC and classified according to Christophers' stages (*176*). A proportion of the females feed in the gravid state; these females were collected in the act of probing or feeding and many of them had fresh blood in the stomach on dissection. The proportion feeding in the gravid state is low in the wet season, and increases in the dry season, especially at the end when the air is hottest and the densities are lowest. This is compatible with the hypothesis that in the wet season the vast majority of females take 1 blood-meal per gonotrophic cycle, while in the dry season, an increasing proportion takes more than 1 (see *43, 82, 130*).

Figure 15 shows the distribution of *A. gambiae* s.l. and *A. funestus* collected by PSC according to the modified Sella stages of the abdomen (*176*). In the wet season the distribution is clearly bimodal, with the second cluster (subgravid and gravid) somewhat smaller than the first (fully and late fed). The first cluster probably fed during the night immediately preceding the PSC, the second cluster on the previous night. These findings combined with those from the ETC (see below) suggest that most vectors leave the hut to oviposit on the second night after feed-

Fig. 15. Distribution by abdominal appearance (modified Stella stages) of *A. gambiae* s.l. and *A. funestus* collected by pyrethrum spray collection, according to season, in 1970-1972, before spraying; all villages combined[a]



[a] E, PF, FF, HG, SG, G = empty, partly fed, fully fed, late fed, half gravid, sub-gravid, gravid, respectively.

ing, but that some leave (or perhaps die) earlier. The proportions between those fed (PF, FF, LF) and those gravid (HG, SG, G) excluding the empty, for the wet season of 1971, were found to be 0.64 and 0.36 for *A. gambiae* s.l., 0.59 and 0.41 for *A. funestus*, suggesting that a higher proportion of *A. gambiae* than *A. funestus* leave the huts before their eggs are mature. In the dry season the distribution of the PSC into Sella stages is more uniform, and the proportion gravid is larger: in the dry hot season it was 0.65 for *A. gambiae* s.l. and 0.56 for *A. funestus*. This, together with the corresponding distribution of the NBC by Christophers' stages, suggests that during the dry season the act of oviposition and probably the maturation of the eggs are delayed and that more than 1 blood meal is taken per gonotrophic cycle. Such feeding is, however, of little consequence epidemiologically on account of the greatly reduced densities during that season.

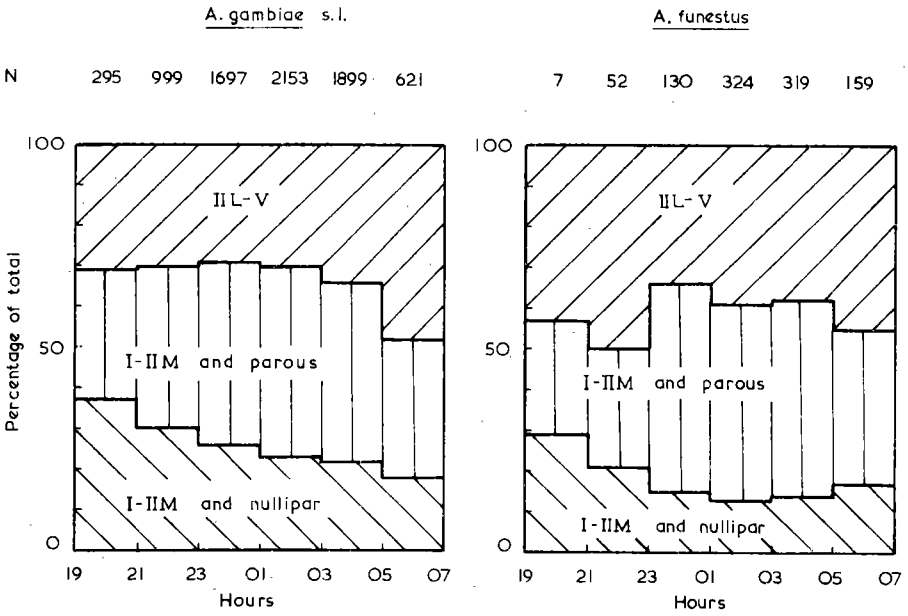
In the exit-trap, during the wet season, the females collected during the early part of the night (before 21h00) are mostly gravid: 74% of the *A. gambiae* s.l. and 67% of the *A. funestus* were classified as gravid or subgravid. The females collected during the remainder of the night are mostly empty or partly fed, amounting to 73% of the *A. gambiae* s.l. and 89% of the *A. funestus*. Most of the first group are probably leaving to

oviposit, while most of the second group are probably leaving in search of blood. In the dry season, the numbers collected by ETC were too small for further analysis.

Only 35 parous *A. gambiae* s.l., collected by NBC at various times and places in the wet season, were examined for ovariolar sacs; in all of them, the sacs were uncontracted, i.e., they had probably oviposited the same night. Together with the distribution of the PSC into Sella stages (see above), this suggests that *A. gambiae* s.l. feed every 2 days, at least in the wet season, when most transmission occurs. The information regarding ovariolar sacs is unfortunately not available for *A. funestus*.

Given that vector mosquitos oviposit and refeed on the same night, it was interesting to find out how the distribution of the NBC into Christophers' stages and into nullipars and parous varied by hour of the night. The results for the wet season of 1971 were analysed (Fig. 16) by classifying the vector females into 3 groups: (1) I–IIM *and* nullipar; (2) I–IIM *and* parous; and (3) IIL–V, the first group being younger than the others

Fig. 16. Distribution of *A. gambiae* s.l. and *A. funestus*, collected by night-bite collection, into 3 groups according to Christophers and Detinova, by hour of the night; wet season of 1971, before spraying, all villages combined[a]



[a] N = numbers dissected. Females were first classified into Christophers' stages; those found in stages I–IIM were further divided into nullipar and parous following Detinova.

(see also p. 72). The proportion of these young mosquitos decreases in the course of the night, in particular for *A. gambiae* s.l., for which it decreases from 37% down to 18%. This is compatible with the hypothesis that gravid vectors oviposit and later feed in the course of the same night. However, considering together the biting cycle (see Fig. 12) and the variation in the composition of the NBC in the course of the night (Fig. 16), it is obvious that the biting cycle varies only to a small extent as a function of age. This is also implicit in the similarity of the biting cycle of sporozoite-positive *A. gambiae* s.l. to the biting cycle of the whole population (see Fig. 13).

The variation by hour of the night of the age composition of *A. gambiae* s.l. and *A. funestus* collected by NBC has been investigated by Hamon (*77*) in the area of Bobo-Dioulasso; he found no significant variation of the proportion parous for *A. gambiae* s.l., while for *A. funestus* the proportion parous went through a maximum in the middle of the night; the latter result is compatible with the finding in Garki of a minimum of young female *A. funestus* in the middle of the night (Fig. 16).

The above distribution of vectors into 3 groups was also used to compare the indoor and outdoor components of the NBC. There was no difference for *A. gambiae* s.l., while for *A. funestus* the proportion of young females was somewhat smaller outdoors. The difference between indoor and outdoor NBC regarding sporozoite rates therefore cannot be explained by a difference in age composition.

Degree of anthropophily

The source of blood meals was investigated by the precipitin test in the PSC and, to a small extent, in the collections made in the artificial outdoor shelters. In the PSC, the percentage of *A. gambiae* s.l. that fed on man was significantly lower in village cluster No. 3 (Sugungum) than in the others: 61% (49/80) versus 91% (673/738). Among the *A. funestus* 97% (229/237) had fed on man, without significant variation between villages. In the artificial outdoor shelters, 23% of the *A. gambiae* s.l. were positive for man (11/48, nearly all from Sugungum). Few of the blood meals taken on man were mixed (4/733). Most of the non-human sources of blood were horses or bovids.

Degree of endophagy

The relative magnitude of the indoor and outdoor biting densities of *A. gambiae* s.l. and *A. funestus*, estimated on human baits made available throughout the night, has been indicated above (see p. 63). This is not sufficient to measure the actual degree of endophagy of vector populations, but it suggests that *A. gambiae* s.l. feeds as readily outdoors as

indoors, while *A. funestus*, while feeding more freely indoors, also feeds quite readily in the open.

The sporozoite rates were greater in those *A. gambiae* s.l. and *A. funestus* biting indoors than in those biting outdoors (see p. 64), and the difference could not be explained by a difference in age composition (see above). It is therefore likely that those feeding on man outdoors have taken a larger proportion of their previous blood meals on animals. This suggests that relative exophagy and zoophily are associated with each other, and that both are stable characteristics of individuals among the *A. gambiae* s.l. and *A. funestus*. Such a behavioural difference is likely to have a genetic basis, and indeed Coluzzi found that within either species of the *A. gambiae* complex, the females biting man indoors differed from those biting man outdoors in the frequency of certain chromosomal inversions, although there was no significant frequency difference between those biting man outdoors and those biting donkeys (see pp. 98-99 and ref. *32*).

Resting behaviour

The small numbers of vectors collected in the artificial outdoor shelters may reflect only the insensitivity of the outdoor-shelter sampling method (see p. 31). Some information about resting behaviour may be deduced from the effect of propoxur spraying (see p. 77), and also from the numerical relationship between the man-biting rate and the indoor resting density (*119*). The proportion of blood meals taken on man by *A. gambiae* s.l. which are followed by rest indoors has been estimated for the wet season of 1971 in the 6 study villages that were followed by NBC and destined to be sprayed the following year, as follows:

Let IRD = the true indoor-resting density
$\hat{\text{IRD}}$ = the indoor-resting density estimated by pyrethrum spray collection
$\hat{\text{IRD}} = b_1 \cdot \text{IRD}$
b_1 = $\hat{\text{IRD}}/\text{IRD}$ = the bias of the estimated indoor-resting density
MBR = the true man-biting rate
$\hat{\text{MBR}}$ = the man-biting rate estimated by night-bite collection
$\hat{\text{MBR}} = b_2 \cdot \text{MBR}$
b_2 = $\hat{\text{MBR}}/\text{MBR}$ = the bias of the estimated man-biting rate
HBI = the proportion of blood meals, in the PSC, positive for man (human blood index)
N = the number of persons per hut (the population of the village, divided by the number of huts)

x = the proportion of blood meals followed by rest indoors (at least until the next morning, time of the pyrethrum spray collection)

T = period of rest indoors after feeding, in days

Then:

$$\mathrm{IRD} \cdot \mathrm{HBI} = \mathrm{MBR} \cdot N \cdot x \cdot T$$

or

$$\frac{\mathrm{I\hat{R}D}}{b_1} \cdot \mathrm{HBI} = \frac{\mathrm{M\hat{B}R}}{b_2} \cdot N \cdot x \cdot T \qquad [1]$$

$\mathrm{I\hat{R}D}$, $\mathrm{M\hat{B}R}$, HBI, N are measured directly. T is estimated by

$$T = 1 + \frac{G}{F},$$

where G, F are the proportions gravid and fed, respectively, in the PSC (if the maturation time is 2 days, as suggested by the clear-cut bimodal distribution of the PSC by abdominal appearance);

x and $\frac{b_2}{b_1}$ (the relative bias of the 2 sampling methods) are unknown. If we know the one, we can compute the other; from [1]:

$$x = \frac{\mathrm{I\hat{R}D}}{\mathrm{M\hat{B}R}} \cdot \frac{b_2}{b_1} \cdot \frac{\mathrm{HBI}}{N \cdot T} \qquad [2]$$

$$\frac{b_2}{b_1} = \frac{\mathrm{M\hat{B}R}}{\mathrm{I\hat{R}D}} \cdot x \cdot \frac{N \cdot T}{\mathrm{HBI}} \qquad [3]$$

Values for the factors in equations [2] or [3] may be derived from the data for *A. gambiae* s.l. and *A. funestus* in the wet season of 1971 (Table 5). For *A. funestus* which is generally believed to be very endophilic, setting its x to 1 we can compute its $\frac{b_2}{b_1}$ ratio by formula [3]; it is equal to 1.16 (i.e., in comparison with the indoor-resting density, the man-biting rate is overestimated by 16%). Taking the same value for *A. gambiae* (i.e., assuming that the relative bias of the 2 sampling methods does not vary between the two species) we can compute its x by formula [2]; it is equal to 0.47. Thus, tentatively, only about half of the blood meals taken on man by *A. gambiae* s.l. are followed by rest indoors (see Discussion).

Age composition and longevity

Age composition can be used either directly (e.g., to compare it ac-

Table 5

Estimation of the proportion of blood meals followed by rest indoors in the wet season of 1971 (21 June—7 November)

Factor[a]	*A. gambiae* s.l.	*A. funestus*
$\hat{MBR}$ (estimated man-biting rate)	10 837/440 = 24.6	2053/440 = 4.67
$\hat{IRD}$ (estimated indoor-resting density)	10 709/410 = 26.1	4103/410 = 10.0
HBI (human blood index) proportion of blood-meals positive for man, in vectors resting in huts	544/624 = 0.872	125/126 = 0.992
N (No. of persons/hut)[b]	2209/1507 = 1.47	1.47
T (duration of rest indoors, days)[c]	1 + 7449/13 042 = 1.57	1 + 2068/3020 = 1.68
x (proportion of blood-meals followed by rest indoors)[d]	0.47	1
b_2/b_1 (relative bias of the 2 sampling methods)[e]	1.16	1.16

[a] Estimated in the 6 villages followed by night-bite collection, and destined to be sprayed the following year, except when otherwise specified.
[b] Estimated in 5 of the 6 villages.
[c] Estimated in 22 villages, including the 6, by the formula $T = 1 + G/F$, where G and F = No. gravid and fed in the pyrethrum spray collections.
[d] Set to 1 for *A. funestus*, computed by formula [2] for *A. gambiae*.
[e] Computed by formula [3] for *A. funestus* and set to the same value for *A. gambiae*.

cording to places, times, interventions) or to compute longevity. Both will be considered for *A. gambiae* s.l. only, the numbers available for *A. funestus* being much lower. Age composition was estimated over a whole wet season to minimize the effect of variable rates of emergence. Two indicators of age composition of the night-bite collection were computed: (1) the proportion parous among those eligible for the method of Detinova (*42*), i.e., among those found in Christophers' stages I–IIM; and (2) the proportion that are either I–IIM and parous, or IIL–V, among all dissected; the vectors included in this second index are not necessarily parous, but they are older than the remainder, i.e., than the I–IIM and nullipars.

Table 6 shows the proportion I–IIM, i.e., eligible for the method of Detinova and the 2 indices of age-composition, for the wet seasons of 1971 to 1975, in villages never sprayed (clusters No. 1–8 in 1971, No. 1–2 in 1972-1973) currently sprayed (clusters No. 3–8 in 1972-1973), and pre-

Table 6

Age-composition of *A. gambiae* s.l. collected by night-bite collection in the wet seasons of 1971 to 1975, in the presence or absence of propoxur

Year	Village clusters	Propoxur	Christophers' stages Proportion I-IIM	(No. classified)	Detinova method Proportion parous	(No. classified)[a]	No. I-IIM and parous, plus No. IIL-V, as a proportion of the total[b]
1971 (21 Jun.-7 Nov.)	1-8	no	0.68	(7 664)	0.64	(5 175)	0.76
1972 (22 May-22 Oct.)	1-2	no	0.40	(594)	0.49	(231)	0.80
	3-8	yes	0.56	(442)	0.39	(247)	0.66
1973 (18 Jun.-4 Nov.)	1-2	no	0.46	(1 780)	0.47	(801)	0.76
	3-8	yes	0.55	(1 707)	0.39	(925)	0.67
1974 (29 Jul.-15 Dec.)	5,7	no	0.63	(1 896)	0.62	(1 130)	0.76
1975 (14 Jul.-30 Nov.)	5,7	no	0.66	(1 233)	0.68	(809)	0.78

[a] Those found in stages I-IIM were examined by the Detinova method; very few of them were not classified.

[b] Assuming that the few found in stages I-IIM and not classified by the Detinova method were not different from the others; the effect of the assumption is negligible.

viously sprayed (clusters No. 5 and 7 in 1974-1975). The effect and after-effect of propoxur are dealt with later (see pp. 86-88). In the villages that were never sprayed, the proportion eligible for the Detinova method and the proportion parous among them (i.e., the first index of age composition) show a rather large change from 1971 to 1972-1973 which is not explicable by pre-existing differences between clusters No. 1–2 and the others. The second index of age composition was more stable, varying only between 0.76 and 0.80 (in the absence of propoxur), and is therefore preferred for further analysis.

The second index may be considered as an estimate of the proportion of the NBC taking their second or a later meal. Given an interval of 2 days between blood meals, p (the probability of surviving 1 day) can be estimated by $\sqrt{P}$, where P = the second index of age composition. In the wet season of 1971, $p = \sqrt{0.76} = 0.87$, and the expectation of life, $1/(-\ln p) = 7.2$ days. An overestimation of the proportion taking their second or a later meal would lead to an overestimation of longevity, and vice versa. The result is also affected by the interval between first and second meals. A fraction of the *A. gambiae* probably take their second

meal, before their first oviposition, one day after their first meal (*69*). If this applies to half the population, the above estimates become:

$p = \sqrt[1.5]{0.76} = 0.83$ and $1/(-\ln p) = 5.4$ days.

Determination of physiological age by the method of Polovodova (*42*) i.e., classification of females by the number of ovipositions, has been applied on a small scale in the study area. The results are presented and discussed in a Technical Note (*43*). The youngest sporozoite-positive *A. gambiae* s.l. examined had oviposited twice, but it was not possible to relate physiological to chronological age.

Vectorial capacity

The entomological factors of transmission[a] are combined into the vectorial capacity $ma^2p^n/(-\ln p)$, which is the entomological component of MacDonald's basic reproduction rate (*97*) from which it was extracted by Garrett-Jones (*66*). The vectorial capacity is a daily rate of potentially infective contact and is the principal input variable for the malaria transmission model (see Chapter 10).

For the baseline period, the vectorial capacity was calculated, for most of the year, on the basis of the following estimates:

ma	=	MBR, averaging between the indoor and outdoor result, and smoothing between data points (see pp. 59-63).
a	=	HBI/FC, where HBI = the human blood index, by PSC (see p. 69), and FC = the interval between blood meals (see p. 68).
p	=	0.819, corresponding to an expectation of life of 5 days. This value was selected on the basis of the project's findings (see pp. 71-73) and the literature review (*20*).
n	=	10 days, calculated from the temperature by Moškovskij's formula (*42*).

The vectorial capacity was thus obtained by multiplying the MBR by the following factors:

	Village clusters	
	No. 3	*Others*
A. gambiae s.l.	0.206	0.308
A. funestus	0.328	0.328

[a] m = the number of vectors/man; a = the number of meals taken on man/vector/day; p = the probability of surviving 1 day; n = the incubation period in the vector, in days.

Note that, in this computation, *A. gambiae* s.l. and *A. funestus* differ only by their human blood index. The vectorial capacity at a given time and place is the sum of the corresponding vectorial capacities of the two species.

During the hot dry season, March to June, several factors of the vectorial capacity may be affected, in addition to the obvious decrease in vector density: (1) the incubation period in the vector is shorter at high temperature; (2) the longevity of *A. gambiae* s.l. may be increased (*82, 130*) or shortened to a lesser extent than the incubation period, by analogy with the effect of a geographical temperature gradient on *A. maculipennis (42)*; (3) feeding continues, in more or less complete gonotrophic dissociation (see p. 66 and refs. *82, 130*). It was assumed that, in combination, these factors produced an increase in vectorial capacity. For convenience, this increase was approximated by increasing the expectation of life, without change in the other factors: the expectation of life was set to 7 days ($p = 0.867$) in the period of March to June. In that period, the vectorial capacity was thus obtained by multiplying the MBR by the following factors:

	Village clusters	
	No. 3	*Others*
A. gambiae s.l.	0.513	0.765
A. funestus	0.814	0.814

The baseline vectorial capacities, calculated as described above for Sugungum and Rafin Marke, the villages with the highest and lowest vector densities, respectively, are shown in Fig. 77 in Chapter 10. The figure shows also the critical vectorial capacity, below which malaria cannot remain endemic (see Chapter 10). In Sugungum, the vectorial capacity stayed well above the critical level throughout the year and reached a peak of about 40, i.e., nearly 2000 times the critical value, in the wet season. In Rafin Marke, the vectorial capacity stayed below the critical level for about 5 months of the year and reached a peak of somewhat more than 200 times the critical value in the wet season.

Effect of Intervention, in particular Residual Spraying with Propoxur

It is unlikely that the very limited larviciding operations conducted in the dry season in and around village clusters No. 5 and 7 (area A1) had any significant effect. The comparison between these village clusters and those where only residual spraying with propoxur was applied provided

no evidence that the larviciding operation modified either the "tail" of the current breeding season of *A. gambiae* s.l. or the onset of the next one. Note that larviciding was not used in those village clusters which had high densities of *A. funestus*. The entomological effects of the intervention phase should probably be explained as entirely due to the residual spraying of propoxur. The small-scale preliminary trial of propoxur is considered in the first section, while the subsequent 5 sections are concerned with the intervention period of the main (large-scale) trial.

Results of the preliminary trial of propoxur

This trial and its results have been reported in detail elsewhere (*104*). The primary objective of the trial was to practise and evaluate the operations (see Chapter 3); the secondary objective was to obtain a preliminary evaluation of the entomological effect of propoxur, although limited by the small scale of the trial. The trial was conducted in 1971 and involved spraying with propoxur (2 g/m^2 of sprayable surface) the villages of Kaya, Gana and Masheme located about 5 km north of Garki (see Fig. 1). The 3 villages were sprayed before the onset of the rains. It was decided *a priori* to evaluate a single round of spraying in Masheme and 2 rounds in Kaya; Gana was included in the first round because of its proximity to the 2 other villages. It was arbitrarily decided to respray Kaya 3 months after the first application or earlier if either the IRD reached 1 vector/hut or if the MBR reached 1 bite/man/night, either indoors or outdoors. Some operational conclusions of the trial were mentioned in Chapter 3.

The entomological evaluation, in the absence of baseline data from Kaya and Masheme, was made by comparison with the contemporary baseline data from the villages selected for entomological follow-up in the main study but excluding Sugungum. Kaya and Masheme were followed by NBC, PSC and ETC in exactly the same way as the other villages (see Chapter 2). The effect of propoxur on *A. funestus* could not be evaluated because, even in the majority of the untreated comparison villages, the density of *A. funestus* barely exceeded the limit of sensitivity of the methods used (see page 56).

A preliminary evaluation of the effect of propoxur on *A. gambiae* s.l. was, however, obtained. The first round of spraying took place when vector density was negligible. In the unsprayed villages, density began to increase about 5 weeks later. In the sprayed villages, this increase was partly controlled, but, in both villages the IRD, MBR(IN) and MBR(OUT) all stayed below 1 for only about 2 months after spraying. Kaya was accordingly resprayed 72 days after the first round. In Masheme, which was not resprayed, both IRD and MBR increased

rapidly to levels within or above the range of the comparison villages, and the total seasonal production of *A. gambiae* s.l. was probably little affected by the single early round of spraying. In Kaya the IRD stayed below 1 for the remainder of the wet season, while in the unsprayed villages it reached peaks of from 7 to 224 according to the village. In Kaya, the rise in MBR was again partially controlled, but the MBR rose above 1 some 3 and 5 weeks after spraying, outdoors and indoors respectively. Peak MBRs of 4 indoors and 17 outdoors were observed 38 days after respraying. Among the comparison villages, the seasonal peaks varied from 26 to 102 indoors, and from 18 to 62 outdoors.

Propoxur thus reduced the IRD more than the MBR, and the MBR (IN) more than the MBR(OUT). No significant difference was detected between sprayed and unsprayed villages with respect to the proportion parous in the NBC, or the proportion gravid in the PSC or ETC. Sporozoite rates were also similar: the seasonal averages combining NBC and PSC were 2.9% in the comparison villages (356/12 162), 1.5% in Mesheme (35/2305), 3.2% (10/317) in Kaya. Sporozoite-positive *A. gambiae* s.l. were found 69 and 75 days after the first round, 12 and 40 days after the second (1 positive *A. funestus* was found 41 days after the first round).

The wall bioassays, conducted in Masheme, showed persistence of a potent insecticidal effect of the sprayed mud walls for periods ranging between 2 and 4 months. The conclusions tentatively drawn from the preliminary trial were that propoxur was effective for 2 months, and that the persistence of relatively high man-biting rates especially outdoors, and the lack of detectable effect on the proportion parous and the sporozoite rate, were due to immigration of unexposed vectors.

On the basis of the preliminary trial, 3 rounds of spraying at 2-month intervals per wet season were planned for the main study.

Effect of propoxur on vector density

Effect of propoxur on the man-biting rate

The effect of propoxur on the MBR of *A. gambiae* s.l. and *A. funestus* may be seen in the graphs already presented (see Fig. 10 and 11) for the 2 villages having respectively the lowest and highest baseline densities. In the wet season of 1972 the MBR of *A. gambiae* s.l. is lower than in the wet season of 1971. In the dry season of 1973 it remains undetectable by the methods used (compare in Fig. 11 the dry seasons of 1971, 1972 and 1973). In the wet season of 1973, the MBR of *A. gambiae* is still lower than in 1971, but higher than in 1972. The averages for the wet seasons of 1971, 1972 and 1973 were 6.1, 0.7 and 1.6 in Rafin Marke; and 67.2, 3.1

and 13.7 in Sugungum.

The MBR of *A. funestus*, on the other hand, decreases from 1971 to 1972 and from 1972 to 1973. The averages for the wet seasons of 1971, 1972 and 1973 were 0.08, 0.04 and 0.01 in Rafin Marke, and 24.1, 0.08 and 0.04 in Sugungum. These changes have to be seen in relation with the contemporary spontaneous changes already mentioned (see p. 60), and illustrated for the untreated village of Kwaru (see Fig. 7). Here the average MBR figures for the wet seasons of 1971, 1972 and 1973 were 7.9, 2.9 and 13.4 for *A. gambiae* and 3.3, 0.6 and 0.2 for *A. funestus*. In the other untreated village, Ajura, the average MBR figures for the wet seasons of 1971, 1972 and 1973 were 10.5, 4.9 and 11.0 for *A. gambiae* s.l. and 0.3, 0.2 and 0.01 for *A. funestus*. Relatively large spontaneous changes were thus occurring in the unsprayed comparison villages, and they were in the same direction as those observed in the sprayed villages.

To measure the impact of propoxur on vector density in a given village, it is therefore necessary to take into account both the prespraying density in the same village and the concurrent changes in untreated villages. An attempt to measure the impact and to relate it to prespraying variables has been reported on elsewhere (*115*).

Table 7 shows the actual numbers of *A. gambiae* s.l. collected by NBC, indoors, outdoors and total, in the wet seasons of 1971, 1972 and 1973 in each of the 8 villages followed by NBC along with the subtotals for the treated and untreated villages. The numbers relating to villages are proportional to the MBR and are directly comparable to each other, because the sampling scheme was the same in the 8 villages and the 3 years. Only a few collections were missed, and only in periods of low to very low density, i.e., they have little effect on the total; this is one reason for using numbers rather than rates, the other being that the actual numbers are more easily amenable to statistical analysis. The numbers actually collected in 1972 and 1973 in a sprayed village were expressed as a fraction of the expected, i.e., the number that would have been collected if propoxur had not been applied. This expected number was calculated on the basis of the baseline (1971) number in the same village and of the relative change observed in the untreated villages between the baseline year (1971) and the intervention year considered (1972 or 1973).

For instance, 61 *A. gambiae* s.l. were collected by indoor NBC in 1972 in Sugungum; in 1971 before spraying the number had been 2439. The corresponding number for the 2 untreated villages combined decreased from 854 in 1971 to 282 in 1972. The "number expected" in Sugungum in 1972 is defined as 2439 x (282/854) = 805, and the "observed" represents 61/805 = 0.076 or 7.6% of the expected. This ratio observed/expected is an adjusted residual density (MBR); a low value indicates a strong impact (a large reduction), and vice versa. An adjusted residual

Table 7

Number[a] of *A. gambiae* s.l. collected by night-bite collection (NBC) indoors (In) and outdoors (Out) in successive wet seasons, by village; and ratio of that number to expected,[b] in the sprayed villages

Village	Treatment	1971 (21 Jun.-7 Nov.)			1972 (22 May-22 Oct.)			1973 (18 Jun.-4 Nov.)		
		In	Out	Total	In	Out	Total	In	Out	Total
1. Kwaru	None	359	275	634	95	138	233	386	689	1 075
2. Ajura	None	495	344	839	187	204	391	438	445	883
Subtotal	None	854	619	1 473	282	342	624	824	1 134	1 958
3. Sugungum	Propoxur in 1972 and 1973	2 439	2 939	5 378	61 (0.076)[c]	186 (0.11)	247 (0.11)	289 (0.12)	804 (0.15)	1 093 (0.15)
4. Ungwar Bako	Propoxur in 1972 and 1973	335	442	777	4 (0.036)	12 (0.049)	16 (0.049)	15 (0.046)	35 (0.043)	50 (0.048)
6. Matsari	Propoxur in 1972 and 1973	943	522	1 465	11 (0.035)	42 (0.15)	53 (0.085)	44 (0.048)	153 (0.16)	197 (0.10)
8. Jaya	Propoxur in 1972 and 1973	670	678	1 348	19 (0.086)	37 (0.099)	56 (0.098)	79 (0.12)	239 (0.19)	318 (0.18)
5. Rafin Marke	Propoxur in 1972 and 1973	288	153	441	16 (0.17)	38 (0.45)	54 (0.29)	42 (0.15)	85 (0.30)	127 (0.22)
7. Nasakar	Propoxur in 1972 and 1973	495	933	1 428	5 (0.031)	27 (0.052)	32 (0.053)	10 (0.021)	64 (0.037)	74 (0.039)
Subtotal	Propoxur in 1972 and 1973	5 170	5 667	10 837	116 (0.068)	342 (0.11)	458 (0.10)	479 (0.096)	1 380 (0.13)	1 859 (0.13)

[a] The actual numbers collected are shown; the number of collections was nearly, but not exactly, constant for each village and wet season; in 1971, villages 1–3 had 10 NBCs, villages 4–8 had 9; in 1972, village 4 had 9, the others 10; in 1973, village 4 had 8, the others 10.

[b] The number expected was computed on the assumption that villages 3–8 would, in the absence of propoxur, have undergone the same natural change as villages 1–2; e.g. the expected No. In, in village 3, in 1972 = 2439 x (282/854) = 805. The numbers expected are not shown.

[c] Ratio of the observed to the expected (i.e., 61/805 = 0.076).

density of 7.6% means that a 92.4% reduction is attributed to the intervention. If one ignores the changes occurring in the unsprayed villages, one gets, in the example above, an unadjusted residual MBR of 61/2439 = 0.025, or 2.5%, i.e., a 97.5% reduction. The adjustment may decrease the calculated impact, as in 1972, or increase it, as in 1973. Such a measurement allows the impact of propoxur to be compared between villages, between years, between indoor and outdoor NBCs, or between vector species.

There was found to be a relatively large and significant variation between villages: in 1972 for the total NBC the ratio observed/expected varied between 4.9% in Ungwar Bako and 29% in Rafin Marke; in 1973 the ratio varied between 3.9% in Nasakar and 22% in Rafin Marke. There was a significant correlation between the 1972 and 1973 results in the same village, suggesting that the impact is related to some relatively stable characteristic of the village.

When the concurrent changes in the unsprayed villages are taken into account, as is done here, there is probably no significant difference in the effect of propoxur between 1972 and 1973: the second year shows a smaller effect in 3 villages, a larger one in the 3 others. The measured effect is significantly and systematically larger indoors than outdoors, but there is a significant correlation between the effect indoors and outdoors in the same village. With respect to species, the effect of propoxur on the MBR of *A. funestus*, even after adjustment for spontaneous changes, is greater than the effect on *A. gambiae* s.l. The MBR observed in Sugungum, adjusted for concurrent changes in Kwaru, was only 2% of the expected in 1972, 3% of the expected in 1973.

The variation between villages in the impact of propoxur on the MBR of *A. gambiae* s.l. was significantly correlated with some of the village's prespraying characteristics, namely, the NBC/PSC ratio and the median biting hour. The variation between villages in their response to propoxur was not clearly associated with variation in the proportions of *A. gambiae* and *A. arabiensis*, but it was clearly associated with variation in the frequency of certain chromosomal inversions in both of these species of the *A. gambiae* complex (see p. 98 and ref. *32*). The same variation between villages was, on the other hand, not significantly correlated with several other prespraying characteristics (vector density, the ratios between NBC indoors and NBC outdoors, between ETC and PSC, between fed and gravid in the PSC or ETC), and was also not significantly correlated with variations in recorded spraying coverage, latitude, or distance from unsprayed villages.

The relationship between the prespraying (1971) NBC/PSC ratio of a village and the residual MBR of the same village in 1972 and 1973 was determined after adjustments of the PSC for the human blood index (HBI)

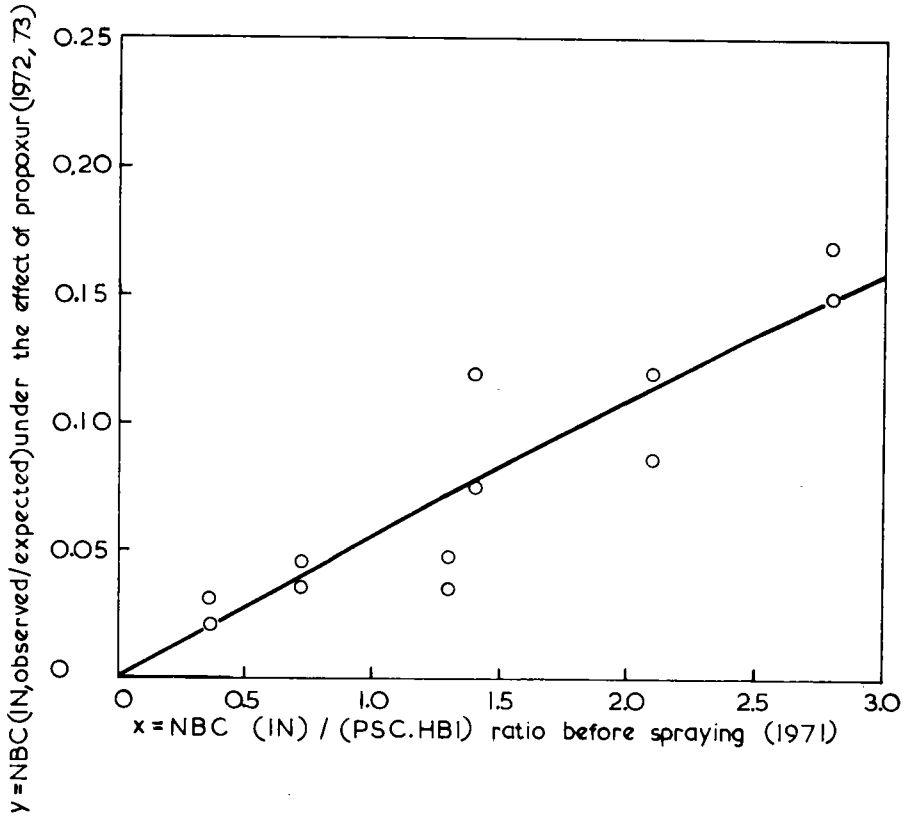
Table 8

Prespraying ratio of the man-biting rate of *A. gambiae* s.l. to its man-fed indoor-resting density, in the wet season of 1971, in various villages, and the residual NBC (observed/expected), under propoxur, in the wet seasons of 1972 and 1973

Village	NBC(In)/man-nights / (PSC/huts) (HBI)[a] in 1971		NBC(In), obs./exp.[b] in 1972	NBC(In), obs./exp.[b] in 1973	NBC(In + Out)/man-nights / (PSC/huts) (HBI)[a] in 1971		NBC(In + Out) obs./exp.[b] in 1972	NBC(In + Out) obs./exp.[b] in 1973
5. Rafin Marke	288/36 / (115/39) (50/51)	= 2.8	0.17	0.15	441/72 / (115/39) (50/51)	= 2.1	0.29	0.22
8. Jaya	670/36 / (810/81) (104/117)	= 2.1	0.086	0.12	1348/72 / (810/81) (104/117)	= 2.1	0.098	0.18
3. Sugungum	2439/40 / (5066/69) (49/80)	= 1.4	0.076	0.12	5378/80 / (5066/69) (49/80)	= 1.5	0.11	0.15
6. Matsari	943/36 / (1422/66) (123/132)	= 1.3	0.035	0.048	1465/72 / (1422/66) (123/132)	= 1.0	0.085	0.10
4. Ungwar Bako	335/36 / (1125/79) (119/132)	= 0.72	0.036	0.046	777/72 / (1125/79) (119/132)	= 0.84	0.049	0.048
7. Nasakar	495/36 / (2019/47) (99/112)	= 0.36	0.031	0.021	1428/72 / (2019/47) (99/112)	= 0.52	0.053	0.039
			[c] r = +0.91 * R = +0.94 *				[c] r = +0.72 R = +0.89 *	
			r = +0.90 * R = +0.94 *				r = +0.97 ** R = +1.00 **	

[a] HBI = human blood index;
[b] from Table 7;
[c] r = correlation coefficient; R = Spearman's rank correlation coefficient;
*, ** : significantly different from zero at 5% and 1% levels, respectively.

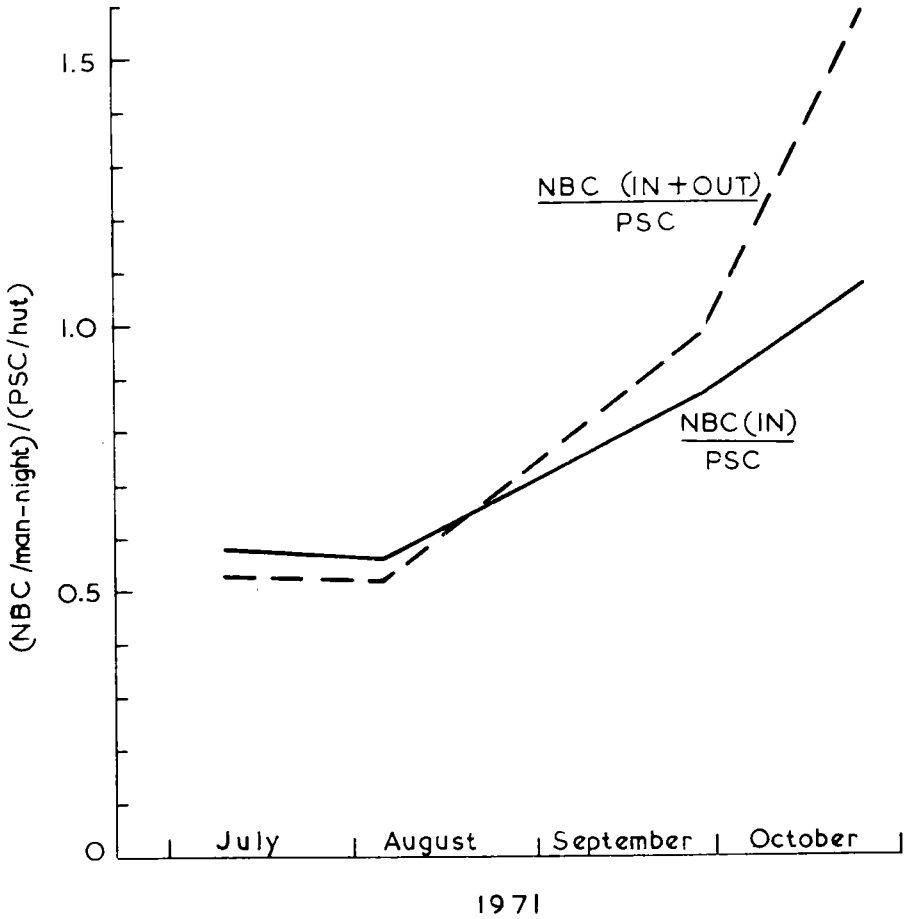
Fig. 17. Man-biting rate of *A. gambiae* s.l., under the effect of propoxur, as a function of the prespraying ratio between man-biting rate and indoor-resting density[a]



[a]Six villages each contributed 1 measurement before spraying (abscissa), 2 measurements after spraying (ordinate); see also Table 8. the curve represents the function $y = 1-e^{-0.058x}$; the value 0.058 was obtained by fitting.

and taking into account either the indoor NBC only (Table 8, left half, and Fig. 17) or the total outdoor and indoor NBC (Table 8, right half). Both the table and the figure show that: (1) the prespraying ratio varies rather widely between villages: NBC(IN)/PSC ranged between 2.8 in Rafin Marke and 0.36 in Nasakar; (2) as already noted (see Table 7), there is a strong positive correlation between the 1972 and 1973 results in the same village; (3) there is a strong and significant positive correlation between the prespraying NBC/PSC ratio and the residual MBR, i.e., the higher the prespraying NBC/PSC ratio, the higher the residual MBR and the poorer the control achieved. These conclusions thus apply equally for the NBC(IN) and for the NBC(IN + OUT). The figure also reveals that

Fig. 18. NBC/PSC ratio of *A. gambiae* s.l., by 4-week periods, during the wet season of 1971 before spraying; all villages combined



an exponential function may be fitted to the 12 data points (6 villages x 2 years), allowing the expected post-spraying MBR to be calculated from the prespraying NBC/PSC ratio.

The 4 species caught biting man in relatively large numbers—namely, the 2 species of *A. gambiae* s.l., *A. funestus* and *A. pharoensis*—were compared with respect to their prespraying NBC/PSC ratio and median biting hour, and their response to propoxur. Here also the adjusted residual MBR showed a positive correlation with the prespraying NBC/PSC ratio, but the correlation with the prespraying median biting hour was negative.

From the above it is likely that the prespraying NBC/PSC ratio is a good indicator of the degree of exophily. As shown by the combined figures for 1971 (Fig. 18), this ratio increases as the wet season progresses. This suggests that exophily increases in the course of the wet season, probably in relation to the increase in vegetation (*63, 80*).

Effect of propoxur on the indoor-resting density, the exit-trap collections, and the outdoor collections

In the sprayed villages, the numbers of vectors collected by PSC were very small: in about 1½ years after the first spraying, i.e., during 2 wet seasons and the intervening dry season, 2197 hut collections yielded 57 *A. gambiae* s.l. females and 8 *A. funestus* females. The huts used as capture stations were sprayed with propoxur like any other hut. Of the 57 *A. gambiae* s.l., 21 were caught in the wet season of 1972, 33 in the wet season of 1973, 3 in the intervening dry season. The captures were concentrated towards the end of the intervals between successive spraying rounds. They were also concentrated in certain villages, and, within villages, in certain huts. The comparatively small variation between villages with respect to PSC after spraying showed no correlation with the large variation in response to propoxur, as assessed from the NBC (see p. 80), and is probably to be explained by random variation between huts with respect to the adequacy of spraying.

The numbers collected by ETC in the sprayed villages were also very small: 1150 trap-nights (in huts which were sprayed like any other hut) yielded 8 *A. gambiae* s.l. females and a single *A. funestus* female.

Village clusters No. 3 and 4 (area B) were also assessed by outdoor collections from artificial shelters. In the wet seasons of 1971 (baseline), 1972 and 1973 (intervention), 160, 176 and 160 collections respectively yielded 172, 15 and 62 *A. gambiae* s.l. females, and 24, 13 and 2 *A. funestus* females. Nearly all were collected in cluster No. 3. In the unsprayed comparison cluster No. 2, there were 80, 88 and 80 collections in 1971, 1972 and 1973 respectively; they yielded only 12, 2 and 18 *A. gambiae* s.l. females and no *A. funestus*.

Sporozoite rates and inoculation rates

During the whole intervention period, only 20 sporozoite-positive vectors, all *A. gambiae* s.l., were collected in the sprayed follow-up villages, 2 in pit-shelters in Sugungum and 18 by NBC. Each of the 6 sprayed villages assessed by NBC contributed to the 18 positives found, and the numbers contributed by different villages were very similar, varying only between 2 and 5. The sporozoite rates of *A. gambiae* s.l. collected by NBC in the wet seasons of 1971, 1972 and 1973 in the treated and un-

Table 9

Sporozoite rate of *A. gambiae* s.l. collected by NBC, before, during and after the application of control measures

Village clusters	Treatment (in 1972-1973)	Sporozoite rate (%) of *A. gambiae* s.l. in the wet season, by NBC				
		1971 (21 Jun.-7 Nov.)	1972 (22 May-22 Oct.)	1973 (18 Jun.-4 Nov.)	1974 (29 Jul.-15 Dec.)	1975 (14 Jul.-30 Nov.)
1, 2	None	1.7 (24/1403)	3.2 (19/594)	1.4 (25/1780)	0.5[a] (1/188)[a]	3.0[a] (10/335)[a]
3, 4	Propoxur	1.4 (35/2580)	0.4 (1/252)	0.7 (7/1005)	—	—
6, 8	Propoxur and low-freq.MDA	2.6 (52/1968)	2.8 (3/107)	0.4 (2/506)	—	—
5, 7	Propoxur and high-freq.MDA	3.8 (65/1713)	2.4 (2/83)	1.5 (3/196)	0.4 (9/1896)	1.3 (16/1233)

[a] In 1974 and 1975, only 2 NBC per wet season in only one village cluster.

treated village clusters (Table 9) do not demonstrate any large effect of propoxur, nor any marked increase in the effect by the addition of mass drug administration; however, it should be noted that these results are based on very small numbers of sporozoite positives. The estimated inoculation rate, on the other hand, was decreased greatly by the treatments, because of the great reduction in the man-biting rate. The cumulative entomological inoculation rates for the wet seasons of 1971, 1972 and 1973 (see Table 4) show a reduction from 132 to 0 and 10 in Sugungum and from 18 to 5 and 2 in Rafin Marke; there was little year-to-year change in the untreated comparison villages.

The capture of 18 positive *A. gambiae* s.l. by NBC in the sprayed villages suggests a large population of positive vectors because the sampling fraction is very small: 48 man-nights of NBC per fortnight out of 50 000 x 14 = 700 000 man-nights per fortnight in the whole of the sprayed area, or a fraction of 7 out of 100 000. The 18 positives captured may therefore correspond to a total of 18 x 100 000/7, i.e., about 250 000 sporozoite-positives.

Vector behaviour

No new information was collected during the intervention phase regarding the frequency of blood meals or the degree of anthropophily. It has already been mentioned that the indoor MBR decreased more than

the outdoor MBR (see p. 80 and Table 7), and this probably reflects selection of the more exophagic fraction of the population (see p. 70).

With respect to resting, there was certainly a marked decrease in the proportion of the population that was found resting indoors. This must be explained to a large extent by a marked increase in indoor mortality. Whether there was also a selection for exophilic behaviour will be discussed below (p. 92).

Age composition and longevity

The effect of propoxur on the age composition as determined by the Detinova method (see Table 6) could be seen by comparison with the 1971 data and with the unsprayed villages. In 1972 and 1973, *A. gambiae* s.l. was, on the average, younger in the sprayed than in the unsprayed villages. The difference was significant but not very large. Only 22 of the *A. gambiae* s.l. captured in the sprayed villages were classified by the Polovodova method; 8/22, or 36%, had oviposited twice or more; in the unsprayed villages, the corresponding proportion was 75/173, or 43%, i.e., very similar ($\chi^2 = 0.16$; $p>0.60$) (*43*).

The interpretation of changes in age composition in terms of longevity is considered in the next section.

Vectorial capacity

Among the components used to estimate the vectorial capacity (see pp. 74-75) the application of propoxur affects *ma*, *p*, and possibly, *a*. The effect on *ma* was measured directly (see pp. 77-80); the effect on *p* has to be deduced from the change in age composition (see the preceding section). A given reduction in average age can, however, be interpreted in more than one way and the method adopted has a large effect on the computed vectorial capacity (*119*). A commonly used method of interpretation assumes implicitly that there is a uniform reduction in longevity, and a new *p* is calculated on that basis. If *P* is the proportion taking their second or later meal and the interval between blood meals is 2 days, then $p = \sqrt{P}$. We get, from Table 6 in 1972, in the unsprayed villages: $p = \sqrt{0.80} = 0.89$; in the sprayed villages: $p = \sqrt{0.66} = 0.81$. This by itself would reduce the vectorial capacity, for $n = 10$, by a factor of $\{(0.89)^{10}/(-\ln 0.89)\} \div \{(0.81)^{10}/(-\ln 0.81)\}$, or about 5. This factor has been called the longevity factor of insecticidal impact by Garrett-Jones & Grab (*67*). If, however, longevity is not reduced uniformly, i.e., if some individual vectors have a higher than average probability of avoiding exposure after every meal, the impact on vectorial capacity is much smaller. If the vector population is composed of 2 fractions, one

completely endophilic (and exposed) and the other completely exophilic (and not exposed), then among the vectors collected by NBC after application of the insecticide the fraction belonging to the unexposed population is approximately P_2/P_1, where P_2, P_1 = the proportion of "old" vectors (e.g., taking their second or later meal) in the presence or absence of the insecticide, respectively.[a] In the same example as above, 0.66/0.80 = 0.83, or 83%, of the vectors collected after spraying would be unexposed; the exposed would make only a negligible contribution to vectorial capacity; and the longevity factor would be: 1/0.83 = 1.2, i.e., practically negligible in comparison with the effect on density.

Nonuniform exposure, due to nonuniform resting behaviour, is *a priori* more likely than the reverse. It fits also better with some other aspects of the data (*119*). In particular, under the hypothesis of nonuniform exposure, in the extreme form outlined, the mosquitos resting outdoors after feeding would always be the same, and the proportion resting indoors after feeding, estimated before spraying, should be equal to the proportion exposed among those taking their first meal, estimated after spraying. The first proportion was estimated as 0.47 (see p. 71); the second proportion is estimated by $(1-P_2/P_1)/(1-P_2)$, i.e., from Table 6, 0.51 in 1972 and 0.36 in 1973, which is not too different from the first proportion.

A direct proof of nonuniform resting behaviour was provided by the finding, in the Garki district, of an association between resting behaviour and the frequency of certain chromosomal inversions within each of the 2 species of the *A. gambiae* complex found in the various localities (see p. 98 and ref. *32*).

The vectorial capacity during the intervention period was calculated twice, i.e., once according to the usual but implicit assumption of uniform exposure and once according to the probably more realistic assumption of nonuniform exposure; the latter was simplified even further by assuming that all vectors collected by NBC in the sprayed villages had a normal expectation of life, i.e., by neglecting the small "longevity effect". This means that the vectorial capacity was obtained by multiplying *ma*, estimated by NBC, by the same factors as before spraying (see pp. 74, 275). Table 32 in Chapter 10 shows the seasonal average vectorial capacities, calculated in this way, for the period 1971 to 1973, i.e., the baseline and intervention phases, in the 4 villages used to test the transmission model previously fitted to baseline data; 2 of the villages

[a] After spraying, very few of the exposed become "old" (e.g., take a second blood meal). Practically all "old" vectors collected after spraying (fraction P_2 of the total) belong to the unexposed fraction: that fraction has the same proportion of "old" individuals as before, i.e., for each "old" individual there are $(1-P_1)/P_1$ young ones. Therefore, in the sample collected after spraying, the proportion belonging to the unexposed population is $(P_2 + \{(1-P_1)/P_1\}P_2)$ which simplifies to P_2/P_1.

were untreated, while 2 (Sugungum and Ungwar Bako) were treated with propoxur alone. During the 3 seasons of the intervention period—i.e., the wet season of 1972, the 1972-1973 dry season, and the wet season of 1973—the vectorial capacity was 0.66, 0.044, and 2.83 in Sugungum, i.e., 30, 2, and 129 times the critical vectorial capacity (see Chapter 10). In Ungwar Bako, the corresponding vectorial capacities were 0.068, 0, and 0.24, or 3, 0 and 11 times the critical value.

When these vectorial capacities, calculated under the assumption of nonuniform exposure, were used as model input, they produced a reasonably realistic parasitological output (see Chapter 10); when the alternative vectorial capacities, calculated under the assumption of uniform exposure, were used instead, the expected impact of propoxur on malaria was systematically and markedly larger than the one actually observed, i.e., the parasitological output was clearly less realistic than under the assumption of nonuniform exposure (*119*).

After-effect of Propoxur, after Discontinuation of Spraying

In 1974-1975, after the end of the intervention phase, observations continued in village clusters No. 2, 5 and 7, i.e., in the village clusters included in the seroimmunological study. In clusters No. 5 and 7 (area A1), previously treated, the entomological observations were limited to the wet season, at the same frequency as previously. In cluster No. 2, untreated throughout, observations were made in the wet season at reduced frequency, but the transition from dry to wet season was observed in some detail in 1975 (see pp. 59-60). The results of these observations are described in the present section.

Vector density

The effect and after-effects of propoxur in 2 villages sprayed in 1972 and 1973 (Rafin Marke and Nasakar), as compared to the untreated village of Ajura (Table 10), were as follows: (1) the man-biting rate (MBR), reduced in 1972 and 1973, has already returned to "normal" in 1974; (2) the ratio between indoor and outdoor MBR, reduced in 1972-1973, is still reduced in 1974 and 1975, relatively more so in Rafin Marke than in Nasakar; (3) the indoor resting density (IRD), reduced to very low levels in 1972-1973, increases in 1974-1975, but returns to "normal" only in Rafin Marke, and only in 1975; (4) the ratio between the biting rate and indoor-resting densities, considerably increased in 1972-1973, decreases

Table 10

Man-biting rates (MBR) and indoor-resting densities (IRD) of *A. gambiae* s.l. in 3 villages in the wet seasons of 1971 through 1975, i.e., before, during and after spraying with propoxur[a]

Village and treatment	MBR and IRD	1971 (21 Jun.-7 Nov.)		1972 (19 Jun.-5 Nov.)		1973 (18 Jun.-4 Nov.)		1974 (29 Jul.-15 Dec.)		1975 (14 Jul.-30 Nov.)	
Ajura (2-553), compact part, untreated throughout[b]	MBR In	12.4	(40)	4.48	(40)	11.0	(40)	13.6	(8)	21.6	(8)
	Out	8.60	(40)	5.00	(40)	11.1	(40)	10.1	(8)	20.4	(8)
	In/Out	1.44		0.90		0.98		1.35		1.06	
	IRD	18.1	(63)	4.53	(68)	7.94	(69)	6.57	(14)	12.7	(13)
	MBR (In)/IRD	0.69		0.99		1.39		2.07		1.70	
Rafin Marke (5-154), sprayed with propoxur in 1972 and 1973	MBR In	8.00	(36)	0.35	(40)	1.05	(40)	4.61	(36)	5.38	(40)
	Out	4.25	(36)	0.90	(40)	2.13	(40)	8.03	(36)	10.1	(40)
	In/Out	1.88		0.39		0.49		0.57		0.53	
	IRD	2.95	(39)	0.02	(48)	0.02	(47)	0.44	(41)	3.76	(46)
	MBR (In)/IRD	2.71		17.5		52.5		10.5		1.43	
Nasakar (7-218), sprayed with propoxur in 1972 and 1973	MBR In	13.8	(36)	0.08	(40)	0.25	(40)	9.73	(40)	3.20	(40)
	Out	25.9	(36)	0.65	(40)	1.60	(40)	27.3	(40)	12.5	(40)
	In/Out	0.53		0.12		0.16		0.36		0.26	
	IRD	39.1	(52)	0.04	(46)	0.08	(48)	2.34	(47)	1.51	(45)
	MBR (In)/IRD	0.35		2.00		3.13		4.16		2.12	

[a] In parentheses: the number of collections (1 collection = 1 man-night or 1 PSC in 1 hut).

[b] In Ajura in 1974-1975 there were only 2 night-biting collections in each wet season; only the corresponding pyrethrum spray collections are used in the table; the inclusion of the 4 PSCs available results in only minor changes.

in 1974-1975, but returns to "normal" only in Rafin Marke and only in 1975.

The relationship of the night-bite catch (NBC), along with the PSC and ETC, to the wet-season rainfall in the 2 sprayed villages (Fig. 19) shows that in 1974 and 1975, when the rains came earlier, there was no corresponding shift in the NBC to earlier dates. This delay of the NBC in relation to the rainfall in 1974-1975 is probably related to spraying with propoxur in 1972-1973 because, in 1971-1973 in the untreated comparison villages, the NBC curve did follow shifts in the rainfall curve. Fig. 19 also shows that in the first half of the wet season of 1974 and 1975 in the villages sprayed in 1972 and 1973, the PSC and ETC were low in relation to the NBC, in comparison with the second half of the same wet seasons or with the wet season of 1971.

Sporozoite rates and inoculation rates

In the villages treated with propoxur (plus high-frequency MDA) the sporozoite rates of the *A. gambiae* s.l. collected by NBC were still low in the 2 years after the intervention period (see Table 9). In 1974 the rate was 0.4%, i.e., even lower than in the intervention period; in 1975, the rate was 1.3%, i.e., about the same as in 1973, the second year of intervention. It should be noted that in the wet season of 1974 4 rounds of chloroquine were administered to those aged less than 10 years (see Chapter 3). The untreated village is of limited value for comparison, because too few samples of the NBC were taken there. There was no significant difference of sporozoite rate between the indoor and outdoor NBCs. The sporozoite rate of the *A. gambiae* s.l. collected by PSC in village clusters No. 5 and 7 was 3.2% (4/126) in 1974-1975, i.e., significantly higher than in the NBC in the same period. There was no significant difference before spraying or in the untreated comparison village. Two positive *A. funestus* were collected by NBC in 1974 in village cluster No. 7.

The cumulative entomological inoculation rate over the entire wet season (see Table 4) scarcely increased in Rafin Marke in the 2 years after the intervention period; but in Nasakar, where it had been relatively much more reduced during the intervention, it increased clearly in 1974 and 1975, although not approaching the very high pretreatment level.

Vector behaviour

The precipitin test was performed on fed *A. gambiae* collected by PSC in 1975. The human blood index was high: 0.93 (357/382). There was no difference between the baseline and post-intervention phases, nor be-

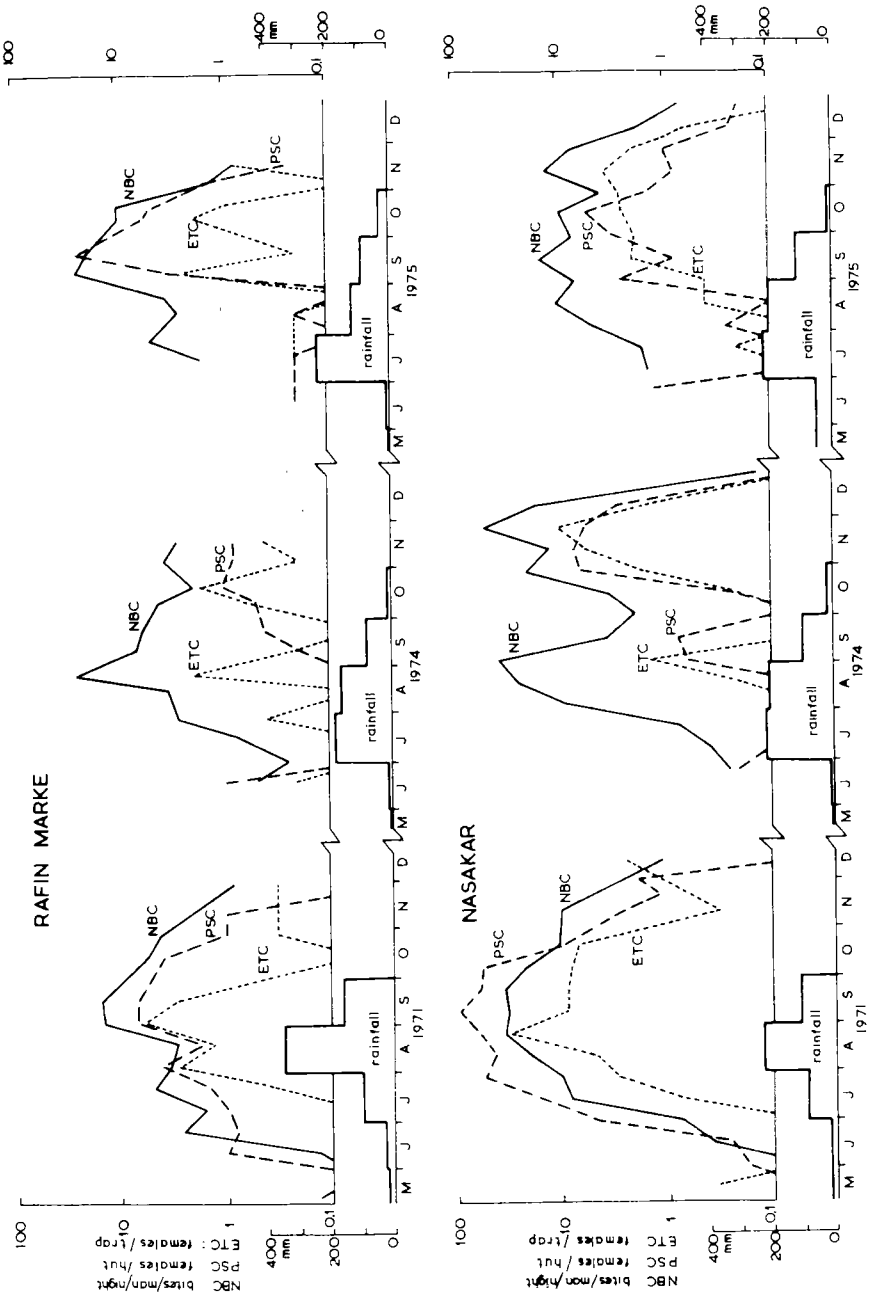


Fig. 19. Rainfall and density of *A. gambiae* s.l. in 2 villages in 1971 before spraying, and in 1974 and 1975 after discontinuation of spraying

tween previously treated and control villages, nor between compact and scattered villages.

The increase in the degree of exophagy, manifested by the decrease of the ratio between indoor and outdoor MBR, which had been observed during the intervention period, persisted in the post-intervention periods of 1974 and 1975 (see Table 10). This effect tended to fade away in the late wet season, when the bioassay indicated little or no insecticidal effect of the sprayed walls (see below). In October-December of 1974 and 1975, in the previously sprayed villages the ratio MBR(IN)/MBR(OUT) was 0.49 and 0.37, versus 0.53 in 1971.

In 1974, in the sprayed villages, there was still a reduction in the proportion of the vector population found resting indoors, as shown by the persistence of a high ratio MBR/IRD (see Table 10). In 1975, this effect had subsided in Rafin Marke, but not yet in Nasakar. This effect of propoxur has to be assessed in comparison with the spontaneous variation of the ratio MBR/IRD in Ajura, shown in the same table. The effect was mainly present in the first half of the wet season (see Fig. 19), when a persistent insecticidal effect of propoxur was demonstrable by bioassay (see below).

Age composition and longevity

In 1974 and 1975, since the age composition of the *A. gambiae* s.l. population was apparently back to normal in the sprayed villages (see Table 6), the longevity of these vectors had probably returned to normal also.

Bioassay and chemical tests

Air and wall bioassay tests were performed in previously sprayed huts and in control huts. The sprayed huts had not been renovated since the last spraying; the control huts had never been sprayed. The bioassay used *A. gambiae* s.l. females caught in villages that had never been sprayed, or their F_1 offspring (Table 11). In the wet seasons of 1974 and 1975, there was still a marked insecticidal effect, both by contact and airborne, which subsided in the latter part of the wet season, when the relative humidity of the air decreases; there was no relationship between mortality and temperature. In the wet season of 1976, the insecticidal effect was somewhat smaller than in 1974 and 1975, but still easily demonstrable.

Chemical analyses of wall-mud specimens for propoxur were performed by the WHO Anopheles Control Research Unit at Kaduna. Only in 1 sample (10 x 10 x 2.5 cm deep), collected on 4 August 1975, was a trace of propoxur (0.028 – 0.093 g/m^2) detected.

Table 11

Bioassay tests (air and wall) conducted with *A. gambiae* s.l.[a] in huts sprayed with propoxur in 1972 and 1973

Date	Days after last spraying (6th round)	Test	Nasakar				Control[b]			
			N	% mortality imm.[c]	% mortality 24 h	RH (%)	*N*	% mortality imm.[c]	% mortality 24 h	RH (%)
5 Sep. 1974	335	air	122	100	—	60-89	83	8.4	10.8	—
2 Oct. 1974	362	air	95	100	—	82-83	31	3.0	16.0	—
2 Oct. 1974	362	wall	120	100	—	82	31	10.0	16.0	—
31 Oct. 1974	391	wall	109	20.0	52.0	32-36	56	0.0	3.5	45
11 Dec. 1974	432	wall	89	10.0	21.0	18	47	0.0	0.0	18
31 Jul. 1975	664	air	96	75.0	100	63-79	70	2.9	12.9	67-80
7 Aug. 1975	671	wall	120	100	—	59	60	0.0	11.7	52
24 Sep. 1975	719	air	90	88.9	98.9	66-76	60	0	1.7	70
24 Sep. 1975	719	wall	120	100	—	76	60	0	8.3	70
30 Oct. 1975	755	wall	118	1.7	11.9	18	64	4.7	10.9	20
24 Aug. 1976	1 053	air	119	49.6	52.9	56-71	66	1.5	7.6	52-64
24 Aug. 1976	1 053	wall	115	84.3	93.0	66	56	3.6	8.9	58-65
30 Sep. 1976	1 090	air	77	39.0	53.2	58-76	48	4.2	4.2	52-64
30 Sep. 1976	1 090	wall	116	75.9	87.9	53	60	0.0	13.3	68

[a] Females caught in the freshly fed state in villages that had never been sprayed, or their F_1 offspring (some of the controls on 24 Aug. 1976, plus all those used on 30 Sep. 1976), also freshly fed, except for the wall bioassay of 30 Sep. 1976, when 70% were unfed.

[b] On 5 Sep. 1974: Garki; on 2 Oct. 1974: Ajura; later: Achauya.

[c] Immediate.

Compact versus Scattered Settlements

In order to interpret any possible parasitological differences between the compact and scattered settlements (see Chapter 5), it was interesting to compare them in terms of entomological findings. NBC, ETC and ORC were performed only in compact settlements, but in 3 of the village clusters the PSC was split between a compact and a scattered settlement (see Chapter 2, Table 1 and Fig. 1). Within each of the 3 clusters, the indoor-resting density (IRD) of *A. gambiae* s.l. was found to be greater in the scattered part: 39 versus 18 females per hut in cluster No. 2, 21 versus 10 in cluster No. 8, and 60 versus 39 in cluster No. 7, all in the wet season of 1971, i.e., before intervention. The difference was very consistent: the scattered settlement had the higher IRD in 23 out of 30 comparisons ($p<0.01$). The difference between the clusters was, however, larger than the difference between compact and scattered settlements in the same cluster. There was no difference between the 2 types of settlement with respect to *A. funestus*, but no comparison was made in the 2 clusters with high densities of *A. funestus*, i.e., clusters No. 1 and 3.

The comparison between the 2 types of settlement with respect to the human blood index (HBI) in the PSC was made only in 1975. There was no significant difference: the HBI(PSC) was 1.00 (40/40) and 0.87 (48/55) in the compact and scattered parts of cluster No. 2 (never treated), 0.95 (160/168) and 0.95 (186/196) in the compact and scattered parts of cluster No. 7 (area A1).

There were, on the average, slightly more sleepers per PSC catching station in the scattered settlements (2.3 per hut) than in the compact settlements (1.8) of the same 3 village clusters. This is unlikely to explain any significant part of the difference in IRD (see p. 54 and Table 3).

We do not know whether the proportion of blood meals taken on man that are followed by rest indoors is the same in the scattered settlements as in the compact ones (see pp. 70-71), but it is unlikely to be larger, because "indoors" represents a much smaller fraction of the environment in the scattered settlements. Therefore, the findings regarding the indoor-resting density and human blood index can probably be interpreted as reflecting a higher man-biting rate in the scattered settlements. The fed/gravid ratio was the same in the scattered as in the compact settlements or slightly lower.

In 1972-1973, cluster No. 2 was left unsprayed; the scattered part continued to have a higher indoor-resting density of *A. gambiae* s.l. than the compact part: 7 versus 4.4 in the wet season of 1972, 27 versus 8 in the wet season of 1973. In clusters No. 7 and 8, which were sprayed, very few mosquitos were caught by PSC, in the scattered as well as in the compact settlements (see p. 84).

Anopheles gambiae and *A. arabiensis*

For a variety of technical and operational reasons, it has not been possible to include the systematic identification of the 2 sibling species of the *A. gambiae* complex in the main entomological sampling scheme. The available information comes mainly from concurrent work by a WHO consultant (*31*) and from preliminary surveys in 1969-1970 (*145*). Further observations by Shidrawi in 1971-1973 were not available at the time of writing. Whereas the preceding sections have treated *A. gambiae* s.l. as a single species, the present section will describe the information available regarding the abundance and characteristics of the two species. The possible consequences of treating *A. gambiae* s.l. as a single species with respect to the understanding of the epidemiology of malaria are considered in the Discussion section.

In the study area, *A. arabiensis* (species B) of the *gambiae* complex was nearly always and everywhere the dominant species. *A. gambiae* s.s. (species A) was, however, found in nearly every single village from which at least 20 mosquitos were identified, and is probably present everywhere. There was some variation between villages with respect to the relative abundance of *A. gambiae* s.s., although no geographical pattern was detected. The abundance of both species increased markedly in the wet season; this increase was more pronounced for *A. gambiae* s.s., which reached its maximum abundance towards the middle of the main breeding season. The proportion of *A. gambiae* s.s. in the total of *A. gambiae* s.l. varied according to method of collection and from year to year (Table 12). In the PSC it ranged between 6% in 1969 and 46% in 1972. The comparison between captures on donkeys (97/1053, or 9.2%, being *A. gambiae* s.s.) and on men (548/1769, or 31.0%, being *A. gambiae* s.s. for all NBC and net-traps, in the absence of propoxur) strongly suggests that this species is more anthropophilic than *A. arabiensis*. The comparison between PSC, NBC, net-traps and ETC, in the absence of propoxur, does not suggest any clear-cut difference between *A. gambiae* and *A. arabiensis*.

Propoxur was applied in 1972 and 1973, and only a few *A. gambiae* s.l. were captured by PSC in the sprayed villages; in the small samples examined, the proportion of *A. gambiae* did not differ significantly from what it was in the unsprayed villages. In 1971, i.e., before spraying, there was also no significant difference in the PSC: 373/1585 or 23.5% of *A. gambiae* s.s. in the villages to be sprayed versus 69/253 or 27.3% in the villages to be left unsprayed. In 1974, i.e., in the first year after spraying, the proportion of species A among *A. gambiae* s.l. was smaller in the previously sprayed villages than in the controls, both in the PSC and in the net-traps. The effect of propoxur on the relative abundance of the

Table 12

Proportion of *A. gambiae* s.s. as percentage of *A. gambiae* s.l. (*A. gambiae* s.s. and *A. arabiensis*), by year, method of collection and treatment[a]

Sampling method	Propoxur[b]	1969 (Sep.-Dec.)		1970 (Jul.-Oct.)		1971 (Aug.)		1972 (Jul.-Sep.)		1973 (Aug.-Sep.)		1974 (Jul.-Sep.)	
PSC	-	5.9	(41/690)	11.8	(244/2 060)	24.0	(442/1 838)	46.1	(967/2 096)	27.4	(120/438)	24.4	(290/1 187)
	+	—	—	—	—	—	—	47.6	(10/21)	20.0	(2/10)	8.9	(5/56)
Net-trap outdoors, human bait	-	—	—	—	—	25.3	(108/427)	39.7	(93/234)	21.3	(112/527)	40.0	(182/455)
	+	—	—	—	—	—	—	—	—	—	—	29.9	(106/355)
NBC, outdoors	-	—	—	—	—	10.0	(3/30)	—	—	—	—	52.1	(50/96)
	+	—	—	—	—	—	—	—	—	—	—	—	—
Capture on donkeys outdoors	-	—	—	—	—	0.0	(0/30)	13.8	(16/116)	3.5	(17/482)	15.1	(64/425)
	+	—	—	—	—	—	—	—	—	—	—	—	—
ETC	-	—	—	—	—	—	—	—	—	—	—	42.2	(106/251)
	+	—	—	—	—	—	—	—	—	—	—	—	—

Sampling method	Propoxur[b]	1969 (Sep.-Dec.)		1970 (Jul.-Oct.)		1971 (Aug.)		1972 (Jul.-Sep.)		1973 (Aug.-Sep.)		1974 (Jul.-Sep.)	
ORC	–	—	—	—	—	0.0	(0/36)	—	—	—	—	5.1	(11/217)
	+	—	—	—	—	—	—	—	—	—	—	—	—
Adult females reared from larvae or pupae	–	—	—	—	—	—	—	—	—	—	—	—	—
	+	—	—	—	—	—	—	34.4	(32/93)	—	—	—	—

[a] Mainly from Coluzzi et al. (*31*) for 1971-1974; from Shidrawi (*145*) for 1969-1970.
[b] Applied in 1972-1973, but still effective in 1974 .

sibling species was thus not very striking; it was, in addition, rather unsystematic when individual villages were considered (*31*). It should, however, be noted that the sampling scheme was not specifically designed to measure this effect and that the samples identified were small.

If the variation between villages with respect to the effectiveness of propoxur could not be explained by variation in the *gambiae/arabiensis* ratio, it was, however, related to the intraspecific frequency of certain chromosomal inversions, which are themselves related to differential resting behaviour (*32*).

Table 13

Percentage of blood-meals found positive for man by the precipitin test in *A. gambiae* s.s. and *A. arabiensis* collected by PSC in Jirima village and in the adjacent nomadic Fulani camp, both unsprayed[a]

Species	Village		Nomadic Fulani camp	
A. gambiae s.s.	100.0	(129/129)	78.6	(66/84)
A. arabiensis	82.9	(165/199)	30.4	(102/335)
Total	89.6	(294/328)	40.1	(168/419)

[a] From Coluzzi et al. (*31*).

Table 14

Sporozoite rates (%) of *A. gambiae* s.s. and *A. arabiensis* collected by PSC in unsprayed villages[a]

Village	*A. gambiae* s.s.		*A. arabiensis*		*p*
Jirima village	4.0	(8/199)	0.0	(0/329)	<0.001
Jirima nomadic Fulani camp	4.3	(3/69)	0.0	(0/182)	0.02
Ajura, Kwaru, Baribari, Gwadawa	9.4	(50/530)	4.0	(11/274)	<0.01
Total	7.6	(61/798)	1.4	(11/785)	<0.001

[a] From Coluzzi et al. (*31*).

In the unsprayed villages, Coluzzi also compared *A. gambiae* s.s. and *A. arabiensis* with respect to the source of blood meals and the sporozoite rates (Table 13). In the compact village of Jirima (unsprayed), where few large animals were present, both species had fed mainly on man, but the proportion fed on man was significantly larger in *A. gambiae* s.s. In the adjacent temporary Fulani camp, where cattle outnumber men by about 10 to 1, 79% of *A. gambiae* had fed on men, versus only 30% of *A. arabiensis*. These results are in obvious agreement with those given above regarding captures on men and donkeys. In examinations of the salivary glands (Table 14), *A. gambiae* was found to have a consistently and significantly higher sporozoite rate than *A. arabiensis*.

Very few hybrid adult females were found: 2 out of 2750, or 0.07%, in 1969-1970 (*145*); 4 out of 9699, or 0.04%, in 1971-1974 (*31*). Hybridization must be very rare, or hybrids must have reduced viability, or both.

In summary, both *A. gambiae* and *A. arabiensis* are present throughout the Garki district; *A. arabiensis* is the dominant species; the relative abundance of *A. gambiae* increases during the main breeding (wet) season; *A. gambiae* is the more anthropophilic and has a higher sporozoite rate. No obvious difference was detected between the species in exophagy and exophily, and no clear-cut difference was demonstrated regarding the reduction of their populations by propoxur.

Discussion

The estimation of the vectorial capacity

Given the presence of a particular malaria parasite in a particular human population, the distribution of the parasite in that population (endemic level, epidemics, etc.), and its consequences (morbidity, mortality, etc.) are determined by the "rate of contact" between persons (see also Chapter 10). This "rate of contact" is best expressed by the vectorial capacity, as defined by Garrett-Jones (*66*), i.e., $ma^2p^n/(-\ln p)$; the vectorial capacity is a daily rate of potentially infective contact between persons through the vectors (here "potentially infective" refers to the survival of the vector through the incubation period or "extrinsic cycle"). The main contributions of entomology to the study of the epidemiology and control of malaria can therefore be classified under the following headings: (1) measuring the vectorial capacity and its natural variation; (2) "explaining" the vectorial capacity in terms of the natural environment and its unspecific modification by man; (3) measuring the reduction in vectorial capacity resulting from defined specific vector control measures (or eventually, measures aiming at a reduction of man-vector

contact, without reduction in the number of vectors). The entomological component of the Garki project was designed in terms of the first and third of these categories. In addition to the above, entomological observations contribute a direct estimation of the inoculation rate.

However important the concept of vectorial capacity, the definition involves certain simplifying assumptions which may be questioned: (1) survival of the vector is not affected by age; (2) survival of the vector is not affected by infection; (3) vector and vertebrate populations mix at random; (4) the vector population is homogeneous with respect to susceptibility to infection, host choice, incubation period, and survival. Furthermore, even if the definition of the vectorial capacity is accepted, its estimation, in this project as well as in others, is open to many questions. The estimation of each of the component factors (*ma, a, p,* and *n,* see p. 74) is subject to bias and random error, e.g., if the vector is partly zoophilic, and if animal blood meals are less likely to be followed by rest indoors, then *a* is systematically overestimated by PSC; indeed the estimation of *ma* by any other method except the NBC is very likely to be even more biased. Still, it would be of great practical importance to be able to relate quantitatively the entomological situation to its malariological consequences, and to attempt this we need to estimate the vectorial capacity (or some alternative "contact rate").

The following approach was therefore adopted: (1) sampling methods were standardized as rigorously as feasible, and a high sampling frequency was adopted; this should fix the biases and reduce the random errors, and thus allow comparison between different times or places; whether success was achieved can, to a certain extent, be tested by comparing independent estimates of the same variable, such as vector density, or by comparing systematic subsamples of the data (e.g., the sums of odd-numbered and even-numbered collection cycles in a given place; a preliminary exploration of this with respect to density showed a strong concordance, suggesting that sampling frequency was adequate); (2) the estimates obtained for the entomological variables are tested for their ability to "explain" any parasitological differences observed (see Chapter 5), and in particular, the estimated vectorial capacity is used as input into a transmission model, which calculates the resulting parasitological situation; comparison with the parasitological situation actually observed will test simultaneously the model's structure and the numerical estimates used (see Chapter 10).

A particular difficulty regarding the estimation of the vectorial capacity in Garki was that, for various technical and operational reasons, the 2 species of the *A. gambiae* complex could not be distinguished in the estimation of the vectorial capacity. This problem is discussed below (see pp. 104-105).

Transmission in Garki and its natural variation

The main vectors of human malaria in Garki are *A. gambiae, A. arabiensis* and *A. funestus. A. pharoensis* is probably a vector also; its contribution to transmission was very small, however.

The level of transmission is certainly very high, whether one judges by the man-biting rate, by the inoculation rate, or by the vectorial capacity. There is a well-known large seasonal variation. There was also a relatively large variation between villages and between successive years. This has to be taken into account in evaluating the effect of control measures. The observed variation in the volume of rainfall could explain the variation in the density of *A. funestus* from year to year but not the concurrent variation in the density of *A. gambiae* s.l., nor the differences between villages. Rainfall was not analysed for patterns, but it could still be done; no detailed information was collected regarding the relief and nature of the soil.

The effect of propoxur

Propoxur produced only mediocre control, in the sense that: (1) the vectorial capacity remained well above its critical level (the minimum level required to maintain endemic *P. falciparum* malaria); and (2) the inoculation rate remained at a readily measurable level. After spraying, transmission was due almost entirely to *A. gambiae* s.l., to the exclusion of *A. funestus*. The epidemiological consequences of this relatively modest impact of residual spraying are considered in Chapters 5 and 10. Possible reasons for the mediocre result could, *a priori*, be the following in any combination: (1) the very high baseline vectorial capacity and inoculation rate; (2) insufficient coverage, either in space (too many huts missed) or in time (delay of first round of spraying or excessive interval between rounds); (3) ineffectiveness of the insecticide; (4) immigration of vectors (*A. gambiae* s.l.) from unsprayed villages; (5) exophilic behaviour of a significant part of the man-biting *A. gambiae* s.l. population. These various possibilities will be considered in turn. The cytogenetic differences which may underlie certain of these explanations, in particular with relation to exophily, are considered in the next section.

The baseline vectorial capacity and inoculation rate were certainly very high, and remained relatively high even despite a large reduction. Coverage in space (proportion of huts sprayed) was very high, by direct independent assessment. Coverage in time (timing of spraying rounds) and effectiveness of the insecticide were also quite good, judging from the PSC, ETC and bioassay tests. Immigration of vectors (*A. gambiae* s.l.) from unsprayed villages was tentatively accepted to explain the results of the small-scale preliminary trial of propoxur (see p. 76), but the

same features—greater reduction in IRD than in NBC (IN), and greater reduction in NBC (IN) than in NBC (OUT)—were observed in the much larger main trial. Furthermore, the variation between villages in the relative effectiveness of propoxur in controlling *A. gambiae* s.l. was independent of the distance from unsprayed villages, and the distribution of sporozoite-positive *A. gambiae* s.l. was also independent of distance from unsprayed villages. Furthermore, the differential effect of propoxur on the *A. gambiae* s.l. populations of different villages is stable from one year to the next and may have a genetic basis (see p. 98; and refs. *32, 115*), which would be incompatible with large-scale migration of *A. gambiae* s.l. between villages. For all these reasons, immigration of *A. gambiae* s.l. from unsprayed areas is unlikely to have been an important factor (it could, however, be very important in cases where control of the *local* vector population is more effective).

This leaves as a determining factor, in addition to the high baseline level of transmission, the possibility of exophilic behaviour of a significant fraction of the *A. gambiae* s.l. population. This is suggested by various observations: (1) the high prespraying ratio of the MBR to the IRD (see p. 70); this ratio has been interpreted in a similar way by Hamon et al. (*79*) in Bobo-Dioulasso; (2) the correlation between the differential effect of propoxur in different villages and their prespraying MBR/IRD ratio (see p. 80 and ref. (*115*); (3) the fact that most of the difference between the effect of propoxur on *A. gambiae* s.l. in Garki and the (much greater) effect of fenitrothion on *A. gambiae* s.l. in Kisumu, Kenya (*64*) can be "explained" by the difference between the two places in the prespraying MBR/IRD ratio of their *A. gambiae* s.l. (*115*). The relevant cytogenetic findings are discussed in the next section. The exophilic and endophilic individuals are probably relatively consistent in their behaviour. This is suggested, indirectly, by the agreement between the estimated prespraying proportion of *A. gambiae* s.l. blood meals followed by rest indoors and the estimated postspraying proportion of vectors exposed after their first meal, under the hypothesis of consistent behaviour (see p. 87 and ref. *119*). But consistent behaviour of individual vectors was also demonstrated directly by cytogenetic findings (see next section). The consistency of the exophilic behaviour of individual vectors affects the impact of an insecticide on transmission and has therefore to be taken into account in the evaluation of insecticidal impact (see *119*).

The consequences of these findings and interpretations with respect to the planning of malaria control will be discussed in Chapter 11. It is already obvious that the following are important (see *114*). (1) Estimation of the baseline vectorial capacity; the development of better methods would be useful. (2) Prediction of insecticidal impact; in Garki (and in

Kisumu) the impact was related to the prespraying ratio between the man-biting density and the indoor-resting density; in Garki, it was also related to intraspecific cytogenetic variation. It is not certain that a method of prediction, which would be both practical and of general value, can be developed, but research in this field, e.g., on field indicators of resting behaviour, would be useful. (3) Evaluation of insecticidal impact, in a field trial or a control programme; this is discussed in a later section (see pp. 105-106).

A. gambiae, A. arabiensis and intraspecific cytogenetic variation

The only members of the *A. gambiae* complex identified in Garki were *A. gambiae* s.s. (species A) and *A. arabiensis* (species B), the latter being more abundant. This corresponds to what was known regarding the geographical distribution of the members of the complex.

According to a recent review of the *A. gambiae* complex (*163*), the main differences between the 2 species, with respect to transmission are the following: (1) *A. gambiae* s.s. is strongly and uniformly anthropophilic and endophilic; *A. arabiensis* is less anthropophilic and endophilic, but with considerable geographical variation (related to cytogenetic variation); (2) where the 2 species are sympatric, *A. arabiensis* is probably the longer-lived, but the evidence is indirect, limited and sometimes discordant; (3) both species have probably the same intrinsic susceptibility to infection with *P. falciparum*, but here also the evidence is limited and sometimes contradictory; (4) residual insecticides readily control populations of *A. gambiae* s.s., but their effect on populations of *A. arabiensis* varies with the degree of endophily; residual insecticides tend to select *A. arabiensis* out of a mixed population of the 2 species, and to select exophilic cytotypes out of a natural *A. arabiensis* population.

In Garki, *A. arabiensis* was indeed less anthropophilic than *A. gambiae* s.s. There was, however, no clear-cut difference regarding endophily and no clear-cut selection of *A. arabiensis* by the application of propoxur, nor could the variation between villages in the *gambiae/arabiensis* ratio explain the differences in effectiveness of propoxur (*115*). The latter was, however, related to the frequency, within each of the 2 species, of certain chromosomal inversions (*32*). In Kisumu, Kenya, *A. gambiae* s.s. was the dominant species (M.W. Service, personal communication), but to explain the magnitude of the difference between the effect of fenitrothion in Kisumu and that of propoxur in Garki on the respective *A. gambiae* s.l. populations it is necessary to postulate that *A. arabiensis* was more endophilic in Kisumu than in

Garki. The intraspecific cytogenetic findings from Kisumu were not available at the time of writing.

The association between resting behaviour and certain chromosomal inversions (*32*) is a direct proof that resting behaviour (e.g., exophily) is a relatively stable characteristic of the individual. This is important for the interpretation of changes observed after spraying (see *119*). The observed differences between the species in human blood indices are insufficient to explain the corresponding differences in sporozoite rates; the discrepancy could be explained by greater longevity of *A. gambiae* s.s., but other explanations cannot be ruled out (e.g., greater susceptibility of *A. gambiae*, greater overestimation of the man-biting habit of *A. arabiensis*).

The continuation of entomological observations after the intervention period provided an opportunity of looking for evidence of behavioural selection. No firm conclusion can, however, be drawn. The persisting increase in the ratio between the outdoor and indoor NBC of *A. gambiae* s.l. probably cannot be explained by a change in the relative abundance of the 2 species (i.e., by interspecific behavioural selection), and could suggest intraspecific behavioural selection, but we cannot rule out the simpler explanation that is due to the actual persistence of propoxur, proven by the bioassay tests.

Since the 2 species of the *A. gambiae* complex that were present could not be distinguished in the estimation of the vectorial capacity in Garki, it is important to try to evaluate the resulting error. The question was explored by simulation as follows. It was assumed that the 2 species did not differ with respect to n, nor in susceptibility to infection. The 2 species could differ with respect to a and p, and also with respect to the proportion of blood meals, taken on either man or animal, that are followed by rest indoors, which would affect the estimation of a from the PSC. Simulations were made under the following assumptions: (1) *A. gambiae* s.s. takes all its blood meals on man and all of them are followed by rest indoors: *A. arabiensis* takes only 40% of its blood meals on man, and half of the blood meals taken on man and one quarter of those taken on animals are followed by rest indoors. (2) The expectations of life, in days, of *A. gambiae* and *A. arabiensis* respectively are 5 and 5 (i.e., $p = \exp(-1/5) = 0.819$), or 4 (i.e., $p = 0.779$) and 6 (i.e., $p = 0.846$), or 6 and 4. (3) The 2 species can be present in any proportion. (4) n is 10, and the number of blood meals per vector per day is 0.5. (5) Parameters are estimated as in this project, i.e., ma is estimated by NBC, a by half the human blood index in the PSC, p by the square root of the proportion of the NBC that are taking their second or later meals. (6) The vectorial capacity is estimated either with or without identification of the two species, in both NBC and PSC, and the two estimates are compared with

each other and with the "true" vectorial capacity, known by construction.

The simulations give the following results. (1) If the 2 species have the same longevity, or if the anthropophilic species (*A. gambiae* s.s.) is also the short-lived species, then both estimates are overestimates, but they do not differ from each other by more than 2%. (2) If the anthropophilic species (*A. gambiae* s.s.) is the longer-lived one, then distinguishing the species leads to overestimation of the vectorial capacity, while failing to distinguish them leads to underestimation, except when the anthropophilic species tends to disappear; the largest difference between the 2 estimates occurs when *A. gambiae* and *A. arabiensis* form 37% and 63% of the NBC, respectively: then failing to distinguish the species leads to an underestimation by 23%, while distinguishing them leads to an overestimation by 7%. (3) Whatever the longevities, the worst estimate is obtained when in fact only the zoophilic species is present: this leads to an overestimation by 43%, because of the bias in estimating a, but in this case, the identification of the species makes no difference.

It is unlikely that the species differ in reality as much as is assumed in these simulations, in particular with respect to longevity. It is thus unlikely that the failure to distinguish between *A. gambiae* s.s. and *A. arabiensis* in the estimation of the vectorial capacity was a major source of error as compared to the other possible sources.

Evaluation of insecticidal impact

The following points are of interest. (1) Among collection methods, PSC, ETC and NBC were all indispensable; in the hands of the project staff CDC miniature light-traps were not a satisfactory substitute for the NBC (*146*), but a reliable trapping substitute for the NBC is obviously desirable; it would not necessarily have to be unbiased. (2) Spontaneous variation between villages and between years was rather large and it is important to take it into account: e.g., ignoring it would lead to the false conclusion that propoxur was less effective in 1973 than in 1972 (see p. 80). This stresses once more the necessity for both adequate baseline data and adequate controls. (3) The insecticidal effect of propoxur (as assessed by bioassay) persisted for at least 3 years after the intervention period, and some changes in the vector populations (e.g., an increase in the ratio of the outdoor NBC to the indoor NBC) persisted for at least 2 years. For operational reasons, different insecticides are sometimes evaluated in succession in the same area, with an interval of 1 or 2 years; this may be insufficient. (4) To translate the change in density and age composition, observed after spraying, in terms of transmission, one has to make an assumption regarding uniformity of exposure, and the usual

(but implicit) assumption of uniform exposure maximizes the calculated reduction in transmission. Nonuniform exposure is, however, more plausible *a priori* and also more apt to explain the changes observed after spraying; nonuniform exposure is also suggested directly by cytogenetic findings (*32*). (5) In evaluating an insecticide, the area required to minimize immigration of vectors is a vexed point; there is, obviously, no general solution. Some observations presented above suggest that in the study zone the migration of vectors between villages was relatively unimportant (see p. 102), and that in that zone the entomological evaluation of an insecticide does not require a very large area.

Summary and Conclusions

The level of transmission in the Sudan savanna is very high indeed: the man-biting rates of *A. gambiae* s.l. and *A. funestus* reached seasonal peaks of 174 and 94 bites/man/night (averages of 8 man-nights); the vectorial capacity (contact rate between persons through the vectors) reached a seasonal peak of about 40, i.e., about 2000 times the critical value required to maintain endemic malaria; the cumulative entomological inoculation rate reached a maximum of 145 sporozoite-positive bites in 1 year (out of which 132 were in the wet season).

In the Sudan savanna there are large seasonal, yearly and local variations in the level of transmission. In most villages, the vectorial capacity drops below its critical level for about half of the year (this does not necessarily prevent transmission, given the large reservoir of parasites), while in some it remains well above the critical level throughout the year. The variations from year to year are relatively important; over a period of 3 years, these variations followed the variations in total rainfall in the case of *A. funestus* but not in the case of *A. gambiae* s.l. Villages, even when not obviously different on inspection and located only a few kilometres apart, differ in vector density, anopheline fauna (*A. funestus* has a very uneven distribution) and probably in vector behaviour and karyotypes. Among 8 villages, the cumulative inoculation rate ranged from 18 to 145 sporozoite-positive bites in one year. The local and yearly variations stress the need for adequate baseline and comparison (control) data for a correct evaluation of the impact of control measures.

Propoxur produced only a mediocre result in terms of transmission in the Sudan savanna: the vectorial capacity remained well above the critical level required to maintain *P. falciparum*, and the inoculation rate remained quite readily measurable. This mediocre result was observed notwithstanding the high standard of the operations, the high coverage achieved and the good bioassay tests. The result was also little affected

by the size of the treated area, as shown by the comparison between the village-scale preliminary trial and the main intervention area covering 165 villages. The mediocre result is due essentially to the high level of the baseline transmission and to the relative exophily of *A. gambiae* s.l. This relative exophily is reflected in a high prespraying ratio between the man-biting and indoor-resting densities. Substituting another insecticide for propoxur would probably not have altered the result very much. In particular, the much greater impact of fenitrothion on *A. gambiae* s.l. in Kisumu, Kenya, than that of propoxur in Garki was largely predictable on the basis of the lower prespraying ratio between the man-biting and indoor-resting densities, and is therefore largely explicable in terms of vector behaviour.

The effect of residual spraying in reducing *A. gambiae* s.l. populations, and hence transmission, varies significantly between villages. The variation is related not to variations in coverage of the spraying operations, nor to distance from unsprayed villages, but probably to variation in the amount of exophily, reflected in the prespraying ratio between man-biting and indoor-resting densities. As stated in the previous paragraph, this prespraying ratio is to a certain extent a predictor of the effectiveness of residual spraying. The amount of exophily is a relatively stable characteristic of villages and is also associated with genetic differences within individual species of the *A. gambiae* complex (see next paragraph). The vector population attached to a village appears thus as relatively isolated genetically most of the time. This would also fit with the observation that the effect of spraying was little affected by the size of the sprayed area. As a consequence of the relative autonomy of the local vector populations, a large area is not required for the entomological evaluation of vector control measures in this particular environment. Possible variations in the amount of exophily should be taken into account in evaluating insecticides.

A. gambiae s.l. in the study area is composed of *A. gambiae* s.s. and *A. arabiensis*. The dominant species is usually *A. arabiensis* but the relative abundance of the 2 species varies between times and places in ways which are not explained. *A. gambiae* s.s. is the more anthropophilic and has higher sporozoite rates; no clear-cut difference was demonstrated regarding exophily or effectiveness of propoxur. The vectorial capacity was estimated as if *A. gambiae* s.l. were a single species; appropriate simulations show that this is unlikely to have introduced a large error in the estimate. The cytogenetic investigations of Coluzzi suggest that resting behaviour and exposure to propoxur are related less to the relative abundance of the 2 species than to the intraspecific frequency of certain chromosomal inversions, some of which may be associated with a relatively stable behaviour pattern of the individual.

A commonly used method of translating the effect of an insecticide on the density and age composition of vector populations into an effect on transmission assumes implicitly a uniform reduction in longevity, i.e., a uniform exposure to the insecticide consequent on a uniform resting behaviour. This tacit assumption is the most optimistic possible regarding insecticidal impact on transmission, but it is not plausible *a priori* nor is it compatible with the finding of an association between resting behaviour and genetic variation. Moreover, the assumption of nonuniform exposure leads to more realistic expectations regarding the effect of the insecticide treatments on the prevalence of *P. falciparum* (see Chapter 10). It is preferable, in the African savanna at least and probably elsewhere, to evaluate insecticidal impact on transmission under the assumption of nonuniform exposure, or even to assume that the vectors caught biting man after spraying have a normal expectation of life. A nonuniform resting behaviour creates the possibility of behavioural selection.

Some effects of spraying with propoxur on vector populations, such as a decrease in the ratio between indoor and outdoor biting densities, or an increase in the ratio between the biting density and the indoor-resting or exit-trap density, remain detectable 2 years after the last application. The sprayed walls demonstrated an insecticidal effect by bioassay in the first half or two-thirds of the breeding season for at least 3 years after the last application. Furthermore, in the post-intervention phase, the sporozoite rate of the man-biting population was low notwithstanding a probably normal longevity and an elevated gametocyte rate (see Chapter 5), and this sporozoite rate was lower than in the indoor-resting population, which was not the case before intervention. Some of these changes could be due to behavioural selection, but the actual persistence of propoxur on the sprayed surfaces may be sufficient to explain them.

Pyrethrum spray collection and exit-trap collections are insufficient for the entomological evaluation of residual spraying, and should be supplemented by collections on human baits (or a substitute collection method, if an adequate one can be found).

Chapter Five

PARASITOLOGY[a]

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 blood 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 the 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 microscopic fields.

After this review, the following methods were selected for a preliminary trial (*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. falciparum* 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 cover.

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 1–8 combined

Parasite	Parasite rates (%)		Increase (%)	
	in 38 258 films examined for 200 fields (*a*)	in 8 821 films examined for 400 fields (*b*)	absolute (*c*) = (*b*) − (*a*)	relative (*d*) = (*c*) / (*a*)
P. falciparum				
asexual stages	48.9	53.7	4.8	9.8
gametocytes	12.7	16.4	3.7	29.1
any stage	50.9	56.2	5.3	10.4
P. malariae	15.3	18.9	3.6	23.5
P. ovale	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 (1–23) combined

Parasite	Slides (%) found positive by:			Parasite rates (%) according to:			Ratios between parasite rates		
	micro-scopists only (a)	super-visors only (b)	both (c)	microscopists (d) = (a) + (c)	supervisors (e) = (b) + (c)	both combined (f) = (a) + (b) + (c)	microscopist / supervisor (g) = (d) / (e)	microscopist / both combined (h) = (d) / (f)	supervisor / both combined (i) = (e) / (f)
P. falciparum, 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. falciparum, P. falciparum* 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

The parasite 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 persons found positive for the species considered (i.e., in the case of *P. falciparum*, 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 × 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).

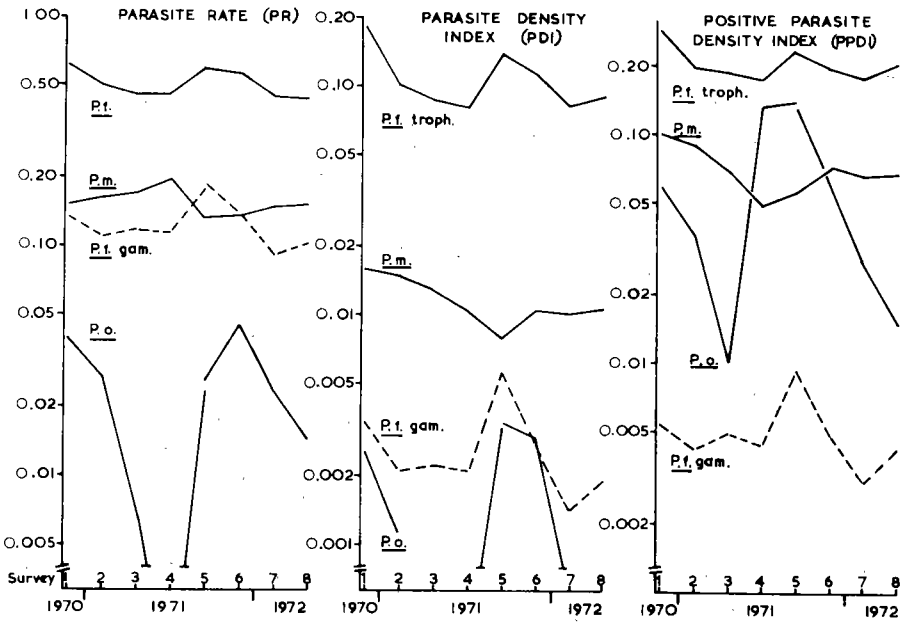
The situation described is really unaffected by antimalarial measures. No insecticides were used in the area in the past. The amount of antimalarials used was negligible: (1) there are only 2 dispensaries in the district, in Garki and in Gwarzo, the latter outside the study area, and an analysis of their 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, all villages combined



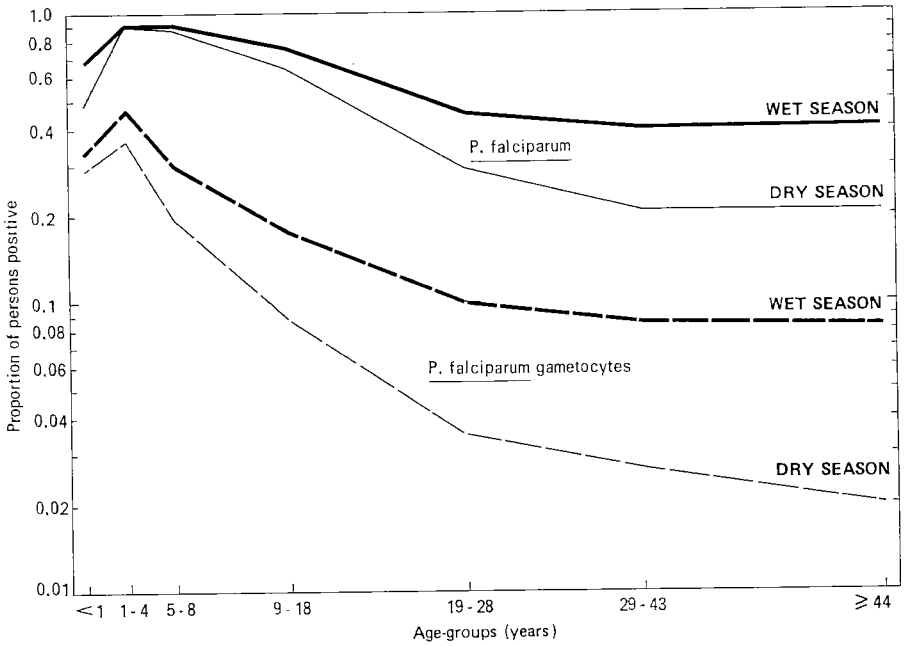
[a] PR = proportion of persons positive; PDI = proportion of fields positive; PPDI = proportion of fields positive in persons positive for the species.

[b] *P.f.* = *P. falciparum*; *P.f.* gam. = *P. falciparum* gametocytes; *P.m.* = *P. malariae*; *P.o.* = *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

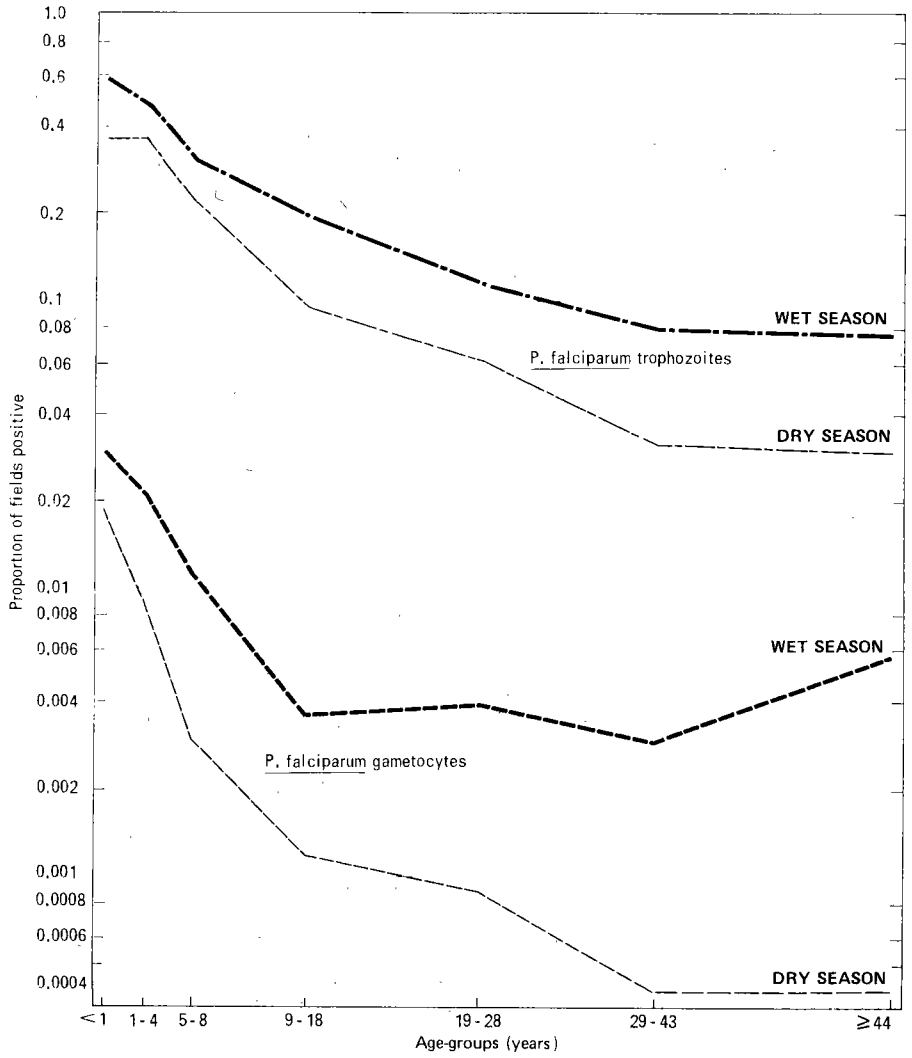
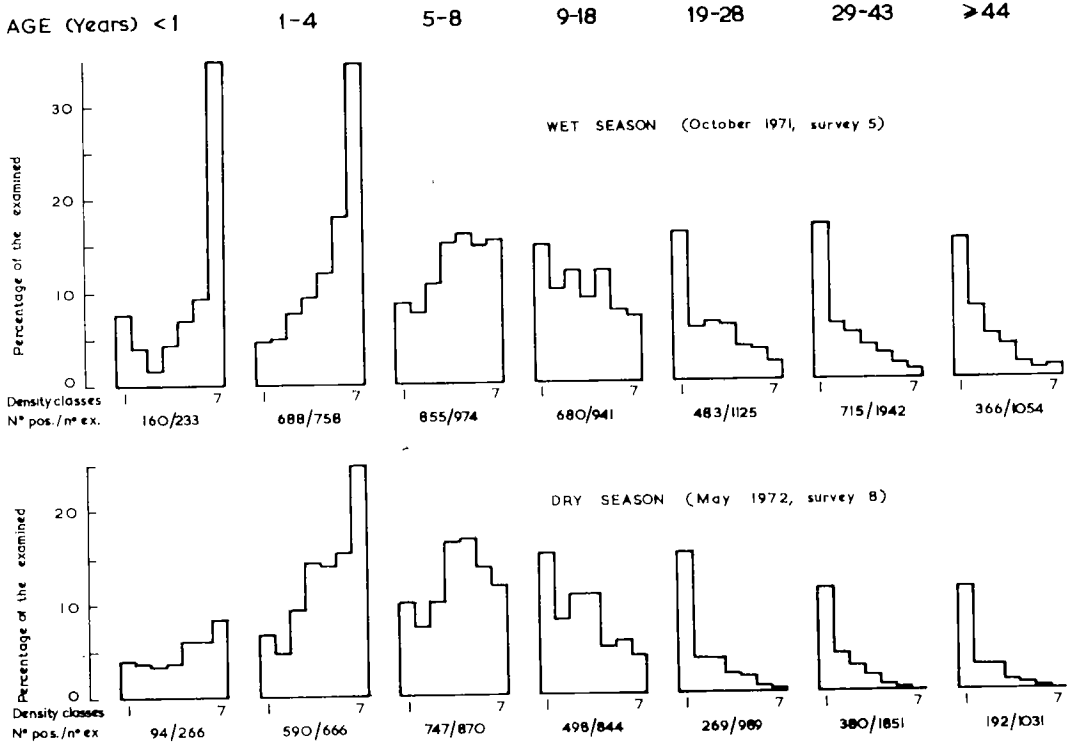


Figure 22 shows only the average densities (PPDI) without telling us anything about the distribution of the population according to density. Therefore Fig. 23 displays the actual frequency distribution of persons into density classes defined in terms of proportions of microscope fields positive. The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

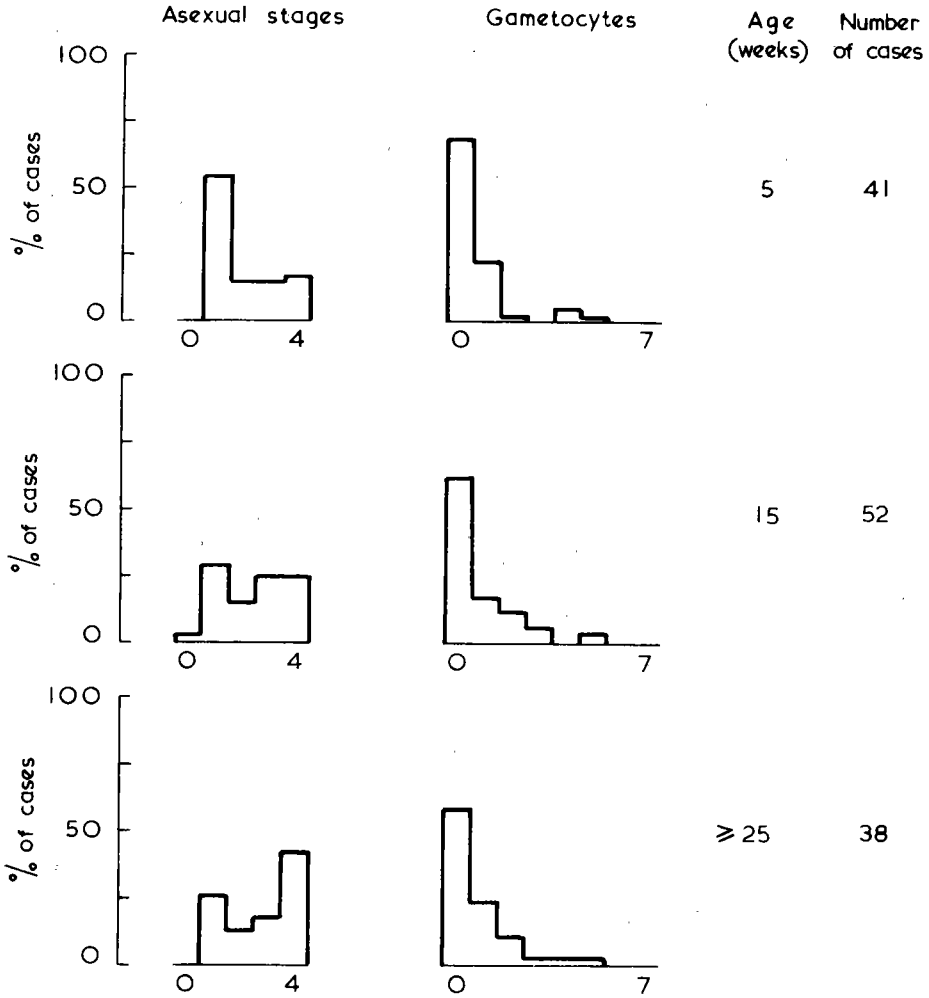
Fig. 23. Age-specific density distribution of *P. falciparum* asexual stages, by season[a]



[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 towards 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]

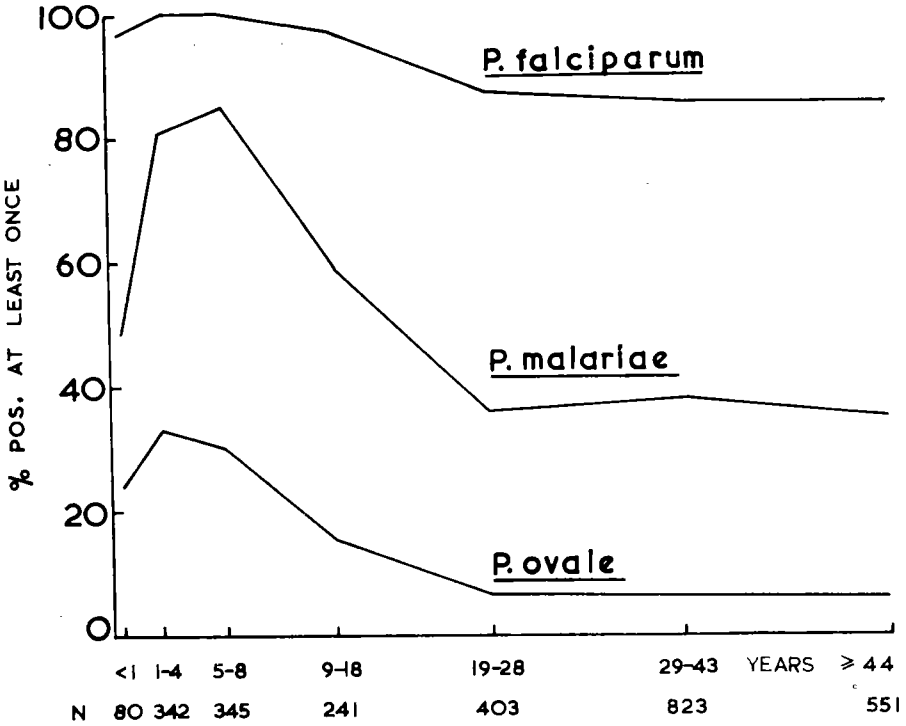


[a] Density of asexual forms: 0 = negative; 1, 2, 3, 4 = up to 4%, 16%, 64%, 100% of fields positive; density of gametocytes: 0 = negative; 1, 2, 3, 4, 5, 6, 7 = up to 2%, 4%, 8%, 16%, 32%, 64%, 100% of fields positive.

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" 2–11 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]



[a] N = number of persons examined at every 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 1–4 and 5–8 years, when it was 100% for *P. falciparum*, more than 80% for *P. malariae* and more than 30% for *P. ovale*.

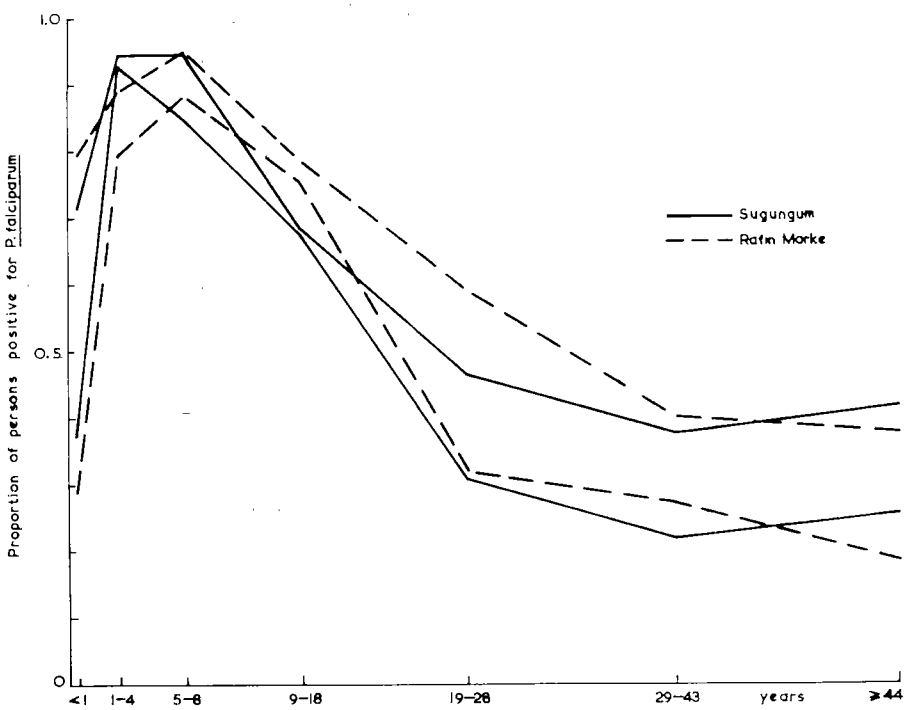
Persons examined at all 8 baseline surveys were classified within specific age-groups by the number of times (0–8) they were found positive. The resulting distribution was compared to the binomial distribution with the same average. This binomial distribution would be expected if results were randomly 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. malariae* *Average*	*P. ovale* *Average*
Compact	15	53.5	(48.8–59.1)	14.6	2.8
Mixed	5	47.5	(45.5–48.5)	13.0	2.7
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

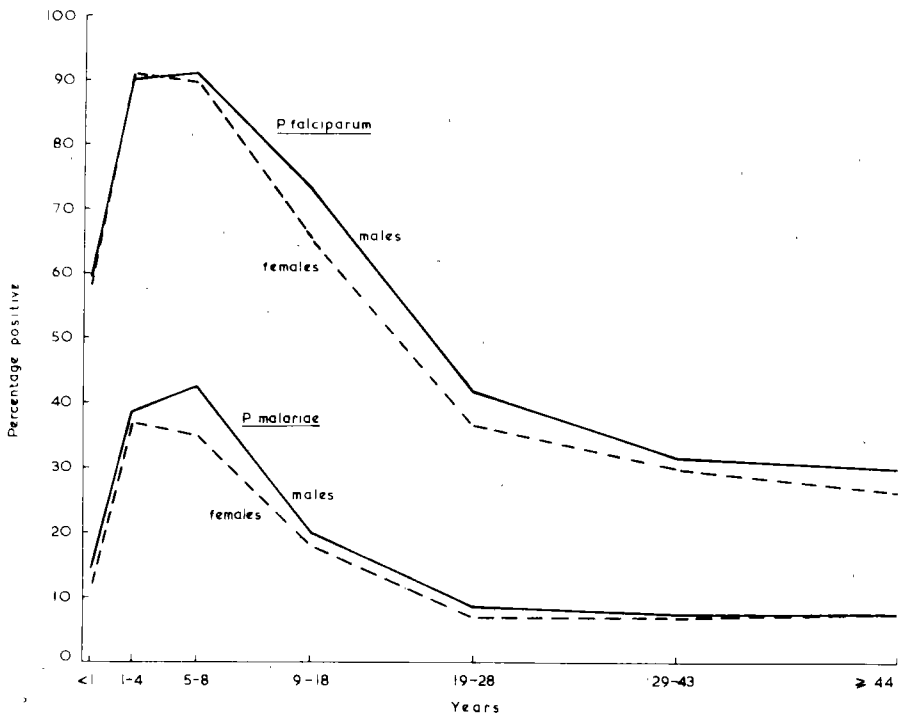
variation between the different types of village is very significant ($p<0.001$).

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
0–8	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 1–5 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 9–18 and 19–28 years, and for *P. malariae*, the crude prevalence or the prevalence 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.

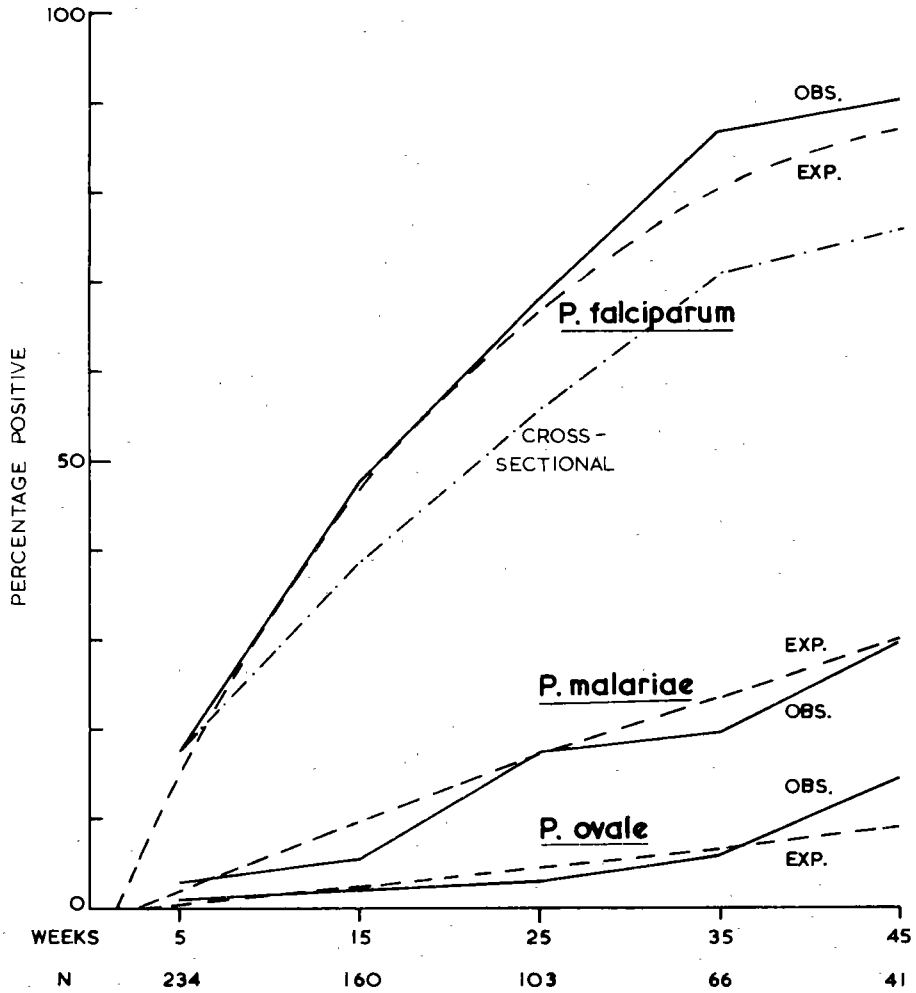
Incidence and recovery rates (conversion and clearance rates)

Infant conversion rates

The daily infant conversion rate (ICR) was estimated by the formula $ICR = -\ln(1-P)/t$, 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

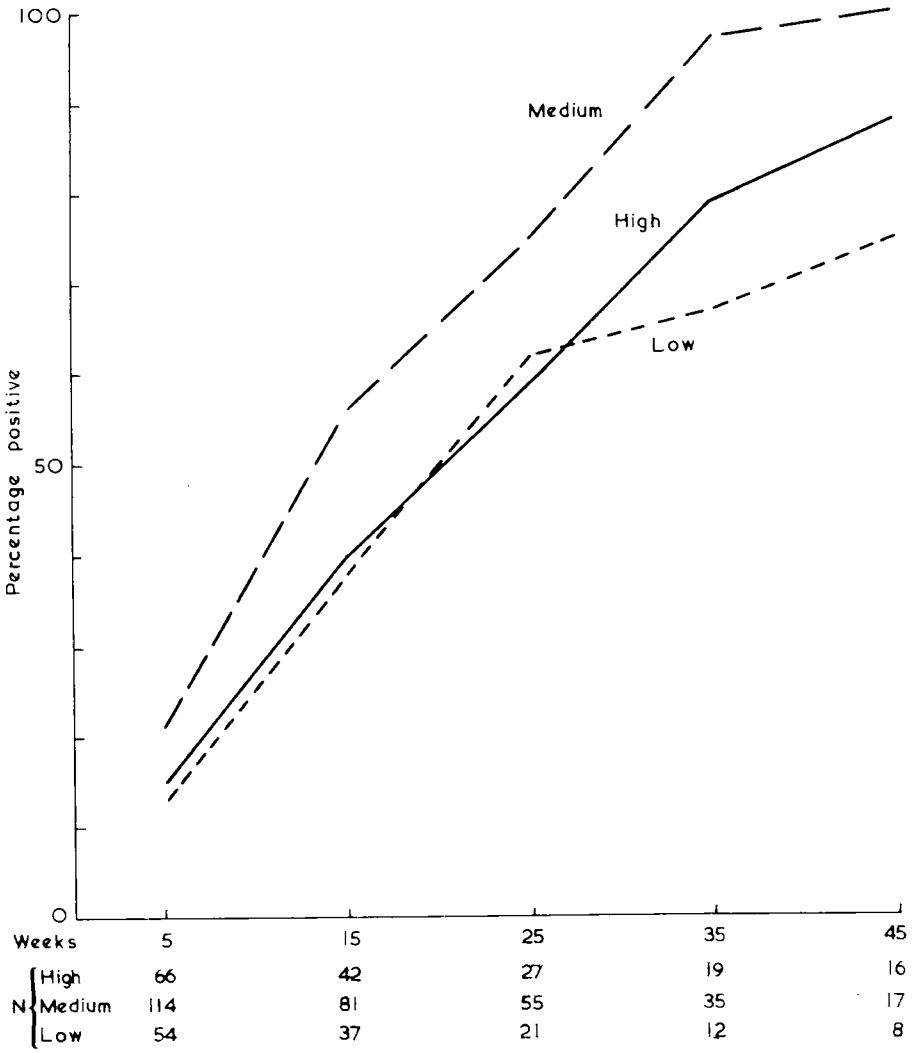
Fig. 28. Observed cumulative prevalence of *P. falciparum*, *P. malariae* and *P. ovale*, by age of infants regularly followed after birth at 10-week intervals compared with the cumulative prevalence expected from the average infant conversion rate ICR;[a] baseline phase, all villages combined



[a] Calculated from: $x(t) = 1-\exp\{-h(t-n)\}$, where h = ICR = 0.0066 for *P. falciparum*, 0.0012 for *P. malariae*, 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 infants followed longitudinally, by age and by the relative level (low, medium, high) of the vectorial capacity of the village; baseline phase, all villages combined



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 10), 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 persons 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 al.(*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 10-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 $\frac{ICR}{EIR}$
Lowest	4, 5 (3 villages)	0.229	0.0128 (16/25) ± 0.0033	0.056
Intermediate	1, 2, 6, 8 (8 villages)	0.252	0.0163 (43/59) ±0.0027	0.065
Highest	3, 7 (5 villages)	0.940	0.0124 (17/27) ±0.0017	0.013

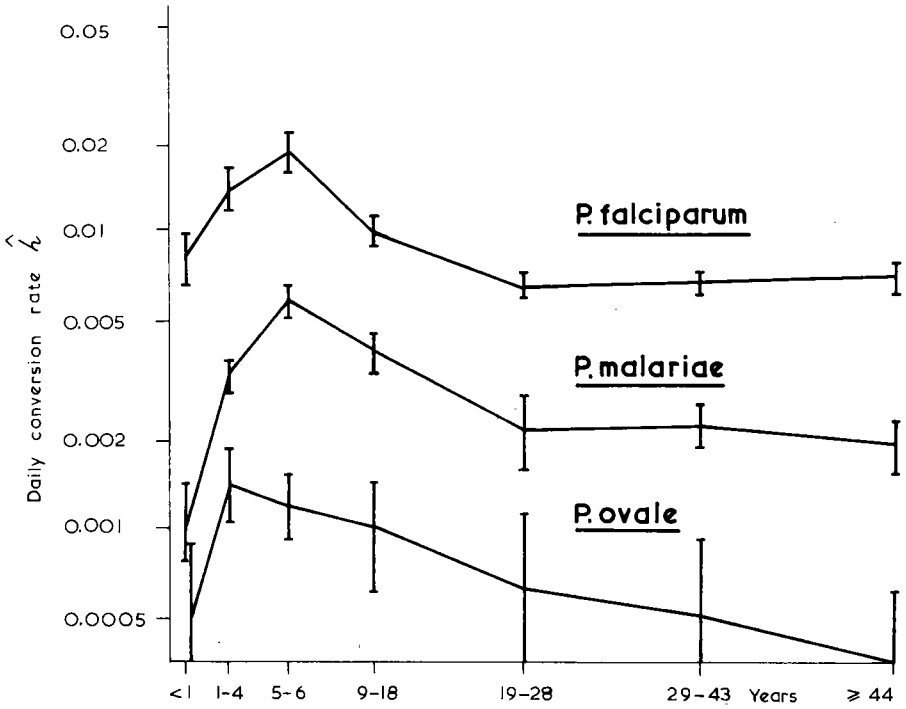
[a] The 6 villages added at the fifth survey are excluded.

[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.
$ICR = -\ln(1-p)/t$, 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

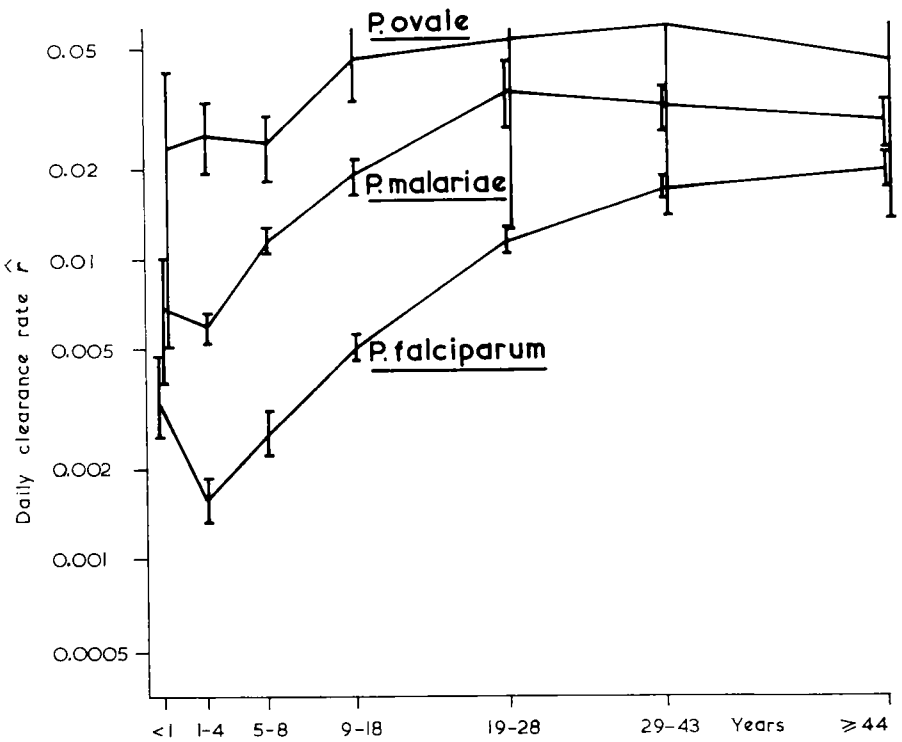


or show no systematic variation as with *P. malariae* and *P. ovale*; later in life, the clearance rate increases towards an adult plateau; the mean clearance rate varies, between age-groups, by factors of about 10, 5 and 2 for *P. falciparum, P. malariae* and *P. ovale* respectively (Fig. 31).

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 the 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.

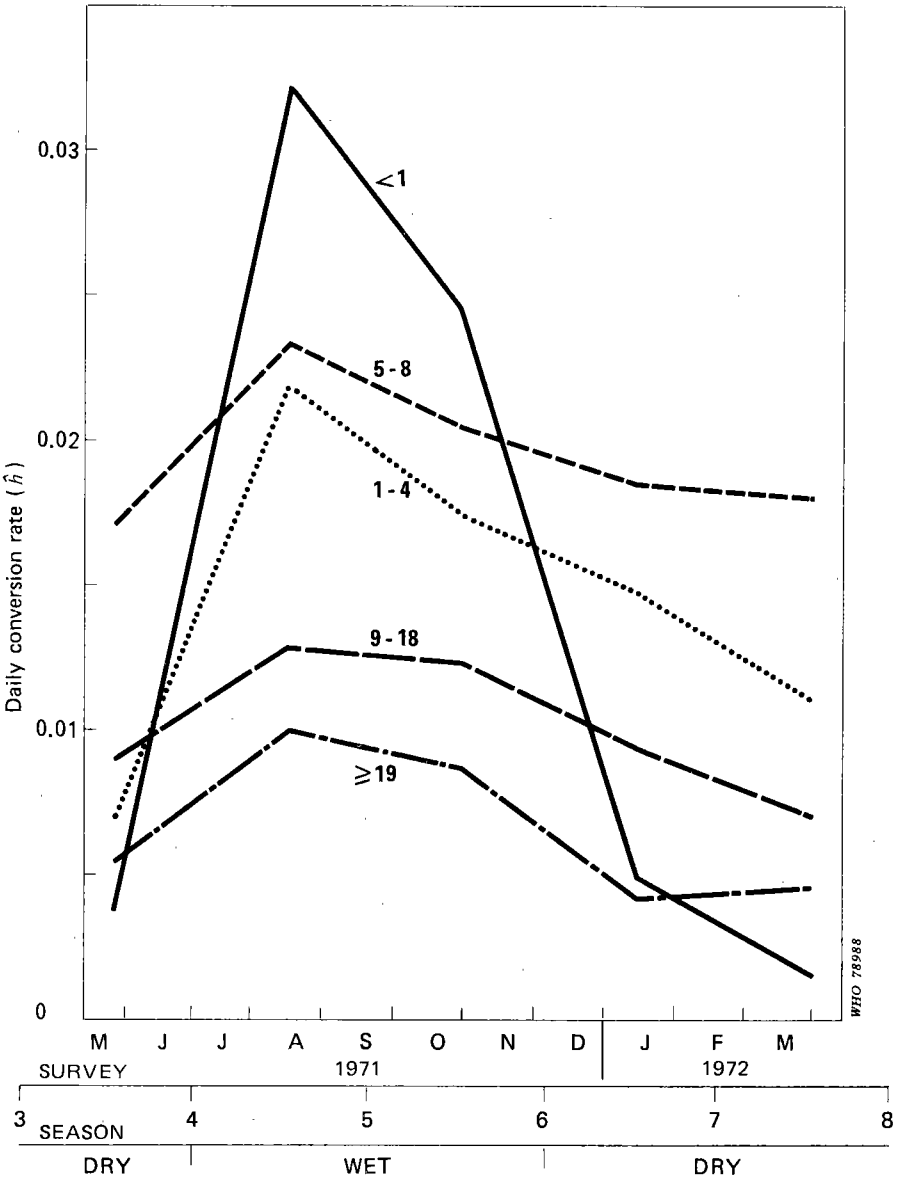
By comparison between villages, it appears that $\hat{r}$ tends to decrease (episodes of parasitaemia tend to be longer) as the vectorial capacity increases; this may be an effect of superinfection.

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).

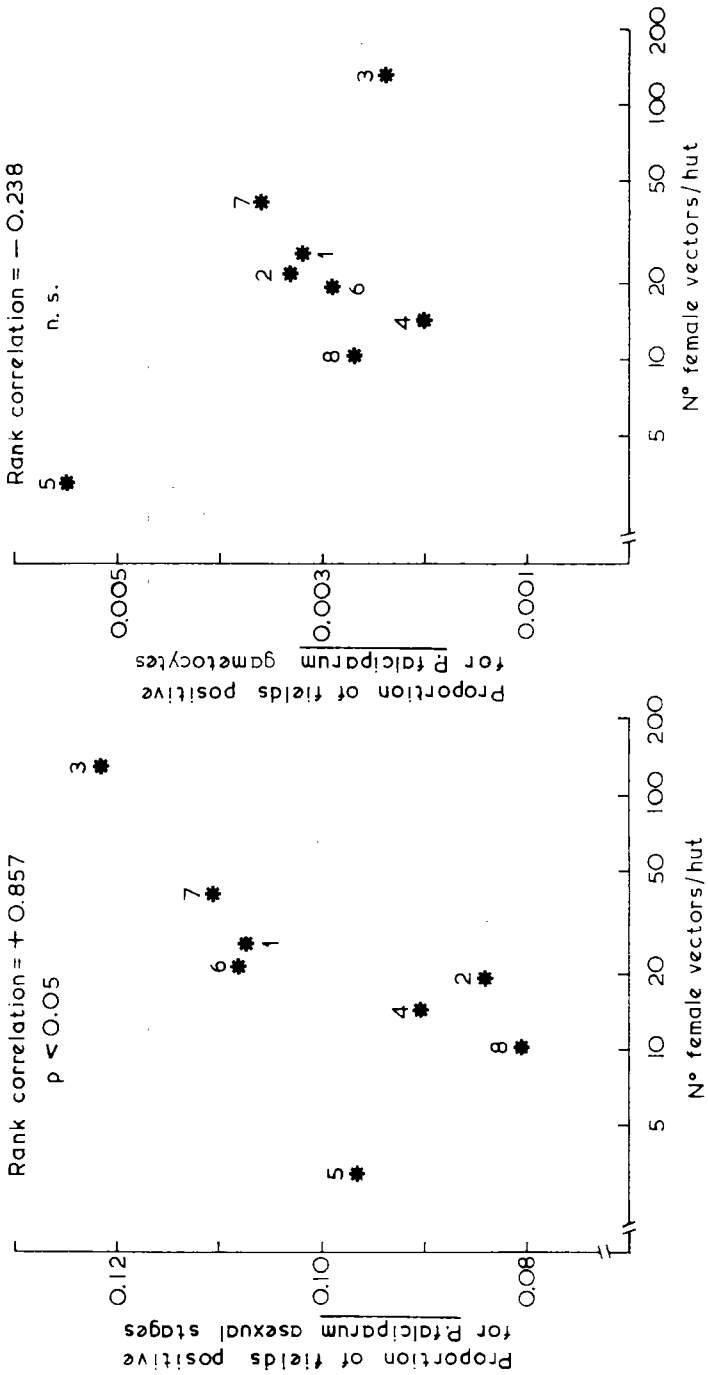


Fig. 33. Correlation between density of vectors and density of *P. falciparum* asexual stages and gametocytes in 8 villages (1–8)[a]

[a] Density of vectors = No. of *A. gambiae* s.l. 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.

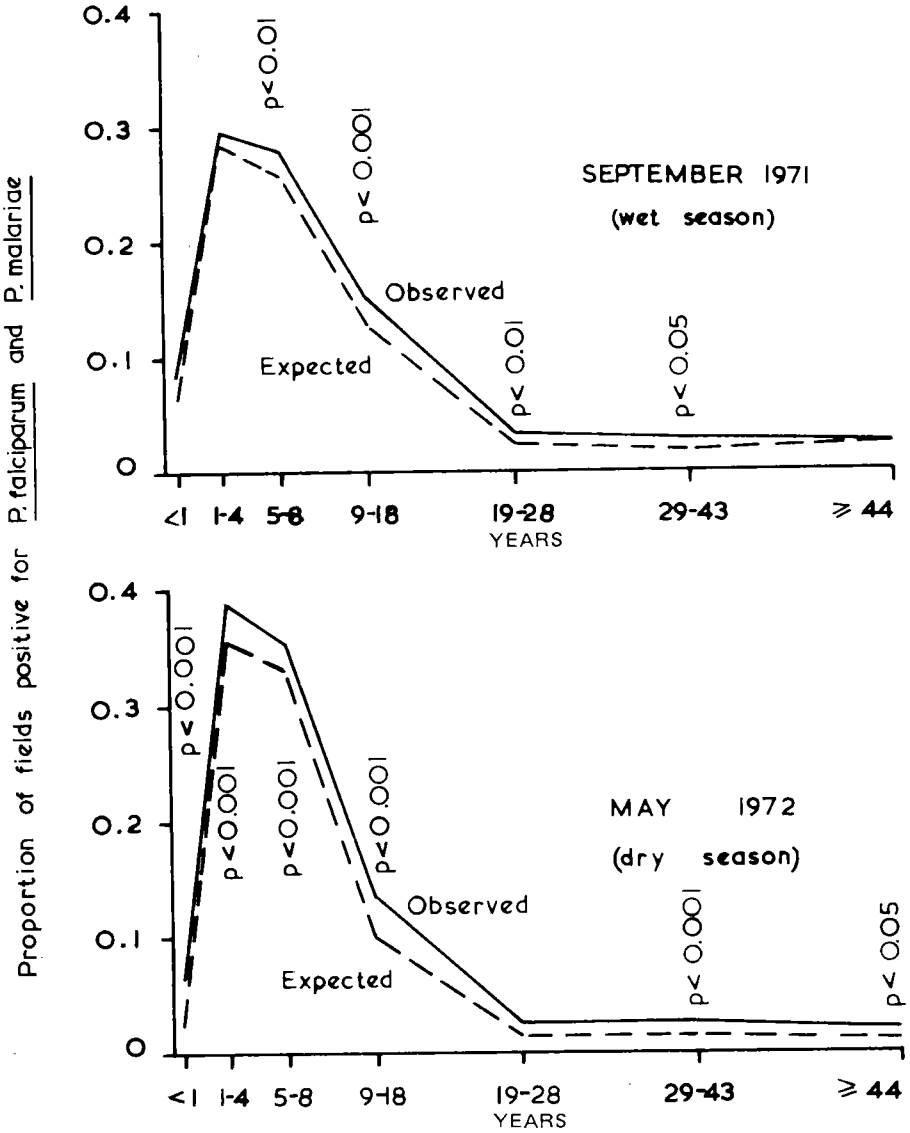
Between the study villages, i.e., between villages with high to very high levels of transmission, an increase in vector densities and vectorial capacities produced an increase in entomological inoculation rates (see Chapter 4), but no increase in infant conversion rates (Table 17, Fig. 29). As a consequence, 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 prevalence 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-

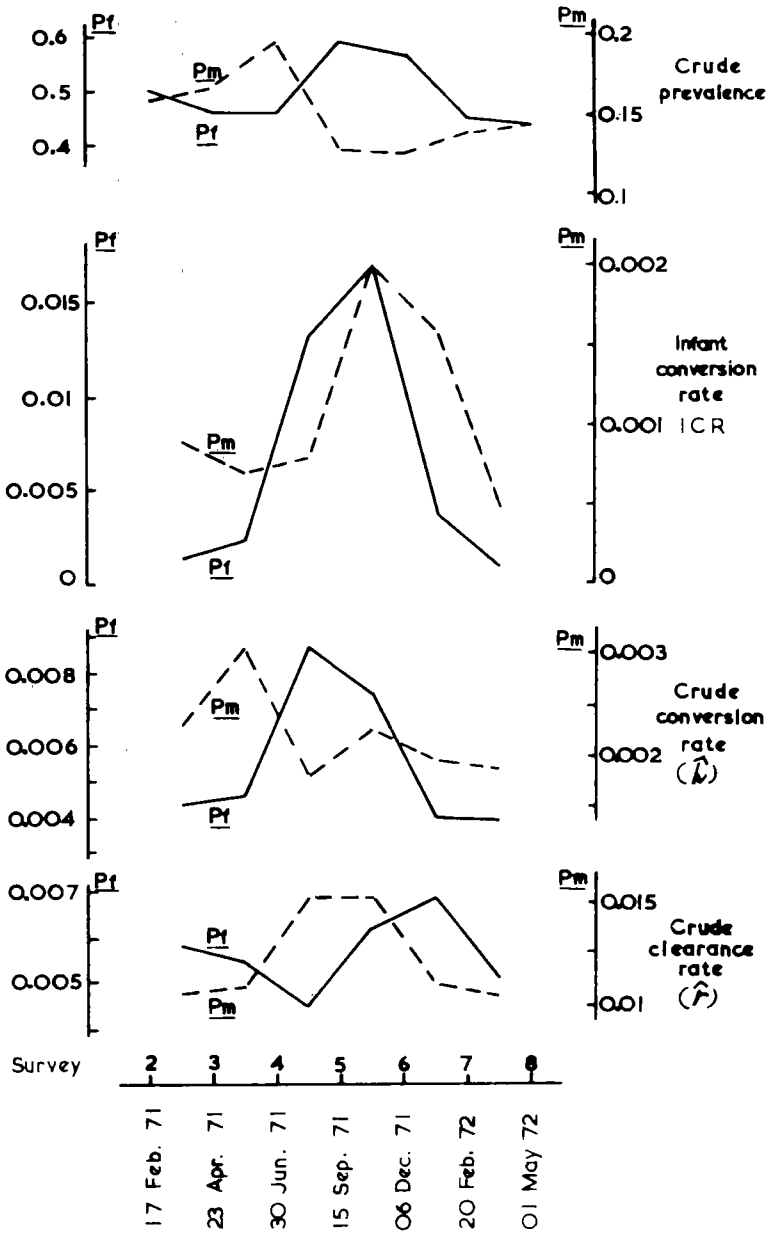
aemias of *P. falciparum* and *P. malariae* in both the wet and the dry seasons (Fig. 34).

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 prevalence, 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 model 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

---

[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 5-18 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 low-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 (A1) produced almost

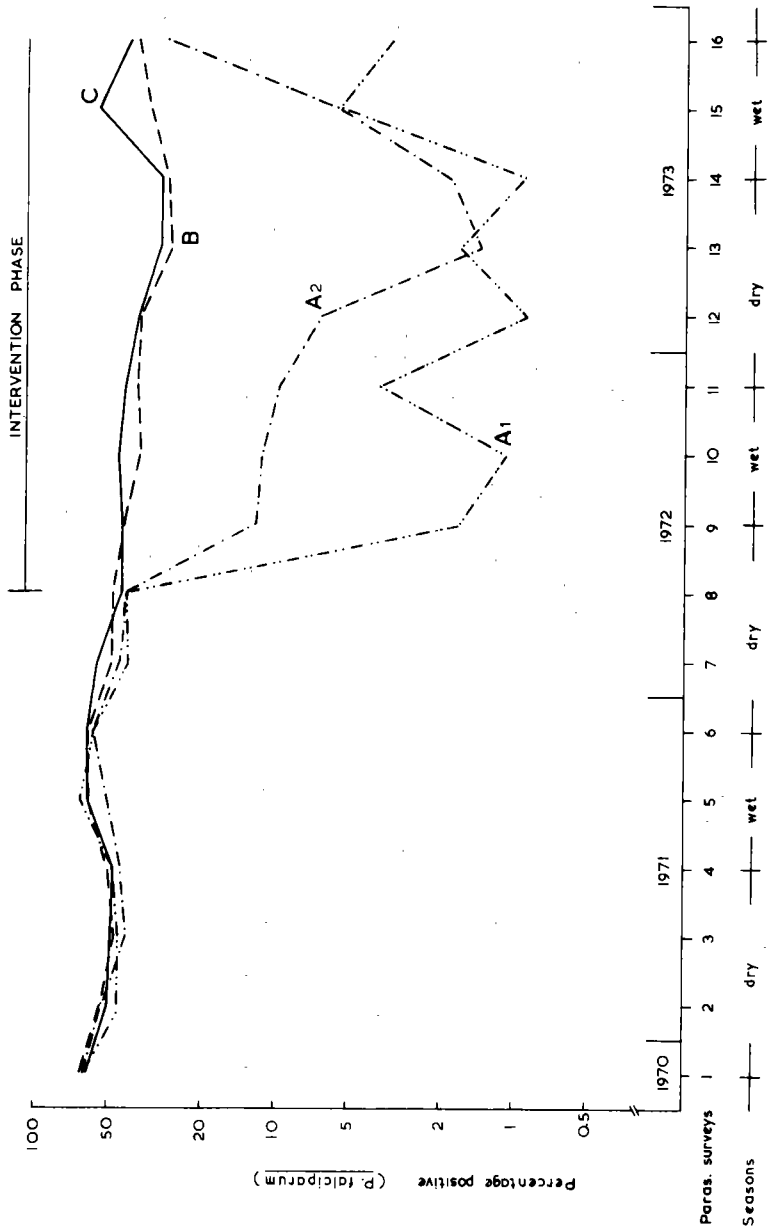
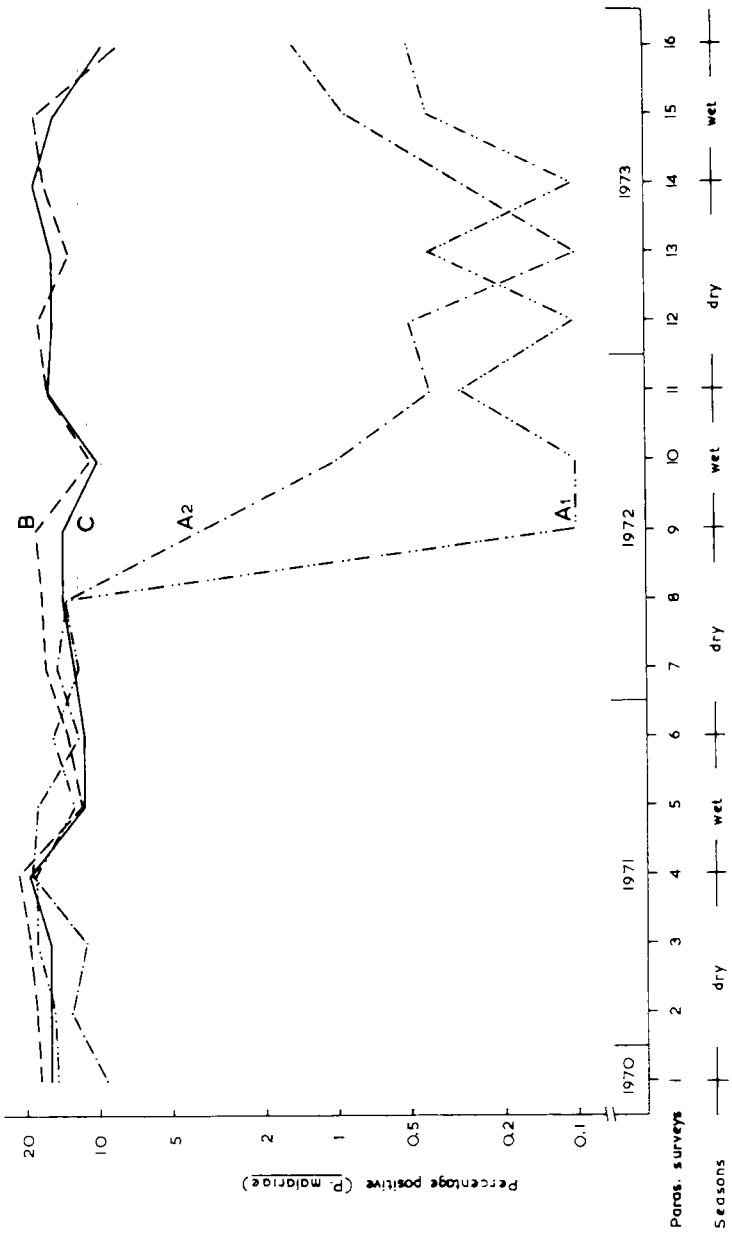


Fig. 36. Crude percentage positive for *P. falciparum*, by survey and treatment throughout the baseline and intervention periods[a]

[a] Interventions: propoxur in A1, A2, B; mass administration of sulfalene-pyrimethamine in A2 every 10 weeks, and in A1 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 A1, A2, B; mass administration of sulfalene-pyrimethamine in A2 every 10 weeks, and in A1 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 A1) 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 A1, 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 A1), 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 prevalence 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-cut pattern.

Certain findings regarding the age-specific prevalence and density of parasitaemia are presented in Fig. 38, 39 and 40. The age-specific prevalence 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, A1) 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 Surveys 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)
	A1	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)
	A1	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)
	A1	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 after survey 8): propoxur in B, A2, A1; MDA in A2 (every 10 weeks) and A1 (every 2 weeks in the wet season, 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 = 0.72).

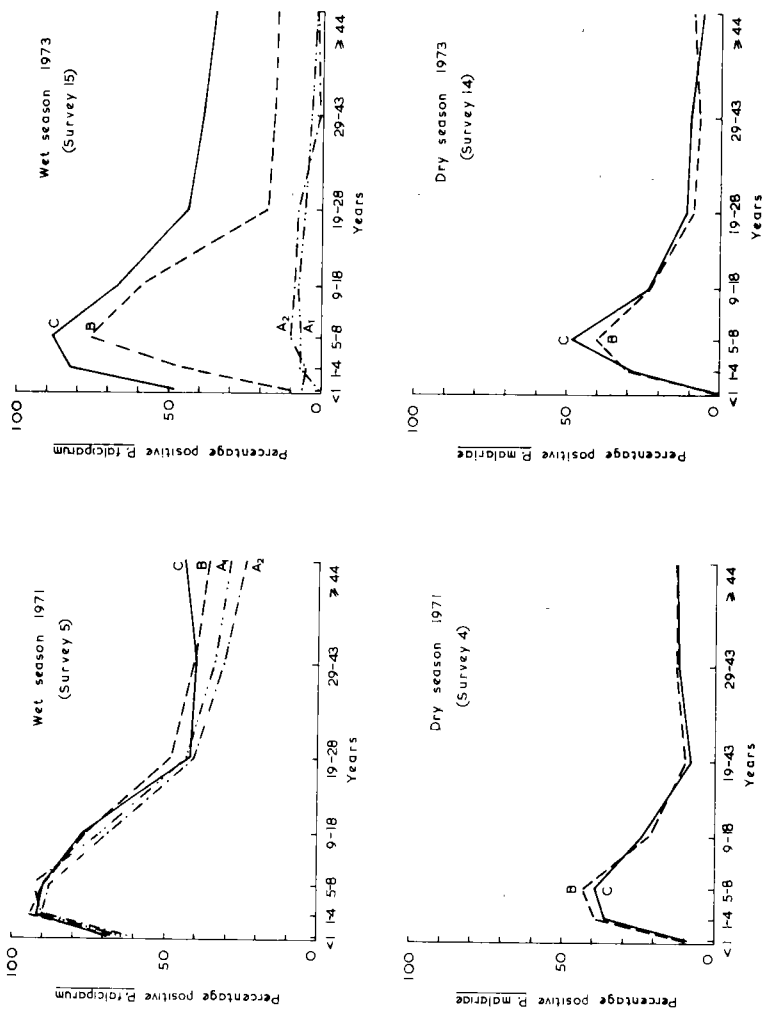
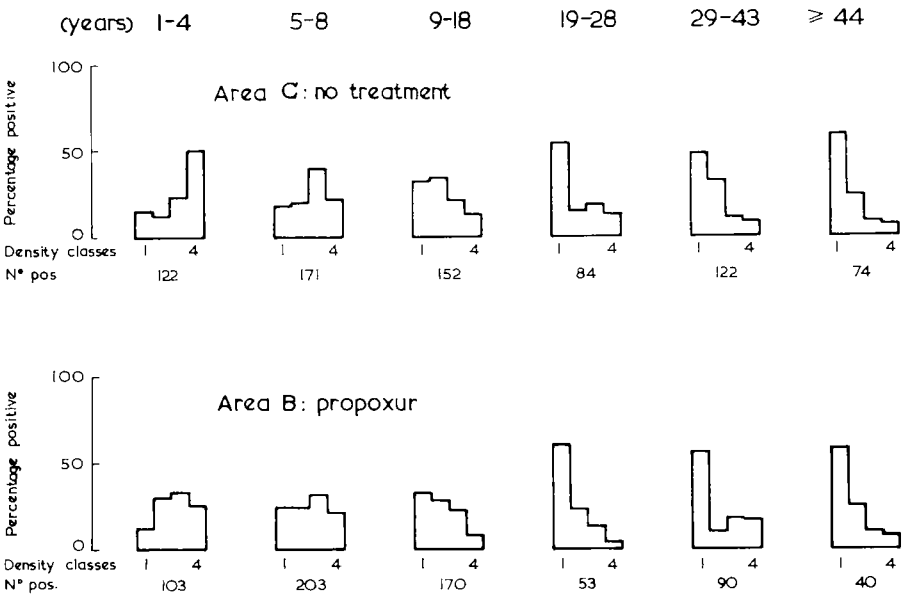


Fig. 38. Age-specific parasite rates for *P. falciparum* at surveys 5 (wet season, baseline period) and 15 (second wet season of intervention period) by treatment (A1, A2, B, C) and for *P. malariae* at surveys 4 (end of dry season, baseline period) and 14 (end of dry season, intervention period), by treatment (B, C)[a]

[a] Interventions: propoxur in A1, A2, B; MDA in A2 every 10 weeks; MDA in A1 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]



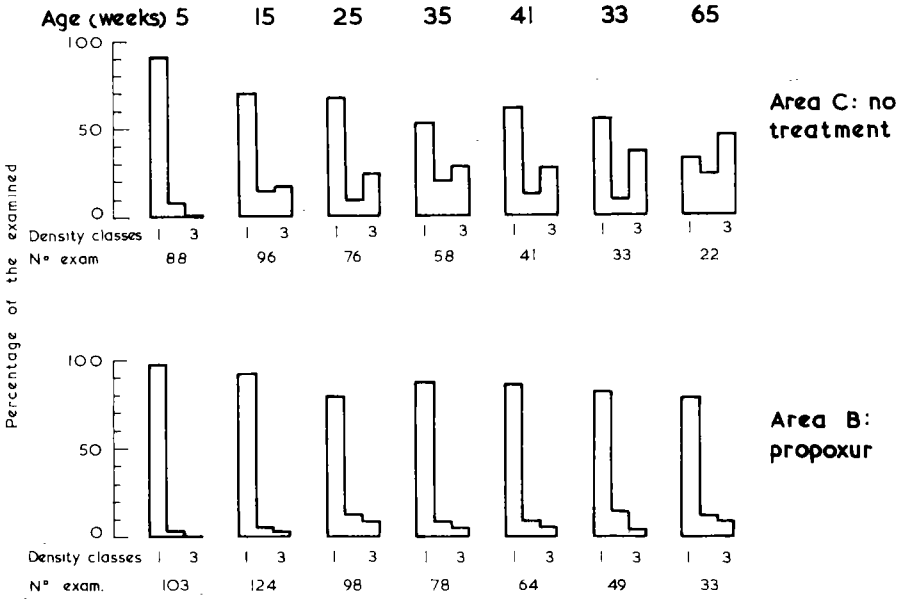
[a] Positives are distributed into classes 1–4 defined by the following upper limits: 4%, 16%, 64% and 100% of microscope fields positive on the thick film.

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 1–4 years to ≥44 years. In the age-group 1–4 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 1st, 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 (A1 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 (A1), 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 (A1 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 (A1), 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 population mobility has been described above (see p. 125). 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. falciparum* 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 10-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 $\geq$19 years.

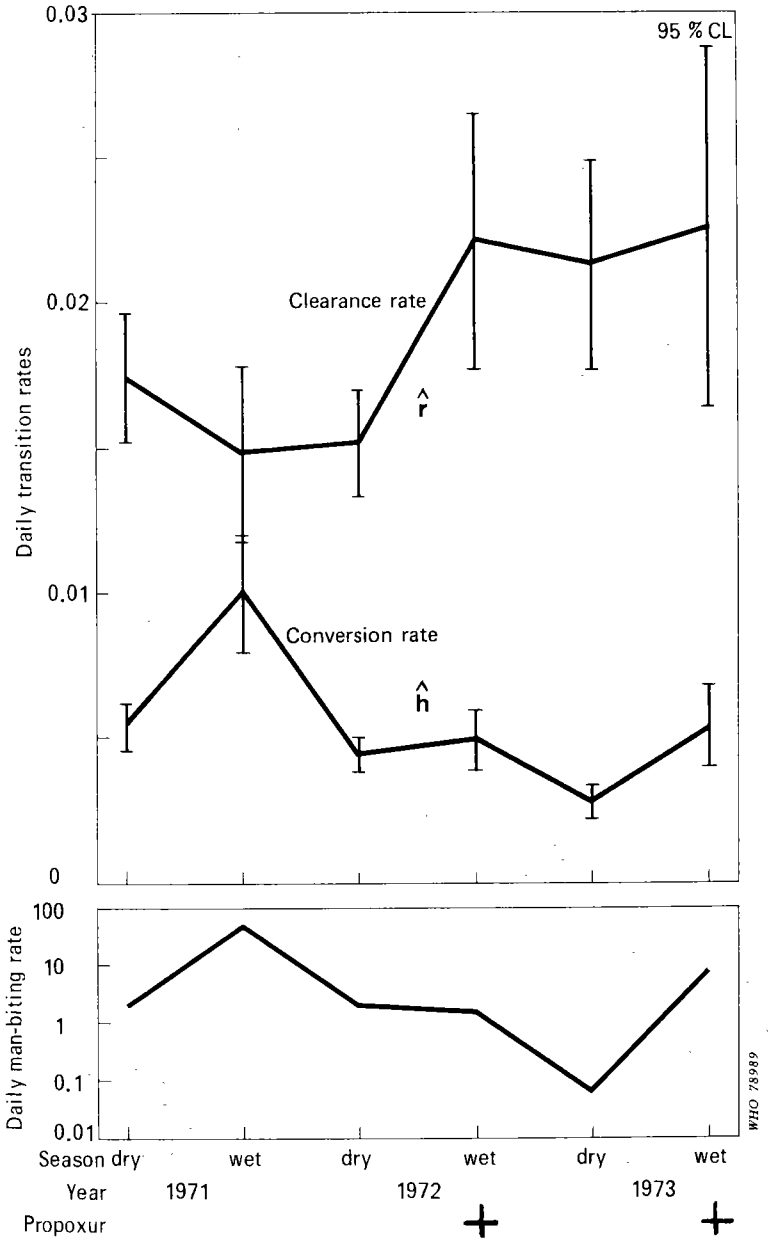
Table 19

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 (34/69)	—	—
		ICR/EIR	0.09	0.04	0.05		
B; 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 = $-\ln(1-p)/t$, where p = conversion ratio (given in parentheses).
[d] When no infant converted, an upper limit for the ICR was calculated as follows: assuming a Poisson distribution of conversions, the probability that n 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 (A1 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.

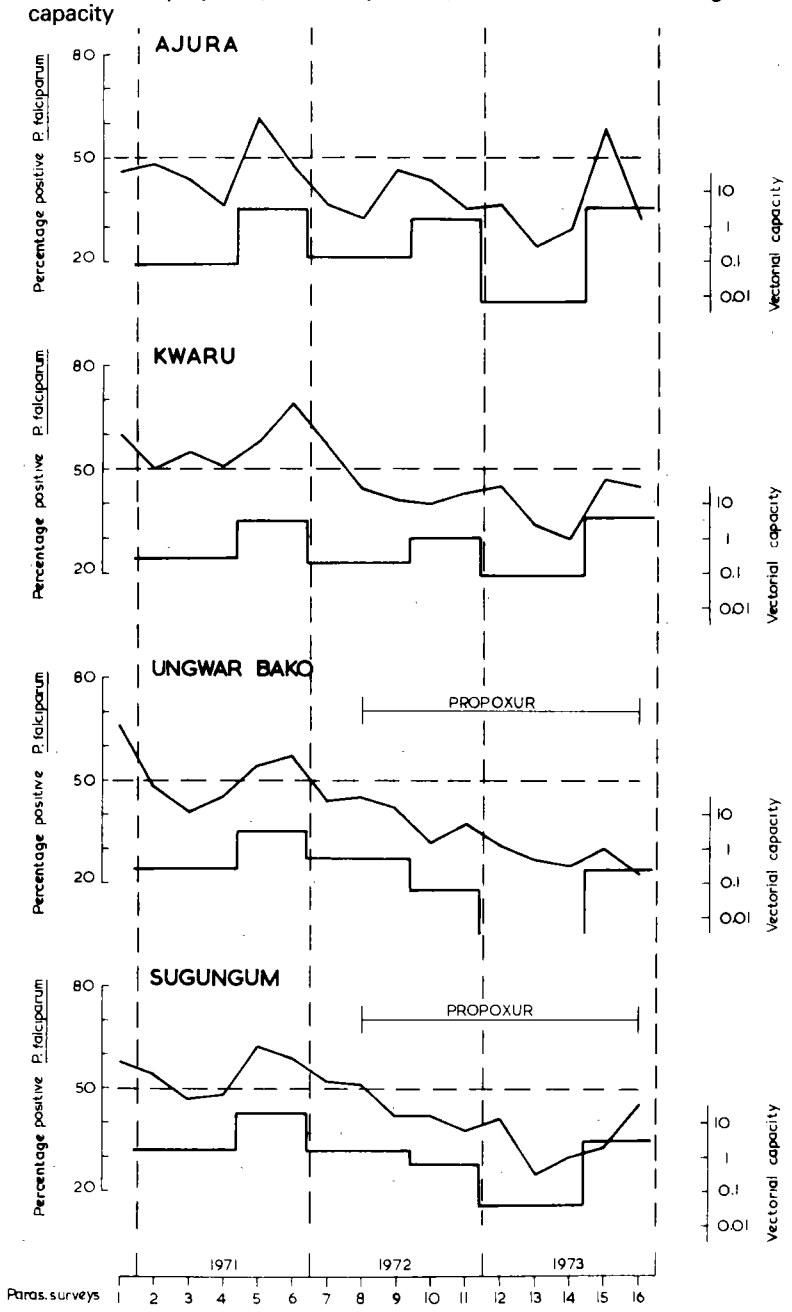
In the villages treated with propoxur alone, the relationship was clearly visible. In the 2 compact villages treated with propoxur alone and also investigated in detail, i.e., Ungwar Bako and Sugungum (see Table 1), the prevalence of *P. falciparum* before, during and after spraying were closely proportional 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, 5–8 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



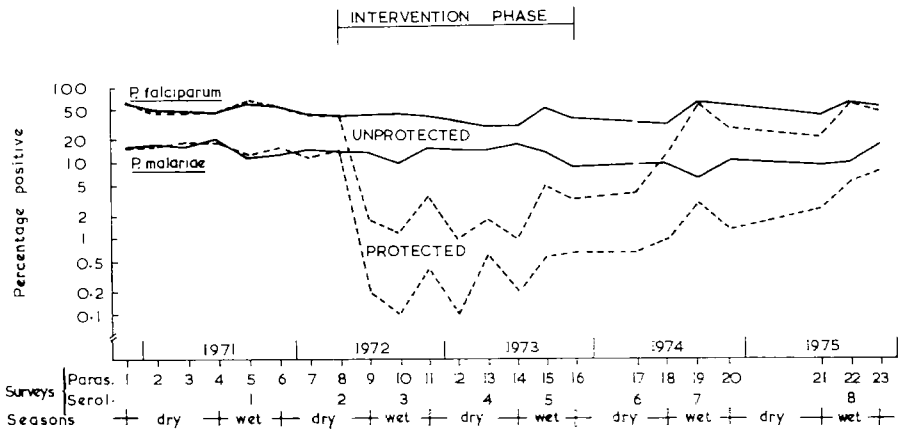
The Post-intervention Phase: The Resurgence of Malaria

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 A1), 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. malariae* 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 (1–23) 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

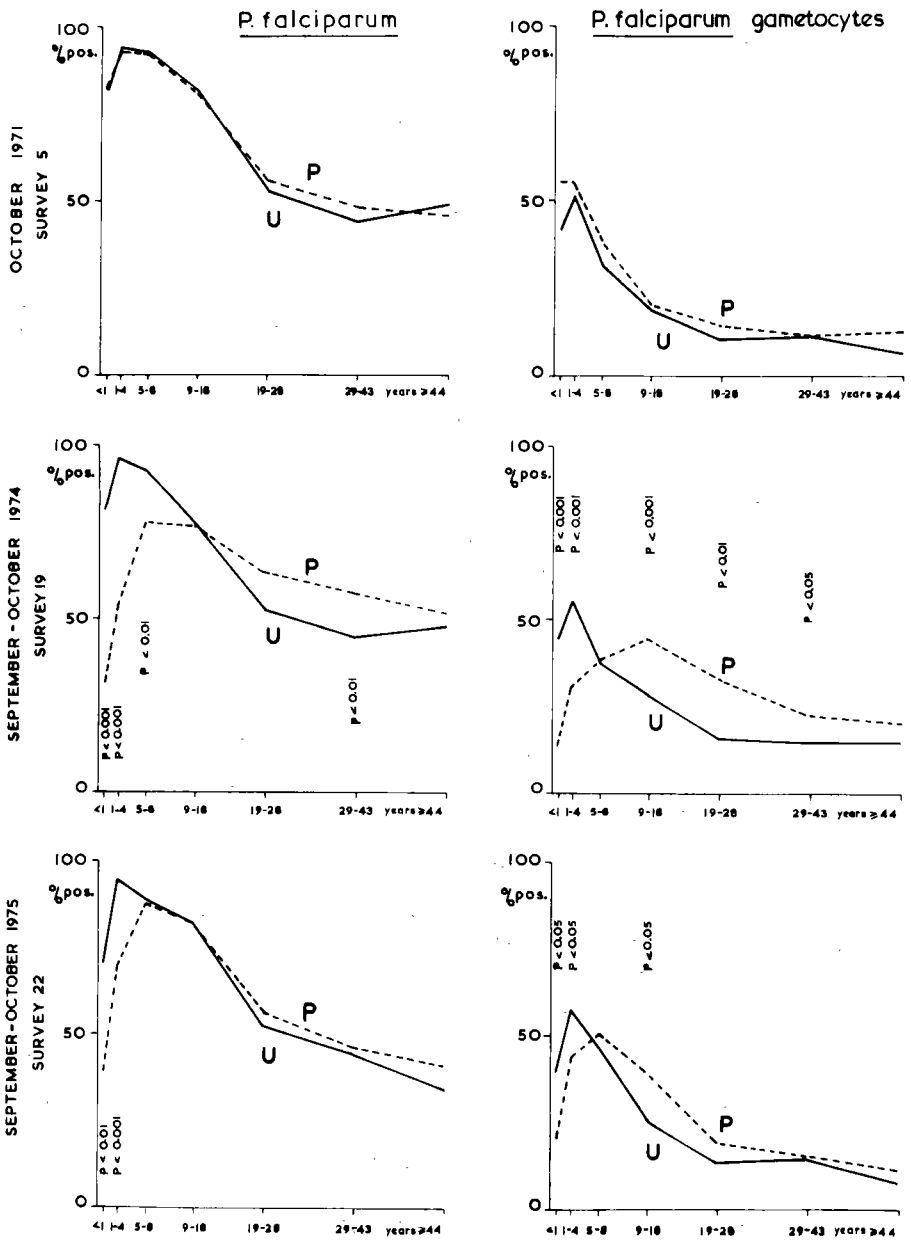
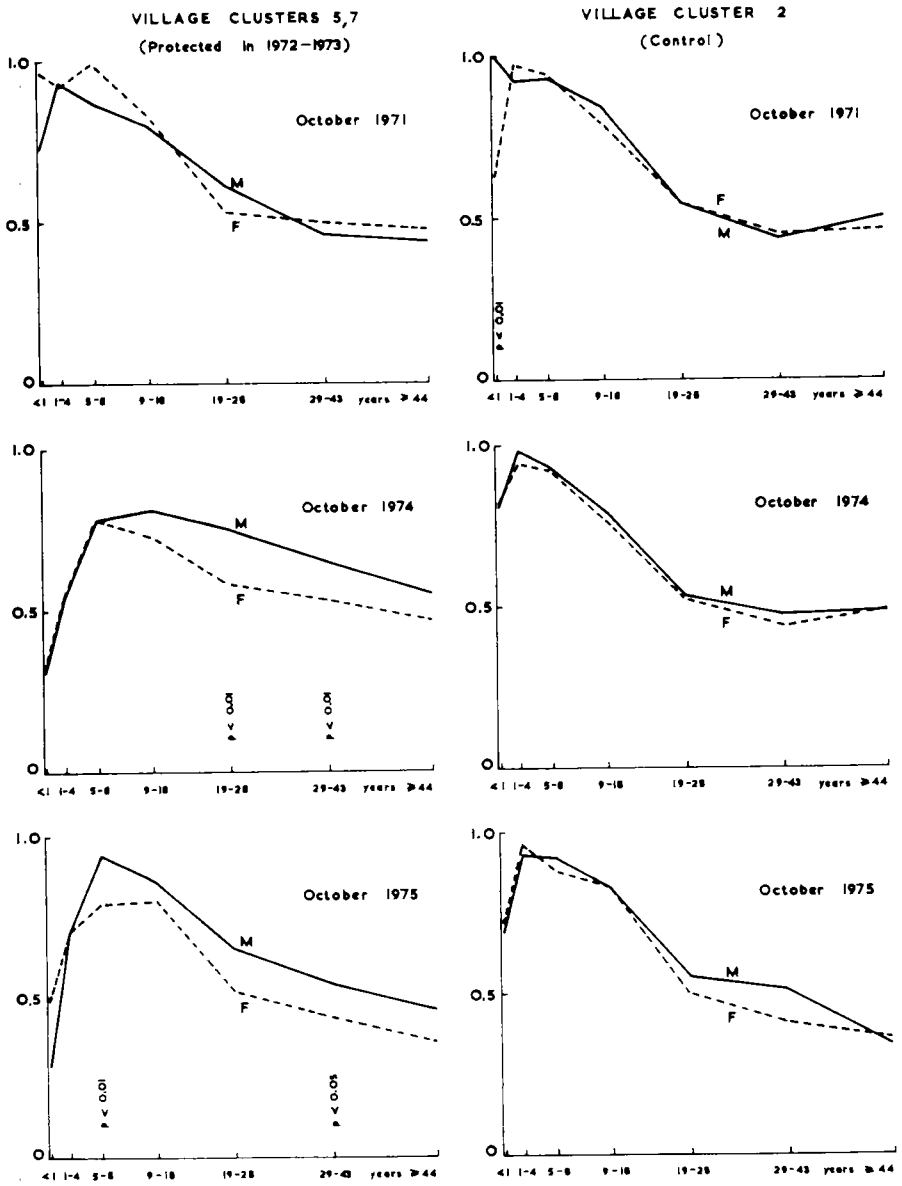


Fig. 45. Proportion of persons positive for *P. falciparum* by age and sex in the wet seasons of 1971, 1974 and 1975 in the unprotected population and the population protected in 1972-73 by propoxur and high-frequency MDA



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 1–8 and at surveys 18–23, in the same persons

Age at survey 1 (years)	Village cluster No. 2 (comparison)			Village clusters No. 5 & 7 (A1)		
	N^a	*r*(*P.f.*)	r(*P.f.* gam.)	N^a	*r*(P.f.)	*r*(*P.f.* gam.)
<1	9	+0.707	+0.444	11	+0.329	+0.482
1–4	37	+0.585	+0.686***	52	+0.157	+0.446*
5–8	44	+0.515***	+0.536*	40	+0.210	+0.224
9–18	24	+0.835**	+0.350	34	+0.500***	+0.341
19–28	41	+0.663***	+0.457	47	+0.309**	+0.329
29–43	90	+0.553**	+0.602***	114	+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 (A1). 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 (A1), 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 the wet season of 1975, in the untreated population (control) and in the population previously protected by propoxur and high-frequency MDA

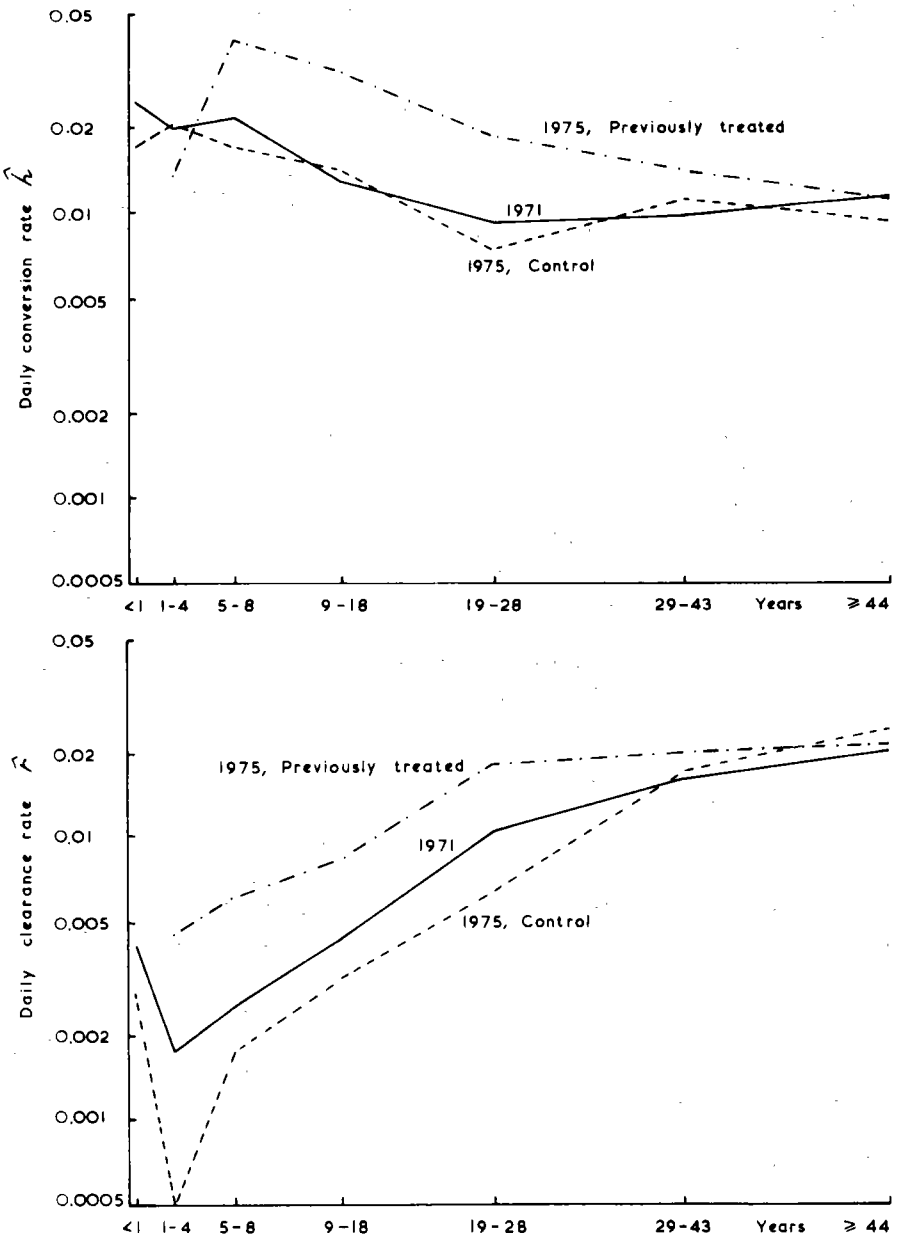
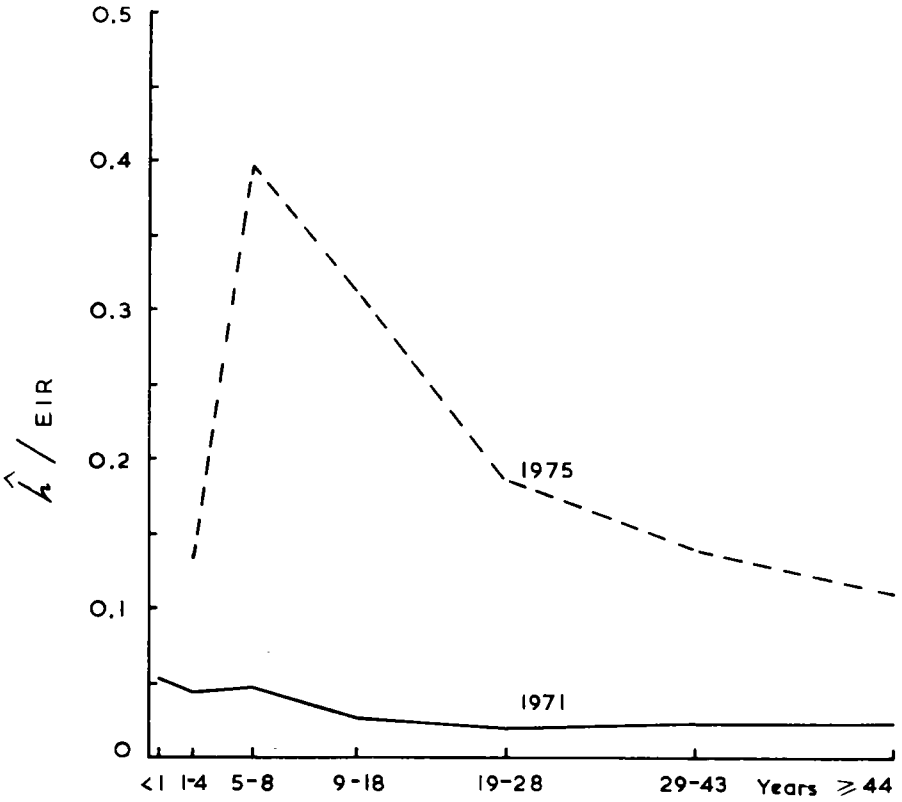


Fig. 47. Ratio between the daily conversion rate for *P. falciparum* (h) and the entomological inoculation rate (EIR) in the wet season of 1971 in the total population, and in the wet season of 1975 in the population previously protected by propoxur and high-frequency MDA



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 prob-

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 1–4 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 (1–4 years) (see p. 128 and Fig. 31). Parasites disappear faster from the blood of infants, either because of maternal antibody (the most likely) or because of some other difference (e.g., milk diet). Maternal 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 maternal 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 maternal 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 1–4 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 ($\hat{r}$) 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.[a]

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 18): $36.8/\{(43.3/60.4)60.1\} = 0.85$; 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 prevalence. 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-

lence 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 A1)

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 mobility, 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 maternal antibodies, they were apparently not protected against acquiring the infection. Maternal 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 1–8 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 markedly 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 model can be formalized by the hypergeometric probability law (*116*). The conceptual model 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 1925-1926 (*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 1925-1926 and of thick films in 1931, 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 1–4 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.

Maternal 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.

Chapter Six

IMMUNOLOGY[a]

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

Material and Methods

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

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

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

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

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

The precipitating antibodies against *P. falciparum* antigen (Ouchterlony test) were detected by a modification of the method of McGregor et al. (*110*). The number of bands of precipitation was recorded. Antigen 672, used for serological surveys 1–5, was procured from the heavily infected placental blood of a Nigerian (Ibadan) woman; antigen P114, used for serological surveys 6–8 was procured from similarly infected placental blood of a Gambian woman. Methodological aspects have been discussed in Technical Notes (*34*, *36*). Occasionally bands of precipitation were observed between adjacent sera on the plates, indicating the presence of soluble antigens; such "S" antigens were first reported from the Gambia (*111*); the cases detected in Garki are reported in a Technical Note (*37*).

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

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

Table 21

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

Parasite species	Test		Definition of "positive"	Prevalence by age-group (in years)[a] 1–4	5–8	9–18	19–28	29–43	≥44
P. falciparum (*P.f.*)	Parasitological surveys No. 1–8		≥1 film positive for *P.f.*	0.98 (272)	0.99 (359)	0.95 (395)	0.76 (392)	0.78 (731)	0.78 (458)
	Serological survey No. 2	Precipitin	≥1 band	0.92 (265)	0.99 (358)	1.00 (395)	0.99 (391)	1.00 (731)	1.00 (454)
		IFA	≥20	1.00 (198)	1.00 (186)	1.00 (123)	1.00 (53)	1.00 (77)	1.00 (51)
		IHA	≥16	0.91 (209)	0.94 (309)	0.96 (369)	0.98 (338)	0.98 (628)	0.99 (367)
		IHA	≥32	0.87	0.91	0.93	0.97	0.98	0.99
P. malariae (*P.m.*)	Parasitological surveys No. 1–8		≥1 film positive for *P.m.*	0.53	0.81	0.61	0.26	0.30	0.30
	Serological survey No. 2	IFA	≥20	0.95	1.00	1.00	1.00	1.00	1.00

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

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

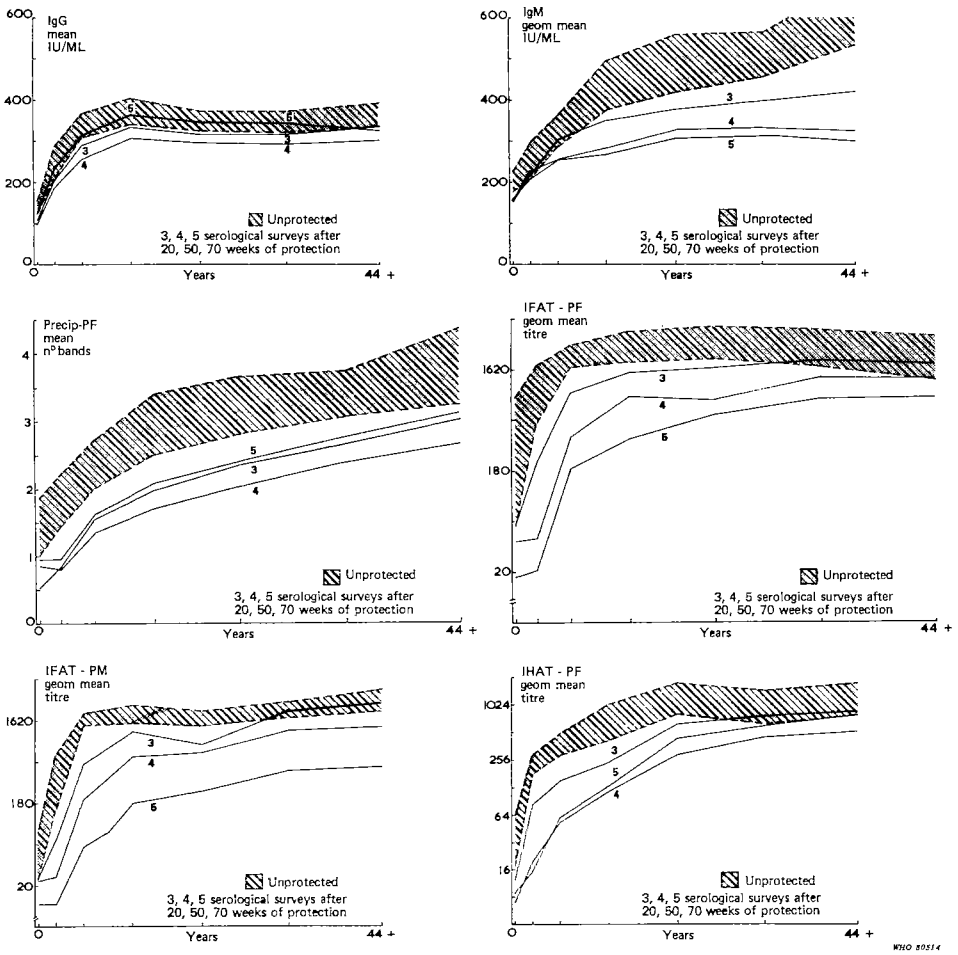
Measurement precision within a survey was monitored by repeated testing either of specimens collected or of standards; comparability between surveys was monitored by the re-examination of specimens from a given survey at subsequent surveys. Some moderate changes in sensitivity were observed, which should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

Serological Results by Age, Survey and Treatment

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

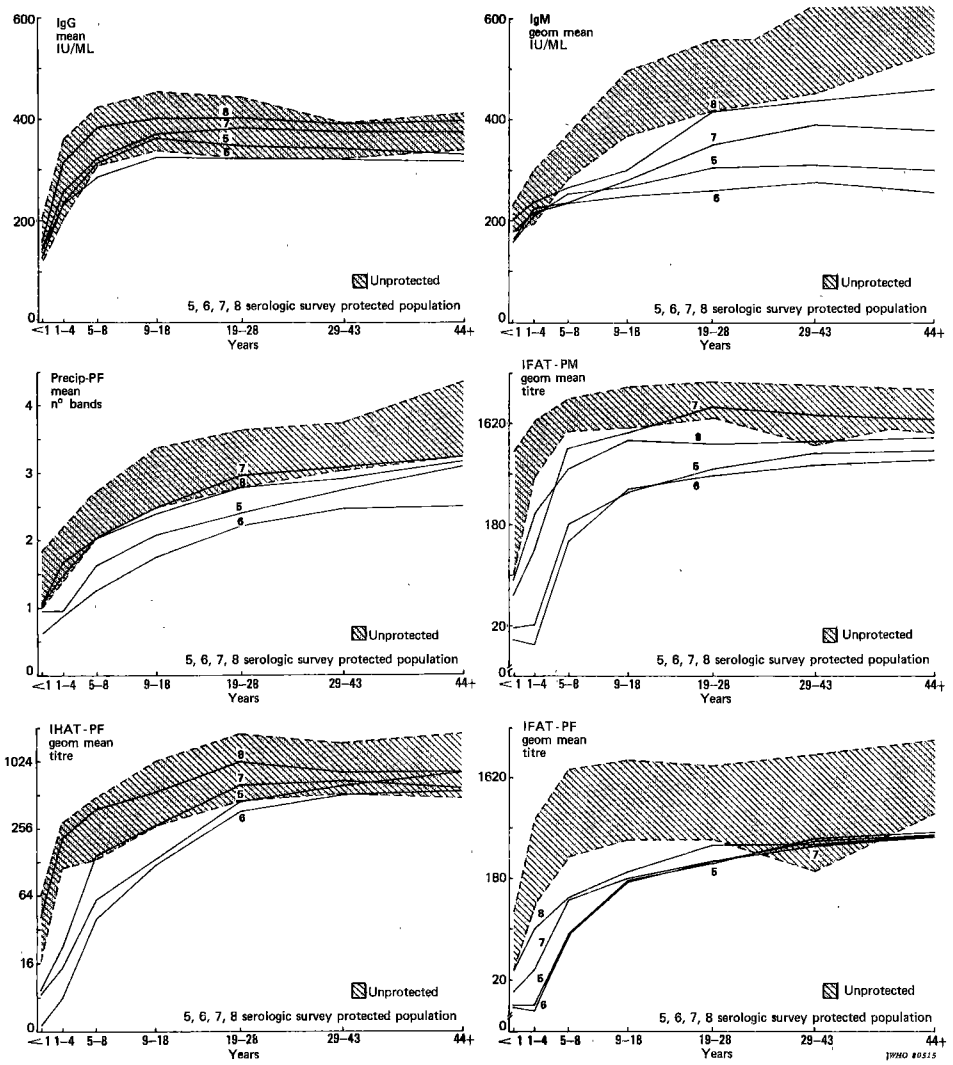
The results for each test are presented in this section and are illustrated in a series of figures and 1 table. Table 21 shows the proportion of the population aged 1 year or older that was positive by each specific serological test before intervention. The numbers examined given in the table are typical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

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



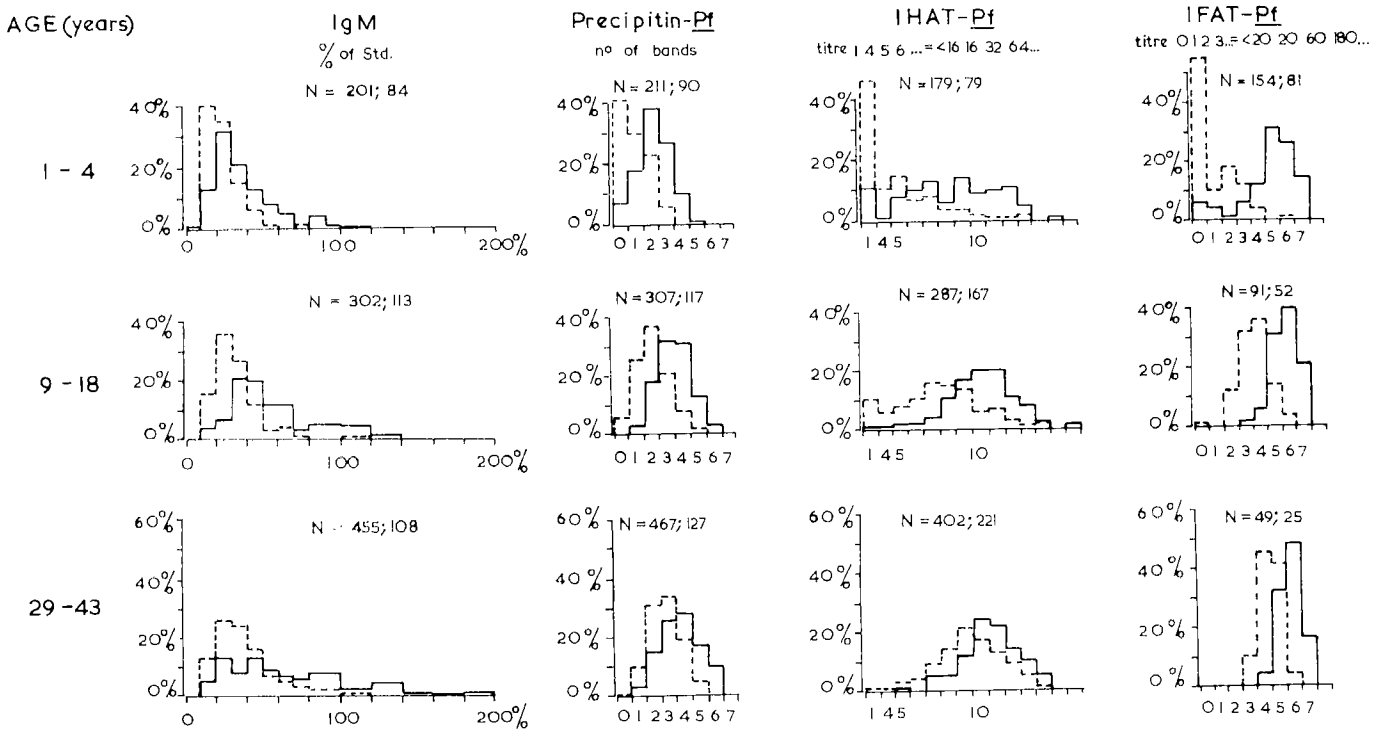
lations, at the end of the intervention period (i.e., at serological survey 5, in the wet season of 1973) and at the end of the follow-up period (i.e., serological survey 8, in the wet season of 1975); the 2 remaining tests—IgG and IFA-Pm or IFA-Pb—are excluded because the first showed very little difference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

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



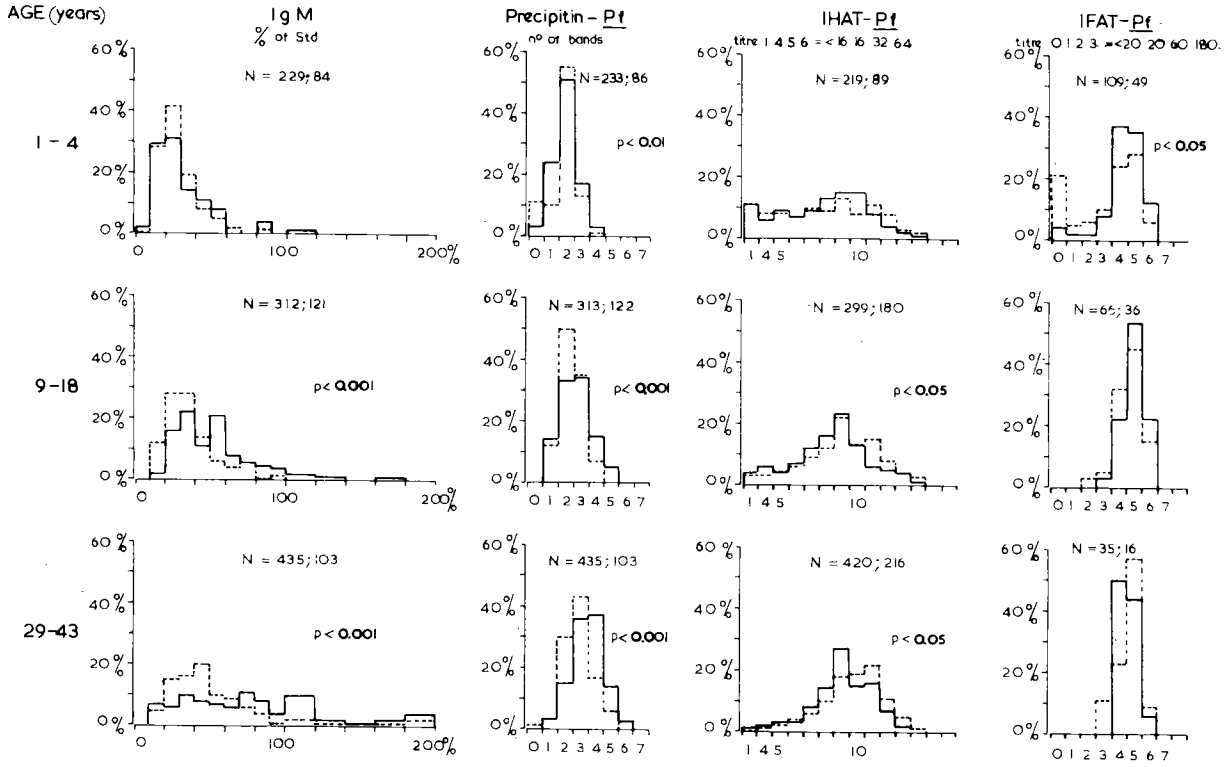
second behaved nearly like the IFA-Pf. Figure 52 shows, by survey, the age-adjusted average and 2 selected age-specific averages of each test in the protected population, expressed as a fraction of the concurrent result in the unprotected population.

Fig. 50. Distribution of IgM levels and results of the precipitin-*P. falciparum*, IHA-*P. falciparum* and IFA-*P. falciparum* tests in the protected (broken lines) and unprotected (solid lines) populations in 3 age-groups, after $1\frac{1}{2}$ years of protection (serological survey 5)[a]



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

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



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

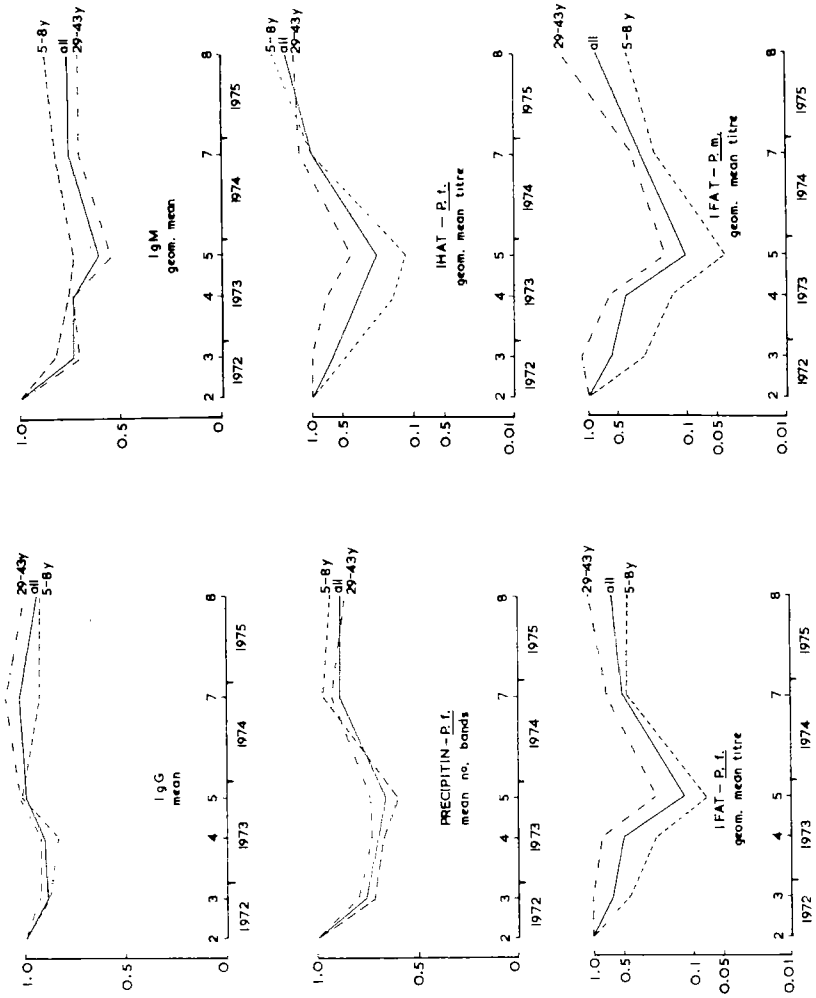


Fig. 52. Serological results during the intervention phase (1972-1973) and 1 and 2 years after the end of the intervention phase, expressed as a fraction of the concurrent results in the untreated control population

Immunoglobulin G

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

Immunoglobulin M

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

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

Precipitin (Ouchterlony) test—*P. falciparum*

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

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

After the end of the intervention phase, the average number of bands of precipitation in the previously protected population showed a time-trend similar to the one shown by the level of IgM, except that it did not increase between surveys 7 and 8 (Fig. 49 and 52). As already mentioned, there was a decrease in the sensitivity (number of bands detected in a given serum) of the test between surveys 7 and 8. At the last survey, the average number of bands was still less than in the unprotected population in all age-groups except the 5–8 years (Fig. 51). In terms of the proportion positive (i.e., having at least 1 band of precipitation) there was still a significant difference in the 3 youngest age-groups: 0.603 versus 0.875 in infants ($p<0.001$); 0.837 versus 0.965 in the 1–4 years old ($p<0.001$); and 0.977 versus 1.000 in the 5-8 years old ($p<0.05$).

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

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

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

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

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

In the unprotected population 100% were positive at or before the age of 5 years (Table 21); the IFA-Pm antibody titres rose with age more slowly than for *P. falciparum*. During the intervention phase, the titres decreased progressively in all age-groups following a pattern similar to that of the IFA-Pf antibody titres (Fig. 48), and the number of "negative" sera increased in the 0–43-year age-groups. After 70 weeks of intervention, almost all sera were negative in the age-groups <1 and 1–4 years and more sera with lower titres were observed in the 5–8-year age-group.

During the post-intervention phase, in the previously protected population, there was no decline in average titre between surveys 5 and 6 (Fig. 49), although there was a decline in test sensitivity (titre of a standard positive serum). There was a very slow increase from surveys 6 to 7 to 8 (Fig. 49 and 52). During the same period, there was a further apparent decrease in test sensitivity and in the titres of the untreated control population. At the last survey, average titres were still lower than in the untreated control population, and the difference was significant at ages 1–18 years.

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

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

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

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

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

The Longitudinal Study of Infants

Definition of 2 populations of infants

The serological results among infants born after parasitological survey 1 were used to establish their average serological history. Because the surveys were conducted at 10-week intervals, an infant was assumed to be 5, 15, 25, 35... weeks old, at the first, second, third, fourth ... parasitological surveys after birth. The following groups were compared:

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

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

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

Among the unprotected infants, the subgroup of those who were born after parasitological survey 8, and who were therefore the exact contemporaries of the protected infants, was compared with the total: differences were neither significant nor systematic, with the possible exception of the IFA-*P. malariae* test for which the 2 sets of results will be presented. Starting with parasitological survey 8, the protection in follow-up clusters No. 5 and 7 (area A1) had consisted of spraying with propoxur and mass drug administration; infants were excluded from drug treatment until found positive, which happened in a very small number of infants, mostly at low densities and without apparent effect on the serological averages. The parasitological conversion rates for *P. falciparum* and *P. malariae* were estimated for the 2 infant populations, and the corresponding cumulative prevalence curves were calculated by the formula

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

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

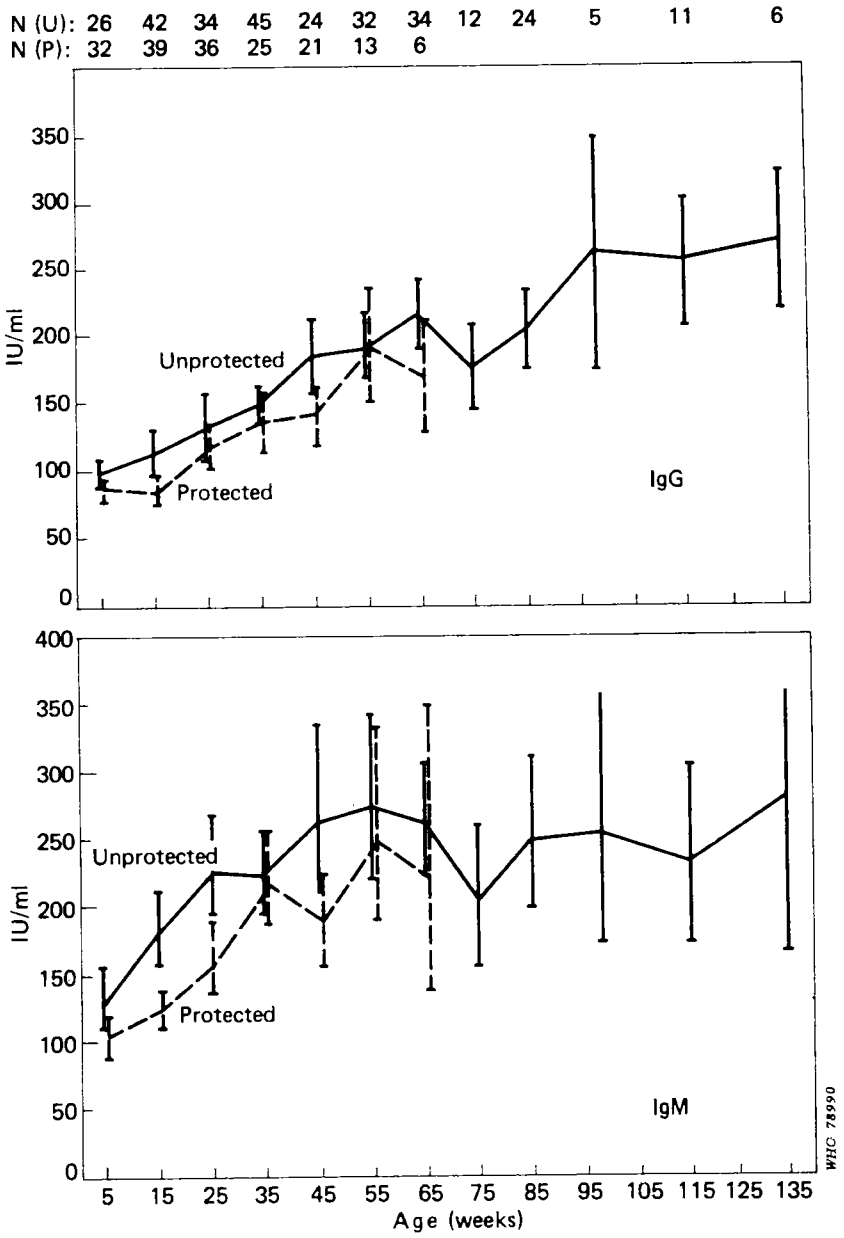
Immunoglobulin levels

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

Malaria antibody levels

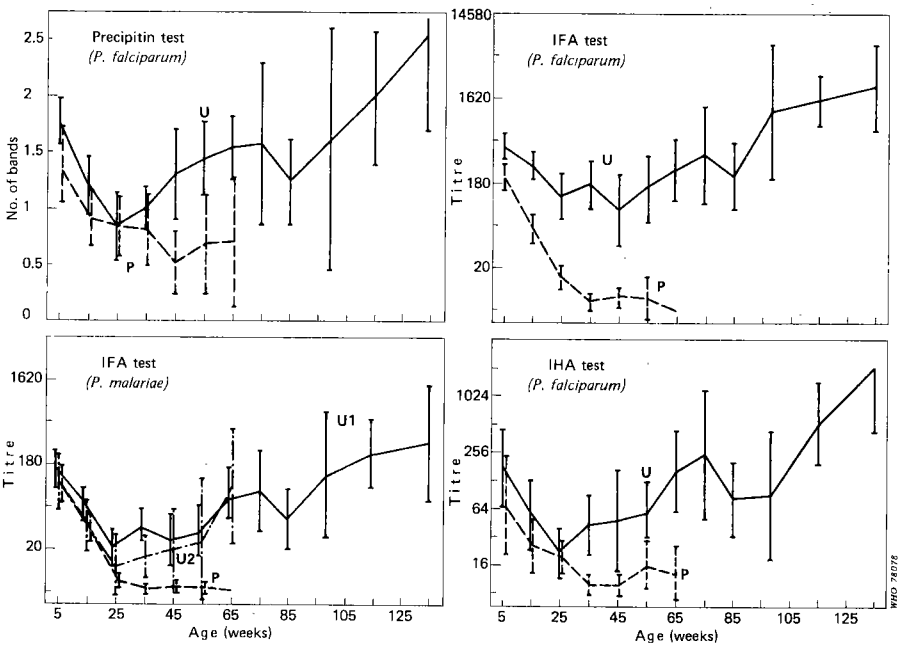
Figure 54 shows the mean antibody levels, by age, in the 2 populations of infants; for computing the geometric means shown for the IFA and IHA titres, an IFA titre of <20 was converted to 6.7 and an IHA titre of <16 was converted to 8. In the unprotected infants the levels of malaria antibodies fell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

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



[a] N(U) = numbers examined in the unprotected population; N(P) = numbers examined in the protected population.

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



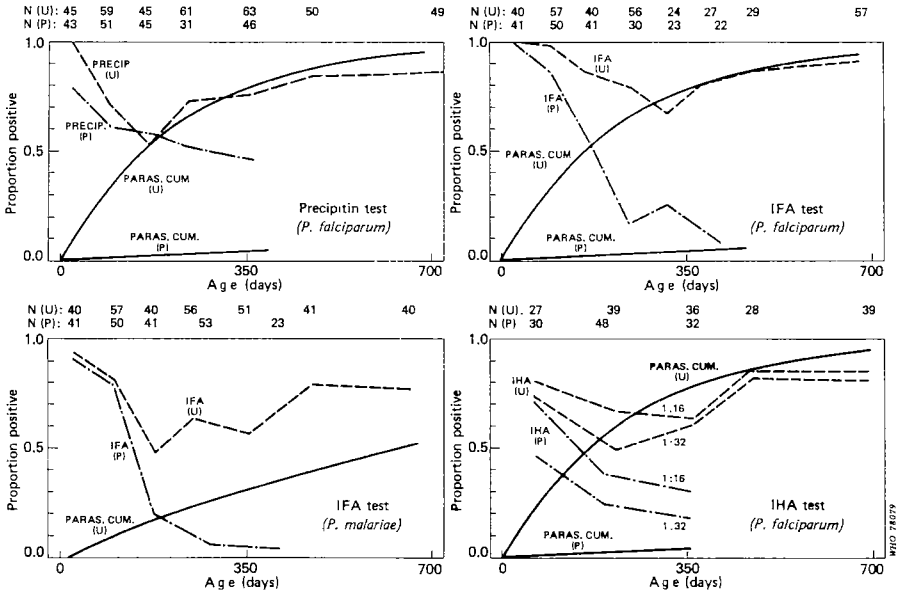
[a] U1 = total unprotected; U2 = unprotected born after onset of intervention phase. See Fig. 55 for the numbers examined in both populations.

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

Proportion positive by the serological tests

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

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



[a] N(U) = numbers examined in the unprotected population; N(P) = numbers examined in the protected population; paras.cum. = cumulative parasite prevalence.

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

In the unprotected infants, the proportion positive for *P. falciparum* by the precipitin test decreased from 1.00 at the age of 5 weeks, to about 0.50 at the age of 25 weeks (175 days), after which it increased; between the ages of 175 and 700 days, the proportion positive was very close to the cumulative prevalence expected from the parasitological infant conversion rate estimated in the same population. In the infants protected from birth, the proportion positive was significantly lower in the youngest group (χ^2 = 8.34) and continued to decrease beyond the age of 175 days.

The proportion positive for *P. falciparum* antibodies by the IFA test decreased in the unprotected infants from 1.00 at the age of 5 weeks to 0.67 at the age of 45 weeks (315 days), after which it increased and was very close to the calculated cumulative prevalence of the infection in the same population. In the infants protected from birth, it was also 1.00 at

Table 22

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

Village clusters		IgG[a]	IgM[a]	Precipitin-Pf[b]	IFA-Pf[c]	IFA-Pm[c]	IHA-Pf[d]
2 (control)	$\bar{x}$	25.2	26.2	1.89	3.44	2.44	6.11
	$S_{\bar{x}}$	5.1	9.1	0.26	0.29	0.18	0.81
	N	9	9	9	9	9	9
5 & 7 (previously protected)	$\bar{x}$	18.1	12.3	1.68	2.78	1.50	6.29
	$S_{\bar{x}}$	1.8	1.3	0.23	0.33	0.20	0.58
	N	16	16	19	18	18	17
		n.s.	n.s.	n.s.	n.s.	$p<0.01$	n.s.

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

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

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

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

Serological results in the newborn in the post-intervention follow-up period

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

Variation by Sex

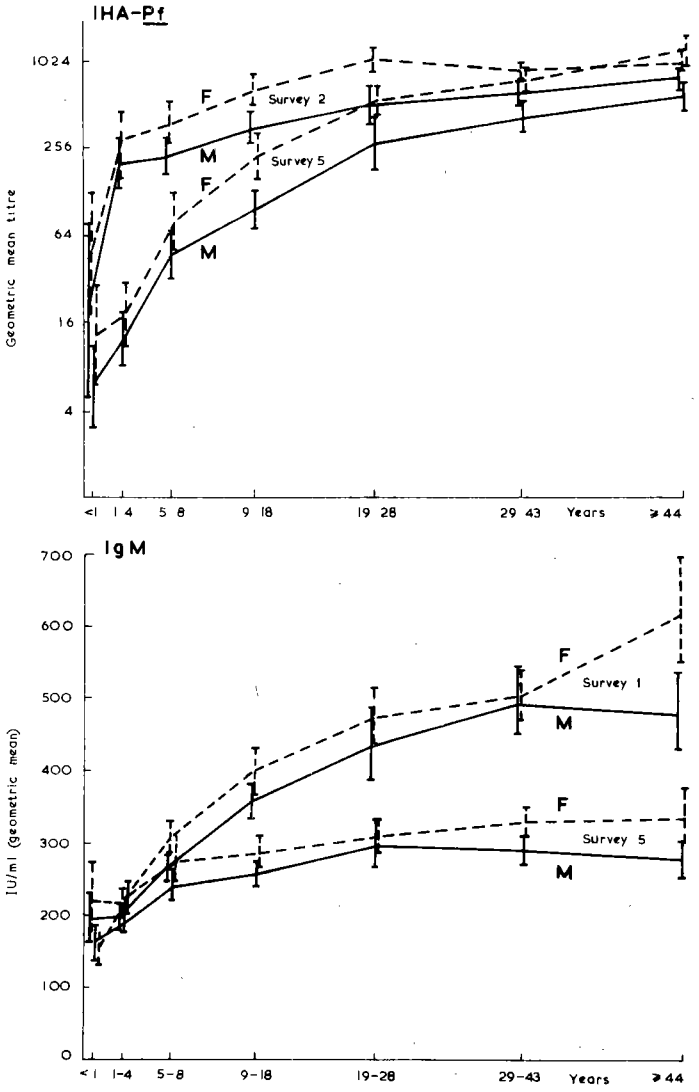
The systematic comparison between the serological results for males and females showed a consistent difference for 2 of the 6 tests: the females had on the average a higher level of IgM and a higher titre of IHA antibodies against *P. falciparum*, both before and after intervention with propoxur and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

Relationship between the Results of the Same Serological Test in the Same Person at Different Surveys

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

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

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



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

Table 23

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

Test	Surveys 2 and 5				Surveys 2 and 8			
	Population				Population			
	Unprotected		Protected		Unprotected		Protected	
	r	N^b	r	N^b	r	N^b	r	N^b
IgG	0.224	460	0.017[c]	1 218	0.052[c]	366	0.041[c]	1 028
IgM	0.676	458	0.630	1 217	0.668	365	0.661	1 027
Precipitin-Pf	0.287	512	0.330	1 285	0.328	371	0.354	1 068
IFA-Pf	0.217	218	0.416	361	0.373	116	0.186	206
IFA-Pm	0.300	218	0.357	361	0.261	116	0.305	206
IHA-Pf	0.667	623	0.686	962	0.618	557	0.514	859

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

[b] N = number examined

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

In summary, the position of a person with respect to the average for his age-group was relatively stable over a period of at least 3½ years for IgM and IHA-Pf, and to a lesser extent also for the precipitin-Pf, IFA-Pf and IFA-Pm. This relative stability was little affected by the intervening period of marked and relatively rapid decrease of the average results during the intervention phase, nor by the subsequent period of increase during the post-intervention phase. For IgM, the strength of the correlation between surveys increased with increasing age, suggesting that the variation within persons decreased with increasing age, while the variation between persons increased (see Fig. 50).

Relationship between the Results of Different Serological Tests in the Same Person at the Same Survey

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

Table 24

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

Pair of tests	Population			
	Unprotected		Protected	
	r	N	r	N
IgG — IgM	0.131	561	0.113	1 710
IgG — Precipitin-Pf	0.141	560	-0.040	1 712
IgG — IFA-Pf	0.223[b]	285	-0.105	527
IgG — IFA-Pm	0.256[b]	285	-0.116	527
IgG — IHA-Pf	0.235[b]	504	0.005	1 526
IgM — Precipitin-Pf	0.278[b]	558	0.170	1 710
IgM — IFA-Pf	0.091	284	0.006	527
IgM — IFA-Pm	0.077	284	-0.044	527
IgM — IHA-Pf	0.270[b]	502	0.175	1 524
Precipitin-Pf — IFA-Pf	0.337[b]	298	0.346[b]	551
Precipitin-Pf — IFA-Pm	0.319[b]	298	0.261[b]	551
Precipitin-Pf — IHA-Pf	0.411[b]	544	0.411[b]	1 537
IFA-Pf — IFA-Pm	0.541[b]	302	0.621[b]	551
IFA-Pf — IHA-Pf	0.445[b]	266	0.539[b]	461
IFA-Pm — IHA-Pf	0.265[b]	266	0.353[b]	461

[a] Adjustment for age consisted in transformation of each result into standard deviations above or below the mean for the corresponding age-group;
[b] Significant at the 1% level.

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

Relationship between Parasitology and Serology

Method of analysis of the baseline data

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

Table 25

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

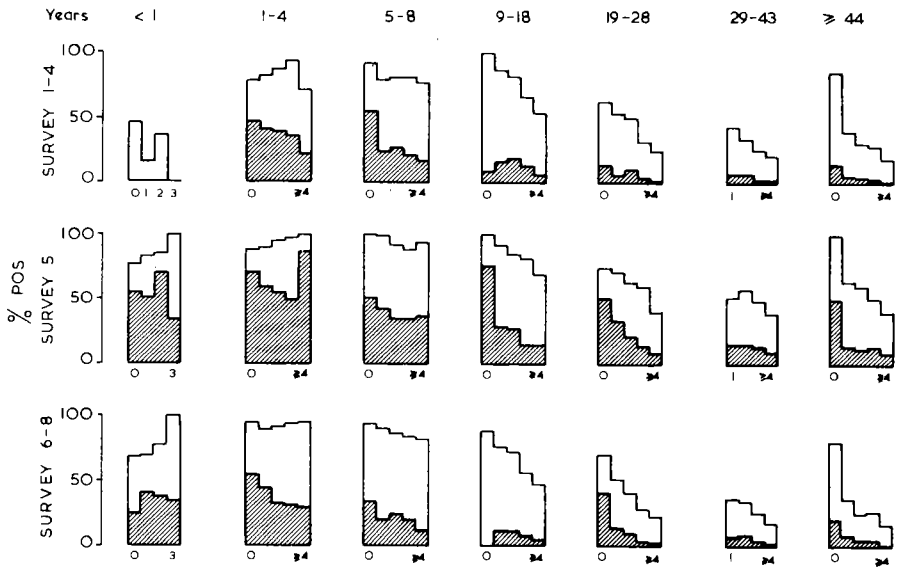
Survey No.	Test	Result	<1 year	1–4 years	5–8 years	9–18 years	19–28 years	29–43 years	≥44 years
5	Precipitin-*P. falciparum* test (No. of bands)	0	13	16	6	4	4	0	2
		1	42	76	71	39	18	27	14
		2	13	109	166	117	71	130	51
		3	3	65	120	163	171	286	156
		≥4	0	7	36	73	133	318	236
5	IHAT-*P. knowlesi* (titre)	<16	4	21	70	43	28	51	26
		16-32	7	65	110	128	92	136	90
		64-128	8	36	57	64	80	151	66
		≥256	5	25	41	66	108	247	154
8	IHAT-*P. falciparum* (titre)	<16	11	19	19	14	6	10	3
		16-128	12	65	78	74	41	49	29
		256-1 024	9	84	159	200	173	362	199
		≥2 048	2	41	53	81	118	207	136
8	IFAT-*P. malariae* (titre)	<20	23	9	0	0	0	0	0
		20-180	48	58	16	2	1	0	0
		540	9	29	17	17	10	7	3
		1 620	3	56	81	54	21	29	20
		≥4 860	0	46	72	50	21	41	28
5	IgM, % IR 0172 Standard	1 = 1-19%	18	86	67	22	22	54	27
		2 = 20-39%	23	110	209	168	120	211	133
		3 = 40-69%	3	17	81	134	146	222	109
		4 = ≥70%	2	3	28	39	98	243	164

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

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

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

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



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

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

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

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

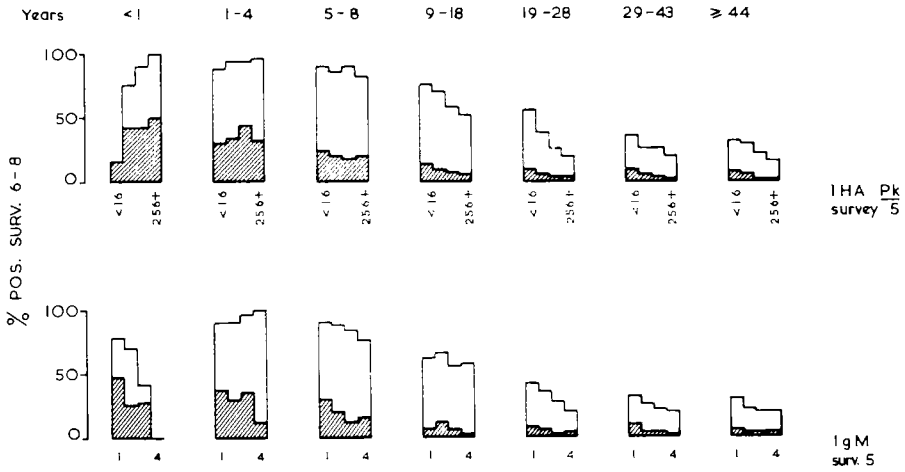
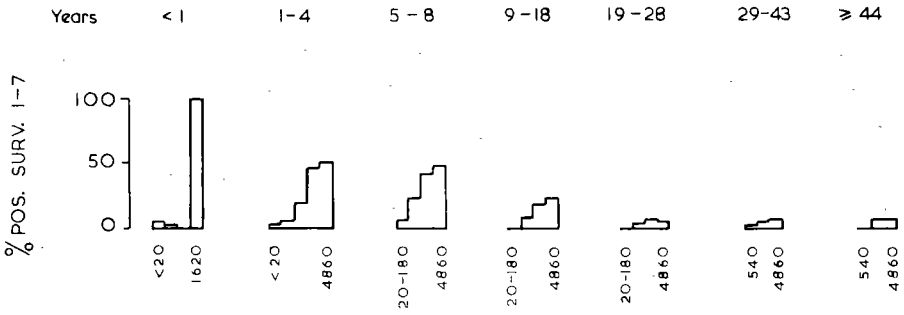


Fig. 59. *P. malariae* parasite rate at parasitological surveys 1–7, according to age and titre (<20, 20–180, 540, 1620, ≥4860) of the IFA-*P. malariae* test at parasitological survey 8



Pk titre and the *P. falciparum* gametocyte rate, in the <1-year age-group and a negative association at ages ≥5 years. The same relationships were again observed before and during the serological survey. A high titre is associated with decreased gametocytaemia, and this association may precede the one with decreased parasitaemia. The association of the IHA-Pk titre with *P. falciparum* trophozoite and gametocyte densities shows the same trend with somewhat more irregularities. The associations between the IHA-Pf titre at the second serological survey and *P. falciparum* parasitaemia are very similar to those just described for the IHA-Pk test at the first survey.

The IFA test and homologous parasitaemia

The associations in this test are less obvious than above. The numbers examined were smaller and several strata were empty or nearly so. In the case of the IFA-Pf test there was a positive association between the test result and concurrent *P. falciparum* parasitaemia up to the age of 5 years and no association for the older ages. In the case of the IFA-Pm test there was a positive association between the test result and *P. malariae* parasitaemia in all age-groups; strong up to the age of 19 years, it was weaker thereafter. This association is very clear between the IFA-Pm titre at parasitological survey 8 and the *P. malariae* parasite rate at surveys 1–7 (Fig. 59), and also between the IFA-Pm titre at survey 8 and the *P. malariae* parasite density at surveys 1–7.

Immunoglobulin levels and parasitaemia

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

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

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

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

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

Discussion

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

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

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

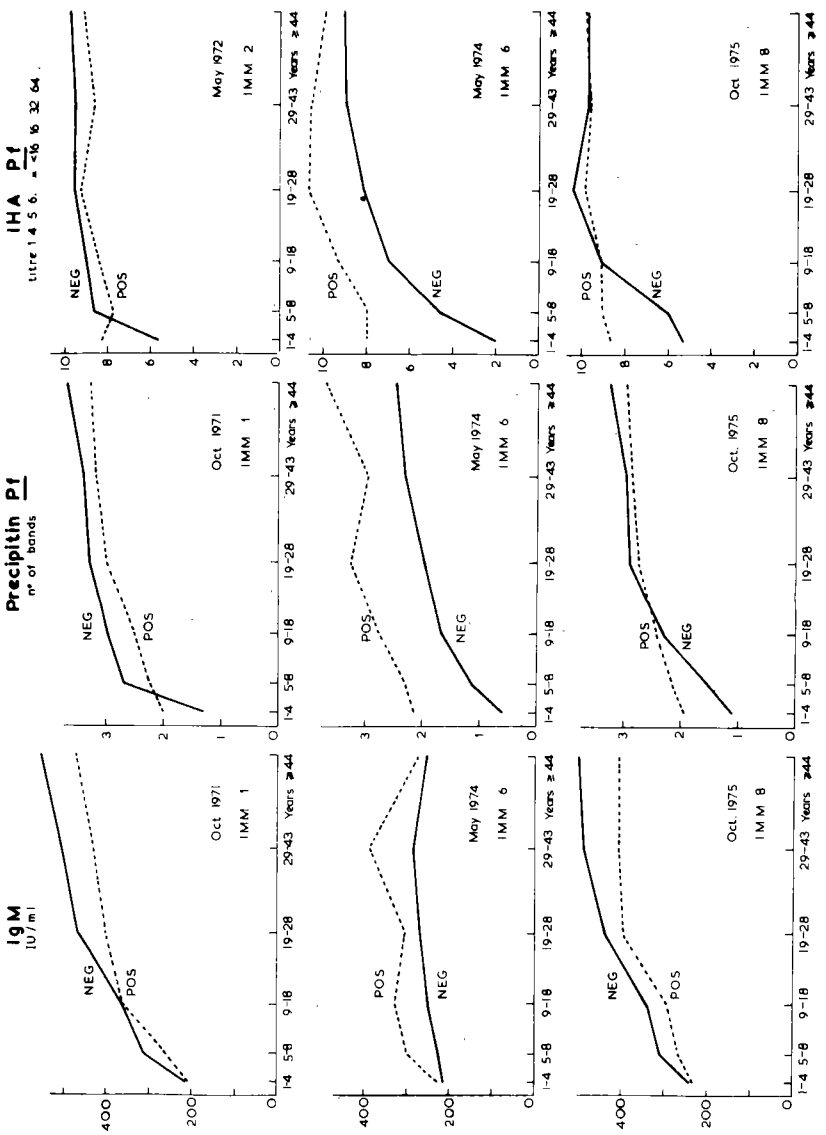


Fig. 60. Serology of parasitological positives and negatives (*P. falciparum*), before intervention (IMM 1–2), after 1½ years of intervention plus one dry season (IMM 6), and in the second wet season after termination of the intervention (IMM 8)

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

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

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

The average level of IgG reached its adult plateau approximately by the age of 5 years in both areas (*140*). It seems, however, that the increase, as a function of age, was steeper in Garki and that the plateau was in fact reached sooner. The average level of the adult plateau in Keneba oscillated between 50% and 60% of the working standard (concentration: 4750mg IgG/100ml), which corresponded to 295-354 IU/ml and was nearly identical to the range of 322-353IU/ml observed for the $\geq$5-years age-groups in Garki at the first serological survey (the conversion factor used was 1IU = 80.4μg IgG). For comparison, sera from normal British adults, studied concurrently with the Gambian sera, had an average IgG level of 1200mg/100ml or 149 IU/ml, i.e., about half the Keneba or Garki level.

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

to an increased dispersion into high levels. The actual average level was probably higher in Garki: in Keneba the (arithmetic) average level of IgM at the age of 15 years was about 35% of the standard, or 260 IU/ml (1IU = 8.47μg IgM), while in Garki the (geometric) average level for the 9–18-year age-group was 372 IU/ml at the first survey. If the same kind of average (arithmetic or geometric) were used for both areas, the difference would be even larger. For comparison, sera from normal British adults, studied concurrently with the Gambian sera, had an average IgM level of 89mg/100ml, or 105 IU/ml, i.e. less than one-third of the Garki level.

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

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

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

Serological changes in the protected population

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

A specific objective of the serological study was to determine which

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

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

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

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

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

Serological changes during the post-intervention phase

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

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

The longitudinal study of infants

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

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

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

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

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

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

Comparison between males and females

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

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

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

The correlation between different surveys in the same person

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

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

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

The correlation between different serological tests in the same person

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

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

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

The relationship between serology and parasitology in the individual

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

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

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

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

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

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

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

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

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

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

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

The correlation between serology and parasitology has also been studied in Keneba (*108*, *109*, *112*, *140*). In both the Garki and Keneba areas the 4 serological variables increased with age, while the parasite rates and densities decreased. The relationship was also studied within specific age-groups: the data from Keneba were analysed by comparing the serological results in persons found positive and negative at the concurrent parasitological examination; the data from Garki were analysed in the same manner and also by stratifying persons by their serological results and comparing the strata with respect to the preceding, concurrent or subsequent parasitological findings. It is the latter analysis which has been presented in this chapter. In Keneba, a positive correlation was found between IgG and the concurrent parasitaemia at 0–20 years, and between IgM and the concurrent parasitaemia at 0–2 years; while in Garki there was no correlation between IgG and parasitaemia and a negative correlation between IgM and concurrent or subsequent parasitaemia. The difference between the two areas with respect to IgG is difficult to explain. The difference with respect to IgM in the young

children could be explained by a higher rate of antigenic stimulation and development of parasitological immunity in Garki, but the difference in adults (no correlation in Keneba, a negative one in Garki) is puzzling; it is possible that in Keneba the situation was modified by the use of drugs to a larger extent than in Garki. With respect to the IFA-Pf test, both areas show a positive association between titre and parasitaemia at 0–4 years and none thereafter. With respect to gametocytes, in Keneba there was no correlation with the IFA-Pf titre at 0–4 years. In the same age-group in Garki, there was also no correlation between gametocytaemia and the IFAT-*P. falciparum* titre, but there was a negative correlation between gametocytaemia and the number of bands in the precipitin-Pf test.

Sensitivity and specificity of the serological tests

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

With respect to *P. malariae*, everybody in the 5–8-year age-group was positive by the IFA test, but only 82% had or had had a patent parasitaemia in the preceding 1½ years, according to the 8 baseline parasitological surveys. This means either that the serological test was relatively nonspecific or that the parasitological test was relatively insensitive; the latter seems likely since parasitaemia could easily have been missed by eight surveys at intervals of 10 weeks, and since *P. malariae* parasitaemia

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

Summary

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

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

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

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

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

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

Chapter Seven

ABNORMAL HAEMOGLOBINS AND ABO BLOOD GROUPS[a]

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

Material and Methods

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

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

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

---

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

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

Table 26

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

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

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

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

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

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

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

Haemoglobin Electrophoresis

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

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

Table 27

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

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

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

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

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

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

Haemoglobins and Genetic Fitness

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

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

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

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

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

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

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

In Garki (Tables 26, 27) this gave

$$\frac{(486/1678)/(126/534)}{(1178/1678)/(394/534)} = \frac{486/126}{1178/394} = 1.290,$$

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

fitness ratio and sufficient to explain it.

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

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

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

The result was:

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

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

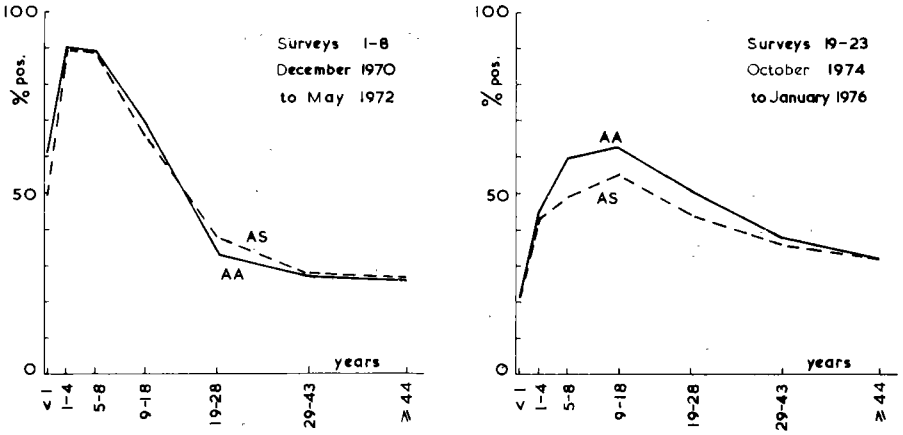
The two fertility rates were remarkably similar ($\chi^2 = 0.03$; $p>0.8$). In order to maintain a prevalence of 29% of sickle-cell trait in adults solely through differential female fertility, the female with sickle-cell trait would have had to contribute to the next generation at a rate that was 1.54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

Sickle-cell Trait and Malaria

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

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

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



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

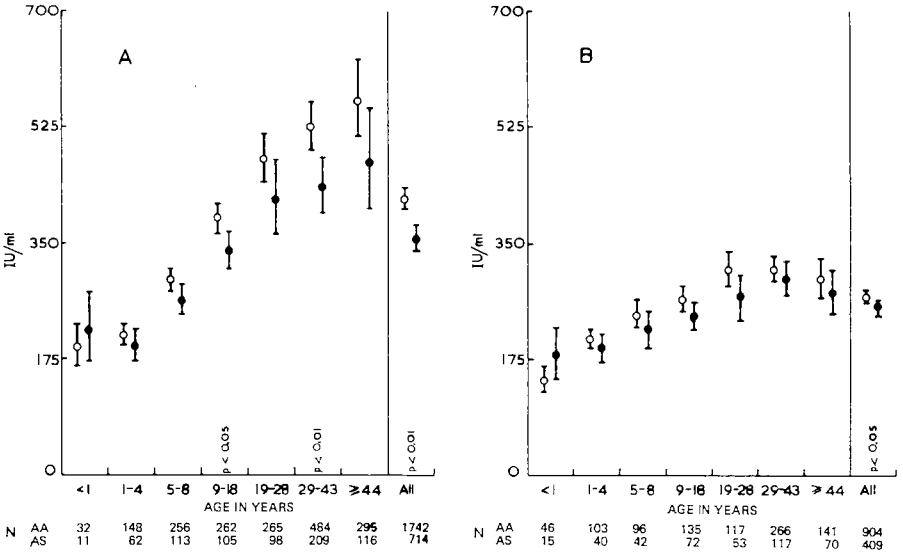
In 1974-1975, after the intervention with propoxur and mass administration of sulfalene-pyrimethamine was terminated, malaria progressively returned to its baseline level. During this period of resurgence, in the previously treated villages, the prevalence of *P. falciparum* tended to remain somewhat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

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

Immunoglobulins

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

Fig. 62 Immunoglobulin M: age-specific geometric mean level of IgM (and 95% confidence limits) in persons with haemoglobin AA (o) and haemoglobin AS (•) genotypes (**A**) in the wet season of 1971 before intervention, and (**B**) in the wet season of 1973 after 70 weeks of antimalarial intervention



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

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

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

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

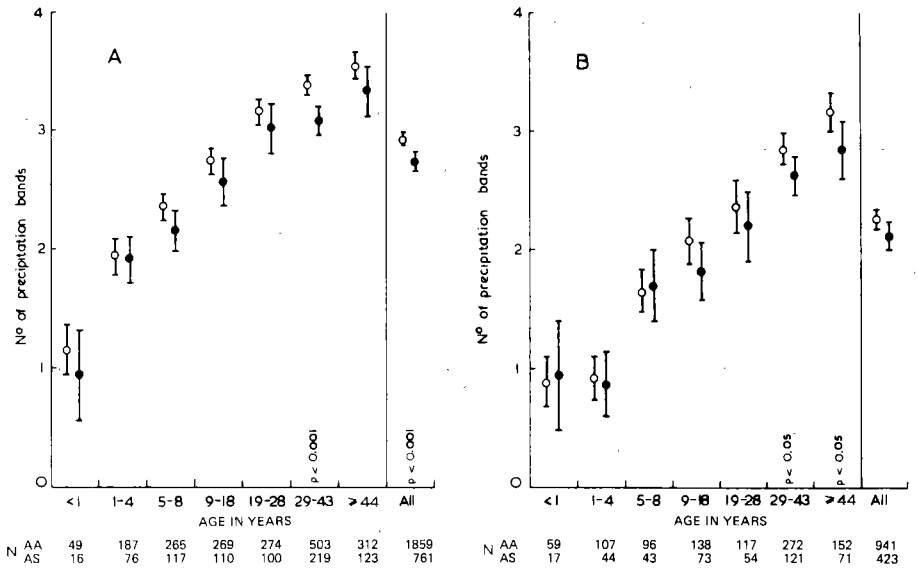
There was a rather clear and consistent difference between the Hb AA and Hb AS. At the first survey there were on the average more precipitin bands in Hb AA subjects in all age-groups, the difference being significant in age-group 29–43 years and for all ages combined (Fig. 63, graph A). At the second survey, the differences between Hb AA and Hb AS were in the same direction (except in the age-group 1–4 years), and were significant in the age-groups 9–18 ($p<0.05$), 19–28 and 29–43 years ($p<0.01$) and for all ages combined ($p<0.001$).

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

Indirect fluorescent antibody test

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

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



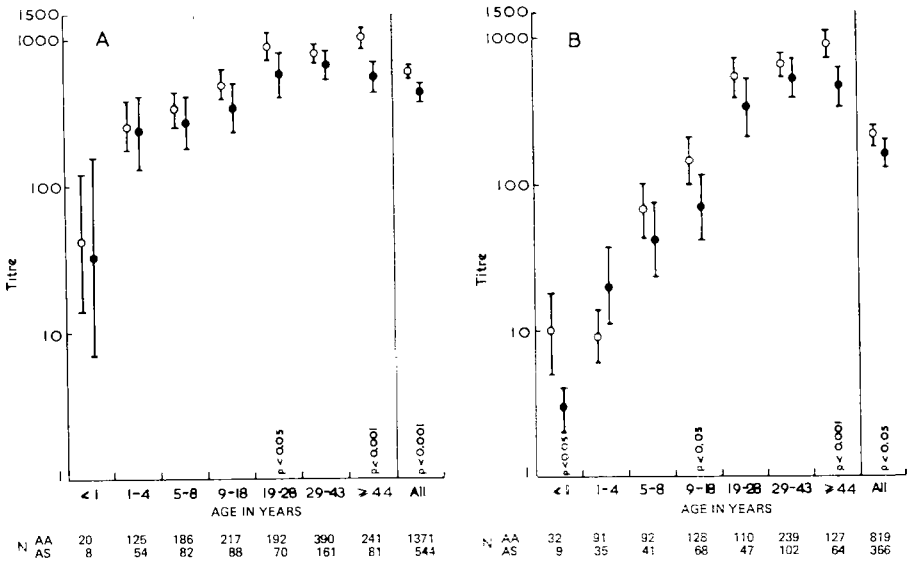
were not very consistent. With a *P. falciparum* antigen, at the first survey, the mean titre was higher in Hb AA subjects except in the age-groups 19–28 and ≥44 years, but none of the differences was significant. However, in the second survey (dry season 1972), the mean titre was higher in Hb AA in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

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

Indirect haemagglutination test with *P. falciparum* antigen

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

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



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

Immunology by sex, tribe and haemoglobin

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

Immunology, malaria parasitology and haemoglobin

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

Findings in the Hb SS Persons (see *120*)

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

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

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

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

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

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

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

Findings in the Hb SC Person

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

Findings in the Hb AC Persons (see *153*)

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

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

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

ABO Blood Groups and Malaria

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

The ABO blood groups were compared in detail with respect to their parasitological and serological results. No significant or systematic difference was detected. In particular, there was no systematic or significant difference between the ABO blood groups regarding the number of times individual persons were positive during the baseline period (parasitological surveys 1–8). There was also no systematic or significant difference between the ABO blood groups during the resurgence of malaria in the post-intervention phase.

Discussion

Haemoglobin genotype frequencies, fitness, and parasitology

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

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

Given the limited viability of the Hb SS, some factor is required to maintain an appreciable frequency of Hb S in a population; even though the mutation rate to this allele is quite high (1.7×10^{-3}) it is inadequate without some additional factor of favourable selection (*155*). There is quite conclusive clinical and epidemiological evidence that Hb S is maintained at high frequency by the partial protection of Hb AS persons against *P. falciparum* (*1*, *93*). The only previous study of malaria and sickling on a sizeable northern Nigerian population was conducted in the even drier climate of Sokoto (*160*); the sickling test was positive in 11% and the prevalence of malaria was significantly lower in sickle-cell children than in normal children, a difference which was pointed out by Allison (*2*), but not by the authors. Partial protection against *P. falciparum* in Hb AS subjects was also clearly demonstrated in the present study. Our findings thus confirm those of most earlier workers elsewhere and are in complete contradiction with the more frequent and dense parasitaemia described in Hb AS children in Accra (*138*).

The most probable mechanism by which Hb AS persons are protected against *P. falciparum* is that oxygen consumption by the parasite in the red cell causes sickling, followed by phagocytosis, thus breaking the malaria cycle (*94*). In addition, with the recently developed continuous culture systems, it has been shown that at a lowered oxygen tension Hb AS and Hb SS cells, when compared with normal cells, exhibit increased resistance to invasion and maturation of *P. falciparum* parasites (*64a*, *131a*). The partial protection is translated into a lower mortality. When transmission is intense, as in Garki, immunity to malaria is acquired early and the advantage of the Hb AS is concentrated in the very young age-groups and consists entirely in a better chance of survival, reflected in the age-specific prevalence of the trait. In areas of low transmission, sickle-cell trait still protects against the complications of malaria in pregnancy (*3*) and so increases female fertility, for example, amongst the Black Caribs (*60*). In Garki, there was no difference in fertility between Hb AA and Hb AS women. It is probable that women of child-bearing age living in areas of high transmission have attained levels of immunity at which sickle-cell trait confers no advantage, or only protection against the development of gross splenomegaly and anaemia in the occasional pregnant subject (*61*). Raper has speculated that, as transmission increases, the frequency of the sickling trait increases up to a maximum frequency beyond which a further increase in transmission would so shorten the period of low immunity, in which the Hb AS have an advantage, that the frequency of the trait would decrease (*137*).

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

Serology of the Hb AA and Hb AS

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

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

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

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

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

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

The Hb SS

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

The Hb AC

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

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

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

The ABO blood groups and malaria

There is a relatively extensive literature on the ABO blood groups and malaria, which has been reviewed by Livingstone (*93*). Certain authors found significant differences (not always the same ones), while others found no difference. Wood et al. (*166*) and Wood (*165*) studied the human host selection of *A. gambiae* s.s. according to ABO blood-group status; they found that O was more attractive than B, which was more attractive than A or AB, in the following ratios: 5:4.3:3.3.

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

Summary

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

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

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

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

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

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

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

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

Chapter Eight

DEMOGRAPHY[a]

The collection of demographic data in the Garki project was geared to the specific purposes of the project, e.g., to allow the calculation of age-specific indices of malaria, in particular among the newborn and the very young children, to ascertain the mortality rates, to trace the trends of immigration and emigration in the follow-up populations, and to facilitate control of the coverage of the survey and MDA operations generally. In this chapter, these demographic data have been summarized to provide an overview of the structure and movements of the study population. Despite their shortcomings, including limitations in time and space, the observations may be of some value to students of African demography, a field in which data are still relatively scarce. It should be added that the potential of the data bank has not yet been fully explored, in particular with regard to analysis of fertility patterns and certain aspects of migration (see also *11*, *180*).

Methods

Demographic data were collected in the village clusters selected for follow-up by 2 methods: namely, through the periodic demographic-parasitological surveys at 10-week intervals, and through fortnightly visits by specially employed itinerant collectors of data on births, deaths and migration. The latter data were incorporated, as appropriate, into the data from the demographic-parasitological surveys (see p. 32). In addition, some demographic information was extracted for a period of 13 months from the mass-drug administration records in area A2, covering a population of about 12 000 persons. The findings in the

[a] The observations presented in this chapter were made by or under the supervision of Mr S. Brøgger, Mr J. Storey and Mr D. Thomas. They have benefited from the advice of Dr A. Benyoussef and Professor M. Prothero.

population receiving MDA are outlined on p. 243, while the rest of the chapter is concerned with the population of the clusters of villages followed up regularly.

Demographic and parasitological (DP) surveys

At the first survey a household roster was established with the name, sex and age of every resident. Age was estimated by questioning the person and his close relatives, and by the field staff's impression; the age recorded was expressed in months for infants, in years for others. At subsequent surveys, each previously registered person was classified as present, absent, moved or dead; new residents were added to the roster and similarly classified. Persons who had moved within the village were traced to their new household (for record linkage). Persons coded "absent" at 4 consecutive surveys were re-coded "moved" with retroactive effect. At a given survey, the registered population includes the present plus the absent. A very high coverage was probably achieved among those persons actually present, except with respect to temporary visitors, especially those not resident in one of the compounds.

Local registrars (pataucis)

From the time of the third survey onwards, three residents of the Garki district were employed as itinerant registrars, known as "pataucis", the Hausa word for a travelling salesman. Each one covered one-third of the follow-up population and visited each village every 2 weeks to record births, deaths and population movements. The village's civil and religious leaders cooperated actively. A preliminary evaluation showed that, in comparison with the pataucis, the DP surveys were missing a significant number of births, but practically no deaths, except the deaths of infants born and dying between 2 successive surveys. Therefore registration of deaths by the pataucis was discontinued, while all births registered by the pataucis were added to the roster of occupants before the following DP survey. Thus from survey 3 onwards the registration of births is more complete, and the age of infants is estimated more accurately. Observations of population movements were much less complete. Efforts to record the whereabouts of persons who were absent at the DP survey had to be abandoned, but reasonably accurate counts were made of temporary visitors to the village at the time of the patauci's visit.

Data-handling

Age was probably estimated with a rather large error, increasing with age itself; the expected "preference" for multiples of 5 and 10 years was

observed. For the analysis of results by age, the following age-groups were used: <1, 1–4, 5–8, 9–18, 19–28, 29–43, ≥44 (completed years). This (non-standard) grouping avoids the use of "preferred" ages (e.g., 10, 12, 17, 20) as limits of age-groups, in the hope of minimizing misclassification.

Each person was given a "date of birth"—the date recorded by the patauci when available, otherwise the 15th of the recorded month of birth for infants and the 15th of a random month in the recorded year of birth for the others. These "dates of birth" were used for finer analysis of data from infants, and for updating the composition of age-groups at any given survey. The data were stored in a way which allows cross-sectional or cohort analysis as required.

The following demographic events were defined in the longitudinal person data: births, deaths, departures from the village (absences lasting through 1 to 3 surveys, moves lasting through 4 or more surveys), and arrivals into the village (i.e., new registrations, minus births). For births, the probable date of the event was recorded; for the other events, a date of registration was recorded, i.e., in principle, the date of the first survey (in the village) after the event. For the sake of counting infant deaths, the age of death was computed under the assumption that the interval between death and its registration was 37 days (i.e., half the average interval between surveys); for computing convenience and consistency, the same rule was applied to all events (except births) and all age-groups (in most cases, it has of course, no effect).

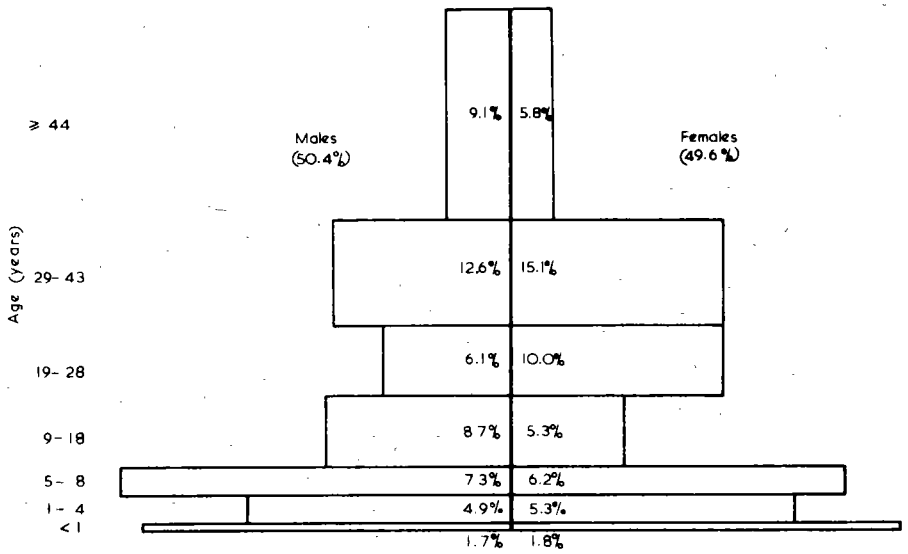
Indices were calculated either by relating the number of events occurring in 1 year to the average registered population (or in the case of the infant mortality rate, to the number of births in the year) or by relating the number of events recorded at a given survey to the registered population at that survey (in relation to absences) or the preceding one (in relation to deaths, moves in and out of the village). The second approach allows the study of seasonal variation, and in certain cases (e.g., infant mortality) may identify more accurately the population at risk.

Results

Distribution of the population by age and sex

The distribution of the registered population by age and sex at survey 7 in February 1972 (Fig. 65) shows that males and females were registered in nearly equal numbers. The age-pyramid is rather irregular, showing

Fig. 65. Distribution of the registered population (N = 7540) by age and sex at survey 7



"excesses" and "deficits" (see p. 244). Variations between village clusters were mostly small, and those between surveys were not statistically significant.

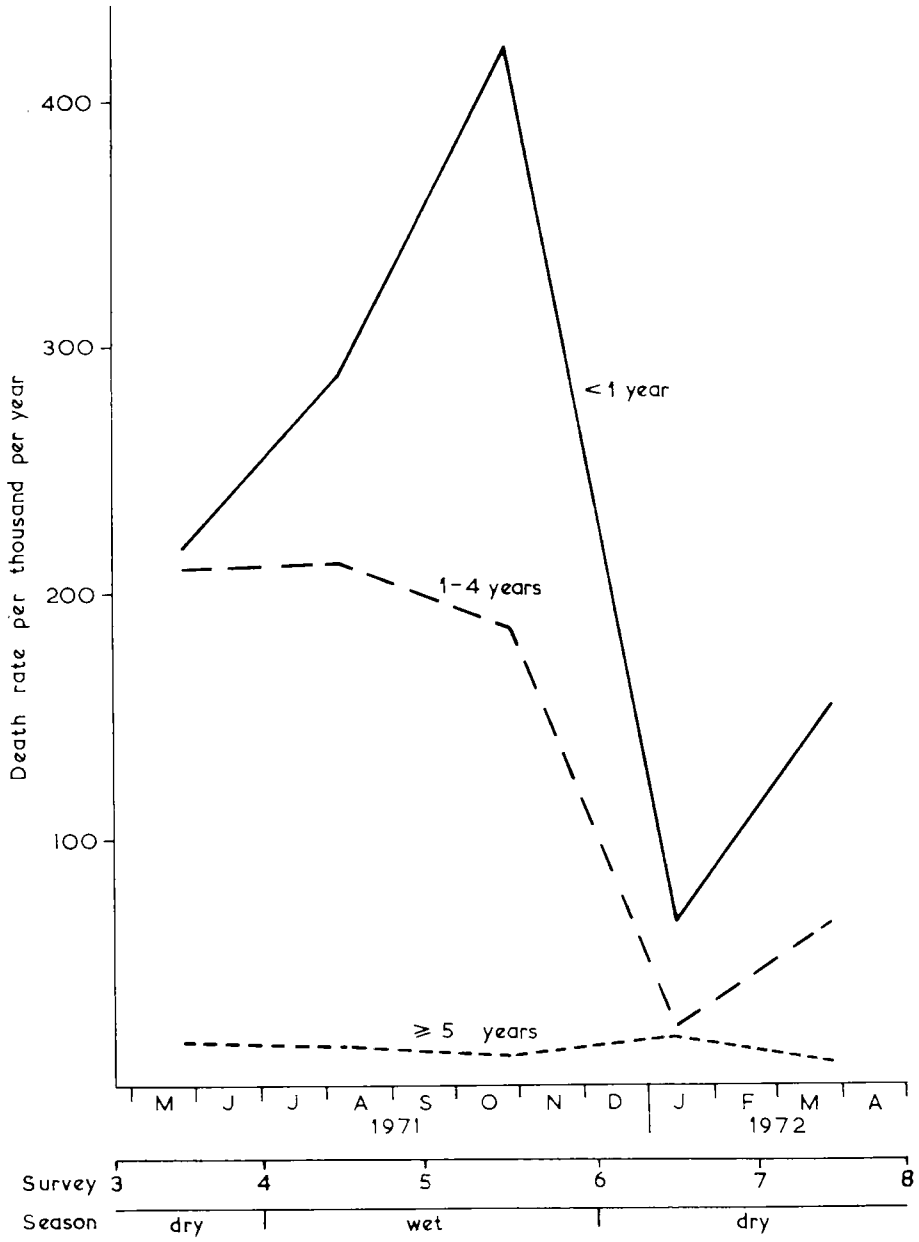
Births

Between surveys 3 and 13 (the last year of the baseline period in 16 villages, plus the first year of the intervention phase in 22 villages) 601 births were registered in 13 050 person years of the population at risk; the estimated crude birth rate (CBR) is, therefore, 46.1 per 1000 per year. There was no significant variation by year, by village or by treatment.

Counting as females of reproductive age those in age-groups 19–28 and 29–43 years plus half those in age-group 9–18 years, their proportion in the registered population was 0.278 and the estimated fertility rate is 46.1/0.278 = 165.8 per 1000 per year. Again, there was no significant variation by year, by village or by treatment in this rate. There was also no significant difference between the Hb AA and Hb AS women (see p. 217).

Birth rates show a marked and relatively regular seasonal variation, with peaks in April-August of 60–70 (annual basis) and lows in October-January of 23–35 (annual basis).

Fig. 66. Seasonal variation in the mortality rate, by age, during the last year of the pre-intervention period



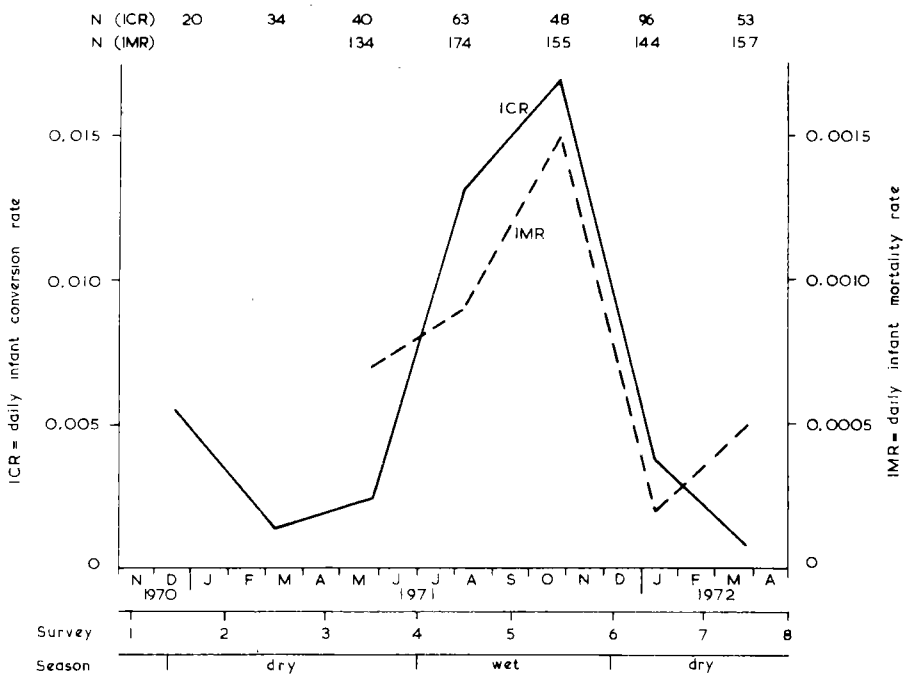
The recorded sex-ratio at birth was 0.92, there being 592 male births and 642 female births between the first and sixteenth parasitological surveys (i.e. over a period of three years) in all the villages surveyed.

Deaths

Crude death rate

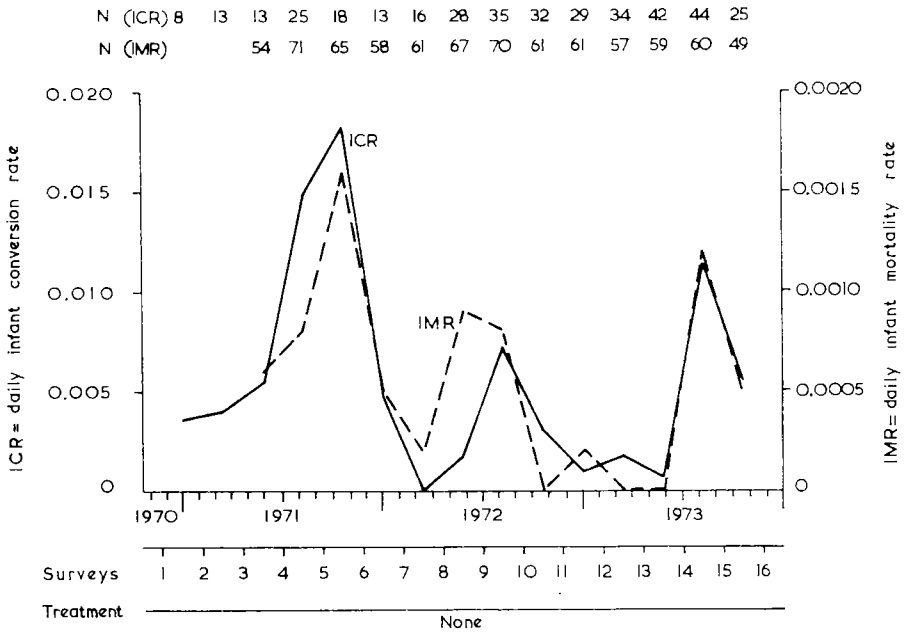
The crude death rate (CDR) in the last year of the baseline period was 37.3 per 1000 (215/5757). There was no significant difference between men and women, nor between the village clusters. There was however a marked seasonal variation, with higher mortality in the wet season, caused almost exclusively by variations in mortality in the children below 5 years of age (Fig. 66). In the first year of intervention, the overall CDR decreased to 20.8, a decrease of about 45%; this was observed, rather unexpectedly, in the untreated village clusters as well as in the treated clusters.

Fig. 67. Daily infant parasitological conversion rate (ICR) for *P. falciparum* and daily infant mortality rate (IMR), by interval (about 10 weeks) between consecutive surveys, during the baseline phase[a]



[a] N(ICR), N(IMR) = number at risk of conversion or death, respectively.

Fig. 68. Daily infant parasitological conversion rate (ICR) for *P. falciparum* and daily infant mortality rate (IMR) by interval (about 10 weeks) between consecutive surveys, in the untreated villages (village clusters No. 1 and 2, area C)[a]



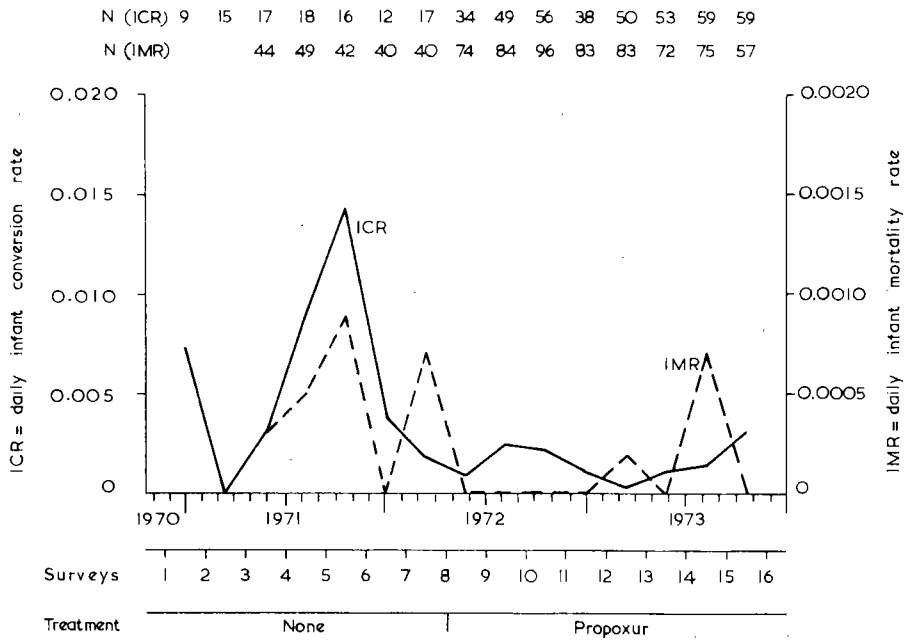
[a] N(ICR), N(IMR) = number at risk of conversion or death, respectively.

Infant mortality rate (IMR)

The IMR, estimated from the number of infant deaths and the number of births in the same period, was 193.1 per 1000 per year in the last year of the baseline period and 105.3 in the first intervention year, with no significant difference between the treated and the untreated village clusters in either period. A more accurate estimate of the IMR in the last baseline year is 245.7, obtained from the proportion of infants dying in each interval between surveys and adjusted to the annual basis. This method results in estimated IMRs for the first intervention year of 135 (9 deaths/316 infant-periods of $\frac{1}{5}$ year)[a] for the untreated village clusters, and 55 (11 deaths/971 infant-periods of $\frac{1}{5}$ year) for the treated clusters, the difference being in the expected direction and statistically significant. For the subsequent 3 surveys, which covered the intervention phase through the wet season of 1973, the corresponding IMRs (per 1000 per year) were 192 (7 deaths/168 infant-periods) and 102 (13 deaths/613 infant-periods) for the untreated and treated village clusters respectively.

[a] $IMR = 1 - (1 - 9/316)^5$

Fig. 69. Daily infant parasitological conversion rate (ICR) for *P. falciparum* and daily infant mortality rate (IMR), by interval (about 10 weeks) between consecutive surveys, in village clusters No. 3 and 4 (area B), before and during the application of propoxur[a]

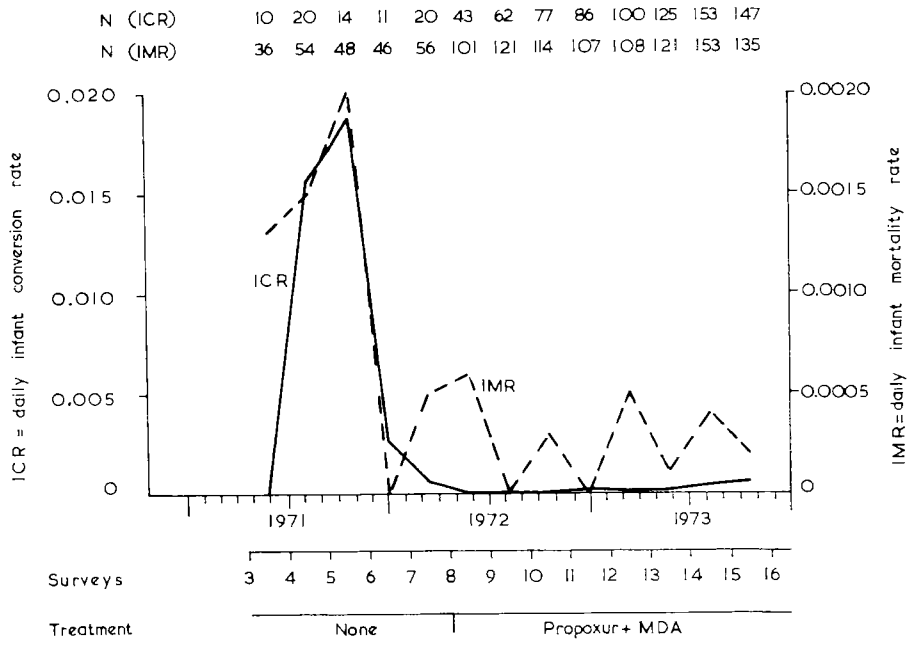


[a] N(ICR), N(IMR) = number at risk of conversion or death, respectively.

The very significant seasonal variation in IMR during the baseline year is shown in Fig. 67, where the IMR is expressed as a daily rate and compared with the estimated daily rate of infant conversion (ICR, see Chapter 5) for *P. falciparum*. The IMR is equal to about one-tenth of the ICR and shows nearly the same seasonal variation. This comparison between IMR and ICR is continued in Fig. 68, 69 and 70, which show their variations over 3 years for treated and untreated village clusters respectively. In the untreated villages (Fig. 69) the daily IMR remains rather consistently equal to about one tenth of the ICR throughout, and the seasonal variations of the two rates are closely correlated. In the treated villages (Fig. 69 and 70) the IMR decreases much less than the ICR, and the seasonal variations of the 2 rates become unrelated after the introduction of malaria control.

Before the introduction of malaria control, the probability of dying within 10 weeks (i.e., before the next survey) was greater (272 per 1000 per year) in infants found positive for *P. falciparum* than in those found

Fig. 70. Daily infant parasitological conversion rate (ICR) for *P. falciparum* and daily infant mortality rate (IMR) by interval (about 10 weeks) between consecutive surveys, in village clusters No. 5–8 (areas A1 and A2) before and during the application of propoxur and of MDA[a]



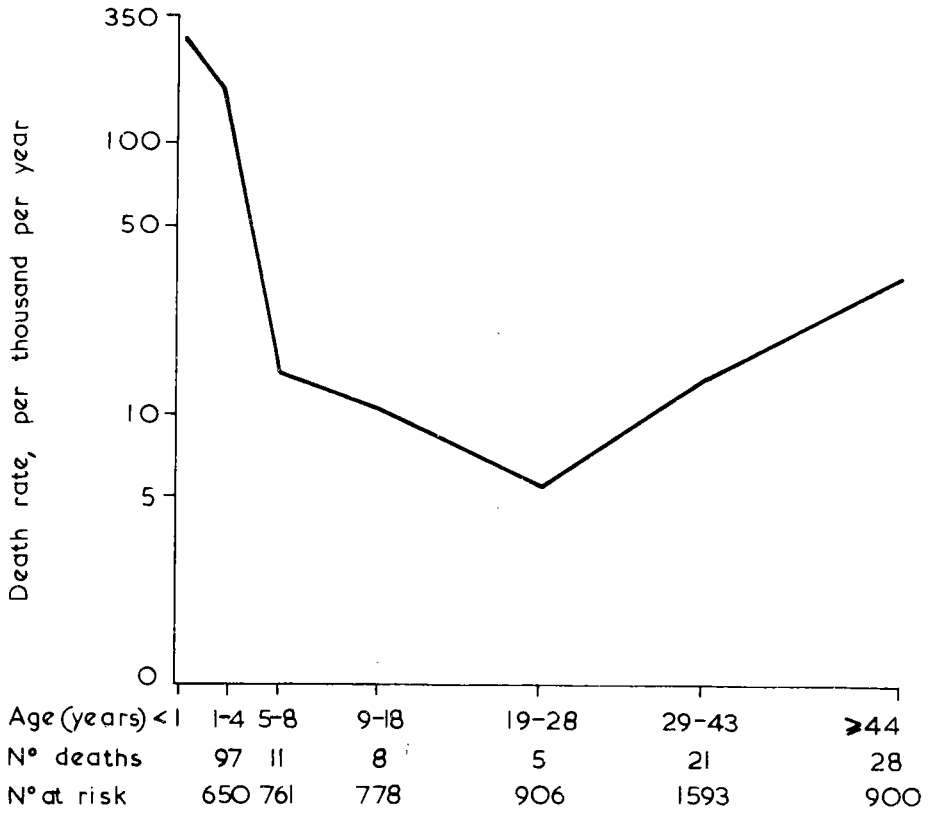
[a] N(ICR), N(IMR) = number at risk of conversion or death, respectively.

negative (202 per 1000 per year); among the positive infants, this probability was greater in those having 25% or more of positive microscopic fields (311 per 1000 per year) than in those having a lower density parasitaemia (231 per 1000 per year). These differences were, however, not statistically significant (see Table 6 in Ref. *180*).

Age-specific death rates

Among the baseline age-specific death rates (Fig. 71), the mortality in the age-group 1–4 years is very high (154 per 1000 per year); at older ages it decreases to 15, 10 and 6 in the age-groups 5–8, 9–18 and 19–28 years respectively, and then increases to 13 and 31 in the age-groups 29–43 and ≥44 years respectively. Mortality in the age-group 1–4 years shows a clear and marked seasonal variation (Fig. 66), with much higher mortalities in the late dry season and in the wet season. The older age-groups do not show a clear seasonal variation. For the combined age-groups

Fig. 71. Baseline age-specific death rate in the last year of the pre-intervention period[a]



[a] The infant mortality rate was estimated as explained in the text.

from 5–8 to ≥44 years, the estimated mortality rates for the 5 intervals are: 17.7, 15.1, 11.9, 19.7 and 9.1 per 1000 per year respectively; thus only the infants and the age-group 1–4 years show a clear-cut and marked seasonal variation of the mortality rate, which was similar in the two groups.

As already mentioned, there is an overall decrease in mortality between the last baseline year and the first year of intervention. Excepting the infants, the largest apparent change associated with treatment occurs in the age-group 1–4 years: whereas in village clusters No. 1, No. 2 (untreated) and No. 3 and 4 (insecticide alone), the mortality in this age-group decreased from 159 to 114 and from 111 to 83, i.e., by 25–30%, in village clusters No. 5–8 (insecticide plus mass drug administration) the mortality decreased from 193 to 61, i.e., by nearly 70%.

During the first year of intervention, the seasonal variation in the death rate in age-group 1–4 years was nearly reversed in the treated village clusters. The mortality in this age-group in the treated villages (i.e., 40 per 1000 on an annual basis) during the wet season (surveys 9–11) was significantly lower than in the untreated villages (i.e., 189), whereas for the first intervention year as a whole the mortality in the treated villages was 70 as against 113 in the untreated villages, a difference which does not attain statistical significance.

Population movements

Migration in and out of the village

The following definitions are used:

Immigration into the village: new registration of a person as resident, at a given survey, excluding those born after the preceding survey. If infants were missed at the first survey at which they are alive, they were counted as "immigrants" at the following survey and the infant immigration rate is thus overestimated.

Emigration out of the village: first coding of a former resident as moved (after changing certain codes from absent to moved, see p. 232). Absences lasting through 1 to 3 surveys were not counted as migrations.

For the baseline year between surveys 3 and 8, the observed migration rates, by age (excepting infants) and by sex (Figure 72) were found to be relatively high. The average immigration rate was 186 per 1000 per year, the average emigration rate 164 per 1000 per year. The rates varied by age and sex: the females showed higher average immigration and emigration rates than the males and equal rates in both directions, while in the males there was a net immigration (at all ages). The highest rates, in and out, were reached in age-groups 9–18 years for the females, in age-group 19–28 years for the males; there is a strong correlation between the two rates, in and out, within age-groups.

Migration rates in infants are a special case: if we use the same method of estimation as above, we obtain an infant immigration rate of 304 per 1000 per year (1000 x (58/191)), an infant emigration rate of 126 per 1000 per year (1000 x (24/191)). The excess of infant "immigrants" results mainly from the fact that an infant first registered at the second survey after his or her date of birth is counted as an immigrant (see definition above).

The migration patterns showed a pronounced variation by season and from one year to the next (Fig. 73). The immigration rate is highest in the latter part of the dry season and the emigration rate is highest just after

Fig. 72. Migration rates in and out of the village, by age (excluding infants) and sex, during the last baseline year

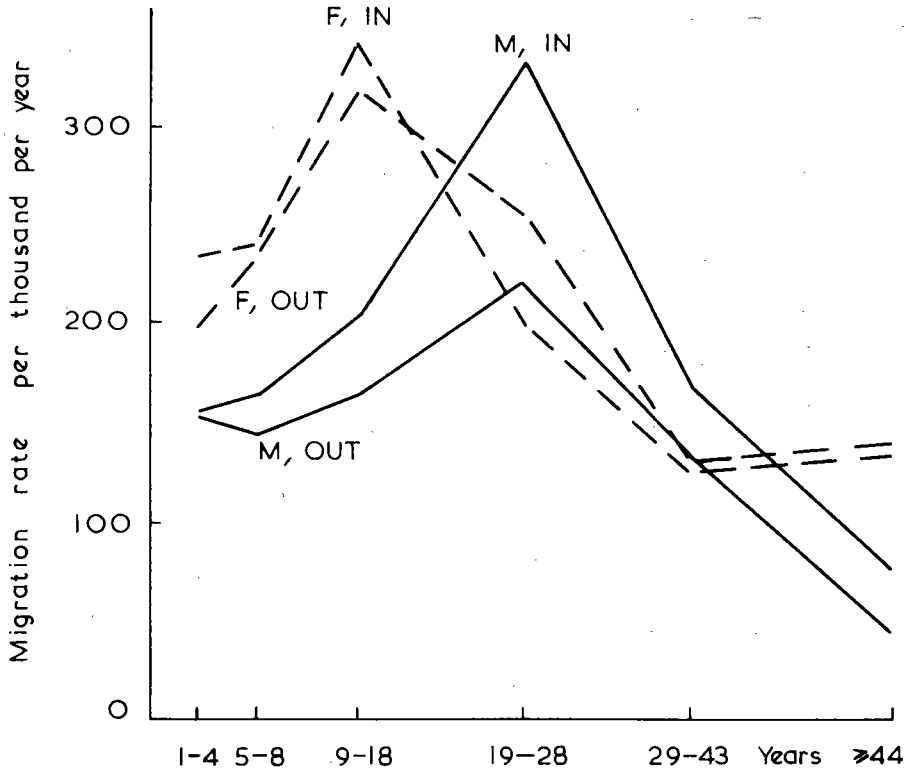
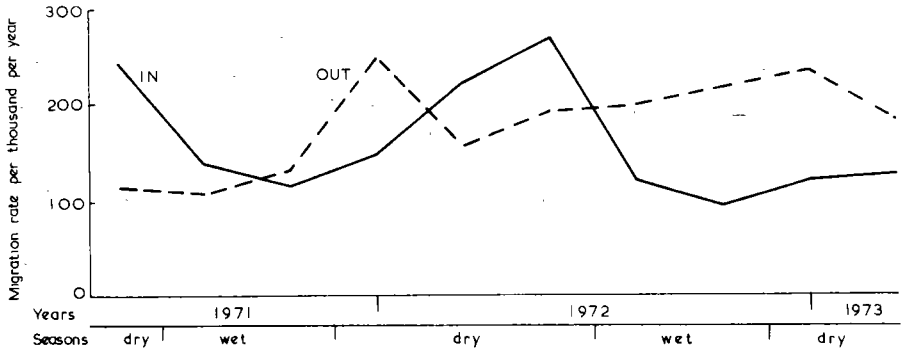


Fig. 73. Migration rates in and out of the village, by interval between consecutive surveys, during the last baseline year and the first intervention year (infants excluded)



the wet season, a pattern repeated in both of the years. In the first intervention year, the net immigration seen in the baseline year changed to a net emigration (158 immigration rate and 224 emigration rate). The seasonal pattern and the change in net migration direction varied little by sex and age. There were some differences in migration patterns from one village to another, repeated in each of the 2 years. The various intervention treatments did not seem to have any influence on the migration.

Absence

As stated above, a person coded "absent" at 1, 2 or 3 consecutive surveys was counted as part of the registered population, but absent. The proportion present out of the registered population ranged from 96.3% (survey 5) to 80.1% (survey 12). The proportion present was lowest in the first part of the dry season, highest at the end of the dry season and in the wet season; the pattern repeated itself in 3 successive years, superimposed on a slight downward trend. In the dry season, the proportion present was higher among females than among males; in the wet season, there was no difference. The proportion present was lower in the age-group 9–43 years than at younger or older ages, and it was also at 9–43 years that the proportion present was lower in males than in females.

The same pattern of absenteeism was seen in all of the villages and in 3 consecutive years. However, there were some differences in the amplitude, corresponding to the differences in levels of migration, and these differences were repeated from year to year. The intervention treatments, or the occasional collection of larger blood samples for seroimmunological tests, had no apparent effect on the proportion absent.

Demographic analysis of the MDA records

In addition to that collected through the demographic-parasitological surveys, demographic information was extracted from the MDA records in area A2, outside the follow-up villages, in a population of about 12 000 (*11*). This analysis confirmed the above findings regarding the distribution of the population by age and sex, the seasonal pattern of migration, the higher migration rates in females than in males, and the existence of a net emigration (in the first year of the intervention phase). The birth rate and the infant and crude mortality rates were somewhat lower than in the follow-up villages, but this is readily explicable by the more thorough investigation of the follow-up villages, in particular through the use of itinerant registrars (pataucis). The rates of migration were higher than in the follow-up villages, but in the MDA population a person was classified as "moved" after only 2 consecutive

absences, at intervals of 70 days (versus 3 consecutive absences, at the same interval, in the follow-up villages).

Discussion

Methods

The periodic surveys (every 10 weeks), conducted primarily to make a longitudinal parasitological study of persons in complete villages selected as natural epidemiological (transmission) units, also yielded relatively detailed demographic information, although the study population was comparatively small for purposes of demographic study. The deployment of the itinerant local registrars (pataucis) added significantly to the completeness of registration of births and of infant deaths.

The 2 methods used for the estimation of demographic rates—i.e., the one using the number of events in a period over the average (or mid-period) population, and the one using transition frequencies between successive surveys— should, in a stationary situation, yield the same results. In small populations, however, random fluctuations (e.g., of the number of births per year) may be relatively large and this may introduce a large error in the estimation of the IMR by the first method, because this method replaces the real population at risk (the births resulting in infant deaths in a given period) by a substitute (the births in the same period in which the infant deaths are counted) that is assumed to be equal. Therefore, in small populations with relatively large migration movements, the method based on transition frequencies between successive surveys (which relates events to the actual population at risk) is undoubtedly superior.

Age and sex composition

The pyramid of ages (see Fig. 65) differs from what would be expected from a stable population: there was an excess in the age-group 5–8 years, a deficit in the age-groups 1–4 and 9–18 years; the ratio of males to females, although equal to 1.01 for the total population, varied greatly by age (extreme values: 1.63 in age-group 9–18; 0.60 in age-group 19–28; 1.56 in age-group $\geq$44). The "irregularities" of the pyramid of ages could, in theory, be explained by: (1) past variations in the birth rates and especially in the death rates; (2) differential net migration rates by age and sex; (3) non-random misclassification of persons into age-groups (too many children misclassified from both directions into the 5–8-year

age-group, too many females misclassified from both directions into the 19–43-year age-group, i.e., the reproductive age); and (4) differential under-registration.

The age and sex composition varied little between follow-up units, so the same factors were probably operating and approximately to the same degree in the whole area. The characteristics of the pyramid of ages listed above have been found also at different times and places in tropical Africa (see Ref. *9*, in particular its Fig. 2.1, 2.6 and 2.7). This is difficult to reconcile with the first 2 "explanations" listed above, and points to the third explanation as the most important. Moreover the observed migration rates by age and sex (see Fig. 72) were no help in explaining the observed age and sex structure.

Birth rate

The high crude birth rate of 46 to 47 per 1000 per year corresponds to expectation. The seasonal variation of the birth rate must be anticipated, with an interval of about 9 months, by a corresponding seasonal variation in the rate of successful conception (i.e. leading to a live birth). The season in which successful conceptions are least numerous is also the period in which the largest numbers of young and middle-aged adult males are absent. The seasonal variation in successful conceptions in the Garki area was similar to that of the risk of acquiring malaria, whereas in the Pare-Taveta area of the United Republic of Tanzania it was the reverse, both in a malarious and in a nonmalarious population (*134*).

Death rates, malaria and malaria control

The small numbers of deaths involved, and their natural fluctuations, impose some caution in drawing conclusions both about baseline values and the possible early effect of malaria control. The crude death rate decreased from 38 per 1000 per year in the last baseline year to 21 per 1000 per year in the first intervention year; as the same proportional decrease occurred in treated and untreated villages, it cannot be attributed to treatment.

During the intervention period (all treatments combined), the IMR became significantly smaller in the treated villages than in the untreated; the death rate in the 1–4-year age-group decreased more in the villages treated with propoxur plus MDA than in the untreated or in those treated with propoxur alone, but the difference either before or under treatment was not significant. The decrease under treatment in both the IMR and the mortality at 1–4 years of age was proportionally much smaller than the corresponding decrease in malaria risk.

In the absence of control measures, the mortality rates among infants and children of 1–4 years and the malaria risk (infant conversion rate for *P. falciparum*) have a strongly correlated seasonal variation; under treatment, the correlation is much weaker or disappears.

The observations are compatible with the hypothesis that malaria is a common precipitating cause of death in infants and in the 1–4-year age-group, and that effective malaria control, even in its early stages, removes this immediate cause in most cases. In a large fraction of cases, however, death has been delayed very little; this is possibly because the infants and children who were at high risk of dying from malaria are also at high risk of dying from one or more other precipitating causes, or from a constitutional (e.g., nutritional) underlying cause. The hypothesis that several causes of death are competing for the same "high-risk" children is also supported by the report, from a Gambian village followed for several years, that an exceptionally high mortality in the dry season, due to an epidemic of measles, was followed by an exceptionally low wet season mortality, presumably due to malaria (*107*).

If chronic malaria affects adversely the general underlying condition of persons, which is likely, death rates may decrease further in the later stages of control; the effect of past malaria experience may be the reason why, in the first year of control, the IMR but not the 1–4-year death rate became significantly smaller in the treated villages than in the untreated.

A relatively weak association between the parasitological status of infants at a point in time and the risk of dying within the next 10 weeks was observed. The finding that such an association is weak is compatible with a high rate of mortality directly caused by malaria: death from malaria in infants is probably related to a rapid increase in parasite density, which would have a high chance of being missed by surveys at intervals of 10 weeks.

It is not known to what extent malaria control affects directly other causes of death. The drugs are probably specific, but the insecticide may reduce other causes of death (e.g., arbovirus infections or fly-transmitted diarrhoeal disease).

The effect of malaria control on mortality has also been studied by Newman (*128*) from the vital statistics of Sri Lanka and Guyana, by Gramiccia & Hempel (*74*) from the vital statistics of American and Asian countries and by Payne et al. (*132*) from the results of the Kisumu (Kenya) project, among others. They all conclude that malaria control produces a marked reduction in mortality.

No attempt has been made here to fit together the various demographic estimates from Garki by means of a population model. This may prove interesting and useful, in view of the relative scarcity of demographic information from the African savanna and the importance of

demography in health and development planning. The natural rate of growth of the population cannot be precisely estimated, given the small sample size. Taking the CBR and CDR at their face values we get rather unlikely high values for the rate of growth: the difference CBR–CDR was 46.1–37.8 = 8.3 per 1000 per year in the last baseline year, and 47.0–20.8 = 26.2 per 1000 per year in the first intervention year. As argued previously, only a small fraction of this apparent increase in rate of growth is probably attributable to malaria control. In periods of drought, the area probably undergoes net emigration. Computation of the expected demographic consequences of malaria control could only be made with a relatively large error. Even so, it may be worth attempting with the limited data available.

Population movements

Only 2 types of movement were measured with any precision: migration in and out of the village, and short-term absences from the village. At least 2 other types of population movements are important in the area: short-term visits to the village, and movements of the semi-nomadic Fulani. The reports of the pataucis and the data collected in the MDA population in area A2 indicate that the amount of short-term visits into the village is substantial and highly variable. On the average, temporary visitors accounted for 3–7% of the population present, more in the dry season than in the wet. The movements of semi-nomads are largely due to the seasonal variation in the distribution of pastures.

Even for the movements that were measured, the geographical distribution of the movements is largely unknown. Admittedly the definitions used for "moved" and "absent" were arbitrary. Moreover, whereas actual rates were estimated for migration, for absences only proportions at a given time were estimated, rather than the rates at which persons leave for or return from a period of absence. Motivations of the population movements are incompletely documented, but it is known that many of the dry season migrations and temporary absences are to seek temporary work to the south. It is also known that on marriage (and divorce, which seemed quite common) it is usually the woman who moves. Furthermore, it is customary for a pregnant woman, especially if she is young, to move to her parents' compound for some months around the time of delivery. The 2 movements which have been measured occur at relatively high rates: 15–20% of the village population are replaced each year; in the dry season, 15–20% of the population are temporarily absent. It is probable that the population is not homogeneous with respect to either type of movement, and that the majority of the population is in fact relatively stable.

Population movements are relevant to the understanding of the epidemiology of malaria and its control. When the parasitology of people coming in (immigrating or returning from absence) and of people going out (emigrating or leaving for a short-term absence) was compared with that of the more stable residents, it was concluded that the parasite reservoir in the more stable part of the population was sufficient to maintain transmission even where MDA was given every 2 weeks in the wet season as in area A1 (see Chapter 5). The impact of temporary visitors and of semi-nomads, although not measurable, does not affect the above conclusion.

Summary

Surveys of total village populations (about 7500 persons in 22 villages) conducted at 10-week intervals for the longitudinal study of malaria yielded information on birth, death and migration rates. The surveys were supplemented by the information gathered by local residents used as itinerant registrars, visiting each village every 2 weeks. Two methods of estimation were used, the usual one of relating events to average population and the theoretically more accurate one making use of the transition frequencies observed between consecutive periodic surveys. Possible early demographic effects of malaria control (propoxur with or without MDA for $1\frac{1}{2}$ years) were investigated.

The distribution of the population by age and sex shows some irregularities frequently observed in tropical Africa and probably best explained by non-random misclassification into age-groups. The crude birth rate (CBR) was 46–47 per 1000 per year and unaffected by treatment. The estimated fertility rate was 166 per 1000 per year. The crude death rate (CDR) was 38 per 1000 per year in the baseline period; it was 21 per 1000 per year in the first year of intervention, but the decrease was the same in the treated and untreated villages. The seasonal variation of the CDR was reversed under treatment. The infant mortality rate (IMR), estimated from the proportion of infants dying between consecutive surveys, dropped from 245 per 1000 per year in the baseline pre-treatment year to 55 per 1000 per year in the treated villages (all treatments combined) in the first intervention year. Although in that year the IMR in the untreated villages was 135 per 1000 per year, the difference between treated and untreated is significant.

The baseline death rate in the 1–4-year age-group was high (154 per 1000 per year). In the wet season this rate became, under treatment, significantly lower in the treated than in the untreated villages, whereas for

the mortality in the entire first year of intervention no significant difference developed; the inversion of the seasonal variation of the CDR was due mostly to the corresponding inversion in the 1–4-year age-group.

In the absence of malaria control, there is a strong correlation between the seasonal distribution of the malaria risk (either infant conversion rate or entomological inoculation rate) and the seasonal distribution of infant deaths. In the first year of control, the malaria risk decreased considerably, but the proportional decrease in the death rates was much smaller and the seasonal distribution of the 2 rates became largely independent. There is an association between the parasitological status of infants and the risk of dying in the next 10 weeks, which although it was in the direction expected, was not statistically significant.

Absence for 4 or more surveys was arbitrarily defined as migration. The yearly migration rates, in and out, are large: 15–20% per year. Migration rates vary by age, sex, place and year; in the second year of observation, there was net emigration. Migration was apparently not affected by the treatments.

Absence from the village for 3 or fewer surveys was arbitrarily defined as absence. Every dry season, the proportion present drops to 80–85%. The proportion absent varies also by age, sex and place; on the average it increased slightly over 3 years of observation but was apparently not affected by the treatments.

Other types of population movements (short-term visits to the village, semi-nomadism) are known to exist, but were not measured with the same precision nor fully analysed.

Chapter Nine

CLINICAL SURVEYS[a]

This chapter describes the results of the limited clinical surveys (nutritional anthropometry, spleens, temperatures) performed in the study population. The surveys are described in Chapter 2 (see p. 33).

Nutritional Anthropometry

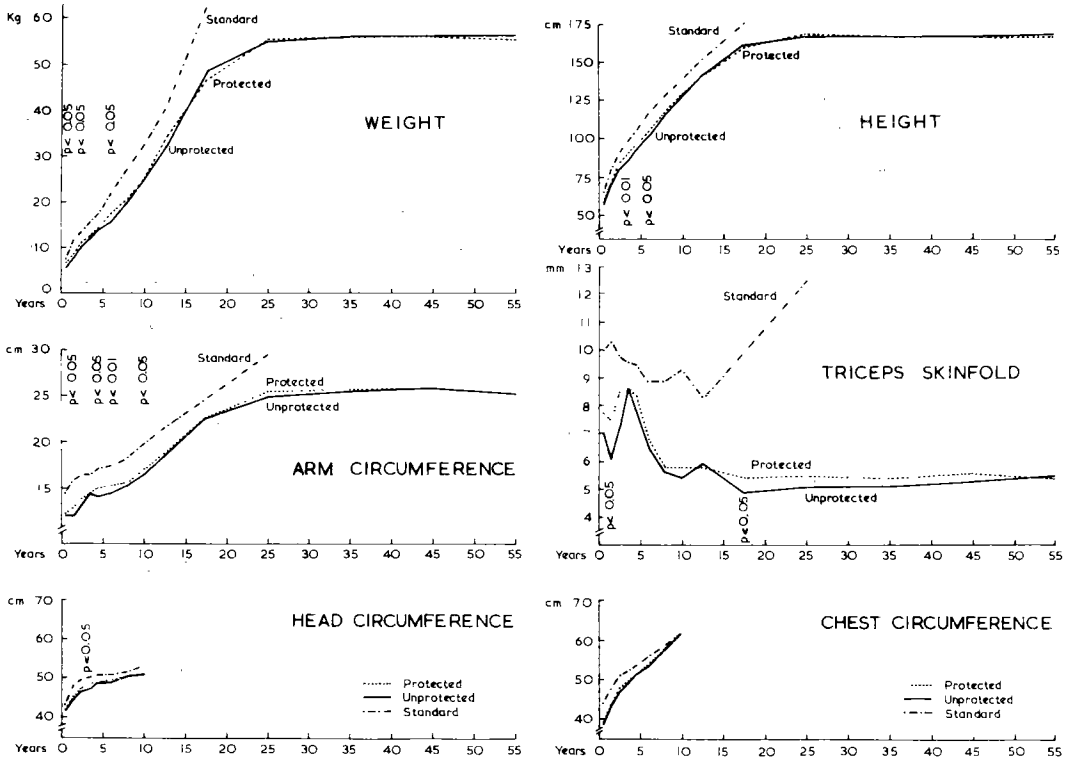
The anthropometric surveys were made in early 1974, 1975 and 1976, i.e., shortly after the second (and last) wet season of the intervention phase and after the first and second wet seasons of the post-intervention phase. The surveys were conducted in the 2 village clusters (No. 5 and No. 7, area A1) which were undergoing the most intensive control treatment (propoxur plus high-frequency MDA) and in 1 untreated comparison cluster (No. 2, area C). In the protected population, the prevalence of malaria (*P. falciparum*) had been reduced to 1–5% for about $1\frac{1}{2}$ years (see Chapter 5).

Figure 74 shows the results of the first nutritional anthropometric surveys, in the protected and unprotected populations, in comparison with certain international standards. The means of all anthropometric measurements were found to be clearly below the international standards. Protected infants and children had on the average slightly better anthropometric measurements. They were somewhat heavier and taller and had somewhat thicker arms and triceps skinfolds. The differences were small to moderate but rather consistent; in several cases, indicated on the graphs by the notations $p<0.05$ or $p<0.01$, the differences were significant. The largest difference was found in the triceps skinfold thickness.

The small differences between the protected and unprotected popu-

[a] The observations presented in this chapter were made by or under the supervision of Dr T. Matsushima and Mr J. Storey.

Fig. 74. Nutritional anthropometric survey, January-February 1974: comparison between unprotected villages, protected villages (prevalence of malaria reduced to 1–5% for 1½ years) and international standards[a]



[a] The Harvard standards were used for weight and height (*156*); various standards were used for arm circumference and triceps skinfold (*86*) and for head and chest circumferences (*162*). The *p* values refer to the comparison between protected and unprotected populations.

lations, detected in the first anthropometric survey, disappeared in the course of the post-intervention phase.

In addition to the surveys described here, the height of children had been studied in the preparatory phase of the project (*13*).

Spleen Surveys

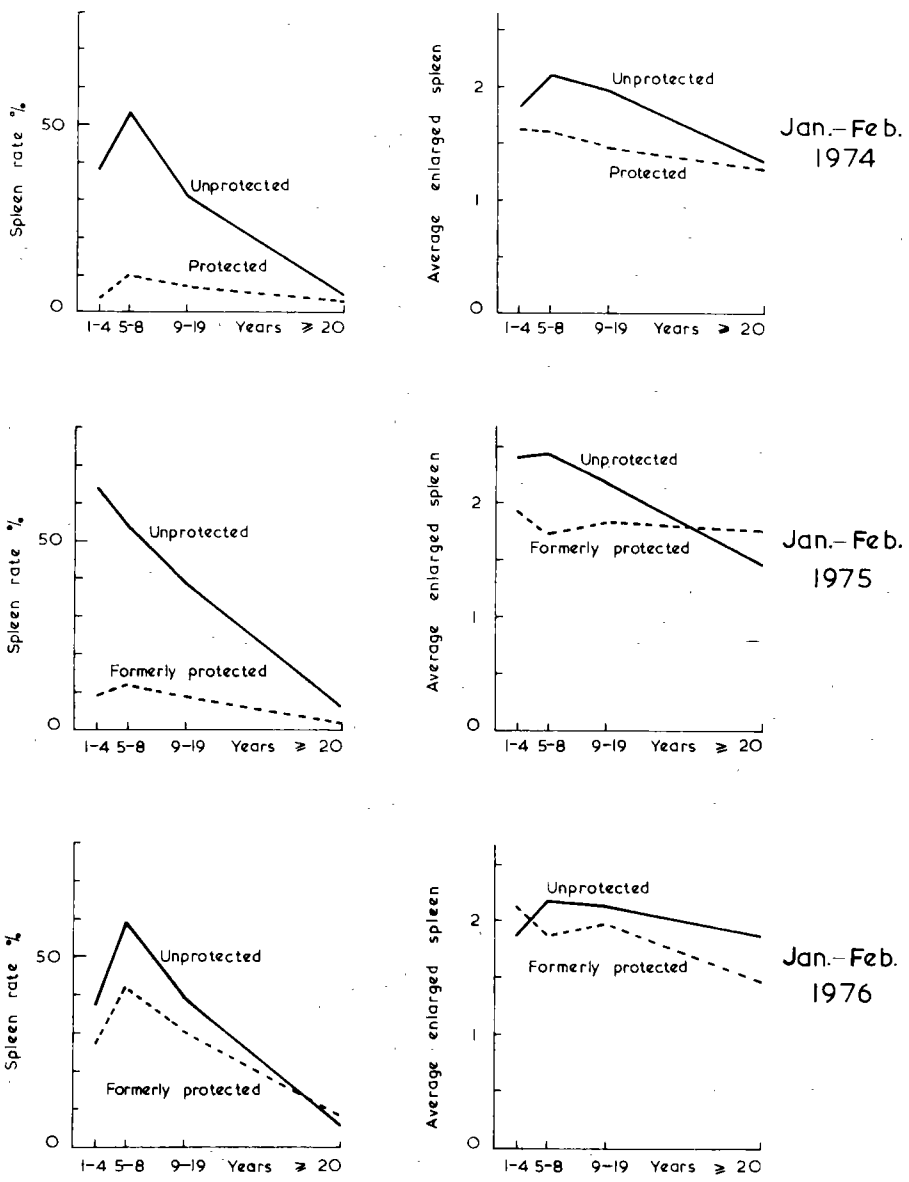
The spleen surveys were conducted simultaneously with the nutritional anthropometric surveys and are also shown graphically (Fig. 75). In the absence of control (village cluster No. 2), the spleen rate was high (around 50%) in young children, low (around 5%) in adults. The distribution of spleen sizes had 2 modes: 0 (not palpable) and class 2 (Hackett); this suggests that a significant number of spleens of class 1 were missed and that the spleen rates were underestimated. The average enlarged spleen (average size of enlarged spleens, where size is represented by Hackett's classification) reached 2–2½ in the age-group 5–8 years and decreased only slightly thereafter. The reduction of malaria to a very low level (1–5%, village clusters No. 5 and No. 7, see Chapter 5) for 1½ years also reduced the spleen rate to a low level, e.g., in the age-groups 1–4 and 5–8 years the spleen rates fell to 4% and 10%, respectively, as compared to 38% and 53% in the untreated comparison group (see the graph for 1974). During the resurgence of malaria in the post-intervention phase, the spleen rate increased towards the same level as in the untreated villages, but while the parasite rate was nearly back to the baseline level in the wet season of 1974 (see Chapter 5), the spleen rate approached the baseline values only about a year later. The control of malaria, and later its resurgence, had apparently only a minor effect on the average enlarged spleen.

Temperature Surveys

Population, study design and methods

Three temperature surveys were conducted: namely, in the middle of the main transmission seasons of 1973, 1974 and 1975 as part of the 15th, 19th and 22nd parasitological surveys, respectively (see Fig. 43). They were conducted in the same population as the anthropometric and spleen surveys, i.e., in village cluster No. 2, untreated throughout, and in village clusters No. 5 and No. 7, treated. The treatment consisted of the

Fig. 75. Spleen rates and the average enlarged spleen in the protected and unprotected populations after $1\frac{1}{2}$ years of protection and 1 and 2 years later



following: (1) in 1972 and 1973: intradomiciliary propoxur plus mass distribution of sulfalene-pyrimethamine every 2 weeks in the wet season, every 10 weeks in the dry season, excluding negative infants; survey 15 was conducted 1 week after the previous round of sulfalene-pyrimethamine; (2) in 1974: 4 rounds of chloroquine, at intervals of 5 weeks, for those below 10 years of age, during the main transmission season; survey 19 was conducted 5 weeks after the previous round of chloroquine; (3) in 1974 and 1975, chloroquine was given to clinical cases, presenting of their own volition, which represented 2.5% of the population in the 5-week period before survey 19 and 7% of the population in the 5-week period before survey 22. In 1972-1973 the following drug dosages were used for those above 10 years of age: 500 mg of sulfalene plus 25 mg of pyrimethamine, 450 mg of chloroquine-base; in younger age-groups, the dosage was appropriately reduced (see Chapters 2 and 3, in particular pp. 23 and 43-49).

Before the application of malaria control measures, the prevalence of *P. falciparum* varied between 41% and 66% in cluster No. 2, and between 42% and 65% in clusters No. 5 and No. 7. At surveys 15, 19, and 22, the crude prevalence of *P. falciparum* was, in the 2 populations respectively, 58% and 5%, 65% and 62%, and 62% and 61%; at survey 19, in clusters No. 5 and No. 7, the prevalence was lower than in the controls below 10 years of age, presumably because of chloroquine, but the prevalence was higher than in the controls above 10 years of age, presumably because of a loss of parasitological immunity (see Chapter 5).

Surveys were conducted in the afternoon by house-to-house visits, guided by the project's own updated census. Axillary thermometers were used, and temperature was recorded to the nearest 0.1 °C. For reasons of acceptability, it was planned not to include adult women in the temperature surveys, and very few were actually included.

Results

Temperature by survey, village cluster (treatment), age, sex and haemoglobin type

Most of the recorded temperatures were rather low: 81% (3759/4647) were below 37 °C, 16% (730) were below 36 °C, while only 6% (270) were above 37.4 °C, and 3% (132) above 37.9 °C.

In 1973, when malaria had been reduced to a very low level in village clusters No. 5 and No. 7, the prevalence of temperatures of 37.5 °C or above was lower in these treated villages than in the control village cluster No. 2 (Table 28); the difference was highly significant in the <9-year age-class. A similar difference, though less pronounced, was

Table 28

Prevalence of body temperatures of 37.5 °C or more, by age, survey and population

Survey	Village clusters	<9 years			≥9 years		
15 (October 1973)	No. 2	11.1%	(25/226)	$p<0.001$	4.2%	(15/361)	n.s.
	No. 5 & 7 (protected)	3.8%	(17/448)		2.5%	(16/634)	
19 (October 1974)	No. 2	17.1%	(36/211)	n.s.	2.7%	(9/329)	n.s.
	No. 5 & 7	12.8%	(55/429)		1.7%	(10/605)	
22 (October 1975)	No. 2	17.4%	(26/149)	$p<0.05$	3%	(9/300)	n.s.
	No. 5 & 7	9.3%	(37/398)		2.7%	(15/557)	

shown in 1975 when the parasitological situation was almost back to the baseline situation. The differences between the two age-groups (<9 and ≥9 years) were significant, except at survey 15 in village clusters No. 5 and No. 7.

Replacing 37.5 °C by 38.0 °C gives smaller numerators, but leads to the same conclusions. At survey 15, the prevalence of temperatures of 38 °C or above in the age-group <9 years was 0.7% (3/448) in the treated population vs. 6.6% (15/226) in the untreated ($p<0.001$). There was no significant difference in the prevalence of elevated temperatures between males and females, either in age-group <9 years or in the age-group 9–18 years (very few older females were examined). There was also no

Table 29

Distribution of persons having a given temperature into density classes for *P. falciparum* asexual stages

Age	Temperature °C	Proportion of fields positive for *P. falciparum* asexual stages				
		0	0.1-4.0	4.1-16.0	16.1-64.0	64.1-100.0
<9 years	<37.5	789 (0.47)	98 (0.06)	84 (0.05)	199 (0.12)	495 (0.30)
	37.5-37.9	28 (0.30)	2 (0.02)	4 (0.04)	15 (0.16)	43 (0.47)
	≥38.0	18 (0.17)	4 (0.04)	3 (0.03)	10 (0.10)	69 (0.66)
≥9 years	<37.5	1 554 (0.57)	380 (0.14)	290 (0.11)	248 (0.09)	240 (0.09)
	37.5-37.9	23 (0.50)	4 (0.09)	5 (0.11)	1 (0.02)	13 (0.28)
	≥38.0	10 (0.36)	4 (0.14)	1 (0.04)	1 (0.04)	12 (0.43)

significant difference in the prevalence of fever between Hb AA and Hb AS persons.

Temperature and P. falciparum

There was a significant relationship between temperature and density of *P. falciparum* asexual stages. There was no consistent difference between surveys or between village clusters in this respect, and the figures were therefore combined (Table 29). It will be seen that, in both age-groups, the parasitological status of persons having a temperature of 38.0 °C or more was very different from the parasitological status of persons having less than 37.5 °C and that those having 37.5 °C to 37.9 °C had an intermediate parasitological status.

Discussion

Nutritional anthropometric surveys

The anthropometric indicators of the nutritional status used in this study were those recommended by Jelliffe (*86*). The nutritional status of the Garki population is not good, even though the international standards that were used may not be ideal as references. Appropriate local standards were unfortunately not available. The nutritional status of infants and young children was slightly better in villages under protection against malaria, than in unprotected villages. The improvement tended to disappear after the end of the intervention period, and it is likely that the difference was caused by the control of malaria. It is generally accepted that infections, including malaria, interfere with nutritional status (*143*). In Pare-Taveta, however, the successful control of malaria by residual spraying produced no significant change in the weight of infants (ages 1–18 months) (*50*). Malaria must be only one of the many factors that determine the poor nutritional status of the population of Garki and other comparable populations.

Spleen rates

The high spleen rate in young children, combined with the low spleen rate in adults, is generally believed to reflect high levels of transmission and of population immunity. Both were certainly present in Garki (see Chapters 4, 5 and 6). After 1½ years of control, the spleen rate was a relatively good indicator of the level of control, but the speed at which the spleen rate declined was not documented. During the resurgence of

malaria, the spleen rate probably increased much more slowly than the parasite rate and was thus not a very good indicator of resurgence. It should be noted, however, that spleens were palpated after the end of the main transmission season.

Temperature surveys

This study of temperature has obvious limitations. There was no baseline survey. The sample (a single cross-sectional temperature survey per year in the middle of the main transmission season, excluding adult women) and the method of measuring temperature were certainly far from ideal. Survey 15 fell about halfway between rounds of drug administration, while survey 19 fell at the very end of the 5-week interval; this may have increased the contrast. While keeping these limitations in mind, the following observations are nevertheless of interest: (1) in the absence of malaria control, high temperatures are more common in children than in adults; (2) the effective control of malaria by propoxur, and especially by the addition of sulfalene-pyrimethamine every 2 weeks, is accompanied by a marked decrease in the prevalence of high temperatures in children, but not in adults (see Table 28, survey 15); (3) the administration of chloroquine every 5 weeks was accompanied by a small, non-significant, reduction in the prevalence of high temperatures (see survey 19, <9 years); it was also accompanied by a lower prevalence of parasitaemia (see p. 156); (4) in the older age-groups, the increase in prevalence of infection above the control levels after interruption of chemoprophylaxis (see Chapter 5) was not accompanied by an increase in the prevalence of high temperatures (see Table 28, survey 19, ≥9 years), suggesting that at ≥9 years there was a loss of parasitological immunity without loss of clinical immunity; (5) there was a positive correlation between temperature and density of *P. falciparum* asexual stages (see Table 29); (6) it can be seen from Table 29 that screening the population for temperatures of 37.5 °C or more would have detected only 14.6% (150/1026) of the positives below 9 years and only 3.4% (41/1199) of the positives above 9 years of age. These findings are probably characteristic of a situation of intense transmission and relatively strong immunity of the survivors.

Summary

Nutritional status, as measured by simple anthropometric indicators, was poor. Infants and young children of the villages in which malaria was controlled for 1½ years by means of residual spraying and MDA had

a slightly better nutritional status than their peers in the unprotected villages. The difference tended to disappear during the post-intervention phase.

In the absence of malaria control, the recorded spleen rates were typical of high levels of transmission and population immunity, namely, about 50% at ages 1–8 years and below 10% in adults. The spleen rate was much lower after 1½ years of malaria control; during the post-intervention phase it returned to the baseline levels more slowly than the prevalence of parasitaemia.

In the unprotected population the wet-season point prevalence of temperatures of 37.5 °C or above was 11% below 9 years of age and 4% above 9 years of age; in the protected population, the prevalence was significantly lower below 9 years of age (4%) but not above that age (2.5%). There was a positive correlation between temperature and *P. falciparum* parasitaemia but it was not very strong, and temperature was a relatively insensitive indicator of parasitaemia. The increase in prevalence of *P. falciparum* above the control levels, detected at ages ≥9 years after the last MDA round, was not accompanied by a corresponding increase in the prevalence of fever.

Chapter Ten

THE MATHEMATICAL MODEL OF TRANSMISSION[a]

The selection of a strategy of control (or eradication) of malaria is, in principle, based on the expected effect of technically feasible intervention methods and on their cost. A mathematical model of the epidemiology of malaria may rationalize this selection by allowing the quantitative comparison of the relative effects of different intervention methods and their combinations, within the expected range of underlying conditions. A fresh attempt to model the epidemiology of malaria was undertaken mainly because previous models do not take into account the effect of immunity on transmission; this may be relatively unimportant for a theory of eradication, but is crucial for a theory of control where the end-point is a new balance of the host and parasite populations.

The model presented here was constructed as an integral part of the project, and was developed in close interaction with the field work (*46*). The specific objectives of the model are: (*a*) to describe the quantitative relationship between the entomological variables and the incidence and prevalence of microscopically detectable *P. falciparum* infections, including their variation by age and season; and (*b*) to compare the expected parasitological effects of specified control measures (larvicides, adulticides, drugs), alone or in combination, at specified expected levels of coverage and effectiveness.

The model attempts to represent the natural history of the infection in man, and its transmission, by a structure which can be manipulated either analytically or by computer simulation. The model, even if it appears complicated to the non-mathematician, is obviously much simpler than malaria itself. Certain epidemiological features are selected while others are neglected, and the selected features are translated into simple and clear-cut assumptions. These assumptions define the struc-

[a] The work reported in this chapter was performed by Dr K. Dietz, Dr L. Molineaux and Mr A. Thomas. They used the data collected in Garki under the direction of Dr S.P. Ramakrishnan and in Kisumu under the direction of Dr R.E. Fontaine.

ture of a model. The model may be discussed *a priori* in terms of what is known about malaria. The model may also be tested *a posteriori* with respect to its capacity to simulate the actual epidemiology of malaria. This discussion and testing of the model was performed in 3 stages. In a first stage, both the structure (the assumptions) and the numerical values of the parameters were varied by informal trial and error until a structure (a set of assumptions) was found which was qualitatively satisfactory in terms of epidemiological behaviour. This was done by a continuous interaction between study of the literature, field observations in Garki (see Chapter 5, in particular pp. 159-162), model building, and computer simulations. The first section below describes the outcome of that first stage. In a second stage, the model was fitted formally to a particular epidemiological situation, namely, the Garki baseline situation, by letting the computer find, for some parameters, the numerical values producing the best fit between model simulations and actual observations; this second stage is described in the section that follows. In a third stage, the entomological observations from different epidemiological situations (e.g., Garki or Kisumu after application of a residual insecticide) were used as input into the model, without change in the other parameters, and the resulting parasitological output was compared to actual observations; this stage is described in the third of the following sections. Once the model has been tested, it may be used as a tool to understand and teach the epidemiology of malaria (see pp. 281-282 and 287) and to plan its control (see p. 286).

The Assumptions of the Model

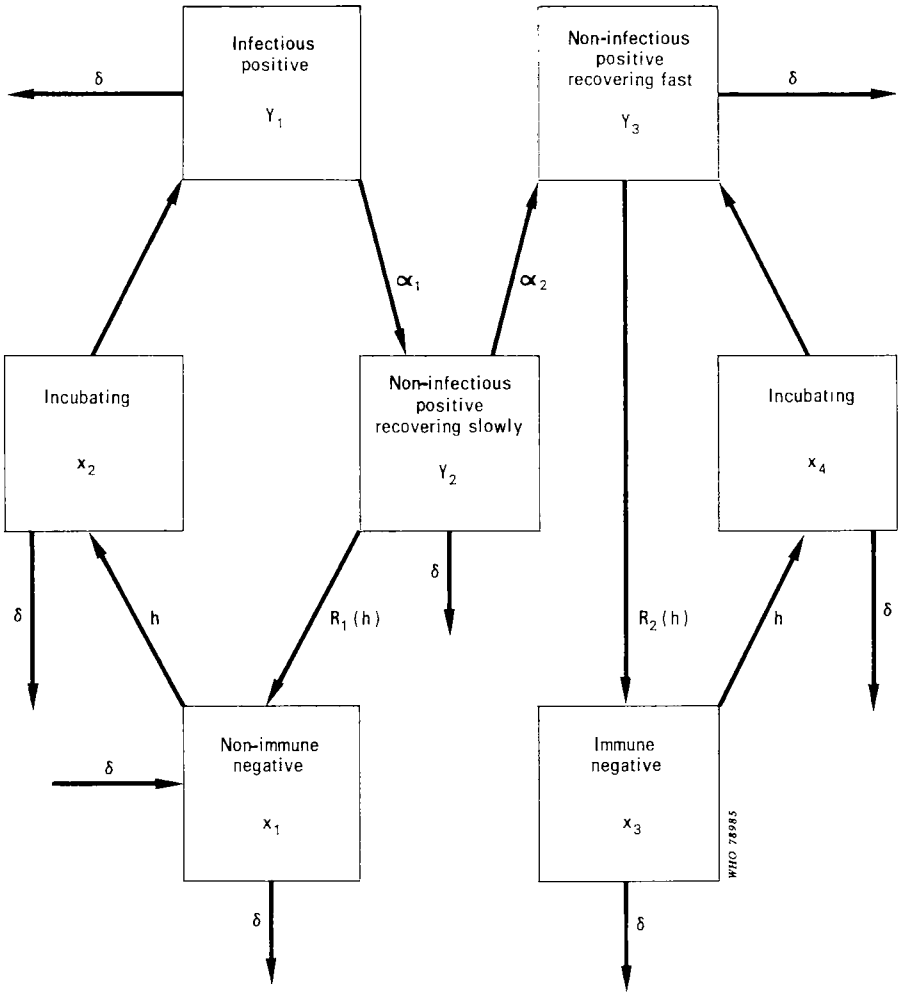
Epidemiological states and transitions

The epidemiological states (or classes) and the transitions between classes by which the model simulates the natural history of *P. falciparum* in man are shown in Fig. 76. The symbols used in the model are listed and defined in Table 30. The letters $x_1, \ldots, x_4$, and $y_1, \ldots, y_3$ are used both to denote classes and the proportions of the population occupying the classes. The population size is thus set to 1:

$$\sum_{i=1}^{4} x_i + \sum_{i=1}^{3} y_i = 1$$

Man is born into the nonimmune negative state x_1; i.e., passive immunity is ignored. Nonimmune negatives are effectively inoculated at a rate h

Fig. 76. States and transitions of the model



(see p. 267) and transferred to the incubating class x_2, in which they stay for a fixed incubation period of N days. After that, they become positive and infectious, in class y_1. While in y_1 the person is infective to mosquitos (see pp. 267-268) and is positive, with a probability q_1, by a standard blood examination, e.g., by the examination of 200 fields of a thick film. Infectivity is lost at a constant rate α_1, at which persons move to the state y_2 in which they are noninfectious but still positive, with a probability q_2, by microscopic examination. Once in state y_2, a person may either recover from infection and return to the nonimmune negative state x_1 at a

Table 30

Symbols and definitions used in the model

Symbol	Name	Definition and/or comments
a	man-biting habit	No. of bloodmeals taken on man by 1 vector in 1 day
C	vectorial capacity	$C = ma^2p^n/(-\ln p)$; see p. 267
g	susceptibility	probability that an infection results, given that at least 1 contact has occurred, in 1 time-unit
h	effective inoculation rate	probability that a negative acquires the infection (becomes incubating), in 1 time-unit; $h = g\{1-\exp(-Cy_1)\}$ see p. 267
m	relative density of vectors	No. of vectors per man, i.e., ratio between the size of the vector population and the size of the human population
n	extrinsic incubation period	incubation in the vector
N	intrinsic incubation period	incubation period in man
p	probability of surviving 1 day	probability that the vector survives 1 day
q_1, q_2, q_3	probability of detection	probabilities that the 3 kinds of positives (y_1, y_2, y_3) are detected by a standard parasitologic examination
r_1, r_2	basic recovery rates of non-immunes, immunes	recovery rate from 1 infection of the y_2, y_3, respectively
$R_1(h), R_2(h)$	actual recovery rates of non-immunes, immunes	actual rate at which the y_2, y_3, recover, taking into account the superinfections resulting from a given h; $R_i(h) = h/\{\exp(h/r_i)-1\}, i = 1,2$; see pp. 265-266
t	time	$x_1(t)$ designates x_1 at time t, $x_1(t-N)$ designates x_1 at time $(t-N)$, etc.
T_1, T_2	expected duration of states y_2, y_3	$T_i = 1/R_i(h), i = 1,2$
x_1, x_3	nonimmune, immune negatives	see pp. 262-265
x_2, x_4	nonimmune, immune incubating	see pp. 262-265
y_1, y_2, y_3	3 kinds of positives	see pp. 262-265
y	true proportion positive	$y_1 + y_2 + y_3$, or $\sum_{i=1}^{3} y_i$
z	observed proportion positive	$q_1y_1 + q_2y_2 + q_3y_3$, or $\sum_{i=1}^{3} q_iy_i$
α_1	rate of loss of infectivity	see p. 263
α_2	rate of acquisition of a high recovery rate	see below
δ	death rate	also birth rate, see p. 266

rate $R_1(h)$, or become an "immune positive" (y_3, see below) at a constant rate α_2. The actual recovery rate $R_1(h)$ is a function of a constant, r_1, which is the basic recovery rate of nonimmunes and of h, the effective

inoculation rate: as the inoculation rate increases, the actual recovery rate decreases, i.e., superinfection prevents recovery (see following subsection) and an increasing proportion of y_2 move to y_3, i.e., become "immune positives". Persons in y_3 are still positive, with a probability q_3, by microscopic examination, and noninfectious; q_3 is smaller than q_1 and q_2; i.e., the probability of diagnosis by a standard blood examination decreases as immunity increases. The "immune positives" in y_3 recover from infection at a rate $R_2(h)$, which is a function of r_2, the basic recovery rate of immunes, and of h; r_2 is larger than r_1, i.e., immunes tend to recover faster than nonimmunes, but here also superinfection reduces the recovery rate $R_2(h)$ (see following subsection). If an immune positive (y_3) recovers from infection he becomes an immune negative (x_3). Immune negatives are successfully inoculated at the same rate (h) as the non-immune negatives, and incubate the infection for the same period of N days, after which they are again "immune positives" (y_3), i.e., detectable with probability q_3, noninfectious, and with the "high" basic recovery rate r_2.

A person may go through several cycles $x_1 \rightarrow x_2 \rightarrow y_1 \rightarrow y_2 \rightarrow x_1$ etc. before moving to y_3. A person may also go through several cycles $y_3 \rightarrow x_3 \rightarrow x_4 \rightarrow y_3$ etc. As the inoculation rate h increases, the recovery rates R_1 and R_2 decrease and an increasing proportion of persons travel the route $x_1 \rightarrow x_2 \rightarrow y_1 \rightarrow y_2 \rightarrow y_3$, without returning to x_1, and, once in y_3, either stay there or, if they recover, are quickly reinoculated and so return, through x_4, to y_3.

Superinfection

The effect of h on $R_1(h)$, $R_2(h)$, i.e., the effect of superinfection on recovery, is handled as follows. We accept Macdonald's assumption: "The existence of infection is no barrier to superinfection, so that two or more broods of organisms may flourish side by side, the duration of infection due to one being unaltered by the others" (*96*). His formula however—i.e., $R = r - h$, for $h<r$; and $R = 0$, for $h \geq r$—represents a quite different assumption, namely, that the durations of the individual inoculations received during one episode are additive, as if an individual could only recover from one inoculation at a time. A new formula was therefore derived for the actual recovery rate in the presence of superinfection, as follows. Inoculations "arrive" according to a Poisson process with rate h. The infection resulting from each inoculation is terminated at a rate r, and has therefore an expected duration of $1/r$. Then, in equilibrium, the number of infections present at any time in a person is a Poisson variable with mean h/r. Hence the probability that an individual has no infection is given by $\exp(-h/r)$. The correct formula

for this probability has been given by Walton (*161*). At equilibrium, this probability is equal to the proportion of infection-free time in any given period. Consider in particular the period between the onsets of 2 consecutive positive episodes; the period is the sum of T, the duration of a positive episode, plus $1/h$, the infection-free waiting time for a new episode. Therefore:

$$\exp(-h/r) = (1/h)/\{T + (1/h)\},$$

which leads to:

$$T = \{\exp(h/r)-1\}/h.$$

The actual recovery rate is the inverse of the expected duration of a positive episode T, i.e.:

$$R(h) = h/\{\exp(h/r)-1\}$$

Substituting the basic recovery rates r_1, r_2 (see previous subsection) into the formula, we get the actual recovery rates $R_1(h)$, $R_2(h)$. For low values of h, this recovery rate is close to the one calculated by Macdonald's formula. But for $h = r$, for instance, the present formula reduces r only by a factor of about 1.7,[a] whereas Macdonald's actual recovery rate would already be 0—i.e., the expected duration of a positive episode would be infinite.

The problem of the mathematical formulation of superinfection has been reviewed by Fine (*58*).

Dynamics of the human population

A very simple demographic model is included in the transmission model. Births are added to class x_1; deaths occur in all classes. There could be as many death rates as there are classes, i.e., mortality could vary with parasitological status or immunity status or both. Age is implicit in the model (see p. 269) and mortality cannot be varied by age *per se* without change in the model structure.

In the simulations described in this chapter, a single death rate has been adopted, i.e., the death rate is independent of age, parasitological status and immune status. The birth rate has been made equal to the death rate (in Fig. 76, both rates are represented by δ). This results in a stable population with an exponential age-distribution.

These very simple demographic assumptions considerably simplify the computations. Note that they are made for the purpose of simulations which explore questions of transmission. For simulations exploring the

[a] $r/R = r\{\exp(h/r) - 1\}/h$; if $h = r$, this becomes $r/R = e - 1 = 2.72 - 1 = 1.72$.

demographic effects of malaria and its control, changes would obviously be required.

Vectorial capacity and inoculation rate

All the information about the vector populations is incorporated into one time-dependent variable, the vectorial capacity C. As in Garrett-Jones (*66*), $C(t)$ is defined here as the number of bites on man that those vectors having bitten an individual on day t distribute after the extrinsic cycle of duration n during the rest of their life. In other words, C is the number of potentially infective contacts an individual person makes, through the vector population, per unit time. For one vector population with density m (i.e., number of vectors alive per human individual), man-biting habit a and daily survival probability p, the formula given by Garrett-Jones is $ma^2p^n/(-\ln p)$.

The formula of the vectorial capacity may be derived as follows: a person is bitten by ma vectors in 1 day; a fraction p^n of the vectors survive the extrinsic cycle (incubation period); they still have an expectation of life of $1/-\ln p$ (the expectation of life is assumed to be independent of age); each of the surviving vectors bites a persons per day.

If there are several, say J, vector populations, either different species or subpopulations with different characteristics, with man-biting habits a_j, daily survival probabilities p_j and possibly time-dependent densities $m_j(t)$, then the total vectorial capacity $C(t)$ is the sum of the vectorial capacities of the individual populations:

$$C(t) = \sum_{j=1}^{J} m_j(t)a_j^2p_j^n/(-\ln p_j)$$

If a_j, p_j, and n are also time-dependent the formula is slightly more complicated. The definition of C depends on entomological variables and on the duration of the extrinsic cycle, which is specific for the parasite considered. It has a meaning independent of the parasite rate, of the sporozoite rate, and of the presence or absence of parasites in a particular population.

The inoculation rate h (effective inoculation rate or incidence rate) is defined as the rate at which negatives are transferred to positive via the incubating state. We assume the following formula for the inoculation rate $h(t)$ on day t:

$$h(t) = g\{1-\exp(-C(t-n)y_1(t-n)\},$$

which is based on the following interpretation. In a stable situation, where C and y_1 are constant, each member of the population receives C

potentially infective contacts per day; a fraction y_1 of these contacts originates from infectives and represents inoculations; therefore the average number of inoculations per person per day is Cy_1; assuming a Poisson distribution, the probability of receiving no inoculation is $\exp(-Cy_1)$, and the probability of receiving at least one is $1-\exp(-Cy_1)$. The parameter g is then defined as the conditional probability that an infection results, given that at least one inoculation has occurred. We shall call g the susceptibility. If the situation is not stable, C, y_1 and h all vary over time, and we have to take into account that the inoculations received on day t originated on day $(t-n)$, or before; it is assumed, however, that they all originated on day $(t-n)$, hence the above formula for $h(t)$. This assumption that all the inoculations originated on day $(t-n)$ considerably simplifies the structure of the model and should be close enough to reality for a relatively short-lived vector. The formula for the inoculation rate implies a strong density-dependent regulation of transmission: the inoculation rate increases linearly with vectorial capacity only for small values of C. For high vectorial capacities the inoculation rate reaches a saturation level. We assume uniform exposure to the inoculation rate.

Equations of the model

The assumptions listed above lead to a set of 7 difference equations, each one expressing the change in the proportion of the population occupying one of the 7 states of the model. The difference equations can easily be derived from Fig. 76 and the assumptions. Considering x_1 for instance during 1 unit of time, i.e., the interval $(t, t+1)$: (1) δ births are added (the birth rate is applied to the whole population, set to 1, as x_1, x_2 etc. are proportions, see above); (2) a fraction $R_1(h)$ of y_2 recover and are also added to x_1; (3) a fraction h of x_1 are effectively inoculated and are subtracted (moved to x_2); (4) a fraction δ of x_1 die and are also subtracted. Hence the first difference equation:

$$\Delta x_1 = \delta + R_1(h)y_2 - (h + \delta)x_1.$$

For simplicity, the difference equations will be written here for an iteration interval of 1 day (in the computer version the iteration interval is variable; for most of the calculations described below, a 5-day iteration interval has been used). As usual, the symbol Δ denotes the difference operator, e.g., $\Delta x_1 = x_1(t+1) - x_1(t)$. The time variable will be omitted except where reference is made to a time different from t.

Considering x_2, during the interval $(t, t+1)$: (1) hx_1 are added; (2) $(1-\delta)^N h(t-N)x_1(t-N)$ are subtracted by moving to x_3, i.e., from

incubating to positive; the expression may be understood as follows: those completing their incubation period at time t entered the incubating state at time $(t - N)$; at that time, $h_1(t - N)x_1\ (t - N)$ entered the incubating state x_2; $(1 - \delta)^N$ of them have survived their incubating state; (3) δx_2 are subtracted by death. Hence the second difference equation:

$$\Delta x_2 = hx_1 - (1 - \delta)^N h(t - N)x_1(t - N) - \delta x_2$$

The 5 remaining difference equations are similarly derived, giving:

$$\begin{aligned}
\Delta x_3 &= R_2(h)y_3 - (h + \delta)x_3, \\
\Delta x_4 &= hx_3 - (1 - \delta)^N h(t - N)x_3(t - N) - \delta x_4, \\
\Delta y_1 &= (1 - \delta)^N h(t - N)x_1(t - N) - (\alpha_1 + \delta)y_1, \\
\Delta y_2 &= \alpha_1 y_1 - \{\alpha_2 + R_1(h) + \delta\}y_2, \text{ and} \\
\Delta y_3 &= \alpha_2 y_2 + (1 - \delta)^N h(t - N)x_3(t - N) - \{R_2(h) + \delta\}y_3.
\end{aligned}$$

In order to stimulate the transmission in a particular population, one has to provide only the yearly pattern of the vectorial capacity $C(t)$. The computer programme reproduces, after a suitable running-in period during which equilibrium is reached, the seasonal changes of any output variable one might wish to study. Among those are the daily inoculation rate h, the observable proportion positive:

$$z = \sum_{i=1}^{3} q_i y_i,$$

the true proportion positive:

$$y = \sum_{i=1}^{3} y_1,$$

the proportion of infectious among the positive y_1/y, etc.

One can also use these equations in a simple way to calculate the age-specific values of these variables under the assumption that a yearly pattern of vectorial capacity is repeating itself. After one has obtained the stable oscillation for the total population according to the equations above, one applies the yearly pattern of the inoculation rate to a cohort formed by 1 individual initially in x_1 representing a newborn child. Time is now interpreted as age of the cohort. The above equations are used as before except that the death (and birth) rate δ is put at 0 and that now the inoculation rate is used as an input parameter instead of the vectorial capacity.

Fitting of the Model

Model simulations have been compared to field observations in order to estimate the parameter values giving the best fit possible, and to evaluate the model by analysing the discrepancies between observed and expected values.

Data selected for fitting

As stated, the first specific objective of the model is to predict the prevalence and incidence of *P. falciparum* from entomological observations. The 2 villages with extreme vectorial densities were selected, and we set ourselves the task of simulating the 2 epidemiological situations as observed during the first year of the project with the same model and the same parameters, with the exception of 2 entomological input parameters (m and a).

Entomological data

Figure 77 shows the observed man-biting rates in the 2 villages; captures were made every 5 weeks in the dry season, every 2 weeks in the wet season; each data point is based on the average of 8 man-nights. From these man-biting rates, from other observations (age composition of the night-bite collection, distribution by abdominal stages, identification of blood meals of pyrethrum spray collections, and temperatures), and from certain assumptions, the vectorial capacities to be used as input were

Fig. 77. Observed man-biting rates (asterisks and circles) and estimated vectorial capacities (solid and broken lines) for *A. gambiae* and *A. funestus* combined

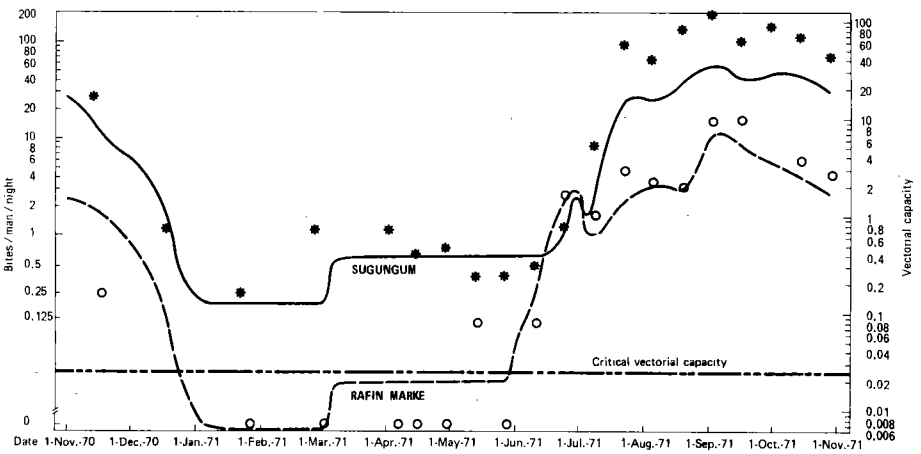
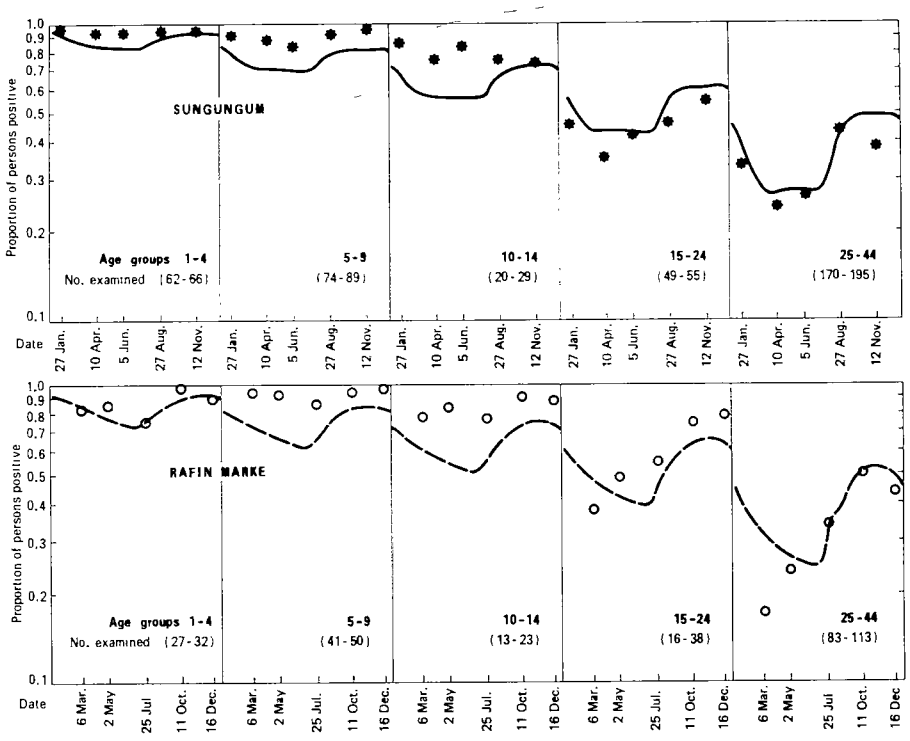


Fig. 78. Comparison of observed (asterisks and circles) and simulated (solid and broken lines) apparent proportions positive



calculated (see pp. 74-75); they are also shown in Fig. 77. It is assumed that this pattern of vectorial capacities was repeating itself year after year.

Parasitological data

Figure 78 shows the observed prevalence of *P. falciparum* in the 2 villages by age and season; here also we assume that the first year of the project was representative of the past; the first survey has been plotted after the fifth for convenience, i.e., in order to show the 5 surveys within a single calendar year. The data show the general pattern already described (see pp. 116-117), with some irregular fluctuation owing to relatively small numbers. The ranges of the numbers examined among the 5 surveys are given (within parentheses) in Fig. 78. The 2 villages have practically the same crude average prevalence; the age of maximum prevalence is lower in Sugungum, which must be the result of a higher average inoculation rate; the seasonal fluctuations are somewhat larger in Rafin Marke, possibly owing to a combination of shorter transmission season

and lower immunity. In addition to these prevalence data (50 data points), the infant conversion rates in the interval between surveys 4 and 5 were also used for fitting (2 data points).

Estimation of model parameters

In order to reduce, as far as possible, the number of parameters to be fitted, we assumed certain values for some of them, on the basis of data from the literature, findings in Garki, and preliminary simulations. These assumed values are listed in Table 31.

Table 31

Parameters corresponding to the fit shown by Fig. 78

(a) *Assumed*		
Birth and death rates of the human population	δ	36.5 per 1000 per year
Vectorial capacity	C	see Fig. 77
Ratio between high and low recovery	r_2/r_1	10
Detectability of positives	q_1, q_2	1
	q_3	0.7
Incubation period in man	N	15 days
Incubation period in vector	n	10 days
Rate of losing infectivity	α_1	0.002/day
(b) *Estimated by fitting*		
Daily rate of acquiring high recovery rate	α_2	0.00019 ± 0.00001
Susceptibility	g	0.097 ± 0.017
Low daily recovery rate	r_1	0.0023 ± 0.0005

For the estimation of δ we calculated the average age of the human population, which was approximately 27 years. On the assumption of an exponential stable age-distribution this corresponds to a daily death rate of about 0.0001 per person.

The ratio between high and low recovery rates r_2/r_1 was set at 10 on the basis of the effect of immunity on the clearance rate of parasitaemia (see p. 128 and Ref. 7)

The estimates for the 3 fitted parameters were obtained by minimizing a χ^2 function that measured the discrepancy between the observed and predicted values. The age-specific apparent parasite rates are calculated, according to the method described above, for the ages of 3, 7, 12, 19 and 32 years; these are the average ages of the observed age-groups 1–4, 5–9, 10–14, 15–24 and 25–44 years, respectively. The age-group ≥45 has been omitted to save computer time, since for every trial set of parameters the computer has first to simulate on the average 30 years of transmission until equilibrium is reached and then apply the inoculation rate thus obtained to a cohort for another 32 years to get the age-specific parasite rates

corresponding to the age-group 25–44 years. Because of the small differences in the parasite rates between the age-groups 25–44 and ≥ 45 years there was no great loss of information. The χ^2 function minimization was done by the CERN (Centre européen pour la Recherche nucléaire) computer programme MINROS. The minimum value obtained on 52 data points is 53.5, 3 parameters being estimated. Their values, together with their standard deviations, are given in Table 31.

As can be seen from Fig. 78, the age-distribution predicted by the model approaches somewhat too rapidly the equilibrium for the adult population, owing to our simple assumption of a constant rate α_2 for acquiring the high recovery rate. The estimate for α_2 implies that it takes an average of about 14.3 years for a positive person to acquire the high level of recovery rate, given that he does not recover before and that he does not die.

Testing of the Model

The malaria model, previously fitted to 1 year of baseline data from the Garki District in the Sudan savanna of northern Nigeria, was tested against data collected in the same area over a period of 3 years, including $1\frac{1}{2}$ years under intradomiciliary propoxur (a carbamate) in certain villages, and against data collected in Kisumu, Kenya, also over a period of 3 years, including 20 months under intradomiciliary fenitrothion (an organophosphorus compound) in part of the area (*64*). The test consisted in using the vectorial capacity, calculated from the entomological observations made in the above places and periods, as input in the Garki model, while keeping the other parameters as fitted to the Garki baseline data, and in comparing the prevalence of *P. falciparum* parasitaemia, as put out by the model, to the one actually observed (*118*).

Method of evaluation

The model calculates the expected proportion of persons found positive, for *P. falciparum*, by the examination of 200 fields of a standard thick blood film, as a function of age and time, given the vectorial capacity and the birth and death rates of the human population. The input parameters of the model fall into 2 categories: (1) constants which govern the interaction between *P. falciparum* and man, e.g., the rate at which a nonimmune person loses infectivity and gains immunity and the recovery rates of nonimmune and immune persons; these parameters were estimated in the process of fitting the model to the Garki baseline

data; they are not expected to vary between epidemiological situations, except if there are relevant genetic differences in either parasite or man; (2) the variables which distinguish one epidemiological situation from another, i.e., the vectorial capacity and to some extent the demographic variables. With respect to the latter, the assumption of equal and constant birth and death rates of 36.5 per 1000 per year reproduces approximately the age-distribution actually observed in Garki. The age-distribution observed in Kisumu was similar and no major change was expected, in the short run, from the application of insecticides. The demographic variables were therefore treated as constants and only the vectorial capacity was varied between the simulation giving the best fit to the Garki baseline data and the simulations described below.

These simulations test, simultaneously: (1) the model's structure; (2) the Garki parameters (except the vectorial capacity); and (3) the possibility of standardizing the estimation of the vectorial capacity in different situations. The criterion of evaluation is the comparison between the parasite rates put out by the model and those actually observed.

Testing of the model against observations made in Garki before and during the application of propoxur

The test is based on the longitudinal study of 4 villages, each one followed as an epidemiological unit, for the 3-year period 1971-1973; 2 of the villages were left untreated throughout; in the 2 other villages, as well as in the villages surrounding them, human dwellings were sprayed indoors with propoxur before and during the wet seasons of 1972 and 1973.

The input vectorial capacity was calculated as follows (see also pp. 74 and 86): (1) ma was estimated by night-bite collections on human baits, taking the average between indoors and outdoors, and over whole seasons (wet and dry), in each of the 4 villages; for the seasons of low density, the average was treated as a constant; for the seasons of high density, the actual seasonal variation was closely approximated by assuming equal periods of linear increase and decrease, while keeping the seasonal average equal to the estimate; (2) a was estimated as on p. 74; (3) n was set to 10 days in the wet season or 17 days in the dry season according to the formula of Moskovskij (in *42*), and the average outdoor temperature in the project villages; (4) the expectation of life $1/(-\ln p)$ was set at 5 days ($p = 0.819$) for both species, in the wet season; in the dry season p^n was assumed to be the same as in the wet season, and the expectation of life was increased accordingly to 8.5 days ($p = 0.889$); it was also assumed that the man-biting rate estimated after spraying was due to unaffected mosquitos, and the same expectation of life was used as before (see pp. 86-88).

Table 32

Vectorial capacities in Garki project, as calculated from field observations, and used as model inputs

Period	Season	Village			
		Kwaru	Ajura	Sugungum	Ungwar Bako
1 Jan. 71 to 20 Jun. 71	dry	0.25	0.084	1.52	0.23
21 Jun. 71 to 7 Nov. 71	wet	3.52	3.34	21.74	3.43
8 Nov. 71 to 21 May 72	dry	0.19	0.13	1.63	0.49
22 May 72 to 22 Oct. 72	wet	1.09	1.57	0.66[a]	0.068[a]
23 Oct. 72 to 17 Jun. 73	dry	0.084	0.006	0.044	0.0
18 Jun. 73 to 4 Nov. 73	wet	4.20	3.40	2.83[a]	0.24[a]
5 Nov. 73 to 31 Dec. 73[b]	dry	0.084	0.006	0.044	0.0

[a] Under propoxur.
[b] Values from the previous dry season.

In summary, the input vectorial capacity, C, was computed by multiplying the estimated man-biting rates by the factor $ap^n/(-\ln p)$; this varied, according to the above, by species, season and place, as follows:

	A. gambiae		*A. funestus*
	Sugungum	*Other villages*	*All villages*
Wet season	0.206	0.308	0.328
Dry season	0.351	0.523	0.558

e.g., in Sugungum, in the wet season, given the estimated man-biting rates, $\hat{ma}$ (*A. gambiae*) and $\hat{ma}$ (*A. funestus*):

$$C = 0.206\,\hat{ma}\,(A.\,gambiae) + 0.328\,\hat{ma}\,(A.\,funestus).^{a}$$

Table 32 shows the vectorial capacity computed in this way and used as input into the model, all other parameter values being identical to those obtained previously in the fitting process. For each village the first year's vectorial capacity was used until a stable pattern of malaria was produced, after which the subsequent 2 years of vectorial capacity were used; the same was done with vectorial capacities 10 times larger and 10 times smaller than the estimated. Fig. 79 and 80 show the prevalence of *P. falciparum* put out by the model, at the 3 levels of vectorial capacity, and also

[a] The "dry season factors" are smaller than on p. 75, and they are not applied over exactly the same period; the effect of the change is negligible, due to low vector densities.

the prevalence actually observed by the examination of 200 fields of a thick-blood film at successive surveys in the 4 villages. Given the estimated vectorial capacity, the model output agrees fairly well with the observations, except for 1972 in the 2 untreated villages. Multiplying or dividing the input vectorial capacity by 10 affected the output relatively little, but the actually estimated vectorial capacity *C* produced an output which was more realistic than the one produced either by 10 *C* (see Ungwar Bako in 1973, Sugungum in 1972 and 1973) or 0.1 *C* (see Sugungum in 1973).

Fig. 79. Prevalence of *P. falciparum* in 2 control villages, as observed (x) and as calculated from the estimated vectorial capacity, C, and from 10 C and 0.1 C

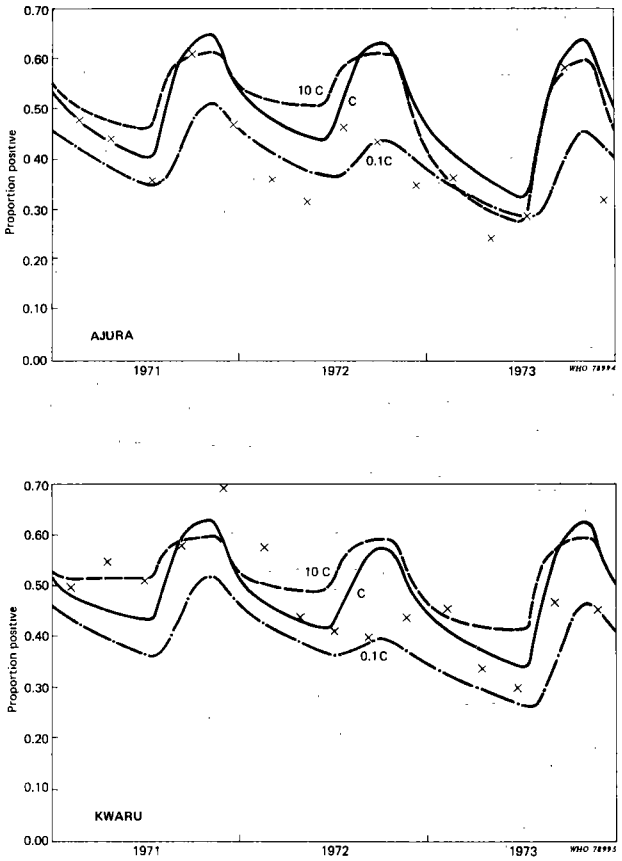
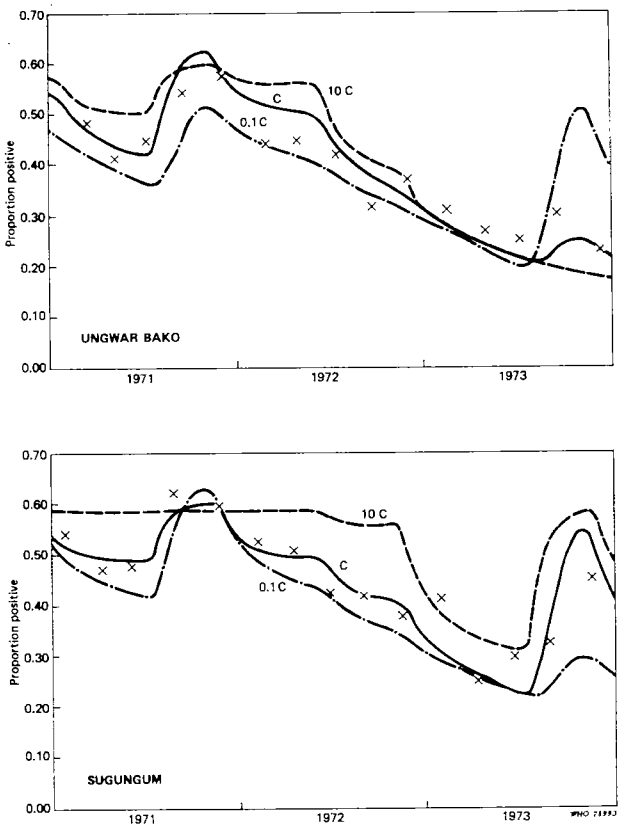


Fig. 80. Prevalence of *P. falciparum* in 2 villages sprayed with propoxur in 1972-1973, as observed (x) and as calculated from the estimated vectorial capacity, C, and from 10 C and 0.1 C



Testing of the model against observations made in Kisumu before and after the application of fenitrothion

The test is based on the longitudinal study of the evaluation area and comparison area from March 1972 to September 1975. Starting in August 1973, the human dwellings of the evaluation area were sprayed indoors with fenitrothion.

The input vectorial capacity was calculated as follows: (1) *ma* was estimated by night-biting collection on human baits indoors, taking monthly averages; (2) *a* was estimated by dividing the human blood index by the interval between blood meals; the human blood index in the baseline pyrethrum spray collections was 0.946 for *A. gambiae*, 0.991 for *A. fun-*

estus; the interval between blood meals was set to 2 days for *A. gambiae* and 3 days for *A. funestus*, based on the local temperature and the findings of Gillies (*68*); (3) n was set to 16 days, according to the formula of Moskovskij and the average outdoor temperature at Kisumu airport; the seasonal variation in temperature was very small; (4) the expectation of life was set at 6 days ($p = 0.846$) for both species, based on the findings of Gillies & Wilkes (*71*) in a relatively similar environment, namely Gonja, United Republic of Tanzania; as in Garki, it was assumed that the man-biting rate estimated after spraying was due to unaffected mosquitos, and the same expectation of life was used as before (see pp. 86-88).

In summary, the factor $ap^n/(-\ln p)$ was, according to the above, equal to 0.197 for *A. gambiae* and 0.138 for *A. funestus*; and the input vectorial capacity C was computed as follows, given the estimated man-biting rates, $\hat{ma}$ (*A. gambiae*) and $\hat{ma}$ (*A. funestus*):

$$C = 0.197\,\hat{ma}\,(A.\,gambiae) + 0.138\,\hat{ma}\,(A.\,funestus).$$

Table 33

Vectorial capacities in Kisumu project, as calculated from field observations, and used as model inputs

Month	1972		1973		1974		1975	
	E[a]	C[b]	E[a]	C[b]	E[a]	C[b]	E[a]	C[b]
Jan.			2.57	2.04	0.0	0.60	0.0	0.16
Feb.			1.70	2.73	0.0	0.36	0.0	0.36
Mar.	1.78	1.73	1.12	0.71	0.0	1.20	0.0	1.22
Apr.	1.71	1.16	1.06	0.50	0.017	22.25		
May	8.19	3.12	4.15	3.68	0.16	8.34		
Jun.	7.57	6.23	2.62	3.76	0.016	0.96		
Jul.	3.28	1.54	1.01	0.61	0.003	0.66		
Aug.	0.64	1.81	0.014	0.93	0.0	0.60		
Sep.	1.36	1.10	0.0	0.74	0.0	0.33		
Oct.	0.38	2.00	0.0	0.85	0.0	0.21		
Nov.	4.37	10.87	0.004	1.81	0.0	0.070		
Dec.	4.93	3.79	0.0	2.90	0.0	0.016		

[a] E: evaluation area, under fenitrothion, starting in August 1973
[b] C: comparison area.

Table 33 shows the vectorial capacity, computed in this way, for each of the 2 areas and used as input; the first year's vectorial capacity was used until a stable pattern of malaria was produced. Fig. 81 shows the age-specific prevalence of *P. falciparum* calculated by the model from the baseline vectorial capacity in the evaluation area, and also the age-specific prevalence actually observed by the examination of 200 fields of a thick blood film at 2 surveys with an interval of 6 months. There is again a fairly good agreement between the model and the observations. The agreement was not quite as good in the comparison area. Fig. 82 shows

Fig. 81. Baseline age-specific prevalence of *P. falciparum* in Kisumu evaluation area, as observed (estimate and 95% confidence limits) and as calculated from the estimated vectorial capacity

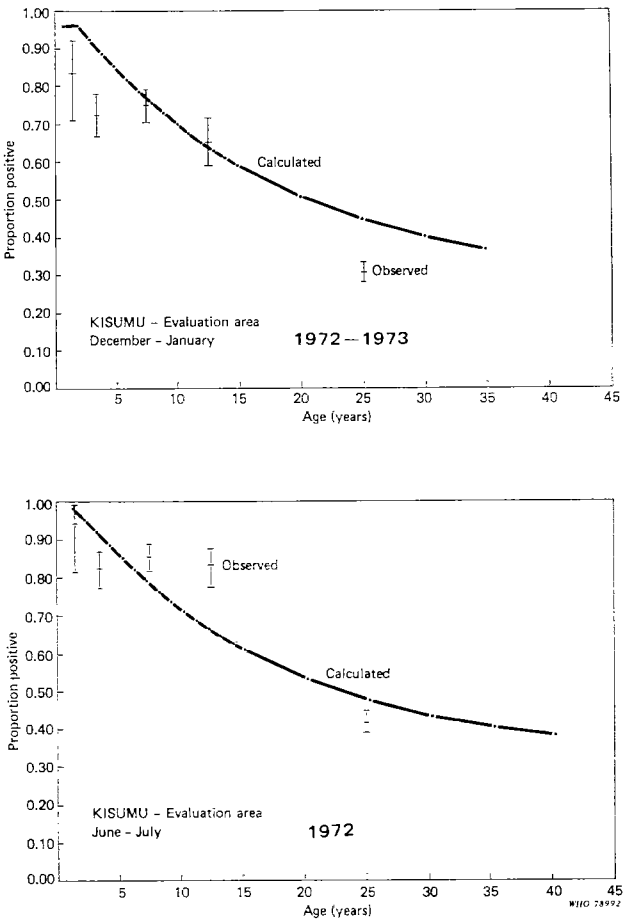
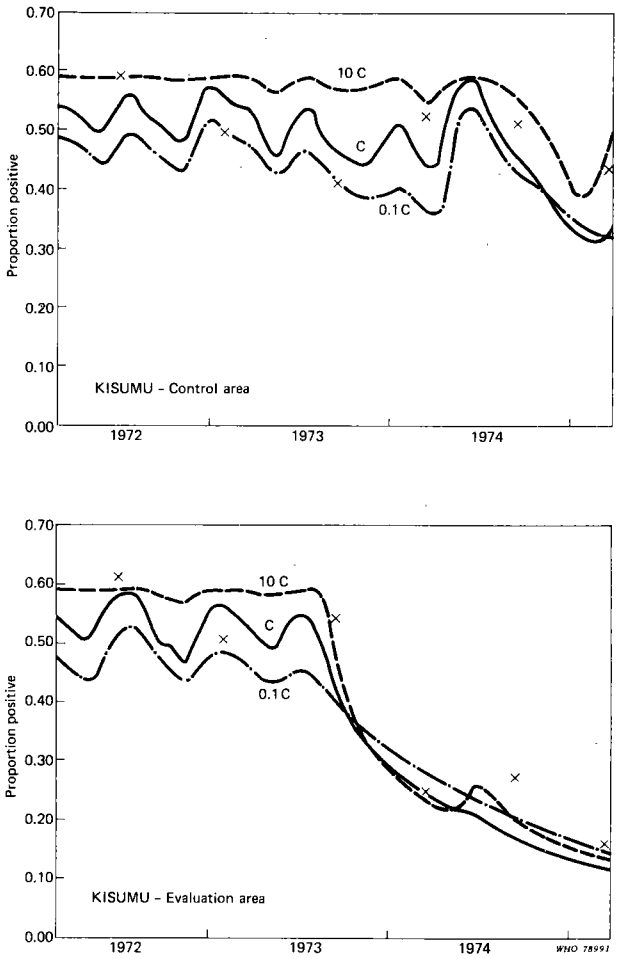


Fig. 82. Prevalence of *P. falciparum* in Kisumu evaluation[a] and control areas, as observed (x) and as calculated from the estimated vectorial capacity, C, and from 10 C and 0.1 C



[a] Fenitrothion was applied in the evaluation area in 1973-1974.

the prevalence of *P. falciparum*, put out by the model and also the prevalence actually observed at successive surveys in both the evaluation and control areas. Once more there is a fairly good agreement between the model and the observations.

Determination of the Endemic Level by the Vectorial Capacity

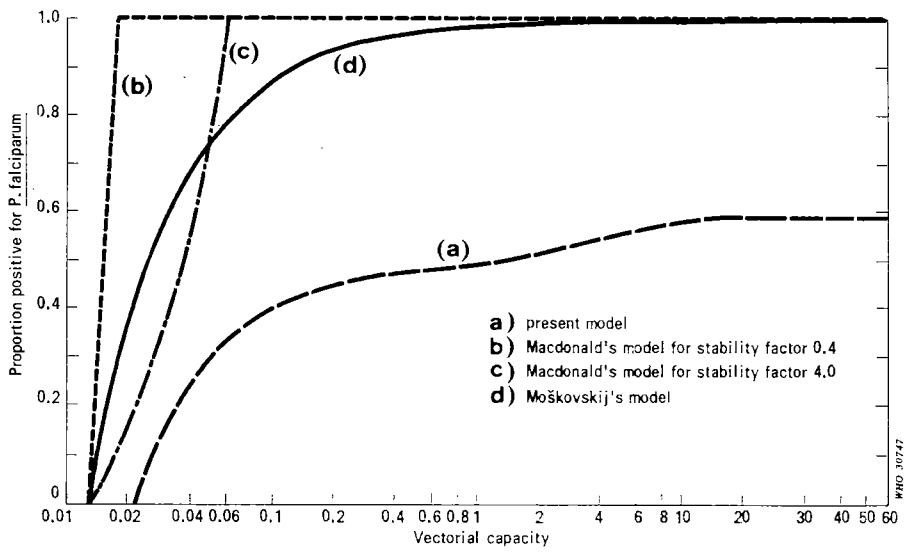
According to this model (*46*) there exists a critical vectorial capacity C^* below which malaria cannot maintain itself at an endemic level. The value of C^* is determined by the condition that the number of secondary cases generated by one infectious case (the basic reproduction rate) equals one. Since a case is infectious for the average period of $(\alpha_1 + \delta)^{-1}$ during which he makes, for small vectorial capacities, approximately gC effective contacts per unit of time, we get

$$C^* = (\alpha_1 + \delta)/g.$$

For the parameters obtained in the previous section this gives a value of 0.022 contact per day as the critical vectorial capacity (see Fig. 77).

The model makes it possible to describe the yearly average crude parasite rate for any level and any seasonal pattern of vectorial capacity. Fig. 83 shows this average for vectorial capacities without seasonal variation. According to this curve, an initial vectorial capacity of 8 (approximately equal to the yearly average vectorial capacity in Sugungum) would have to be reduced by a factor of more than 170 to reduce the yearly average crude parasite rate to half its original value. Propoxur reduced the vectorial capacity by a factor of about 10 only (see Chapter 4)

Fig. 83. Yearly average crude parasite rate as a function of yearly average vectorial capacity



and the prevalence of *P. falciparum* in Sugungum was only slightly reduced (see Chapter 5).

To demonstrate the difference between certain previous models and the present one, Fig. 83 shows the endemic level as a function of vectorial capacity for the models of Macdonald (*98*) and Moškovskij (*121*) as well as the present model. For Macdonald's model we use his formula (5)

$$L_X = (z_0 - 1)(-\ln p)/a.$$

If we take into account that the basic reproduction rate z_0 equals C/r, it follows that the endemic level L_X is a linear function of the vectorial capacity, starting at 0 for $C = r$ and reaching the level 100% for $C = \{1 + a/(-\ln p)\}r$. This shows that according to Macdonald the endemic level is not uniquely determined by the vectorial capacity but depends also on his "stability factor" $a/(-\ln p)$. In Fig. 83 the endemic level according to Macdonald was calculated for his value of the duration of infective gametocytaemia (r^{-1}) in nonimmune persons (80 days) and for stability factors 0.4 and 4 (lines b and c, respectively). His endemic level L_X is zero for $z_0 = 1$, or $C = r$. (His critical basic reproduction rate of 1 corresponds to a critical vectorial capacity of 0.0125, which is very similar to the value we have obtained. Within a narrow range of vectorial capacity above the critical value, his endemic level is higher for a lower stability factor. The reason for this dependence lies in his assumption that superinfections in the mosquito are wasted, i.e., a mosquito once infected cannot increase its infectivity by further infections. This implies that for a given vectorial capacity the inoculation rate is higher for vectors with fewer superinfections, i.e., with a lower average number of human blood meals.

The endemic level according to Moškovskij was calculated using his formula $M = 1 - \tau/a$, and equating his "communicability" a with vectorial capacity, and setting his "exhaustibility" τ to 0.0125 (Macdonald's daily recovery rate from infective gametocytaemia).

The 3 models agree with respect to the existence of a critical level of vectorial capacity below which *P. falciparum* cannot maintain itself in a human population; the actual estimates of this critical level, made according to 2 of the models (Macdonald's and the present one), are also in close agreement. Above the critical level of vectorial capacity, however, the endemic level rapidly reaches 100% according to Macdonald or close to 100% according to Moškovskij, whereas according to the present model the observable endemic level (the yearly average parasite rate) increases less rapidly and only up to a plateau of approximately 60%; the present model also calculates a "true" endemic level (not shown in Fig. 83), which is however not directly comparable to any available observations.

Variants of the Model

The model described above is the basic model, describing the prevalence of *P. falciparum* as a function of vectorial capacity and age in a human population, for a given birth (and death) rate.

The simulations described above were done with a deterministic version of the model, i.e., $h(t)$, $R_i(h)$, α_i, δ, q_i are used as determined proportions. The model can be made stochastic by using $h(t)$, $R_i(h)$, α_i, δ, q_i as probabilities.

For the simulation of mass drug administration (MDA), 2 additional states are used: successful treatment transfers the x_1, x_2, y_1, y_2 to the nonimmune protected state, while y_3, x_3, x_4 are transferred to the immune protected state. While in the protected state, persons are negative and refractory to inoculation. Protection is lost at a constant rate and protected nonimmunes and immunes are returned to x_1 and x_3, respectively. Any pattern of mass drug administration can be put in. It is assumed that a fixed fraction of the population does not participate at all in the MDA and that, in addition, each MDA misses a random fraction of the participators. The assumption of nonrandom participation reduces the expected effect of MDA; it is also more realistic (see Chapter 3).

Note on the Computer Programmes

The programming of the malaria model was done in the computer language FORTRAN IV. The computer used has changed over the years; early work was performed on an IBM 360/40, while the most recent work has been done on an IBM 370/158. Also, a version of the programme in the computer language BASIC has been written for the Hewlett-Packard 9830 minicomputer.

One year of simulation requires about 0.1 second of computer time on the IBM 370/158, or about 70 seconds on the HP 9830. Simulations of drug interventions, in which there is a parallel, nonparticipating population, require about twice as long. For the study of interventions in a particular situation, the required equilibrium can be found in a preliminary simulation and does not have to be repeated for every simulation.

In the case of fitting the model to the field data, 4 factors complicated the programming. The first was that 3 different types of results were required, namely, (1) the age-specific prevalence, (2) the infant conversion rate over a specified time interval, and (3) the crude prevalence 2 years after interruption of transmission. This last result was ultimately not used owing to the lack of observations for comparison. The second

complication was that the model was being fitted to data from 2 places simultaneously. The third difficulty was that the programme was being frequently modified in order to test various structures with differing numbers of parameters. Finally, since the equilibrium of the model is a function of its parameters, it was necessary to provide a period of running-in, to reach equilibrium, for each trial set of parameters. A special subroutine was written to monitor and assist the running-in. Thus the fitting was accomplished with a rather complicated and cumbersome programme. The programme which is used to study alternative interventions is much simpler and quite easy to use.

Discussion

The model and reality

The model, as fitted to 1 year of baseline data from 2 villages in Garki and given the relevant entomological data, simulated fairly realistically the prevalence of *P. falciparum* in 4 villages in Garki, for 3 years including 1½ years under propoxur in 2 of the villages, and also in 2 areas of Kisumu for 3 years including 20 months under fenitrothion in 1 area. Some discrepancies remain; this is not surprising, considering the simplifying assumptions included in the model, and the sampling and measurement errors involved in the estimation of the input vectorial capacity and of the parasite rates to which the model outputs are compared. In particular, the baseline parasitology may reflect unknown changes in vectorial capacity over the preceding years.

Unbiased estimates are and may remain impossible to obtain, but a model is epidemiologically satisfactory if it predicts reliably the relationship between variables estimated in a standardized way, even if this way is biased; the present model did this fairly well. A better fit could probably be obtained only at the cost of an increase in the number of parameters. On the other hand, in the process of fitting the model to the baseline data, it was found that any further simplification of the model structure decreased significantly the quality of the fit.

It is obvious that in fitting a model to reality, certain aspects of the latter are selected. We selected primarily the observed prevalence by age, time and place (times and places differing in vectorial capacity), and secondarily the incidence in infants in the transmission season. This selection was not arbitrary: we considered that if the model was realistic in the aspects selected, it would constitute a useful planning tool.

The model's performance was about equal in 2 rather different environments. It may be expected to simulate the epidemiology of

P. falciparum in other situations as well, but not necessarily in all, as there may be genetically determined differences between geographical strains of *P. falciparum*, e.g., with respect to duration of parasitaemia or infectivity to the vector (*85*, *147*).

To adapt the model to the epidemiology of other human malarias, some structural changes would probably be required. The Garki project produced good epidemiological information on *P. malariae* and *P. ovale*, but it is probable that at least *P. malariae* is significantly affected by the presence of *P. falciparum* in the same host; the reverse is probably not true (see Chapter 5). It is probably acceptable to model the transmission of *P. falciparum* as if no *P. malariae* were present, as was done here, while it would not be acceptable to do the reverse.

The present model and others

The application of mathematics to the problem of malaria transmission was initiated by Ross (see *59*) and pursued by many others. A recent review (*18*) lists most relevant references, to which the following could be added: Dutertre (*51*), Olaofe & Olaofe (*129*), Radcliffe (*135*), and Rao et al. (*136*). The subject was also reviewed by Bailey (*5*). Such a review is outside the scope of the present work. Selected comparisons with the models of Macdonald and Moskovskij were made above (pp. 281-282). One of the main reasons for constructing a new malaria model is that the previous models did not take into account the known characteristics of immunity to malaria. This is what the present model attempts to do; Dutertre (*51*) has since made an independent attempt.

When Macdonald (*98*) applied his model to data from East Africa he discussed the role of immunity as the regulating mechanism of transmission. He found that as a result of immunity the infectivity of positive mosquitos is reduced and that the recovery rate is increased. He stressed the importance of this regulation for the strategy of control ("control which is only partially effective can only reduce the stimulus to immunity and by adjustment the reproduction rate will remain unaltered") and concluded that "the only escape is by control ... without the incidental help of immunity". His theory of control (reduction of the basic reproduction rate for nonimmune persons to less than 1) is in fact a theory of eradication, which is a particular case of control. The present model, however, attempts to describe the actually observable endemic levels for the whole range of vectorial capacities in a dynamic way, i.e., taking into account the regulation of the endemic level through the immune mechanism. Formulation of a general theory of control, including eradication, requires such a model.

The present model enables one to compare, for example, the reduction in vectorial capacity necessary to go from a hyperendemic level to a mesoendemic level with the reduction necessary to go from mesoendemic to eradication. The conclusions obtained by the present model coincide qualitatively with those obtained by Moškovskij's, namely that a higher reduction in the vectorial capacity (or "communicability" in Moškovskij's terminology) is required in the first case. But the quantitative statement of that conclusion would be different.

No other model has probably been tested to the same extent with actual data (*5*). This was possible essentially because the development of the model was an integral part of a relatively comprehensive field project. There was a continual interchange between field work and theoretical work: actual observations imposed changes in the model, work with preliminary versions of the model influenced the study design and the collection of data.

In comparing their models to reality, other aspects of the latter have been selected by other authors; e.g., Dutertre (*51*) concentrates exclusively on the infant conversion rate and its evolution throughout the year.

The model and the planning of malaria control

To what extent can this or any other transmission model predict the future? It predicts the parasitological consequences of a change in vectorial capacity. No model predicts the spontaneous changes in vectorial capacity, incidentally illustrated in this chapter, nor the extent to which the application of a specified control measure will change the vectorial capacity. With respect to the latter, it was shown in Garki that the prespraying ratio between the man-biting density and the indoor-resting density has some predictive value regarding the entomological effect of a residual insecticide (*115*), but to know the actual effect of a control measure in a specified situation and in specified hands an *ad hoc* empirical trial is required.

How much information is required for using the model in a particular situation? The Kisumu simulations used only 2 estimates made by the project itself, namely the man-biting rate and the human blood index; all other inputs were available independently of the project. In many situations, the information already available is sufficient to conduct preliminary simulations; they may identify which additional data, if any, are required for selecting a plan of action.

Considering the long-term objectives of the Garki project, what is the use of an "epidemiologically satisfactory" model for the planning of malaria control? Simulations should, in defined situations, assist decisions, by exploring questions such as: (1) to what extent can the

infection be controlled by available measures? (2) within stated resources, what is the best strategy? (3) what baseline information, or what pilot trial, is necessary for decision? (4) what could be expected from a new tool (e.g., a long-acting drug or a vaccine)?

The simulations should be conducted under a range of assumptions regarding spontaneous changes in the underlying situation and regarding the effect of control measures on their direct targets. Other things being equal, the use of an epidemiologically reliable model should, on the average, increase the reliability of the answers to the above questions. The epidemiological benefits to be expected from a *P. falciparum* vaccine (with hypothetical characteristics) have been the object of a simulation exercise (*183*).

The investigation of specific questions may require some changes in the computer programmes. This would be the case, for instance, for the simulation of age-specific interventions.

The model was developed with the transmission and control of the infection in mind. Stimulation of the effect of malaria and its control on morbidity or mortality would obviously require structural changes; it might be possible to develop a morbidity or a mortality index giving different weights to the different kinds of positives (y_1, y_2, y_3); the data to validate such an index may, however, be inadequate.

The model and the teaching of the epidemiology of malaria

Simulation exercises with the model may illustrate in a didactically effective way several important features of the epidemiology and control of malaria, such as the following: the interaction between vectorial capacity, endemic level, age-specific prevalence and immunity; the effect of vector control as a function of initial and final vectorial capacities; the effect of nonrandom participation of the population in MDA; the existence of critical levels, e.g., of vectorial capacity, MDA frequency or coverage; the cumulative effect of combining various control measures as a function of the initial vectorial capacity, etc. A teaching version of the model and teaching exercises based on its use have been developed and are available upon request. They will be revised on the basis of the feedback from their actual use in teaching.

Summary

A new model of the transmission of *P. falciparum* has been developed, taking into account the special characteristics of immunity to malaria. The model calculates the prevalence of *P. falciparum* as a function of the

vectorial capacity and of its spontaneous and man-made changes. The model also calculates the effect of mass drug administration on prevalence.

The model was fitted to the baseline data from the Garki project. This involved the selection of a model structure by trial and error, and the estimation of certain model parameters, which were not directly measurable, by minimization, i.e., by letting the computer find the values which gave the best fit. An acceptable fit was obtained.

The model thus fitted was further tested as follows: 3 years of entomological observations from Garki before and after spraying with propoxur, and also from Kisumu, Kenya, before and after spraying with fenitrothion, were used to calculate vectorial capacities, which were used as input in the model; and the patterns of prevalence of *P. falciparum* put out by the model were compared to the actual observations. The fit was quite good on the whole.

It is concluded that the model simulates the epidemiology of *P. falciparum* infections with acceptable realism and can be used both for planning malaria control and for teaching the epidemiology and control of malaria.

Chapter Eleven

PRACTICAL CONCLUSIONS FOR THE FUTURE OF MALARIA CONTROL

This chapter draws the practical conclusions from the field observations made in Garki and the theoretical work involved in making a coherent interpretation of these observations, both the observations and the interpretation being viewed in the light of the results obtained by previous workers.

The first group of conclusions (pp. 290-294) refers to the importance of malaria as a public health problem and to its control in a specific ecological area of Africa, the Sudan savanna. The results obtained in the Garki project can confidently be extrapolated to the rural areas of the whole Sudan savanna, which cuts across the African continent and has a numerous population (see Fig. 2 for West Africa; the Sudan savanna extends further east across the United Republic of Cameroon, Chad, the Central African Republic and Sudan). The main vectors (*A. gambiae*, *A. arabiensis* and *A. funestus*) are the same throughout that zone, and so are the ecological and climatic factors. The surveys carried out so far, as well as the epidemiological studies and control trials, have shown remarkable similarities in parasite rates, vectorial capacity and type of response to residual insecticides, drugs, or both. The results may probably be extrapolated also to the Guinea savanna, which extends between the Sudan savanna and the forest and which also has a numerous population; since the Guinea savanna is wetter, *A. arabiensis* is probably absent from the rural areas of its southern half. The area of Bobo-Dioulasso, Upper Volta, with which certain comparisons are made below, lies at the limit between the Sudan and Guinea savanna vegetation zones. The findings made in Garki relate to a community which did not yet have the benefit of modern medical care in the area, where practically nothing was being done to reduce the contact between malaria vectors and man, and where antimalarial drugs were practically not available either from the health services or through commerce.

The prevalence of malaria, the risk of transmission and the actual incidence of new infections in the Garki area were all among the highest

documented from any area of endemic malaria. In the dry season, transmission was very limited in most of the study area but remained easily measurable where some surface water persisted, as around the village of Sugungum; this suggests that the development of irrigation will prolong the season of significant malaria transmission, unless appropriate measures are taken.

The second group of conclusions (pp. 294-296) refers to the planning and to the evaluation of malaria control in general, i.e., outside the particular situation and environment directly investigated in Garki.

The Control of Malaria in the Sudan Savanna of Africa

Residual insecticides

The domiciliary spraying of propoxur alone had a very limited impact on malaria. The vectorial capacity (i.e., the risk of transmission) was reduced by about 90%, but the prevalence of *P. falciparum* was reduced only by about 25% on the average and in the villages with the highest baseline transmission, a new equilibrium already had been reached after 2 years (i.e., further spraying would not improve the result). These findings confirm the poor results obtained previously by others with the application of various residual insecticides in the Sudan savanna of Africa, in particular in Western Sokoto (*19*, *47*), in northern Cameroon (*23*, *24*) and in the area of Bobo-Dioulasso, Upper Volta (*26*).

The possible causes for these poor results obtained in the past have been reviewed and discussed by several authors (see *inter alia 16*, *78*, *80*, *122*, *169*). The following have been considered as causes for failure: (1) operational inadequacies (in particular incomplete coverage) and the underlying administrative difficulties (*16*, *99*); (2) ineffectiveness of the insecticide, because of resistance, in particular to dieldrin (*19*, *24*), irritation of the vector limiting its exposure (see *inter alia 89*, *123*, *124*), or insufficient vector mortality (related to the dosage applied, to the interval between successive applications and to the high mortality required when the baseline level of transmission is high (see Ref. *99*, *144*, and the text below); (3) loss of insecticide through sorption into mud walls (*99*), wearing off and decay of straw or thatch walls and roofs (*24*) and replastering of walls; (4) immigration of infected people, of vectors, or of both from unsprayed into sprayed areas, a source of failure caused by the limited extent of the treated areas (*167*); (5) the very high baseline level of transmission and the relative exophily of the vector (*26*).

In Garki, the resources invested in the control operations and in the evaluation allow a firm and exhaustive interpretation of the incomplete-

ness of malaria control achieved by propoxur alone. Geographical reconnaissance was thorough and regularly updated; propoxur was sprayed at short intervals and under strict supervision, and the coverage achieved in time and space was probably as nearly complete as possible. The extremely low catches by pyrethrum spray collection and exit-trap collection, and the high mortality observed in the wall and air bioassays, demonstrated that residual spraying with propoxur was very effective in killing the exposed vectors. Thus categories 1 to 3 of those listed above can be ruled out. This does not imply that in other situations they are not important factors of failure. Category 4, the limited size of the sprayed area, was not a significant factor. This was because the entomological effect of propoxur was about the same in the main trial (covering 165 villages) as in the preliminary village-scale trial; because the variation in the entomological effect of propoxur was not related to distance from unsprayed villages; and because the parasitological comparison between the stable and mobile parts of the human population demonstrated that mobility did not cause a significant increase in the parasite reservoir. This leaves only the fifth category of causes of failure. The baseline vectorial capacity (i.e., the risk of transmission) was very high indeed, and the local *A. gambiae* s.l. rested outdoors to a significant degree; this applies both to *A. gambiae* s.s. (species A) and to *A. arabiensis* (species B), the two members of the *gambiae* complex present in the area. This exophilic behaviour is probably genetically determined, at least in part. Hence a significant fraction of the vector populations avoids exposure altogether and has a normal longevity. This has the following consequences: (1) transmission may continue, even if the insecticide is 100% effective at a single exposure; (2) the actual reduction in vectorial capacity (risk of transmission), corresponding to a given reduction in density and average age of a vector population, is smaller than the reduction calculated in the usual way, which implicitly assumes a uniform exposure of the vectors to the insecticide.

In all likelihood other and cheaper insecticides would have given results inferior to those obtained with propoxur, which has a very high and rapid knock-down effect and also a fumigant toxicity. It is likely that the much greater impact achieved by fenitrothion on malaria transmission by *A. gambiae* s.l. in Kisumu, Kenya (*64*), than by propoxur in Garki is in fact due to the greater endophily of the vector in Kisumu, rather than to a greater effectiveness of fenitrothion against the exposed vectors.

Even the modest gains due to propoxur may be nullified by the development of mosquito resistance to propoxur after a few years. It may be concluded that in the rural areas of the Sudan savanna of Africa residual spraying is not to be recommended as a malaria control method. Its possible combination with mass drug administration is discussed in the

next section.

The outdoor resting places of *A. gambiae* s.l. are little known, and therefore the possibility of controlling the vector by outdoor spraying at an acceptable cost and without selecting resistant genotypes appears remote.

The combination of mass drug administration with residual insecticides

The combination of mass administration of sulfalene-pyrimethamine with residual spraying of propoxur failed to interrupt transmission for any length of time. This was true even when drugs were given every 2 weeks in the wet season and every 10 weeks in the dry season with a coverage of 85%. A high level of control was, however, achieved at that frequency of MDA: the prevalence of parasitaemia decreased very rapidly and varied in the 1–5% range, according to season. When drugs were given every 10 weeks throughout the year, the prevalence was also considerably reduced but rose to 30% in the wet season of the second year, when conditions were favourable for vector breeding. MDA probably reduced infant and childhood mortality; temperature and nutritional anthropometric surveys, conducted in the population treated at high frequency, demonstrated a decrease in morbidity in children. In the first wet season after the period of intervention of 1½ years with high-frequency MDA, the prevalence of *P. falciparum* rose temporarily above the baseline or comparison levels, demonstrating a loss of parasitological immunity without a corresponding increase in the point-prevalence of fever in the middle of the wet season. This suggests that there was no significant loss of clinical immunity. However, one cannot state what would have happened if the period of effective control had been prolonged further.

Previous trials using the combination of MDA and residual spraying in the Sudan savanna of Africa had achieved variable degrees of control, but all had failed to interrupt transmission (*24*, *53*, *54*, *80*, *126*, *169*). A failure to interrupt transmission by the addition of MDA to residual spraying could be due to many causes: resistance to the drug, inadequate coverage, dosage or frequency, immigration of infected persons. As was the case for the effect of residual spraying, the resources invested in the implementation and evaluation of the Garki project allow a definite interpretation of the results. The local parasites were fully susceptible and the dosage used was adequate, as demonstrated by a special trial. The coverage was probably about as high as is possible in favourable circumstances (well qualified and motivated staff, good supervision), and it is

unlikely that at a higher frequency good coverage could be maintained for a long time. Immigration of infected persons contributed to transmission but was not necessary to maintain it. This is fairly certain at the lower frequency of MDA and probably also at the higher frequency. The most likely explanation of the persistence of local transmission is that effective coverage is never total while the vectorial capacity (risk of transmission) remains relatively high, even after the application of propoxur. Coverage was nonrandom, i.e., certain persons were consistently missed by the MDA. From the point of view of reducing transmission, nonrandom coverage is much worse than random coverage. Actual coverage is probably always nonrandom, in contrast to the random coverage implicitly assumed in computing the expected effect of MDA in a previous trial (*100*).

The combination of propoxur and MDA, which achieved a high level of control by means of adequate planning, staff and supervision, is, however, too expensive to be used on a large scale or for a long period. In addition, its prolonged use could lead to the selection of resistant parasites or to a loss of immunity in older children and adults, with dangerous consequences in case of interruption of the programme of MDA. The use of such methods was justified in a research project limited in time and space, for the sake of measuring what can and what cannot be achieved by presently available control methods and in order to study the effect of a drastic reduction in antigenic stimulus on the immune response.

In conclusion, MDA either alone or in combination with residual insecticides, is not recommended in the rural areas of the Sudan savanna of Africa.

Selective chemotherapy and chemoprophylaxis

It may not be feasible at an acceptable cost at the present time to control malaria in the rural areas of the Sudan savanna by an attack on transmission. It should, however, be possible to reduce the morbidity and mortality due to malaria by the treatment of clinical cases. At the age-group-related dosages tried in Kano, a single dose of chloroquine can cure a *P. falciparum* infection that has not been allowed to develop into a pernicious form. When the aim is not a reduction of transmission, the addition of pyrimethamine or primaquine would be redundant. Although the reproduction rate and the parasite rate of malaria would remain unchanged, the number of early deaths and the duration of incapacitating morbidity could be greatly reduced, without affecting the build-up of immunological processes. For this the requirements are: (1) the making available and constant replenishment of 4-aminoquinolines in suitable form and at cost price in every village; (2) an intense and simple

health education campaign stating the effect of the drugs, the dosage required for each age-group, and the advantages of self-administration of the drug in case of fever.

This method does not exclude the use of chemoprophylaxis for the most vulnerable groups of population, wherever facilities exist, nor the use of other control methods where the prevailing conditions make their use profitable (e.g., antilarval operations in cities, combined operations in selected communities or development areas). The cost of the application of this method would be obviously very limited, especially if the drugs were sold at cost price to the people. The availability and replenishment of the drugs could greatly benefit from the support of the existing primary health services organization or from the assistance of local residents.

In Garki, chloroquine treatment of fever cases by a voluntary worker designated by the village and given very simple instruction was used in 1974-1975, i.e., after the end of the intervention phase of the project in the villages previously protected by propoxur and MDA. The itinerant workers employed at a small fee for the registration of births were also used to ensure the supply of chloroquine to the villages. The chloroquine itself was supplied free of charge. This modest programme was well received by the village population, but the project's resources in 1974-1975 did not allow for its adequate evaluation. The socioeconomic conditions of the success of such a programme and the actual results it can achieve in terms of morbidity and mortality are imperfectly known, but several trials have been in progress for a number of years (e.g., *44*, *45*).

Selective chemotherapy and chemoprophylaxis are unlikely to exert sufficient pressure for the selection of resistant strains of *Plasmodium* parasites.

The Use of the Malaria Transmission Model for the Planning of Malaria Control

The model simulates fairly realistically the epidemiology of *P. falciparum*. It can calculate the parasitological consequences of: (1) specified changes in the vectorial capacity (risk of transmission) or its component entomological factors (density, longevity, man-biting habit) and (2) MDA at a specified effective coverage and frequency, with a drug protecting the individual for a specified period. The model can be used to compare the outcomes of alternative plans of intervention, as follows: (1) a range of baseline situations is simulated; all that is required for this is

a range of vectorial capacities; the precise estimation of the vectorial capacity is complicated, expensive and subject to a large error; however, in many situations, a plausible range can be calculated on the basis of available entomological information; at low levels of endemicity, in the absence of the large-scale use of drugs, the vectorial capacity corresponding to a given endemic level may be read off the graph in Fig. 83; (2) the intervention strategies which are operationally and financially feasible are listed; (3) the range of expected effect of each single control method on its immediate target is specified (e.g., "larviciding will reduce emergence by 60–80%"; "residual spraying will reduce longevity of exposed vectors by 50–75%, but 5–10% of vectors avoid exposure altogether"; MDA will cover 60–80% of the participating population at each round, but 1–5% of the population are never reached"; etc.); (4) simulations are made, applying the various strategies (comprising one or more single control methods, with the above specified ranges of direct effects) to the range of baseline situations; (5) the outcomes of the simulations using the different strategies are compared; if one strategy gives a better outcome over the whole range of assumptions explored, it is obviously preferred; (6) if, within the range of assumptions explored, it is not always the same strategy that gives the best result, or if a threshold result (e.g., eradication) is reached under some assumptions only, more information is required to make the best decision; the simulations will have helped to identify the crucial missing information or to specify the minimum level of effectiveness to be reached by a given control method to achieve a specified result; (7) if none of the strategies gives an acceptable result, one may look for other strategies or new control methods, or additional resources, or one may devote the available resources to the solution of some other problem.

It is clear from the above that the predictions of the model are conditional and comparative. The possibility of predicting the future in absolute terms is limited by ignorance about spontaneous changes (e.g., in breeding conditions for the vector) and uncertainty about the precise impact of a control method on its direct target (e.g., the precise reduction in vectorial capacity that will follow from the use of a given insecticide by a given team in a given situation), or about the actual effective coverage that will be achieved by a control method (e.g., MDA). The aim of model simulations for planning, however, is not so much to predict the future as to choose the best line of action among a limited number of possible interventions. A major effort has, however, been made in this project to test the model against actual field observations, because it is likely that among different models the one which is capable of simulating the epidemiology of malaria most realistically will also be the safest guide for action, even in situations for which only limited information is available.

The model's output is in terms of *P. falciparum* parasitaemia. To simulate the epidemiology of other malaria parasites or to use other end-points than parasitaemia, e.g., morbidity and/or mortality, various adaptations would be required. Adequate data against which to test such adaptations may not be available, but this would not necessarily invalidate their use in planning.

Computer programmes representing the model have been written in FORTRAN IV (for IBM 370) and in interactive BASIC (for HP 9830). These programmes and the corresponding documentation may be requested from WHO. Requests will be satisfied according to their merits and as resources permit, at a fee to cover costs.

REFERENCES

1. Allison, A. C. Protection afforded by sickle cell trait against subtertian malarial infection. *British medical journal*, **1**: 290-297 (1954).
2. Allison, A. C. Malaria in carriers of the sickle cell trait and in newborn children. *Experimental parasitology*, **6**: 417-447 (1957).
3. Allison, A. C. Polymorphism and natural selection in human populations. *Cold Spring Harbor symposia on quantitative biology*, **29**: 137-149 (1964).
4. Ashkar, T. S., Cornille-Brøgger, R., Storey, J., Nambiar, R. V. & Molineaux, L. ABO blood grouping of an indigenous population in rural northern Nigeria. Unpublished WHO Technical Note No. 29, MPD/TN/75.1, 64-68 (1975).
5. Bailey, N. T. J. *The mathematical theory of infectious diseases and its applications.* 2nd Ed. London, Griffin, 1975.
6. Beet, E. A. Sickle cell disease in the Balovale district of Northern Rhodesia. *East African medical journal*, **23**: 75-86 (1946).
7. Bekessy, A., Molineaux, L. & Storey, J. Estimation of incidence and recovery rates of *Plasmodium falciparum* parasitaemia from longitudinal data. *Bulletin of the World Health Organization*, **54**: 685-693 (1976).
8. Boyd, M. F. (ed.) *Malariology*. Saunders, Philadelphia and London, 1949, 2 vols.
9. Brass, W., Coale, A. J., Demeny, P., Heisel, D. F., Lorimer, F., Romaniuk, A. & Van de Walle, E. *The demography of tropical Africa.* Princeton, Princeton University Press, 1968.
10. Bryceson, A. D., Fleming, A. F. & Edington, G. M. Splenomegaly in northern Nigeria. *Acta tropica*, **33**: 185 (1976).
11. Brøgger, S. Some demographic observations of births, deaths and migration in a rural area of Kano State, northern Nigeria. Unpublished WHO Technical Note No. 20, MPD/TN/74.1, pp. 35-45 (1974).
12. Brøgger, S. & Cornille R. L. Precision and accuracy in the determination of immunoglobulin concentration in a large series of plasma specimens examined under field conditions. Unpublished WHO Technical Note No. 6, ME/TN/72/1, pp. 45-59 (1972).
13. Brøgger, S., Storey, J. & Thomas, D. Height of the children in the study population. Unpublished WHO Technical Note No. 1, ME/TN/72/1, pp. 14-16 (1972).
14. Brøgger, S., Lietaert, P., Matsushima, T. & Ramos Camacho, E. Estimation of sprayable surfaces. Unpublished WHO Technical Note No. 2, ME/TN/72/1, pp. 17-23 (1972).
15. Brøgger, S., Storey, J., Bekessy, A. Some observations on distribution of malaria parasites in thick blood films. Unpublished WHO Technical Note No. 10, MPD/TN/73.1, pp. 39-43 (1973).
16. Bruce-Chwatt, L. J. Problems of malaria control in tropical Africa, *British medical journal*, **1**: 169-174 (1954).
17. Bruce-Chwatt, L. J. Parasite density index in malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **52**: 389 (1958).
18. Bruce-Chwatt, L. J. Mathematical models in the epidemiology and control of malaria. *Tropical and geographical medicine*, **28**: 1-8 (1976).
19. Bruce-Chwatt, L. J. & Archibald, M. Malaria control pilot project in western Sokoto, northern Nigeria: a report on four years' results. *Proceedings of the 6th International Congresses of Tropical Medicine and Malaria*, **7**: 347-361 (1959).

20. Brun, L. O. Contribution à l'étude biologique et écologique des vecteurs majeurs de paludisme en Afrique de l'ouest. Thèse, Université de Rennes, 1973.
21. Cao, A., Melis, M. A. & Galanello, R. Fetal haemoglobin and malaria. *Lancet*, **1**: 202 (1977).
22. Carnevale, J., Frézil, M., Bosseno, M. F., Le Pont, F. & Lancien, J. Etude de l'agressivité d'*Anopheles gambiae* A en fonction de l'âge et du sexe des sujets humains. *Bulletin of the World Health Organization*, **56**: 147-154 (1978).
23. Cavalié, Ph. & Mouchet, J. Les campagnes expérimentales d'éradication du paludisme dans le nord de la République du Cameroun. I. Les vecteurs de l'épidémiologie du paludisme dans le Nord-Cameroun. *Médecine tropicale,* **21**: 846-869 (1961).
24. Cavalié, Ph. & Mouchet, J. Les campagnes expérimentales d'éradication du paludisme dans le nord de la République du Cameroun. II. Les opérations de lutte antipaludique et leurs résultats. *Médecine tropicale* **22**: 95-118 (1962).
25. Cavalli-Sforza, L. L. & Bodmer, W. F. *The genetics of human populations*, San Francisco, Freeman, 1971, p. 137.
26. Choumara, R., Hamon, J., Ricosse, J. & Bailly, H. Le paludisme dans la zone pilote antipaludique de Bobo-Dioulasso (Haute Volta, A.O.F.) Première partie: Présentation de la zone pilote. Quatrième partie: Epidémiologie du paludisme dans les zones traitées ou non traitées. *Cahiers de l'ORSTOM*, No. 1, pp. 17-20, 99-105 (1959).
27. Coatney, G. R. Simian malarias in man: facts, implications and predictions. *American journal of tropical medicine and hygiene*, **17**: 147-155 (1968).
28. Coatney, G. R., Collins, W. E., Warren, M. W. & Contacos, P. G. *The primate malarias*. US Department of Health, Education and Welfare, NIH, Bethesda, 1971.
29. Cohen, J. E. Heterologous immunity in human malaria. *Quarterly review of biology*, **48**: 467-489 (1973).
30. Cohen, S. Immune effector mechanisms. In: Cohen, S. & Sadun, E. H. ed. *Immunology of parasitic infections*, Oxford, Blackwell, 1976. pp. 18-34.
31. Coluzzi, M., Sabatini, A. & Petrarca, V. Chromosomal investigations on species *A* and *B* of the *Anopheles gambiae* complex in the Garki district (Kano State, Nigeria)—results of species identification from 1971 to 1974. Unpublished WHO Technical Note No. 24, MPD/TN/75.1, pp. 16-25 (1975).
32. Coluzzi, M., Sabatini, A., Petrarca, V. & Di Deco, M. A. Behavioural divergencies between mosquitos with different inversion karyotypes in polymorphic populations of the *Anopheles gambiae* complex. *Nature*, **266**: 832-833 (1977).
33. Cornille-Brøgger, R. Titration of IgM antibodies to *Plasmodium falciparum* by the indirect fluorescent antibody test. Unpublished document WHO/MAL/77.886 (1977).
34. Cornille, R. L. & Brøgger, S. Evaluation of veronal and phosphate buffered gel for the detection of malaria precipitins by double diffusion test. Unpublished WHO Technical Note No. 3, ME/TN/72/1, pp. 21-23 (1972).
35. Cornille-Brøgger, R. & Mathews, H. M. Field application to malaria studies of the passive haemagglutination (PHA) test with lyophilized cells. *Bulletin of the World Health Organization*, **52**: 39-42 (1975).
36. Cornille-Brøgger, R., Brøgger, S., Storey, J. & Thomas, D. P. The precipitin test for malaria antibodies as applied in the immunological studies of project IR-0172. Unpublished WHO Technical Note No. 12, MPD/TN/73.1, pp. 50-56 (1973).
37. Cornille-Brøgger, R., Storey, J. & Thomas, D. P. "S antigen-antibody" systems associated with *Plasmodium falciparum* infections in northern Nigeria. Unpublished WHO Technical Note No. 14, MPD/TN/73.1 pp. 65-68 (1973).

38. Cornille-Brøgger, R., Mathews, H. M., Storey, J., Ashkar, T. S., Brøgger, S. & Molineaux, L. Longitudinal serological study of malaria in the rural west African Sudan Savanna, in relation to the application of control measures. I. Study design, parasitological situation and serological methods. Unpublished document WHO/MAL/76.870 (1976).
39. Cornille-Brøgger, R., Mathews, H. M., Ashkar, T. S., Storey, J. & Molineaux, L. Longitudinal serological study of malaria in the rural west African Sudan Savanna, in relation to the application of control measures. V. Follow-up after the period of malaria control. Unpublished document WHO/MAL/77.890 (1977).
40. Cornille-Brøgger, R., Mathews, H. M., Storey, J., Ashkar, T. S., Brøgger, S., & Molineaux, L. Changing patterns in the humoral immune response to malaria, before, during and after the application of control measures: a longitudinal study in the West African Savanna. *Bulletin of the World Health Organization*, **56**: 579-600 (1978).
41. Cornille-Brøgger, R., Fleming, A. E., Kagan, I., Matsushima, T. & Molineaux, L. Abnormal haemoglobins in the Sudan savanna of Nigeria. II. Immunological response to malaria in normals and subjects with sickle cell trait, *Annals of tropical medicine and parasitology*, **73**: 173-183 (1979).
42. Detinova, T. S. *Age-grouping methods in Diptera of medical importance: with special reference to some vectors of malaria,* Geneva, World Health Organization Monograph Series No. 47, 216 pp. (1962).
43. Detinova, T. S. & Shidrawi, G. R. Observation on age determination of *A. gambiae* Giles S.L. and *A. funestus* Giles in the savanna zone, Kano, Nigeria. Unpublished WHO Technical Note No. 25, MPD/TN/75.1 pp. 26-36 (1975).
44. Diallo, S., Coulibaly, A. & Konaté, L. Effets de l'administration sulfadoxine-pyriméthamine sur l'accès palustre en zone d'hyperendémie. *Bulletin de la Société médicale d'Afrique noire de Langue française,* **21**: 398-401 (1976).
45. Diallo, S., Coulibaly, A., Konaté, M. & Samba, O. Chimioprévention à la chloroquine et prévalence du paludisme, *Médecine d'Afrique noire*, **24**: 117-125 (1977).
46. Dietz, K., Molineaux, L. & Thomas, A. A malaria model tested in African savanna. *Bulletin of the World Health Organization*, **50**: 347-357 (1974).
47. Dodge, J. S. Outdoor malaria transmission in a DDT-sprayed area of Western Sokoto, northern Nigeria, Unpublished document WHO/MAL/520.65 (1965).
48. Dowling, M. A. C. Improvements in techniques for diagnosis of malaria parasites in routine blood examination. Unpublished document WHO/ME/SGPM/WP/68.15 (1968).
49. Dowling, M. A. C. & Shute, G. T. A comparative study of thick and thin blood films in the diagnosis of scanty malaria parasitaemia. *Bulletin of the World Health Organization*, **34**: 249-267 (1966).
50. Draper, K. C. & Draper, C. C. Observations on the growth of African infants, with special reference to the effects of malaria control. *Journal of tropical medicine and hygiene*, **63**: 165-171 (1960).
51 Dutertre, J. Etude d'un modèle épidémiologique appliqué au paludisme. *Annales de la Société belge de Médecine tropicale*, **56**: 127-141 (1976).
52. Edozien, J. C., Boyd, A. E. & Morley, D. C. The relationship of serum gamma globulin concentration to malaria and sickling. *Journal of clinical pathology,* **13**: 118-123 (1960).
53. Escudié, A., Hamon, J., Ricosse, J. -H. & Chartol, A. Résultats de deux années de chimioprophylaxie antipaludique en milieu rural africain dans la zone pilote de Bobo Dioulasso (Haute Volta). *Médecine tropicale*, **21**: 689-728 (1961).

54. Escudié, A., Hamon, J. & Schneider, J. Résultats d'une chimioprophylaxie antipaludique de masse par l'association amino-4-quinoléine/amino-8-quinoléine en milieu rural africain de la région de Bobo-Dioulasso (Haute Volta). *Médicine tropicale*, **22**: 268-305 (1962).
55. Espenshade, E. B. Jr. *Goode's world atlas*. 12th ed. Chicago, Rand McNally, 1964.
56. Field, J. W. & Shute, P. G. The microscopic diagnosis of human malaria. II. A morphological study of the erythrocytic parasites. *Studies from the Institute for Medical Research, Federation of Malaya*, No. 24, p. 251 (1956).
57. Field, J. W., Sandosham, A. A. & Fong, Y. L. The microscopical diagnosis of human malaria. I. A morphological study of the erythrocytic parasites in thick blood films (Second edition). *Studies from the Institute for Medical Research, Federation of Malaya*, No. 30, p. 236 (1963).
58. Fine, P. E. M. Superinfection—A problem in formulating a problem (an historical critique of McDonald's theory). *Tropical diseases bulletin*, **72**: 475-488 (1975).
59. Fine, P. E. M. Ross's *a priori* pathometry—a perspective. *Proceedings of the Royal Society of Medicine*, **68**: 547-551 (1975).
60. Firschein, I. L. Population dynamics of the sickle-cell trait in the Black Caribs of British Honduras, Central America. *American journal of human genetics*, **13**: 233-254 (1961).
61. Fleming, A. F., Allan, N. C. & Stenhouse, N. S. Splenomegaly and sickle cell trait. *Lancet*, **2**: 574-575 (1968).
62. Fleming, A. F., Storey, J., Molineaux, L., Iroko, E. A. & Attai, E. D. E. Abnormal haemoglobins in the Sudan savanna of Nigeria. I. Prevalence of haemoglobins and relationships between sickle cell trait, malaria and survival. *Annals of tropical medicine and parasitology*, **73**: 161-172 (1979).
63. Foll, C. V., Pant, C. P. & Lietaert, P. E. A large-scale field trial with dichlorvos as a residual fumigant insecticide in northern Nigeria. *Bulletin of the World Health Organization*, **32**: 531-550 (1965).
64. Fontaine, R. E., Pull, J., Payne, D., Pradhan, G. D., Joshi, G., Pearson, J. A., Thymakis, M. K. & Ramos Camacho, M. E. Evaluation of fenitrothion for the control of malaria, *Bulletin of the World Health Organization*, **56**: 445-452 (1978).
64a. Friedman, M. J. Erythrocyte mechanism of sickle-cell resistance to malaria, *Proceedings of the National Academy of Science*, **75**: 1994-1997 (1978).
65. Garnham, P. C. C. *Malaria parasites, and other haemosporidia*. Oxford, Blackwell, 1966.
66. Garrett-Jones, C. The human blood index of malaria vectors in relation to epidemiological assessment. *Bulletin of the World Health Organization*, **30**: 241-261 (1964).
67. Garrett-Jones, C. & Grab, B. The assessment of insecticidal impact on the malaria mosquito's vectorial capacity, from data on the proportion of parous females. *Bulletin of the World Health Organization*, **31**: 71-86 (1964).
68. Gillies, M. T. The duration of the gonotrophic cycle in *Anopheles gambiae* and *Anopheles funestus*, with a note on the efficiency of hand catching, *East African medical journal*, **30**: 129-135 (1953).
69. Gillies, M. T. A modified technique for the age-grading of populations of *Anopheles gambiae*, *Annals of tropical medicine and parasitology* **52**: 261-273 (1958).
70. Gillies, M. T. & de Meillon, B. *The anophelinae of Africa south of the Sahara (Ethiopian Zoogeographical Region)*. South African Institute for Medical Research, Publication No. 54, 2nd ed., 1968, p. 343.
71. Gillies, M. T. & Wilkes, T. J. A study of the age-composition of populations of *Anopheles gambiae* Giles and *A. funestus* Giles in north-eastern Tanzania. *Bulletin of entomological research*, **56**: 237-262 (1965).

72. Gordon, R. M. & Davey, T. H. *P. malariae* in Freetown, Sierra Leone. *Annals of tropical medicine and parasitology*, **26**: 65-84 (1932).
73. Gordon, R. M. & Davey, T. H. A further note on the increase of *P. malariae* in Freetown, Sierra Leone, *Annals of tropical medicine and parasitology*, **27**: 53-55 (1933).
74. Gramiccia, G. & Hempel, J. Mortality and morbidity from malaria in countries where malaria eradication is not making satisfactory progress. *Journal of tropical medicine and hygiene*, **75**: 187-192 (1972)
75. Greenwood, B. M. Possible role of B-cell mitogen in hypergammaglobulinaemia in malaria and trypanosomiasis. *Lancet*, **1**: 435-436 (1974).
76. Hamilton, P. J. S., Morrow, R. H., Ziegler, J. L., Pike, M. C., Wood, J. B., Banyikidde, S. K. & Hutt, M. S. R. Absence of sickle-cell trait in patients with tropical splenomegaly syndrome. *Lancet*, **2**: 109 (1969).
77. Hamon, J. Etude de l'âge physiologique des femelles d'anophèles dans les zones traitées au DDT, et non traitées, de la région de Bobo-Dioulasso, Haute-Volta. *Bulletin of the World Health Organization*, **28**: 83-109 (1963).
78. Hamon, J. Malaria: Tropical Africa. In: Cockburn, A., ed., *Infectious diseases: their evaluation and eradication*. Springfield, Thomas, 1967, pp. 276-291.
79. Hamon, J., Choumara, R., Adam, D. & Bailly, H. Le paludisme dans la zone pilote antipaludique de Bobo-Dioulasso (Haute-Volta, A.O.F.) 2ème partie: Enquêtes entomologiques; 3ème partie: Résultats des enquêtes entomologiques. *Cahiers de l'ORSTOM*, No. 1, 37-61, 63-72 (1959).
80. Hamon, J., Mouchet, J., Chauvet, G. & Lumaret, R. Bilan de quatorze années de lutte contre le paludisme dans les pays francophones d'Afrique tropicale et à Madagascar. Considérations sur la persistence de la transmission et perspectives d'avenir. *Bulletin de la Société de pathologie exotique*, **56**: 933-971 (1963).
81. Harrison Church, R. J. West Africa: a study of the environment and of man's use of it. In: Beaver, S. H. ed. *Geographies for advanced study*. 6th ed. London, Longmans, 1968.
82. Holstein, M. H. *Biology of* Anopheles gambiae: *research in French West Africa*. Geneva, World Health Organization Monograph Series No. 9, 1954, p. 172.
83. Huehns, E. R. & Beaven, G. H. Developmental changes in human haemoglobins. In: Benson, P. ed. *The biochemistry of development*. London, Heinemann, 1971, p. 175.
84. Iloeje, N. P. *A new geography of West Africa*. London, Longmans, 1972.
85. James, S. P., Nicol, W. D. & Shute, P. G. A study of induced malignant tertian malaria. *Proceedings of the Royal Society of Medicine*, **25**: 1153-1186 (1932).
86. Jelliffe, D. B. *The assessment of the nutritional status of the community (with special reference to field surveys in developing regions of the world)*. Geneva, World Health Organization Monograph Series No. 53 (1966).
87. Kouznetsov, R. L. Malaria control by application of indoor spraying of residual insecticides in tropical Africa and its impact on community health. *Tropical doctor*, **7**: 81-91 (1977).
88. Kowal, J. M. & Knabe, D. T. *An agroclimatological atlas of the northern states of Nigeria*. Zaria, Ahmadu Bello University Press, 1972.
89. Kuhlow, F. Field experiments on the behaviour of malaria vectors in an unsprayed hut and in a hut sprayed with DDT in northern Nigeria. Unpublished document WHO/MAL/310 (1961).
90. Lacan, A. Le *Plasmodium ovale* dans les territoires africains d'expression française. *Bulletin of the World Health Organization*, **29**: 415-417 (1963).
91. Lehmann, H. & Huntsman, R. G. Laboratory detection of haemoglobinopathies. *Association of Clinical Pathologists broadsheet*, **33**: 10 (1975).

92. Livingstone, F. B. *Abnormal haemoglobins in human populations.* Chicago, Aldine, 1967, p. 343.
93. Livingstone, F. B. Malaria and human polymorphisms. *Annual review of genetics,* **5**: 33-64 1971.
94. Luzzato, L., Nwachuku-Jarret, E. S., & Reddy, S. Increased sickling of parasitised erythrocytes as mechanism of resistance against malaria in the sickle-cell trait. *Lancet,* **1**: 319-321 (1970).
95. Macdonald, G. Malaria in the children of Freetown, Sierra Leone. *Annals of tropical medicine and parasitology* **20**: 239-263 (1926).
96. Macdonald, G. The analysis of infection rates in diseases in which superinfections occur. *Tropical diseases bulletin,* **47**: 907-915 (1950).
97. Macdonald, G. The analysis of equilibrium in malaria. *Tropical diseases bulletin,* **49**: 813-829 (1952).
98. Macdonald, G. The measurement of malaria transmission. *Proceedings of the Royal Society of Medicine,* **48**: 295-301 (1955).
99. Macdonald, G. *The epidemiology and control of malaria.* London, Oxford University Press, 1957, p. 201.
100. Macdonald, G., Foll, C. V. & Cuellar, C. B. The potential value of mass treatment in malaria eradication. Unpublished document WHO/MAL/67.615 (1967).
101. Mancini, G., Carbonara, A. O. & Heremans, J. F. Immunochemical quantitation of antigens by single radial diffusion. *Immunochemistry,* **2**: 235-254 (1965).
102. Mathews, H. M., Fried, J. A. & Kagan, I. G. The indirect haemagglutination test for malaria evaluation of antigens prepared from *Plasmodium falciparum* and *Plasmodium vivax. American journal of tropical medicine and hygiene,* **24**: 417-422 (1975).
103. Matsushima, T., Shidrawi, G. R., Clarke, J. L., Lietaert, P., Ramos-Camacho, E., Boulzaguet, J. R. & Ashkar, T. S. A village scale field trial with propoxur (OMS-33) against *A. gambiae* and *A. funestus.* Unpublished WHO Technical Note No. 7, MPD/TN 73.1, pp. 5-17 (1973).
104. Matsushima, T., Ramos-Camacho, E., & Nambiar, R. V. Report on first year's (1972) spraying operation of propoxur. Unpublished WHO Technical Note No. 13, MPD/TN/73.1, pp. 57-64 (1973).
105. Matsushima, T., Brøgger, S., Storey, J., Ramos-Camacho, E., Nambiar, R. V. & Petrides, J. Mass drug administration using a combination of sulfalene and pyrimethamine applied to a rural population in part of Garki District, Kano State, northern Nigeria. (Report on the mass drug administration carried out for the first year of operations (1972)). Unpublished WHO Technical Note No. 18, MPD/TN/74.1, pp. 19-26 (1974).
106. Matsushima, T., Brøgger, S., Nair, V. R. & Nambiar, R. V. Mass drug administration using a combination of sulfamethoxypyrazine and pyrimethamine applied to a rural population in part of Garki district, Kano State, northern Nigeria (Second Report to Technical Note No. 18). Unpublished WHO Technical Note No. 28, MPD/TN/75.1, pp. 53-63 (1975).
107. McGregor, I. A. Measles and child mortality in the Gambia. *West African medical journal,* **13**: 251-257 (1964).
108. McGregor, I. A. & Wilson, R. J. M. Precipitating antibodies and immunoglobulins in *P. falciparum* infections in the Gambia, West Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **65**: 136-145 (1971).
109. McGregor I. A., Williams, K., Voller, A. & Billewicz, W. Z. Immunofluorescence and the measurement of immune response to hyperendemic malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **59**: 395-414 (1965).

110. McGregor, I. A., Hall, P. J., Williams, K., Hardy, C. L. S. & Turner, M. N. Demonstration of circulating antibodies to *Plasmodium falciparum* by gel diffusion techniques. *Nature,* **210**: 1384 (1966).
111. McGregor, I. A., Turner, M. W., Williams, K. & Hall, P. Soluble antigens in the blood of African patients with severe *Plasmodium falciparum* malaria. *Lancet,* **1**: 881-884 (1968).
112. McGregor, I. A., Rowe, D. S., Wilson, M. E. & Billewicz, W. Z. Plasma immunoglobulin concentrations in an African (Gambian) community in relation to season, malaria and other infections and pregnancy. *Clinical and experimental immunology,* **7**: 51-74 (1970).
113. Meuwissen, J. H. E. T. The indirect haemagglutination test for malaria and its application to epidemiological surveillance. *Bulletin of the World Health Organization,* **50**: 277-286 (1974).
114. Molineaux, L. Entomological parameters in the epidemiology and control of vector-borne diseases. In: *Medical Entomology Centenary, Symposium proceedings.* Royal Society of Tropical Medicine and Hygiene, London, 1978, pp. 100-105.
115. Molineaux, L., Shidrawi, G. R., Clarke, J. L., Boulzaguet, R., Ashkar, T. & Dietz, K. The impact of propoxur on *Anopheles gambiae* s.l. and some other anopheline populations, and its relationship with some pre-spraying variables. *Bulletin of the World Health Organization,* **54**: 379-389 (1976).
116. Molineaux, L., Storey, J., Cohen, J. & Thomas, A. Longitudinal study of *P. falciparum*, *P. malariae* and *P. ovale*, in the West African savanna, in the absence of control measures: II. Relationships between the species of plasmodium, in particular *P. falciparum* and *P. malariae*. Unpublished document MPD-012/78.17 (1977).
117. Molineaux, L., Cornille-Brøgger, R., Mathews, H. M. & Storey, J. Longitudinal serological study of malaria in infants in the West African savanna. *Bulletin of the World Health Organization,* **56**: 573-578 (1978).
118. Molineaux, L., Dietz, K. & Thomas, A. Further epidemiological evaluation of a malaria model. *Bulletin of the World Health Organization,* **56**: 565-571 (1978).
119. Molineaux, L., Shidrawi, G. R., Clarke, J. L., Boulzaguet, J. R. & Ashkar, T. S. (1978c). The assessment of insecticidal impact on the malaria mosquito's vectorial capacity, from data on the man-biting rate and age-composition. *Bulletin of the World Health Organization,* **57**: 265-274 (1979).
120. Molineaux, L., Fleming, A. F., Cornille-Brøgger, R., Kagan, I. G. & Storey, J. Abnormal haemoglobins in the Sudan savanna of Nigeria. III. Malaria immunoglobulins and antimalarial antibodies in sickle cell disease. *Annals of tropical medicine and parasitology,* **73**: 301-310 (1979).
121. Moškovskij, S. D. A further contribution to the theory of malaria eradication. *Bulletin of the World Health Organization,* **36**: 992-996 (1967).
122. Mouchet, J. & Hamon, J. Les problèmes techniques de l'éradication du paludisme en Afrique. *Cahiers de l'ORSTOM, Entomologie médicale* **1**: 39-48 (1963).
123. Muirhead Thomson, R. C. The effects of house spraying with pyrethrum and with DDT on *Anopheles gambiae* and *A. melas* in West Africa. *Bulletin of entomological research,* **38**: 449-464 (1974).
124. Muirhead Thomson, R. C. Recent knowledge about malaria vectors in West Africa and their control. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **40**: 511-536 (1974).
125. Najera, J. A. A critical review of the field application of a mathematical model of malaria eradication. *Bulletin of the World Health Organization,* **50**: 449-457 (1974).
126. Najera, J. A., Shidrawi, G. R., Storey, J. & Lietaert, P. E. A. Mass drug administration and DDT indoor-spraying as antimalarial measures in the northern savanna of Nigeria. Unpublished document WHO/MAL/73.817 (1973).

127. Nambiar, R. V., Ramos-Camacho, E., & Matsushima, T. Report on second year's (1973) spraying operation of propoxur. Unpublished WHO Technical Note No. 26, MPD/TN/75.1, pp. 39-42 (1975).
128. Newman, P. *Malaria eradication and population growth, with special reference to Ceylon and British Guiana*. Bureau of Public Health Economics, University of Michigan, Ann Arbor, Research Series No. 10, 1965.
129. Olaofe, G. O. & Olaofe, K. A simple model for tropical malaria epidemics. *Mathematical biosciences,* **25**: 205-215 (1975).
130. Omer, S. M. & Cloudsley-Thompson, J. L. Survival of female *Anopheles gambiae* Giles through a nine-month dry season in Sudan. *Bulletin of the World Health Organization,* **42**: 319-330 (1970).
131. Onori, E. Distribution of *Plasmodium ovale* in the eastern, western and northern Regions of Uganda. *Bulletin of the World Health Organization,* **37**: 665-668 (1967).
131a. Pasvol, G., Weatherall, D. J. & Wilson, R. J. M. Cellular mechanism for the protective effect of haemoglobin S against *P. falciparum* malaria, *Nature*, **274**: 701-703 (1978).
132. Payne, D., Grab, B., Fontaine, R. E. & Hempel, J. H. G. Impact of control measures on malaria transmission and general mortality. *Bulletin of the World Health Organization,* **54**: 369-377 (1976).
133. Peaston, N. & Renner, E. A. Report on an examination of the spleen and parasite rates in school-children in Freetown, Sierra Leone. *Annals of tropical medicine and parasitology,* **33**: 49-61 (1939).
134. Pringle, G. Experimental malaria control and demography in a rural East African community: a retrospect. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **63**, No. 4, suppl., pp. S2-S18 (1969).
135. Radcliffe, J. The periodicity of endemic malaria. *Journal of applied probability,* **11**: 562-567 (1974).
136. Rao, N. R., Vig., O. P. & Agarwala, S. N. Transmission dynamics of malaria. *Bulletin of the Haffkine Institute,* **2**: 71-78, 112-116 (1974).
137. Raper, A. B. Sickling and malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **54**: 503-504 (1960).
138. Ringelhann, B., Hathorn, M. K. S., Jully, P., Grant, F. & Parniczky, G. A new look at the protection of haemoglobin AS and AC genotypes against *Plasmodium falciparum* infection: a census tract approach. *American journal of human genetics,* **28**: 270-279 (1976).
139. Rogers, N. A., Fried, J. A. & Kagan, I. G. A modified indirect microhaemagglutination test for malaria. *American journal of tropical medicine and hygiene,* **17**: 804-809 (1968).
140. Rowe, D. S., McGregor, I. A., Smith, S. J., Hall, P. & Williams, K. Plasma immunoglobulin concentrations in a West African (Gambian) community and in a group of healthy British adults. *Clinical and experimental immunology,* **3**: 63-79 (1968).
141. Rowe, D. S., Grab, B. & Anderson, S. An international reference preparation for human serum immunoglobulins G, A and M: content of immunoglobulin by weight. *Bulletin of the World Health Organization,* **46**: 67-69 (1972).
142. Rucknagel, D. L. & Neel, J. V. The haemoglobinopathies. In: Steinberg, G. ed. *Progress in medical genetics*. New York, Grune & Stratton, 1961, p. 158.
143. Scrimshaw, N. S., Taylor, C. E. & Gordon, J. E. *Interactions of nutrition and infection*. Geneva, World Health Organization Monograph Series No. 57, 1968.
144. Service, M. W. Some basic entomological factors concerned with the transmission and control of malaria in northern Nigeria. *Transactions of the Royal Society for Tropical Medicine and Hygiene,* **59**: 291-296 (1965).

145. Shidrawi, G. R. The distribution and seasonal prevalence of members of the *Anopheles gambiae* species complex (species A and B) in Garki District, northern Nigeria. Unpublished document WHO/MAL/72.776—WHO/VBC/72.387 (1972).
146. Shidrawi, G. R., Clarke, J. L. & Boulzaguet, J. R. Assessment of the CDC miniature light trap for sampling malaria vectors in Garki District, northern Nigeria. Unpublished WHO Technical Note No. 11, MPD/TN/73.1, pp. 44-49 (1973).
147. Shute, P. G. & Maryon, M. A contribution to the problem of strains of human plasmodium. *Rivista di malariologia,* **33**: 1-21 (1954).
148. Storey, J. & Matsushima, T. Observations on patient attendance at dispensaries in Garki district, Kano State, Nigeria. Unpublished WHO Technical Note No. 8, MPD/TN/73.1, pp. 18-28 (1973).
149. Storey, J., Brøgger, S. & Molineaux, L. A trial of methods for the microscopic detection of malaria in man. Unpublished WHO Technical Note No. 9, MPD/TN/73.1, pp. 29-38 (1973).
150. Storey, J., Rossi-Espagnet, A., Mandel, S. P. H., Matsushima, T., Lietaert, P., Thomas, D., Brøgger, S., Duby, C. & Gramiccia, G. Sulfalene with pyrimethamine and chloroquine with pyrimethamine in single-dose treatment of *Plasmodium falciparum* infections: a trial in a rural population in northern Nigeria. *Bulletin of the World Health Organization,* **49**: 275-282 (1973).
151. Storey, J., Nambiar, R. V. & Matushima, T. Drug administration to self-reported fever cases through village and hamlet heads in Garki district, Kano State. Unpublished WHO Technical Note No. 27, MPD/TN/75.1, pp. 43-52 (1975).
152. Storey, J., Brøgger, S. & Molineaux, L. Longitudinal study of *P. falciparum, P. malariae* and *P. ovale*, in the west African savanna in the absence of control measures. I. Prevalence, incidence of and recovery from patent parasitaemia. Unpublished document MPD-012/78.16 (1977).
153. Storey, J., Fleming, A. F., Cornille-Brøgger, R., Molineaux, L., Matsushima, T, & Kagan, I. Abnormal haemoglobins in the Sudan savanna of Nigeria. IV. Malaria, immunoglobulins and antimalarial antibodies in haemoglobin AC individuals. *Annals of tropical medicine and parasitology,* **73**: 311-315 (1979).
154. Sulzer, A. J., Cantella, R., Colichan, A., Gleason, N. N. & Walls, K. W. A focus of hyperendemic *Plasmodium malariae—P. vivax* malaria with no *P. falciparum* in a primitive population in the Peruvian Amazon jungle. Studies by means of immunofluorescence and blood smear. Unpublished document WHO/MAL/75.858 (1975).
155. Vandepitte, J. M., Zuelzer, W. W., Neel, J. V. & Colaert, J. Evidence concerning the inadequacy of mutation as an explanation of the frequency of the sickle cell gene in the Belgian Congo. *Blood,* **10**: 341-350 (1955).
156. Vaughan, V. C. Growth and development. In: Vaughan, V. C. & McKay, R. J. eds. *Nelson textbook of pediatrics,* 10th ed. Philadelphia, Saunders, 1975.
157. Voller, A. & O'Neill, P. Immunofluorescence method suitable for large-scale application. *Bulletin of the World Health Organization,* **45**: 524-529 (1971).
158. Voller, A., Bidwell, D., Huldt, G & Engvall, E. A microplate method of enzyme-linked immunosorbent assay and its application to malaria. *Bulletin of the World Health Organization,* **51**: 209-211 (1974).
159. Voller, A., Bidwell, D. E. & Bartlett, A. Enzyme immunoassays in diagnostic medicine; theory and practice. *Bulletin of the World Health Organization,* **53**: 55-65 (1976).
160. Walters, J. H. & Bruce-Chwatt, L. J. Sickle-cell anaemia and falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **50**: 511-514 (1956).
161. Walton, G. A. *Plasmodium falciparum* and *Anopheles gambiae* in relation to malaria occurring in infants: on the control of malaria in Freetown, Sierra Leone. *Annals of tropical medicine and parasitology,* **41**: 380-407 (1947).

162. Watson, E. H. & Lowrey, G. H. *Growth and development of children.* 5th ed. Chicago, Year Book Medical Publishers, 1967.
163. White, G. B. *Anopheles gambiae* complex and disease transmission in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **68**: 278-298 (1974).
164. Wilson, B. (1949) Malaria incidence in Central and South Africa. In: Boyd, M. D. ed. *Malariology,* Philadelphia, Saunders, 1949, pp. 800-809.
165. Wood, C. S. The relationship between ABO polymorphisms and malaria vectors. *Human biology,* **46**: 385-404 (1974).
166. Wood, C. S., Harrison, G. A., Doré, C. & Weiner, J. S. Selective feeding of *A. gambiae* according to ABO blood group status, *Nature,* **239**: 165 (1972).
167. WHO. *Report on the malaria conference in equatorial Africa.* Geneva, World Health Organization Technical Report Series No. 38, p. 72 (1951).
168. WHO. Manual for processing and examination of blood slides in malaria eradication programmes (2nd edition), Unpublished document WHO/PA/262.61, p. 127 (1961).
169. WHO. Malaria eradication. Report on the Third African Malaria Conference, Yaoundé, 3-13 July 1962. Unpublished document WHO/MAL/376—AFR/MAL/9/62 (1963).
170. WHO. Informal meeting on the priority needs of field research in malaria in tropical Africa. Unpublished document MPD 012/78.18 (1969).
171. WHO. Parasitology baseline report (1971-1972). Unpublished document MPD 012/78.01 (1974).
172. WHO. Parasitology—intervention phase report (1971-1972), Unpublished document MPD 012/78.02 (1974).
173. WHO. Immunology baseline report (1971-1972). Unpublished document MPD 012/78.03 (1974).
174. WHO. Immunology—intervention phase report (1972-1973). Unpublished document MPD 012/78.04 (1974).
175. WHO. Entomology baseline report (1971-1972). Unpublished document MPD 012/78.05 (1974).
176. WHO. *Manual on practical entomology in Malaria.* Geneva, WHO offset publication, No. 13, 1975, Part I, 160 pp. Part II, 191 pp.
177. WHO. Entomology intervention phase report (1971-1973). Unpublished document MPD 012/78.06 (1975).
178. WHO. Meteorology, baseline and intervention period, including relationship of entomological findings (1971-1973). Unpublished document MPD 012/78.07 (1975).
179. WHO. The intervention period: the control operations (1972-1973). Unpublished document MPD 012/78.08 (1975).
180. WHO. Demographic report (1971-1973). Unpublished document MPD 012/78.09 (1975).
181. WHO. Informal consultation on project MPD 012: research in the epidemiology and control of malaria in African savanna. Unpublished document MPD 012/78.11 (1975).
182. WHO Scientific Group on Developments in Malaria Immunology. *Report.* Geneva, World Health Organization Technical Report Series No. 579 (1975).
183. WHO. The epidemiological benefits expected from a *P. falciparum* vaccine: a simulation exercise. Unpublished document MPD 012/78.12 (1976).
184. WHO. Garki Project: Entomology 1974-1975. Unpublished document MPD 012/78.13 (1976).
185. WHO. Garki Project: Parasitology 1974-1975. Unpublished document MPD 012/78.14 (1976).

ACKNOWLEDGEMENTS

The work carried out in the Garki project would not have been possible without the support and dedication of the national authorities and of all the project staff at Federal, State, District and village levels. The understanding and the co-operation of the population, often put to inconvenience in their own homes, made it possible to satisfy the requirements of the research work.

It would be impossible to mention them all individually, but we would like to single out the following:

Federal Government of Nigeria

Emirate and Local Government Authority

His Highness Alhaji Audu Bayero, Emir of Kano
Alhaji Umaru Sarkin Fulani J.aidinawa, District Head, Garki

State Government, Ministry of Health

Alhaji Mohammed Ibrahim, Permanent Secretary
Dr I. Imam, Director Medical Services
Dr Qureshi, Principal Medical Officer, Preventive
Dr Baisu, Principal Medical Officer, Curative

The University of Ibadan and especially the Staff of the Department of Clinical Pathology (Host to the WHO-Immunology Research and Training Centre, Dr V. Houba) under the leadership of Prof. B. K. Adadevoh, gave tremendous assistance in the early setting-up of the serological part of the project.

The continuous help provided by the WHO representatives in Nigeria at Lagos (Dr D. A. W. Nugent, Dr S. Adrien and finally Dr E. S. W. Bidwell) and by their staff, in clearing and forwarding the imported goods for the project and in discussing administrative questions with representatives of the Federal Government, is acknowledged with gratitude.

The preparation of the anti-immunoglobulins antisera for the project fell on Miss K. Hug of the Institut de Biochimie of the University of Lausanne, Switzerland, directed by Professor H. Isliker. The Institute were the host to the WHO Immunology Research and Training Centre, headed by Dr D. S. Rowe, who supervised Miss Hug's work. Their very important contribution to the serological aspects of the research is acknowledged.

Finally, special thanks should be given to Mrs M. Escribano-Matty for revising and preparing all the figures and tables and the references, and to Miss Frances Bonello for typing the text of this publication.

Appendix 1

STAFFING OF THE PROJECT

WHO project field staff

Name	*Capacity*	*Period of work with the project* *Started*	*Ended*
Dr S. P. Ramakrishnan	Project director	1 Sep. 70	11 Dec. 73
Dr T. Matsushima	Team leader	total duration of project	
Mr G. R. Shidrawi	Entomologist	from start	1 Jun. 75
Mr J. L. Clarke	Entomologist	12 Aug. 70	30 Apr. 72
Mr S. Brøgger	Statistician	1 Jun. 70	10 Oct. 73
Dr R. L. Cornille-Brøgger	Immunologist	17 Aug. 70	31 Dec. 73
Mr J. Storey	Laboratory technician (parasitology)	total duration of project	
Mr T. S. Ashkar	Laboratory technician (serology)	1 Feb. 71	to end
Mr J. R. Boulzaguet	Laboratory technician (entomology)	total duration of project	
Mr P. E. Lietaert	Sanitarian	from start	1 Jun. 71
Mr J. Petrides	Sanitarian	from start	1 Mar. 70
Mr D. Thomas	Sanitarian	from start	30 Apr. 74
Mr E. Ramos-Camacho	Sanitarian	8 Mar. 71	31 Mar. 75
Mr V. R. Nair	Sanitarian	3 Dec. 72	30 Nov. 73
Mr R. V. Nambiar	Sanitarian	1 May 72	to end
Mr A. Abrar	Administrative officer	1 Apr. 70	1 Jul. 74
Mrs J. Marcroft	Clerk-typist	1 Apr. 70	30 Apr. 74

Nigerian field staff (see also below)

Name	*Capacity*
Mr Hussaini Yacim	Senior laboratory technician (parasitology)
Mr Barau Mohammed	Laboratory technician (parasitology)
Mr Daniel Ejeh	Laboratory technician (parasitology)
Mr Elijah Zakary	Laboratory technician (serology)
Mr Sani Galadima	Laboratory technician (entomology)
Mr Garba Yaro	Field supervisor
Mr Raphael Odeh	Field supervisor
Mr Alhaji Anmusa Sadami	Field supervisor

Short-term consultants, advisers and collaborating scientists

Name	*Responsibility*	*Institution*
Dr R. L. Cornille-Brøgger	Serology	—
Professor M. Coluzzi	Vector genetics	Istituto di Parassitologia, Rome
Dr A. Sabatini	Vector genetics	Istituto di Parassitologia, Rome

Name	*Responsibility*	*Institution*
Professor M. Prothero	Human geography migrations	Institute of Geography, Liverpool
Dr I. A. McGregor	Serology, antigen preparation	Medical Research Council, London
Professor B. O. Osunkoya	Serology, antigen preparation	University of Ibadan
Dr A. I. O. Williams	Serology, antigen preparation	University of Ibadan
Dr A. Voller	Serology	Nuffield Institute, London
Professor A. F. Fleming	Human genetics (Hb types)	Ahmadu Bello University, Zaria
Dr I. G. Kagan	IHA test, serology	Centre for Disease Control, Atlanta
Dr H. M. Mathews	IHA test, serology	Centre for Disease Control, Atlanta
Dr H. O. Lobel	IHA test, serology	Centre for Disease Control, Atlanta
Professor P. G. Jenssens	Research orientation	Institute of Tropical Medicine, Antwerp
Professor A. O. Lucas	Research orientation	Department of Preventive and Social Medicine, University of Ibadan
Dr N. Detinova	Entomology	Martsinovsky Institute of Medical Parasitology and Tropical Medicine, Moscow
Dr D. S. Rowe	Immunology	University of Lausanne, Lausanne

WHO headquarters staff, full-time, for various durations

Mr A. S. Thomas	Programmer analyst
Mrs M. Escribano-Matty	Technical assistant—graphics
Mrs P. Rozé	Programmer analyst
Miss J. Richards	Programmer analyst
Miss R. Prieto	Technical assistant

WHO headquarters staff, part-time

Name	*Division or unit*[a]	*Responsibility*
Dr G. Sambasivan	Director, ME	Coordination, budget
Dr K. W. Newell	Director, RECS	Coordination, budget
Dr T. Lepes	Director, MPD	Coordination, budget
Dr H. C. Goodman	Chief, IMM	Coordination, budget
Mr J. W. Wright	Chief, VBC	Coordination, budget
Mr J. Hamon	Chief, VBC	Coordination, budget
Dr G. Gramiccia	ME—MPD	Planning, coordination with field

[a] HST = Division of Health Statistics; IMM = Immunology unit; ME = Division of Malaria Eradication; MPD = Division of Malaria and other Parasitic Diseases; RECS = Division of Research on Epidemiology and Communication Science; SHS = Division of Strengthening of Health Services; VBC = Division of Vector Biology and Control.

Name	*Division or unit*	*Responsibility*
Dr L. Molineaux	RECS—MPD	Planning, evaluation, epidemiology, model
Dr A. Rossi-Espagnet	RECS—SHS	Study design
Dr K. Dietz	RECS—HST	Mathematical model
Dr N. T. J. Bailey	RECS—HST	Statistics, study design
Mr R. F. Fritz	VBC	Planning (entomology), insecticides
Dr K. S. Hocking	VBC	Planning (entomology)
Dr A. Szenberg	IMM	Planning (serology), evaluation
Dr P. A. Falk	IMM	Evaluation (serology)
Dr S. Mandel	RECS	Statistics, epidemiology
Dr A. Bekessy	RECS	Statistical evaluation
Dr A. Benyoussef	RECS	Sociology, demography
Dr A. Weston	RECS	Social behaviour
Mr C. Garrett-Jones	ME	Planning (entomology)
Dr A. R. Zahar	ME—MPD	Evaluation (entomology)
Dr J. H. Pull	ME—MPD	Coordination with field, evaluation
Dr C. Alff-Steinberger	RECS	Numerical analysis
Miss N. Jenny	ME—MPD	Administrative assistant
Miss F. Bonnello	MPD	Secretary

Nigerian staff trained and working with the project

The total number of staff deputed or recruited by the Nigerian Government to work with the project varied from 113 to 143 during the period of more intense activity (collection of basic data and intervention phase) in 1971-1973. This number was gradually reduced to 68 during the course of the post-intervention phase in 1974-1975.

By category, the following numbers of Nigerian staff were trained for the project during the period of maximum activity (1971-1973):

Chief microscopist	1	Pump mechanic	1
Microscopists	5	Draughtsman	1
Microscopist trainees/ blood collectors	17	Clerk typist	1
		Drivers	17
Laboratory assistant (entomology)	17	Mechanics	2
		Storekeeper	2
Laboratory attendants (entomology)	9	General labourers	3
		Machine-operator	2
Field supervisors	6	Watchmen	4
Mosquito scouts (spraymen)	65	Office cleaner	1
Squad leaders	14	Statistical clerk	2
Geographical reconnaissance agents	15 (in 1970-1971 only)	Stewards	2

It is understood that, on account of the turnover or redeployment of staff, the number of people trained was in excess of that of people employed at any one time for any category of post. In addition, 13 Nigerian technical health staff were given a fellowship by the Federal or State Government for training with the project comprising a higher health superintendent, 3 health inspectors, 7 rural health inspectors, a laboratory technologist, and a microscopist.

Appendix 2

COST OF THE PROJECT

The field research project was financed by WHO (Regular Budget and Malaria Special Account) and by the Federal Government of Nigeria, in the proportion of about 85% and 15% respectively.

The costs borne by the Federal Government included the salary of the Governmental staff working for the project, the cost of building and maintenance of the project's accommodation in Kano and Garki. WHO covered all other costs, including an annual subsidy towards the costs borne by the Government. Details of the expenditure by year are given in Appendix Tables 1 and 2. Over the 7 years of activity of the project, the total cost was slightly over US $6 000 000, or an average of $870 000 per year. Of these, about 60% were for the staff, including travel and consultants, 13% for supplies, 12% for buildings and accommodation, about 8% for computer processing, and about 7% for miscellaneous expenses. A Plan of Operations, stating the objectives pursued and the methods followed by the project, as well as the responsibilities of the Government and WHO, was prepared and signed by the parties in 1970 and revised in 1975.

Appendix Table 1: Summary of Government costs for the project, 1970-1976

Commitment	1970 US$	1971 US$	1972 US$	1973 US$	1974 US$	1975 US$	1976 US$	Total US$
Government staff	15 998	15 998	15 998	15 999	15 999	136 820[a]	nil after April	216 812
Office accommodation[b]	268 832	–	–	–	–	–	–	268 832
Staff accommodation[c]	65 807	77 009	77 009	83 612	64 609	31 924	63 849	463 819
Total	350 637	93 007	93 007	99 611	80 608	168 744	63 849	949 463

[a] $76 000 were covered by Federal Government funds.
[b] Consisting of two complexes built for the purpose and from capital expenditure, one at Garki (field station) and one at Kano Project HQ (original source of funds not known).
[c] 1970 – 1974: 13 staff members
1975 – 1976: 5 staff members, 2 in Government quarters.

Appendix Table 2: Summary of all costs for the project, 1970-1976

Source	1970 US$	1971 US$	1972 US$	1973 US$	1974 US$	1975 US$	1976 US$	Total US$
Government	350 638	93 007	93 007	99 610	80 608	168 744	63 849	949 463
WHO	614 799	870 520	1 027 852	1 150 924	529 796	575 861	372 517	5 142 269
Total	965 437	963 527	1 120 859	1 250 534	610 404	744 605	436 366	6 091 732