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IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE

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THE EPIDEMIOLOGY AND MATHEMATICAL MODELLING OF MALARIA TRANSMISSION

By

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To my family and friends
- past and present.

" All theory, dear friend, is grey, but the golden tree of actual life springs ever green. "

Goethe.

ABSTRACT

The epidemiology of human malaria is reviewed, focusing on falciparum malaria in areas where the disease is stable and endemic, for which the majority of detailed information is available. A combination of the examination of field data, laboratory based investigations and the development of a variety of simple mathematical models is used to further the understanding of the epidemiology of malaria in an attempt to more reliably model the transmission dynamics.

The validity of laboratory based models is examined, including ways in which modifications in experimental design might more closely relate to naturally occurring malaria. A brief investigation is made of the effect on patent parasitaemia of re-infection during various phases of the initial infection.

A review is made of the transmission dynamics of malaria. A simple age-structured model is presented based on the early deterministic models of Ross and Macdonald in which the original framework has been extended and developed to include a number of the more recent ideas. Analysis of available epidemiological data and comparison with model results is used to discuss the shortcomings of this approach and also highlight which parts of the model structure is essentially correct and give results in good agreement with observed trends.

Age-prevalence profiles are a standard measure of the pattern of transmission of malaria and in endemic areas the observed profiles show highly characteristic patterns. The simple age-structured model based on the Ross/Macdonald model investigated how assumptions about the acquisition and maintenance of immunity affected age-prevalence profiles. Additional simple compartmental models are introduced and developed to investigate further the effects of these and other determinants such as the existence of multiple sub-populations of parasites, genetic heterogeneity in the human community with regard to susceptibility and development of immunity and the gradual development of a "higher" level of immunity through continued exposure to infection.

Finally, a completely different approach is examined which includes several new ideas concerning the development of immunity and attempts to overcome many of the shortcomings associated with the Ross/Macdonald type models. The model also examines the ways in which age-related exposure rates, relapses, superinfections and the acquisition of immunity help to define the shape of age-prevalence profiles.

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CHAPTER 1 Introduction

Parasites of the genus Plasmodium are responsible for the disease " malaria " in mammals, birds and lizards. Along with other members of the sub-order haemosporina they are the only protozoan parasites capable of invading the red blood cells of vertebrates. More than 100 plasmodial species have been described, causing malaria in a wide range of vertebrates and exhibiting narrowly-defined host specificities. Four species of Plasmodium infect man; P.falciparum (most common in the tropics, accounting for approximately 80 % of all infections and is by far the most clinically severe), P.vivax (most common in temperate areas), P.malariae and P.ovale. A considerable amount of attention has focussed on human malaria because of the debilitating and often fatal effects of infection, and its widespread distribution particularly amongst tropical and sub-tropical areas. It is estimated that approximately 15 % of all clinical illness can be attributed directly to malaria (Cohen & Lambert,1982), in certain regions of the world.

All known forms of Plasmodium have obligate heteroxeneous life-cycles involving alternate vertebrate and arthropod hosts. Infection in man is initiated through the bite of an infected female Anopheline mosquito, which injects anti-coagulant salivary fluid containing sporozoites into the blood capillaries, during a blood-meal. There are four phases to the life-cycle, 3 asexual (2 in the vertebrate, 1 in the vector) and 1 sexual (in the vector). Each phase ends with development of an invasive form, which is extracellular, mobile and able to enter a host cell before the succeeding phase is established. Each of the 3 asexual phases includes considerable multiplication of parasite numbers; by a factor 8-32 (repeated) during erythrocytic schizogony, by a factor 1 000 during oocyst development and by a factor 10-30 000 during hepatic schizogony (see Appendix A.1 for a full description of the life-cycle). During a typical infection the density of asexual forms of P.falciparum in a peripheral blood smear is usually 4 000-11 000 per µl. If infection is severe it may be as high as 5 % of erythrocytes (more than 250 000 parasites per µl at normal red cell counts, (WHO,1986)).

Typical symptoms of infection include chills and fever, usually lasting 6-8 hours, which reoccur as successive broods of merozoites are released from the red blood cells. These pathological effects are associated with the destruction of erythrocytes and the release of parasite-derived toxins into the blood-stream. As the disease develops destruction of

erythrocytes often results in anaemia and enlargement of the spleen. The essential pathology of severe falciparum malaria is the sequestration of erythrocytes containing mature forms of the parasite in deep vascular beds. Sequestration is greatest in the brain (MacPherson et al,1985) so that coma and other cerebral dysfunction is a prominent feature in severe cases, and accounts for 80 % of all fatal cases (Spitz,1946). Sequestration also causes impairment of hepatic and renal function. Other symptoms of severe malaria are haematological and coagulation abnormalities, including severe anaemia, and gastrointestinal problems which are especially common in children (McGregor et al,1956). Severe infections are most frequently found among children in endemic areas. In adults attacks may be about as troublesome as the common cold. A recent study in Gambia (Marsh, unpublished) suggests that roughly 10 % of cases (exclusively children) are admitted to hospital as severe cases and, of these 10 % die. Of those dying, the under 3 year olds die mainly as a result of anaemia, whereas cerebral malaria was found to be rare before the third year of life. Some more unusual reactions to chronic malaria are related to impaired immune responses, these include Tropical splenomegaly syndrome, Nephrotic syndrome and Burkitt's lymphoma.

The world malaria situation has remained static over recent years, both in the number of reported cases and in its global distribution. Despite the widespread use of insecticides and anti-malarial drugs, the pattern of infection is considerably worse than it was in the early 1970's. Some 100 countries or areas are recorded as still having indigenous malaria (fig. 1.1). Of a total world population of 4 818 million in 1985, an estimated 2 316 million (48 %) lived in areas which were under malaria risk and where anti-malaria measures were being applied. Roughly 405 million (8.5 %) still lived in malarious areas without specific protection and where the prevalence of malaria has remained virtually unchanged (this area includes a large part of tropical Africa - population 400 million).

Notification of cases is uncertain and unreliable in many tropical areas, and the number of cases reported to the WHO (4.8 million in 1985) does not include data from the WHO African region where little impact has been made in malaria control. The official estimate from the WHO for this area is a "conservative" 100 million cases and 1 million deaths, mainly in children under the age of 5 years (WHO,1987).

In tropical Africa P.falciparum is the dominant species and the principle vectors are Anopheles gambiae and A.funestus. Most of tropical Africa is affected by stable holo- or hyperendemic malaria. Malaria is described as holoendemic when the prevalence of infection in children (2-9 years) is constantly over 75 % , but where the prevalence

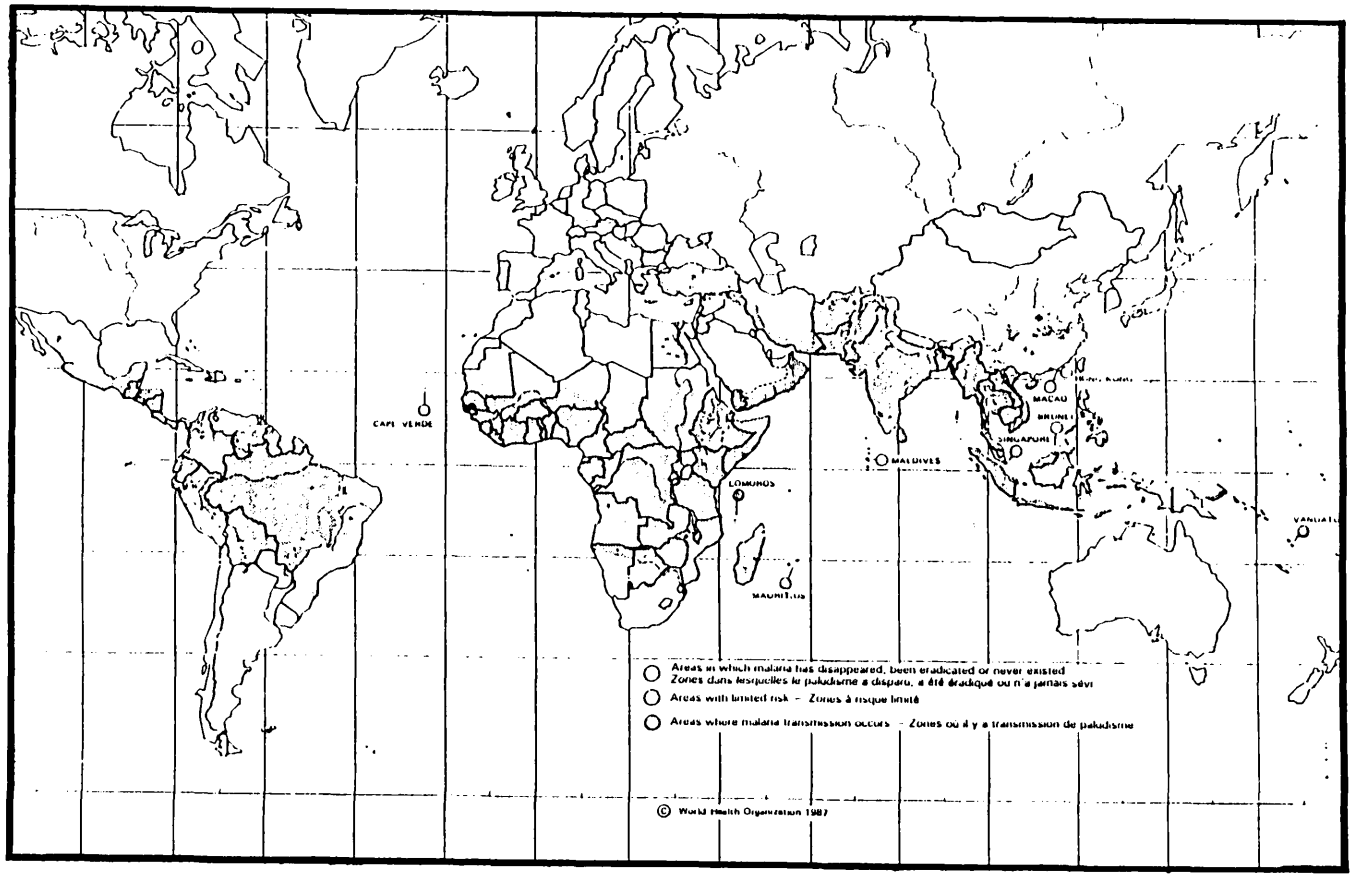


Fig. 1.1. Epidemiological assessment of the status of malaria, 1985 (WHO,1987).

among adults is low (less than 25 %). The prevalence of infection in hyperendemic areas is typically over 50 % in children (aged 2-9 years) and over 25 % among adults (refer to Appendix A.2 for a more detailed description of the classification of endemicity). Transmission in these areas is intense but stable, so that these patterns do not vary to any great extent from year to year. In hyperendemic areas however, transmission is usually seasonal so that regular seasonal variations occur to the prevalence of infection in the various age-groups. In the semi-arid fringe areas malaria transmission tends to be less intense, and is described as hypoendemic (prevalences less than 10 % among all age groups) or mesoendemic (prevalences between 11 % and 50 % among children aged 2-9 years). Transmission is also less stable, so that the amount of malaria in a locality may vary considerably from year to year and the pattern of disease may, at times become epidemic in character (refer to Appendix A.2 for a more detailed description of stability). Such patterns are not seen in areas with highly stable malaria. Depending on environmental conditions, the malaria situation may vary widely within the same country, and from season to season. WHO estimates indicate that 40 million people in tropical Africa live in hypoendemic areas, about 120 million in mesoendemic areas and 240 million in areas where malaria is hyperendemic or holoendemic.

Three major problems exist concerning the wide-scale control of malaria. The first is the current lack of any type of anti-malarial control over large areas of tropical Africa, where the malaria situation is most acute. The most recent WHO World Malaria Situation (1985) states,

> "For a variety of technical, operational, administrative and financial reasons, a reduction of malaria prevalence, especially in rural areas, is not feasible in many (African) countries. The only action that could be currently attempted in these countries is the reduction of mortality and morbidity through the use of antimalarial drugs..... in many countries, the setting up of realistic plans of action and coverage is very slow. Drug shortages are not infrequent and there is no epidemiological and operational monitoring and evaluation of activities. One of the major constraints hampering the implementation of anti-malarial action is the lack or shortage of trained personnel.."

(WHO,1987).

Further problems include poor public acceptance of repeated spraying and drug taking, and the lack of knowledge

of the possible side-effects of long-term drug treatment (Thompson & Werbel,1972; Thong et al,1979; Ponnampalam,1979). In other areas the malaria situation continues to be influenced by anti-malarial efforts. Large-scale use of anti-malarials however can cause problems, in the form of resistance by vectors to insecticides and by parasites to drugs. Insecticides in the past have been widely and often indiscriminately used, mainly in agriculture, which has led to the development of insecticide resistance. Many Anopheline species, of which 8 may be considered major vectors of malarial parasites over large areas, are now resistant to more than one insecticide. The resistance of P.falciparum to drugs continues to affect antimalarial programmes. It has been detected in more than 50 countries (fig. 1.2), and is particularly prevalent in those countries located in S.E.Asia, S.America and E.Africa. The problem is becoming increasingly important in S.E.Asia where multiple-resistant strains are appearing.

Because vaccines have been effective against other diseases, and in the light of growing resistance by both vector and parasite, the development of a vaccine has been considered an important and highly desirable complement to the control of malaria. It has been envisaged that malaria vaccines could be used to solve problems in areas subject to resistance and in areas where control has not been possible. However because of the complex life-cycle of the parasite and problems associated with antigenic variation, antigenic and phenotypic diversity and other immune evasion mechanisms used by malaria parasites, it has been questioned whether the development of such a vaccine is possible. Various techniques developed in the last 10 years such as; in vitro cultivation of parasites, methods for the production of monoclonal antibodies, cloning and sequencing of genes encoding specific antigenic proteins and the synthesis of antigenic polypeptides, have however brought the reality of a vaccine considerably closer. Techniques now exist for both the recognition and production of suitable antigens. However, selection and adaption of an appropriate system still remains to be solved.

Three types of malaria vaccines are currently being developed, namely; sporozoite vaccines, blood-stage (merozoite and schizont) vaccines and sexual stage (gametocyte and ookinete) vaccines. Only the sporozoite and blood-stage vaccines have reached the stage of being tested on human volunteers, where results have been variable (Ballou et al,1987; Herrington et al,1987; Pattarroyo et al,1988). Each type of vaccine will possess its own functional characteristics, an effective sporozoite vaccine would restrict/prevent the establishment of infection. Ideally the host would remain aparasitaemic, asymptomatic and incapable of infecting mosquitoes. This type of vaccine might be of use

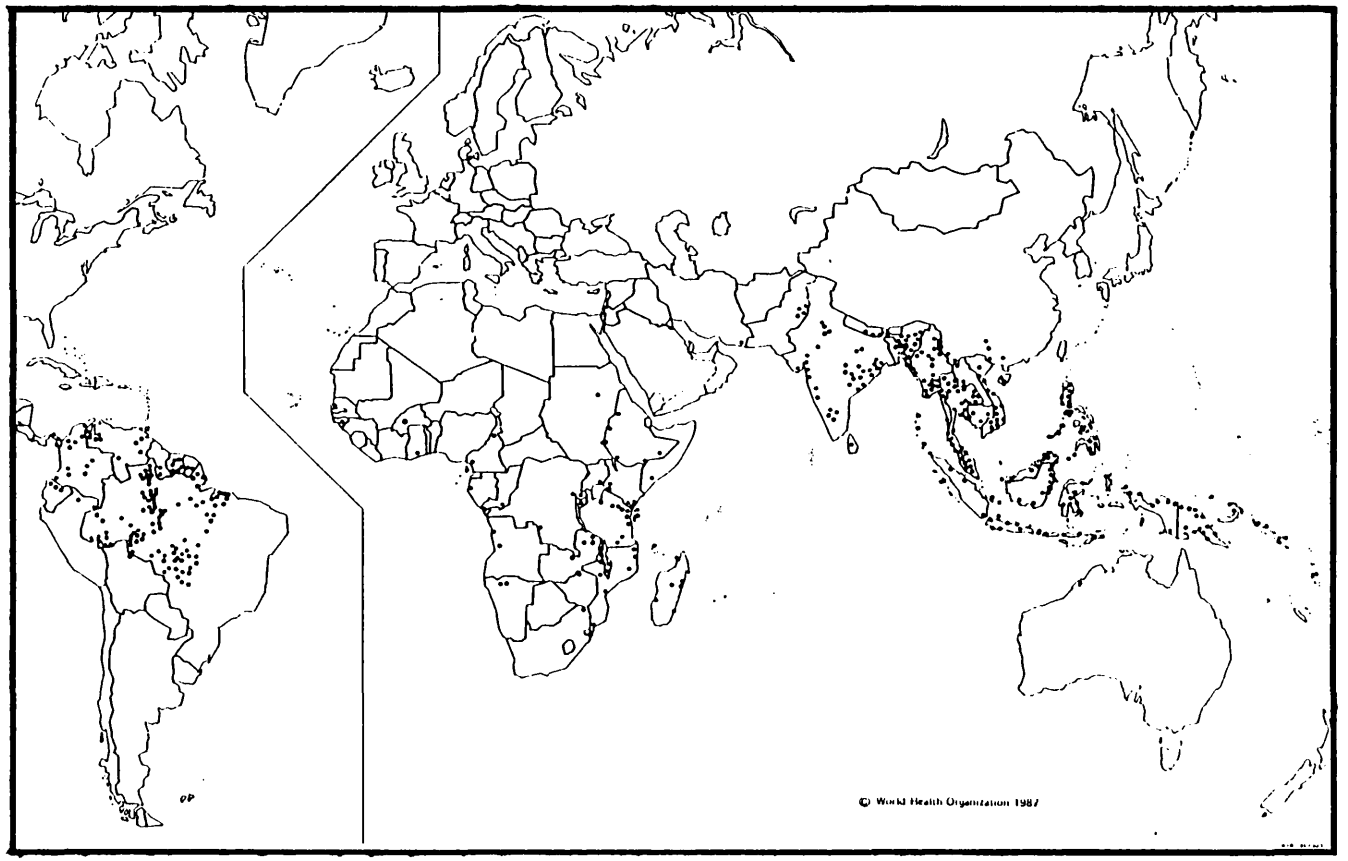


Fig. 1.2. Areas where chloroquine-resistant P.falciparum has been reported, 1985 (WHO,1987).

in the interruption of transmission and in the reduction of mortality/morbidity in those who have been vaccinated. Blood-stage vaccines would be effective at controlling the multiplication of the parasite within the host, and would therefore limit mortality/morbidity. The vaccine, however, is likely to be ineffective in interrupting transmission, and immunized hosts would remain infective to mosquitoes. Sexual stage vaccines are simply transmission blockers, having no effect on the course of infection in the immunized host. The benefits of the vaccine would be a reduction in transmission. Reduction in the incidence of mortality/morbidity would require the inclusion of the sporozoite and/or a blood-stage vaccine in a " vaccine cocktail ".

Other issues and problems determining the usefulness of malaria vaccines also exist, such as the questions of vaccine stability and cost, the need for and practicability of boosters, and the design of an initial vaccine regime. The effect of mass vaccination on the natural immunity of the community in terms of reduced antigenic stimulus, caused by either less severe/abbreviated infections and/or reduced transmission also needs to be considered. Carefully conducted field trials are necessary to elucidate the efficiency of vaccines under natural conditions. However, the pattern of disease varies considerably between areas under different epidemiological situations so that extensive clinical and field trials in a great variety of situations may be necessary to determine the type of vaccine and the vaccination regime appropriate to specific situations.

Suitable mathematical models, constructed to mimic the dynamics of acquired immunity and other determinants of infection such as genetic heterogeneity in host/parasite populations could however be used to investigate many of the problems mentioned above. Models may be of additional use to identify the effects of different factors on the acquisition of functional immunity to infection and on indices such as the incidence and prevalence of infection. Since mathematical models are critically dependent upon a quantitative understanding of the epidemiology of disease, they may also be used to identify uncertainties and areas lacking vital quantitative information. Even if the development of a vaccine remains on the horizon for some years to come, immunity in the population, and the identification of the major determinants of infection in the human population are of importance because they influence the efficiency of any intervention strategy.

The aim of the research described in this thesis is to further understanding of the population dynamics of malaria by the development of simple mathematical models. The focus is on the factors determining age-specific prevalences and

the acquisition of functional immunity. The majority of the work is based on falciparum malaria in areas where the disease is stable and endemic. The majority of detailed information is available for such areas.

Numerous models have been developed to investigate the dynamics of disease transmission, which are reviewed in the thesis. The emphasis for these models has however focussed on the vectorial components of transmission and many fail to describe the effects of immunity in man. Now that vector control is no longer viewed as a viable strategy for disease control on its own, and in view of the recent progress towards vaccine development, interest is now directed at the immunological aspects of infection. These include the development of functional immunity, and its impact on the age-specific prevalence and the intensity of transmission.

The thesis is organised as follows. Chapter 2 reviews the epidemiology of malaria, which provides the background biological and ecological information required for the construction of the mathematical models. The chapter firstly focuses on the major vectorial, parasite, human and environmental determinants of human malaria, and secondly gives a brief account of the effects and patterns of infection among human and vector populations.

The potential use and validity of laboratory based host-parasite systems as models for human malaria is discussed in Chapter 3. An investigation is made of the effects of superinfection on patent parasitaemia in a rodent malaria model. Suggestions are made for future work in this area.

The basic malaria models of Ross(1911,1916) and Macdonald(1952a,1952b,1957) are described in Chapter 4. An account is made of the various extensions and developments that have been made to the original models. For example, to include a more accurate description of the effects of superinfection on the magnitude of the recovery rate (Dietz, Molineaux & Thomas,1974; Aron and May,1982), variable mosquito densities (Aron,1981; Dietz, Molineaux & Thomas,1974), heterogeneous contact rates (Dye & Hasibeder,1986) and more complex models that describe immunity in man (Dietz, Molineaux & Thomas,1974; Aron,1983; Dutertre,1976; Elderkin et al,1977).

A simple age-structured model is presented in Chapter 5, based on the malaria models of Ross (1911,1916) and Macdonald (1952a,1952b,1957). The original Ross/Macdonald framework is extended to include an age-structure and is developed to investigate a variety of assumptions concerning the development of immunity in man. The effects of these assumptions on age-prevalence profiles and the shortcomings of this type of model is discussed.

Chapter 6 describes various simple compartmental models which are used to investigate the affects of a number of

factors on the shape of age-prevalence profiles. These factors include the existence of multiple parasite sub-populations and genetic heterogeneity in the human population with regard to susceptibility and the development of immunity. Section 6.4 of Chapter 6 describes a simple model which allows functional immunity to be gradually acquired by the human population. Specific assumptions concerning the rate at which this immunity is developed are made. Their effect on age-prevalence profiles are assessed and their validity examined by comparison with epidemiological data.

Chapter 7 introduces a completely different approach to modeling the development and maintenance of immunity to malaria. Functional immunity is assumed to develop gradually and be dependent on an individual's accumulated past experience of infection and/or parasite antigen. The model demonstrates the critical relationship between transmission and " immune status " and explores the role of acquired immunity in determining infection and recovery rates and in shaping age-prevalence profiles. The model considers diverse issues, which include, age-dependent vector-man contact rates, immunity acquired at different rates to different aspects of infection, loss of immunity and maintenance of immunity dependent on exposure rates, immunodepression, superinfection and the effects of fluctuating mosquito populations.

Finally, Chapter 8 discusses the results of the proceeding chapters, and suggests possible directions for future research.

CHAPTER 2 A Review of malaria epidemiology

2.1 Determinants of infection

In order to survive as a species, malaria parasites must remain in the human host sufficient time to produce mature infective gametocytes, and to be able to produce sufficient gametocytes, (both female and male), with which to infect suitable vectors. The parasite must also be adapted to be able to infect vectors that are sufficiently anthrophilic and can live long enough to permit the completion of the extrinsic phase of the parasite life cycle and infect further human hosts. In other words the basic reproductive rate (refer to section 4.2 (i)) of the parasite must exceed unity in value. Tables 2.1 (a) and (b) summarise the major vectorial, parasite and human determinants of human malaria which are recognised as having a major impact on (i) the vector populations potential to spread malaria (see Chapter 4), (ii) the net reproductive rate of the disease (see Chapter 4), and (iii) the stability of transmission (see Appendix A.2 and Chapter 4). These, and several additional factors, many of which cannot easily be quantified, are discussed in detail in this chapter.

2.1 (i) Vector Factors

The relative frequency of feeding on man, rather than some other animal, and the susceptibility to infection, determines the probability of a mosquito acquiring infection, and, in conjunction with vector longevity, the probability of transmitting infection to a new host. The relative frequency of feeding on man is a composite factor reflecting the mosquitoes attraction to man, the frequency of biting - determined by the gonotrophic cycle - and the densities of various potential hosts.

2.1 (i) (a) HBI

The main source of recent information are the reviews by Zahar(1985a,1985b,1985c) and by Garrett-Jones, Boreham & Pant(1980). The review by Garrett-Jones, Boreham & Pant(1980) consolidates the data of the WHO precipitin testing of Anopheline blood smears 1971-1978. The reviews by Zahar provide regional summaries (1985a, West Africa; 1985b, Equatorial and Southern Africa; 1985c, East Africa, Eastern Outer Islands and S.W.Arabia) of the above results with additional data for 1978-1985. All the results appear to

Table 2.1 (a)

Determinants of infection in human malaria - Vectorial Factors

Parameter	Definition	Common name of index
IMBR	Rate of biting on man by a single mosquito	Man-biting rate by a single mosquito
MBR	Bites per human host by the vector population	Total man-biting rate
b"	Probability that a blood meal from an infected host results in an infection	Susceptibility to infection
γ"	Duration of the extrinsic cycle in days	Latent period in mosquito
μ"	Per capita vector mortality rate	Reciprocal of the average lifespan of a mosquito
U	Average number of blood meals taken per week by a single mosquito	Feeding frequency
P	Proportion of blood meals taken on man	Human blood index

Method of estimation

The product of the vector feeding frequency (U below) and the human blood index (P below) based on the observations in the field under conditions of natural transmission, IMBR = U X P.

Number of mosquitoes caught on human baits per unit time as a function of the number of humans present (WHO,1975).

Number of captive mosquitoes developing sporozoite infections having fed on a demographically representative human population who are known to be infected (Graves et al,1988)

Calculated from the environmental temperature by Mošk ovskij's formula (Detinova,1962)

Mortality can be estimated by indirect method under field conditions based on morphological changes to the tracheoles of the ovaries after the first oviposition (Detinova,1962). The estimate is based on age-grading or from a knowledge of the proportion of the vector population that is parous and the value of U (eq.(2.1)).

The reciprocal of the gonotrophic cycle (mean time required for egg maturation and laying and also location of the next blood meal). The most accurate field estimates can be made using the formula developed by Carnevale et al(1979, cited by Zahar,1985b).

Estimated from the analysis of blood-meal samples by the precipitin test (WHO,1975, Part II, p129), according to the guidelines stated by Garrett-Jones et al(1980).

Table 2.1 (b)

Determinants of infection in malaria - Parasite and Human Factors

Parameter	Definition of index	Method of estimation
σ	The reciprocal of the of the pre-patent period	The interval between an infective bite and the appearance of patent parasitaemia, as determined by examination of peripheral by a thick blood smear (WHO,1986).
r	The rate of recovery from a single infection (or the reciprocal of the duration of infection)	Estimated from subjects who have been artificially inoculated or by longitudinal observation of malaria cases where further sources of infection are absent
b	Probability that a sporozoite positive bite results in a patent infection (human susceptibility to infection)	Normally estimated from the ratio of the parasitological: entomological inoculation rates (Davidson & Draper,1953; Pull & Grab,1974)

indicate that no anopheline species feeds exclusively on human blood. There do appear to be a number, such as the important African vector A.gambiae, that prefer to feed on man even when other hosts are available. The high preference of this species for human blood is clearly indicated by Bruce-Chwatt & Göckel(1960) who found that 68 % of specimens caught in animal sheds contained human blood in their stomachs. The precipitin test allows the determination of the human blood index (P) (also known as the degree of anthrophily) defined in table 2.1 (a). This index may initially appear qualitatively and quantitatively sound, but it may give seriously misleading results unless one interprets them in relation to (a) the type of shelter where the mosquito was captured, (b) whether the shelter had been sprayed with insecticide and (c) the availability or non-availability of other hosts. Mosquitoes captured inside huts used by humans are likely to have higher human blood indexes than those caught in animal huts where human hosts are not readily available. Residual spraying will complicate interpretation of results and also reduce sample sizes. To obtain unbiased results it seems necessary to examine specimens from all possible resting places, including outdoor ones, and possibly at different times of the day and night. However, evidence does suggest that many vector species are opportunistic feeds, so that variations exist between villages and at different times of the year according to the changing availability of alternative hosts, particularly cattle. Highton(1979) found that the proportion of A.arabiensis feeding on man in the Kano Plain, Kenya varied between villages. In one village where the man:cattle ratio was 1:2.5 the value of P was 0.18, in a neighboring village where the ratio was 1:0.6 the proportion of this species that fed on man was 0.56. Similar results from other parts of Africa were found by White et al(1972), although no correlation was found by Joshi, Service & Pradhan(1975) in the same area of Kenya.

Table 2.2 summarizes some of the precipitin results for the major African vector species, A.funestus and A.gambiae from which the estimated value of P was made for the model in Chapter 5. A number of precipitin results from Asian countries are also included.

2.1 (i) (b) Selective feeding

Much evidence suggests that members of the human population are not uniformly exposed to, or bitten with equal frequency by, members of the vector population. The age or size of the human host appears to be an important determinant (Port et al,1980; Carnevale et al,1978; Muirhead-Thomson,1951) although variations between individuals of the

Table 2.2

Estimated Human Blood Indices recorded for several major vector species in various African and Asian countries (unsprayed areas)

Location		HBI (prop. +ve for man)	Species
EAST AFRICA			
Ethiopia			
Garrett-Jones, Boreham & Pant(1980)			
	H	1.00	A.gambiae
	H	0.63	A.gambiae
	H	0.94	A.pharoensis
White(1980)			
	H	0.50	A.arabiensis
	Various	<0.01	A.quadriannulatus
Uganda			
White(1973)			
	Various	0.94	A.gambiae (Sp.A)
	Various	0.74	A.gambiae (Sp.B)
	Various	0.87	A.gambiae (Sp.D)
Kenya			
Garret-Jones, Boreham & Pant(1980)			
	H	0.67	A.gambiae
	H	0.95	A.gambiae
	O	0.21	A.gambiae
	H	0.924	A.arabiensis
	O	0.12	A.arabiensis
	H	0.989	A.funestus
	O	0	A.funestus
	H	0.932	A.funestus
Chandler(1975)			
	H	0.81	A.gambiae
	H	0.94	A.funestus
Joshi, Service & Pradhan(1975)		0.958	A.gambiae
	H	0.924	A.arabiensis
	H		
Tanzania			
White(1970)		0.98	A.funestus
Various (foothills)		0.84	A.gambiae
Various (foothills)		0.89	A.funestus
Various (Swamp)		0.65	A.gambiae
Various (Swamp)			
White, Magayka & Boreham(1972)		0.912 (.76-1.0)	A.gambiae (Sp.A)
	H	0.609 (.14-1.0)	A.gambiae (Sp.B)
	H	0.975 (.84-1.0)	A.funestus
	H	0.02	A.gambiae (Sp.A)
	O	0.07	A.gambiae (Sp.B)
	O	0.24	A.funestus
	O		
EQUATORIAL AFRICA			
(All Garrett-Jones, Boreham & Pant,1980)			
		0.553	A.gambiae
Cameroon	C	1.0	A.gambiae
Central African Republic	H	1.0	A.gambiae
Congo	H	0.991	A.gambiae
Gabon	H	0.97	A.gambiae
Rwanda	H	0.899	A.gambiae
	H	0.984	A.funestus
	H		

Location		HBI (prop. +ve for man)	Species
WEST AFRICA			
Gambia			
Boreham & Port(1982)			
Bed nets		0.989	A.gambiae
	H	0.70	A.gambiae
Bryan et al(1981), cited in Zahar,1985a			
	H	0.13	A.melas
	H	0.45	A.gambiae
Nigeria			
Molineaux & Gramiccia(1980)			
	H	0.97	A.funestus
	H	0.87 (0.61-0.91)	A.gambiae
	O	0.23	A.gambiae
Garrett-Jones & Shidrawi(1969)			
	H	0.75	A.gambiae
Service(1969/1970)			
	H	0.876	A.gambiae
	H	0.833	A.gambiae
Garrett-Jones, Boreham & Pant(1980)			
	H	0.975	A.funestus
	H	1.0	A.funestus
	H	0.85	A.gambiae
	H	0.996	A.gambiae
	H	0.991	A.gambiae
	H	0	A.hargreavsi
Burkina Faso			
Garret-Jones, Boreham & Pant(1980)			
	H	0.99	A.gambiae
Senegal			
Garrett-Jones, Boreham & Pant(1980)			
	H	0.675	A.gambiae
ASIA			
Bangladesh			
Khan & Talibi(1972)			
	H	0.93	A.minimus
Garrett-Jones, Boreham & Pant(1980)			
	H	0.32	A.balbacensis
Papua New Guinea			
Garrett-Jones, Boreham & Pant(1980)			
	H	0.98	A.farauti
	O	0.43	A.farauti
	H	0.98	A.punctulatus
	H	0.992	A.punctulatus
Khmer			
Sloof & Verdrager (1972)			
	H	0.75	A.balabacensis

KEY H - Human dwellings
O - Animal dwellings
C - Experimental feeding

same age also occur. These variations appear to be based on a range of factors including race (Harrison,1978), quality of bed net (Port & Boreham,1982), proximity to mosquito breeding sites, blood group (Wood et al,1972) and health (Day & Edman,1983; Rossignol et al,1985; Bryan & Smalley,1978).

Wood et al(1972) found that A.gambiae preferentially selected hosts of the blood group O. The mean number of bites taken on individuals with blood group O was 5.045 (42 subjects) compared to 3.503 (60 subjects) for all non-O subjects, the difference is significant at the 0.1% probability level. The experiments strongly suggest that A.gambiae is able to recognize ABO blood group variations, and is selective in feeding.

Bryan & Smalley(1978) found a striking change in the feeding behaviour of the mosquitoes on a pair of human hosts (one a woman the other a child aged 3 years) when one of them became ill with fever (origin unknown). In the 10 days prior to the illness only 2 mosquitoes were recorded as having fed on the child compared to 40 on the woman. During the 4 day fever however, 23 mosquitoes fed on the child, 3 on the woman and a further 2 were found to have fed on both woman and child (blood meal identification by ABO blood group type). Further evidence for the affect of illness includes several laboratory based experiments using murine hosts. Day & Edman(1983) showed that laboratory mice infected with P.chabaudi, P,berghei or P.yoelii became lethargic and ceased to display normal anti-mosquito behaviour. Periods of reduced defensiveness corresponded with maximum mosquito engorgement and with period of maximum gametocyte infectivity to mosquitoes. Rossignol et al(1985) found that P.chabaudi infections in mice enhanced the ability of vectors to locate blood capillaries and consequently the mean duration of probing (blood location) on infected animals was reduced by at least 1 minute as compared to non-infected mice. It is not known whether comparable vector-parasite mutualism occurs among the human malarias. However, defensive behaviour similar to that seen among murine hosts which will affect the feeding success of the vector has often been recorded among many other animals (Blackmore & Dow,1958; Kale et al,1972) including children (Muirhead-Thomson,1951). Parasite-disrupted hemostasis is thought to be a feature common to may vector-borne diseases. Increased feeding success of mosquitoes on infected children, who frequently have the highest gametocyte densities (see section 2.2), particularly during periods of peak gametocyte infectivity may be important to the maintenance of human malaria in nature.

Some heterogeneity in the rate of contact between man and vector might also be expected due to the differential sleeping habits of the people compared to the feeding habits of the vectors (Shidrawi,1970; Dodge,1965). The major African

vectors A.gambiae and A.funestus both attain peak biting rates during the night. However, whereas A.gambiae bites exclusively during the night (Shidrawi(1970) found that 93 % of females fed after 22.00), A.funestus has been recorded to bite during the day (in woods or dark places) (Service,1963; Krafsur,1977; Smith,1961). Certain portions of the population (particularly men) are found outdoors after 22.00 and therefore might be expected to have a greater risk of infection. Typical observations of vector feeding habits show that less than 4% of biting by A.gambiae takes place outdoors after 22.00 (Shidrawi,1970; Dodge,1965; Mouchet & Gariou,1957), from which it may be inferred that the vast proportion of local transmission normally takes place indoors. Factors such as sleeping habits therefore may not have a significant effect on contact rates. Similar results have been found for A.funestus (Cavalie & Mouchet,1961). It is interesting to note that outdoor biting becomes more frequent during the dry season (Shidrawi,1970; Rishikesh,1966). It has been suggested that this change in behaviour depends on climatic conditions. However, it is also possible that the change in human sleeping habits during the dry season, is an important factor when an increasing number of people remain outdoors throughout the night.

Several studies have attempted to assess the affect of size and/or age on human host on the rate of contact with the vector population (see table 2.3). The studies include the investigation of selective feeding among either family groups (4-8 persons) or pairs of individuals. As far as possible the groups comprised of people who normally slept together. A few studies concluded that there was no selective feeding (Clyde & Shute,1958; Smith,1956) whereas the majority seem to indicate that adults are considerably more "attractive" to mosquitoes than children (Muirhead-Thomson,1951; Thomas,1951; Spencer,1967; Carnevale et al,1978; Boreham et al,1978; Bryan & Smalley,1978; Port et al,1980). Mother and infant pairs and large family groups show the age-dependent biting habits of vectors most clearly and it has been suggested that the relationship is dependent on the exposed skin area available for feeding (Downe,1960,1962; Edman & Webber,1975). Individuals that contribute a large amount to the total exposed skin area, receive a higher proportion of total number of mosquito feeds. This is particularly apparent in mothers sleeping alone with their infant. Studies by Boreham et al(1978), Port et al(1980) and Bryan & Smalley(1978) involving mother-infant pairs showed that the mother was between 3 and 7 times more "attractive" to the biting mosquitoes. A further study by Spencer(1967) recorded that none of the infants were bitten, even though the mothers sustained between 106 and 1 bite per night. Port et al(1980) suggests that the tendency for mosquitoes to select their

Table 2.3

A Summary of Field Studies on Selective Feeding by various Anopheline Vector Species, according to age of host

Study location	Anopheline sp.	Reference
Tanzania	A.gambiae	Smith (1956)
Tanzania	A.gambiae A.funestus	Clyde & Shute(1958)
Kenya	A.gambiae	Boreham et al (1978)
Congo	A.gambiae	Carnevale et al (1978)
Gambia	A.gambiae	Bryan & Smalley (1978)
Gambia	A.gambiae A.melas	Port et al (1980)
Sierra Leone	A.gambiae	Thomas (1951)
West Indies	A.albimanus A.aquasalis	Muirhead-Thomson (1951)
Papua New Guinea	A.farauti A.punctulatus	Spencer (1967)

Study Groups (persons/grp)	Conclusions and Method
1 Man/1 Boy	No apparent differences in the number of bites per person per hour, (BCH).
19 Groups (4/5 pers.)	No consistent deviation of Anophelines to certain age groups was found, (L)BM.
3 Pairs (M/I)	In mother/infant (M/I) pairs mothers were found to be considerably more "attractive" to biting mosquitoes, (H)BM.
Family Groups (7/9 pers.)	Anophelines bit both females and males in equal amounts, adults found to be X 3 more "attractive" than infants, BCH.
7 Pairs (M/I)	The mothers were found to be X 7 more "attractive" than infants, (ABO)BM.
20 Groups (2/3 pers.)	Proportion of feeds per person correlated with the contribution made the total surface area of the group, (ABO)/(H)BM.
6 Groups (2/8 pers.)	Consistent preference for the adult members of family groups, BCH.
Family Groups (5/8 pers.)	Infants found to be bitten much less frequently than adults and older children, BCH.
6 Pairs (M/I)	0 bites on infants, compared to an average of 39 bites for the mother, BCH.

KEY BCH - Biting catches, (L)BM - Blood-meal typing (leukocytes), (H)BM - Blood-meal typing (haptoglobins), (ABO)BM - Blood-meal typing (ABO blood groups).

hosts by skin area results in age-dependent contact rates only if the hosts sleep closely together in a hut, as is the case for mother and infant pairs and for a large number of people in one hut. If the distance between hosts is large, mosquitoes entering the hut are equally likely to attack and feed on any host present.

The studies by Port et al(1980) and Carnevale et al(1978) provide the most detailed information on age-dependent contact rates. Both studies include individuals from a reasonable range of ages, and the investigations were carried out over a considerable time period with numerous mosquito biting sessions. The distribution of bites among the age-groups; 0-2 years, 2-10 years, 10-20 years and >20 years, for both studies (fig. 2.1) clearly show that the age/size of the host may be an important factor influencing the "attraction" of mosquitoes to certain hosts. Further evidence for age-dependent biting rates is given by Gill(1938) who observed that during the early stages of a malaria epidemic in Sri Lanka (then Ceylon) 1934-35, infection was almost exclusively confined to adults. Higher vector-man contact rates among adults which results in a greater risk of acquiring infection could well produce this pattern of infection.

The examples of selective feeding by vectors given above could be of great significance not only for the development of acquired immunity and natural selection of ABO blood groups in malarious areas but they may also significantly affect the level, the stability and/or the persistance of transmission. Dye & Hasibeder(1986) and Dietz(1980) have both illustrated the possible effects of selective feeding on the dynamics of infection using simple mathematical models which incorporate heterogeneous contact rates. These are discussed in more detail in Chapter 4.

2.1 (i) (c) Gonotrophic cycle

The gonotrophic cycle or feeding cycle is the time period between blood meals, and therefore the reciprocal of the gonotrophic cycle is often used as an estimation of the frequency of biting. During this interval the blood meal is digested, the ovaries develop, the female mosquito locates a suitable breeding place, egg-laying takes place, and then the female mosquito locates another blood-meal. The average duration of the cycle is between 2 and 4 days, the exact time being dependent on both the species involved and the environmental temperature (see section 2.1 (iv)).

There is no easy practical method of determining mosquito biting habits, although it is possible to make educated estimates from laboratory observations under varying

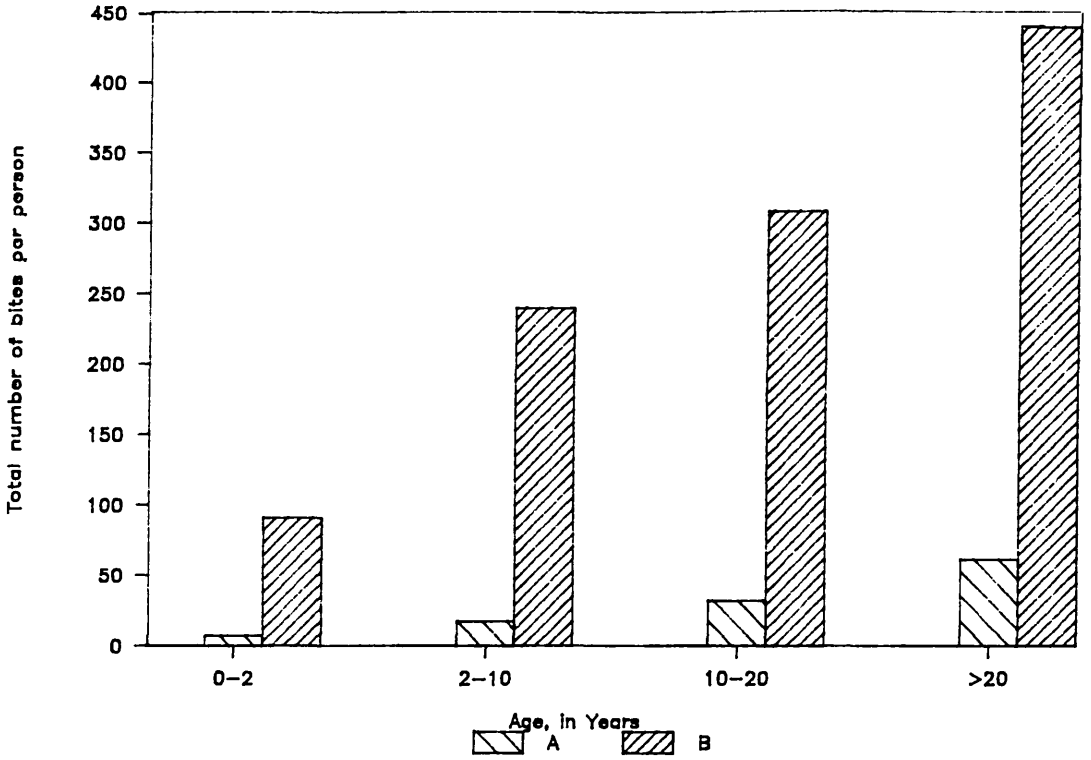


Fig. 2.1. Selective feeding by female A.gambiae according to age of host.
A. Aggregated data from a study by Port et al(1980) from two villages in Gambia, investigating biting-rates on 35 people sleeping in small family groups (2/3 persons). Number of feeds estimated from haptoglobin and ABO typing of blood meals (Port et al,1980), B. Data from a study by Carnevale et al(1978) from a village in the Congo, investigating biting-rates on 24 people sleeping in large family groups of 9, 8 or 7 persons. Number of feeds estimated from biting catches.

conditions of humidity and temperature (these estimates however make no allowance for the fact that not all female re-feed on the day of oviposition). Field estimates are also possible by observing the proportions of different stages of blood digestion and ovarian development. The dispersal of mosquito populations and the low percentage of recaptures may make estimates from the direct observation of biting of marked released mosquitoes impracticable and unreliable (Reisen & Aslamkhan,1979; Gilles & Wilkes,1965; Quraishi et al,1966).

A more accurate method of estimating the average duration of the gonotrophic cycle was proposed by Carnevale et al((1979) cited in Zahar,1985b). The study compiled and analyzed the results of observations presented in unpublished reports during 1975-1977 for an area in the Congo. A formula was suggested for the duration of the gonotrophic cycle (GC) in parous females (females that have laid at least one batch of eggs), based on the mean time required for both egg maturation and laying and also location of the next blood meal. The gonotrophic cycle was found to be 2.5 days for A.gambiae. Using the same formula field observations in Upper Volta (Brengues & Coz,1973 cited by Zahar,1985a) estimate the GC for both A.funestus and also A.gambiae to be 2.43 days.

Table 2.4 summarises field estimates for the duration of the gonotrophic cycle in A.gambiae and A.funestus from which the estimated value of U was made for the model in Chapter 5.

The GC of nulliparous females is generally 1 day more, since newly emerged females wait one day before feeding.

2.1 (i) (d) Susceptibility

Mosquitoes present obvious variations in susceptibility to malarial infections, not simply because only anophelines are susceptible to human malaria, but also because there is considerable evidence that within-species variation in the susceptibility of Anophelines to particular malaria parasites occurs naturally in vector populations (Collins et al,1979; Warren et al,1977,1979). Susceptibility of an anopheline to human malaria is a complex biological phenomena. The mosquito host may present barriers against any of several developmental stages in the malaria parasite including; exflagellation, fertilization, ookinete penetration of the midgut, oocyst development and sporozoite migration. Partial/total failure at one or more of these stages can affect susceptibility. Particular reference is often made to the "gut barrier" which prevents/restricts penetration of the parasite through the gut wall (Micks et al,1948). Warren et al(1977) however emphasise the importance of exflagellation,

Table 2.4

Reported Mean Duration of Gonotrophic Cycle for the Two Major African Vector species.

Location	Gonotrophic Cycle (in days)	Reference
A.gambiae		
Tanzania	3	Gilles & Wilkes(1965)
Tanzania	3	Gilles & Wilkes(1963)
Nigeria	3	Garrett-Jones & Shidrawi(1969)
Nigeria	2	Molineaux et al(1979)
Sudan	2	Zahar(1974)
Upper Volta	2.43*	Brengues & Coz(1973), (cited in Zahar,1985a)
Congo	2.50*	Carnevale et al(1979), (cited in Zahar,1985a)
Various locations	2	Muirhead-Thomson(1951)
Laboratory	4	Najera et al(1967)
A.funestus		
Cameroon	3	Mouchet & Gariou(1960)
Upper Volta	2.43*	Brengues & Coz(1973), (cited in Zahar,1985a)

(* denotes gonotrophic cycle calculated using the method by Carnevale et al(1979), cited by Zahar,1985a mentioned in the main text above).

fertilization and/or midgut penetration in determining susceptibility to malaria parasites, by affecting both the number of mosquitoes that become infected and also in the level of infection obtained. More recently, encapsulation of the ookinete after it completes its passage through the midgut, has also been shown in strains of A.gambiae which can both reduce susceptibility and also produce complete refractoriness (Collins et al,1986).

Among potential human vector species (i.e. species that have been shown not to be refractory to infection), the majority of the variation appears to be genetically based, although extrinsic factors can also be important. Analysis of the variation falls into four main categories: intra/inter-specific differences in susceptibility among laboratory and field populations (Eyles & Young,1950; Warren et al,1977; Collins et al,1977; Pringle,1962); highly-specific geographic strain-to-strain relationships between vectors and parasites (Young et al,1946; Boyd et al,1938; Collins et al,1977; Rutledge et al,1970); experimental work in which the overall susceptibility of the population is altered by genetic selection (Graves & Curtis,1982; Macdonald,1967; Wakelin,1978; Collins et al,1986; Ward,1963); and experimental infections in which the intensity of infection is altered by extrinsic factors (Klinger & Mer,1937; Terzian et al,1956; Hovanitz,1947).

There is considerable evidence to support the idea that there is active natural immunity of a genetically induced character distributed heterogeneously throughout vector populations. It is possible, by genetic selection, to derive highly resistant strains of mosquitoes from relatively susceptible strains. Ward(1963) attributes most of the susceptibility/refractoriness to the effects of single genetic factors which lack dominance. Collins et al(1986), however, mentions the possibility of mono-factorial inheritance of refractoriness, with intermediate forms caused by minor genes. Double feeding experiments in the same mosquito at different times (Huff,1930) confirmed that adult vectors retain "a state of specificity" to infection with a particular parasite species/strain throughout life.

Additionally there is non-inherited variation to malaria infection, some of which is related to the "quality" or quantity of the infective gametocytes (see section 2.1.(ii)(c)). However other factors have also been implicated. The age of the mosquito at the time of infection has been shown to have a significant effect on the intensity of infection in Ae.aegypti with P.gallinaceum (Terzian et al,1956). Hovanitz(1947) found a relationship between the amount of blood ingested by Ae.aegypti and the number of P.gallinaceum

oocysts that developed. Klinger & Mer (1937) noted significant seasonal variations in the degree of susceptibility to infection in colonies of A.elutus maintained in outdoor cages. Low temperatures seemed to have a negative effect. Rutledge et al(1969,1970) found that mosquitoes reared and maintained under constant conditions also gave seasonal variations in susceptibility. They concluded that gametocyte density/"quality", and hence human infectivity were probably the variable factor and not vector susceptibility.

Quantitative estimates for the proportion of bites on infectious individuals (gametocyte carriers) which infect vectors is restricted to a small number of studies which suggest that an accurate estimate is between 0.072 and 0.64. The studies include: Muirhead-Thomson(1957) who found that individuals that were able to infect any mosquitoes, infected on average 0.24 of the feeding A.gambiae. Boyd(1949a) recorded that during the infective phase of an induced infection, on average 0.48 of the mosquitoes became infected. Graves et al(1988) found that 0.38 of mosquitoes (range 0.091-0.75) feeding on infectious individuals became infected. Data presented by Draper(1953) suggests that an average of 0.09 of feeding mosquitoes on children acquire infection, similarly Barber & Olinger(1931), suggested that the figure be 0.47, and Robertson(1945), suggested 0.51. All three studies however illustrate the general effects of increased gametocyte density on the proportion of mosquitoes infected. Furthermore, Smalley & Sinden(1977) observed for four patients with P.falciparum gametocytaemia, that on days 1-4 of gametocytaemia, an average of 0.64 biting mosquitoes were infected; on days 11-12, an average of 0.072 were infected. However, these values are not accurate estimates for b" (the proportion of bites on infected individuals that produce an infection in the vector) used in the mathematical models in later chapters, since not all infected individuals are potentially infectious (have gametocytes in the peripheral blood). An accurate estimate for b" needs to account not only for the natural heterogeneity in susceptibility within the vector population (which might vary considerably between locations), but also factors such as the frequency distribution of gametocyte densities within the community and also the variations in infectivity during the courses of natural infection. Quantitative data of this sort is not available, as yet.

2.1 (i) (e) Mortality

Of the factors that determine whether a species is a potent vector, longevity is of considerable importance. No matter how susceptible a particular vector species is to infection or how strong the preference for human blood, if the vector does not naturally have a life expectancy longer than the duration of the extrinsic parasite cycle, the species cannot act as a vector. For the species where females do survive until sporozoites appear in the salivary glands, the greater the proportion that survive and the longer the infective life, the more potent the species will be as a vector.

The length of life for mosquito is quite difficult to assess since controlled insectary environments are probably quite different to those experienced in natural surroundings. Direct estimates, for example, from wild populations maintained in large cages or measurement of the survival of a generation of newly emerged mosquitoes by marking the emerging mosquitoes with stains on radio-isotopes are also likely to give erroneous results.

The most widely used indirect method for estimating longevity under field conditions is based on morphological changes to the tracheoles of the ovaries after the first oviposition. An assessment of the proportion of the vector population which is parous and its ratio to that fraction which is nulliparous allows an estimate for the daily mortality rate to be made (Detinova,1962). The method is however critically dependent on the estimate of the duration of gonotrophic cycle, so that, the probability, p, of survival through 1 day, is given as,

$$p = (\text{ proportion parous})^{1/n} \qquad (2.1)$$

where,

$$\mu'' = - \ln p \qquad (2.2)$$

The parameter n is the duration of the gonotrophic cycle and $1/\mu''$ is the mean expectation of life.

In addition to the practical problems of estimating mortality among natural populations, longevity has been found to be dependent on both species-specific inherent factors and environmental and climatic conditions, especially the relative humidity. As such seasonal variations in mortality frequently occur. Table 2.5 summarises mortality rates

Table 2.5

Estimated Daily Survival Probabilities (p) and Expectation of Life for Several Major Malarial Vector Species.

Location	p	Expectation of life, in days ($\mu'' = -\ln p$)
EAST AFRICA		
Ethiopia (Krafsur,1970)		
A.gambiae		
Wet	0.89	8.58 (0.116)
Dry	0.79	4.24 (0.235)
A.funestus		
Dry	0.79	4.24 (0.235)
Uganda (Najera et al,1967)		
A.gambiae	0.687	2.66 (0.375)
A.funestus	0.826	5.23 (0.191)
Tanzania (Gilles & Wilkes,1963)		
A.funestus	0.830	5.38 (0.186)
EQUATORIAL AFRICA		
Congo (Carnevale et al,1982b)		
A.gambiae (Savanna)		
Dry	0.874	7.49 (0.134)
Wet	0.966	28.90 (0.035)
Mean	0.937	15.37 (0.065)
A.gambiae (Forest)		
Dry	0.895	9.01 (0.111)
Wet	0.968	30.75 (0.032)
Mean	0.935	14.88 (0.067)
Zaire (Vincke,1965)		
A.gambiae	0.79	4.24 (0.236)
WEST AFRICA		
Kaduna, N.Nigeria (Service,1965)		
A.funestus	0.89	8.58 (0.116)
Kaduna, N.Nigeria (Rishikesh et al,1977)		
A.funestus	0.93	13.78 (0.073)

Location	p	Expectation of life, in days ($\mu'' = -\ln p$)
WEST AFRICA		
N.Nigeria		
Kankiya (Garrett-Jones & Shidrawi,1969)		
A.gambiae	0.94	16.16 (0.062)
Kankiya (Najera et al,1973)		
A.gambiae	0.938	15.62 (0.064)
Garki (Molineaux et al,1979)		
A.gambiae	0.89	8.58 (0.116)
Ghana (Brady,1963)		
A.funestus		
Coast (March)	0.932	14.20 (0.070)
Bush (Feb-Mar)	0.95	19.49 (0.051)
(July-Aug)	0.96	24.50 (0.041)
Grassland (July-Aug)	0.88	7.82 (0.128)
A.gambiae		
Coast (March)	0.86	6.63 (0.151)
(July-Aug)	0.90	9.49 (0.105)
Bush (Feb-Mar)	0.896	9.10 (0.110)
(July-Aug)	0.91	10.60 (0.094)
Grassland (July-Aug)	0.896	9.10 (0.110)
Forest (Aug)	0.872	7.30 (0.137)
ASIA		
Iran (Quraishi et al,1966)		
A.stephensi	0.51-0.60	1.5-2 (0.67-0.51)
Pakistan (Reisen & Ashlamkhn,1979)		
A.stephensi	0.71-0.75	3-3.5 (0.34-0.29)
Pakistan (Russell et al,1944)		
A.culicifacies	0.735	3.25 (0.308)

estimated for the two major African vectors A.gambiae and A.funestus in various Afrotropical regions, and also compares the mortality rates for several major vector species found in the Indian sub-continent. The life-span of the vectors in India and Pakistan are considerably shorter than those of the major vectors in Africa. In addition vector species in India prefer feeding on cattle than on man. Malaria under these conditions is maintained by force of numbers of vectors. If the density of vectors declines, the numbers may be insufficient to enable the infection to persist. Malaria in these areas is characteristically unstable and subject to seasonal or periodic epidemics.

The logarithmic graph of the cumulative weekly survival of A.quadrimaculatus (Keener,1945) under laboratory conditions formed almost a straight line demonstrating that mortality was in the form of a geometric progression unaltered by age (Macdonald,1952a). Based on this and other observations, Macdonald (1952a) suggested that under natural conditions, mosquito mortality does not change with age.

2.1 (ii) Parasite Factors

2.1 (ii) (a) The pre-patent period

Pre-patency is defined as the interval between the infective mosquito bite and the appearance patent of parasitaemia. It is not to be confused with the incubation period, which is the interval between the infective mosquito bite and the appearance of symptoms of disease such as fever. Pre-patent periods for P.falciparum vary between a maximum of 24 days to a minimum of 5 days , from 7 to 26 days for P.vivax, from 16 to 39 days for P.malariae and 15 to 27 days for P.ovale (Grab & Pull,1974; Pampana,1969; Bruce-Chwatt,1980; Boyd,1949a; Fairley,1947; Wernsdorfer,1980). The average pre-patent period of P.falciparum infection is generally accepted as about 11 days; for P.vivax infections, 13 days; P.ovale infections 14 days and 3-4 weeks for P.malariae infections (Pampana,1969). Studies on sporozoite-induced malaria in man have also suggested an inverse relationship between the size of the sporozoite inoculum and the duration of the pre-patent period (Jeffery et al,1959; Shute et al,1976; Boyd,1941 cited in Herrington et al,1987).

Protracted pre-patent periods however often occurs in P.vivax infections in certain parts of the world, and may last up to 12 months (Garnham et al,1975; Coatney et al,1950a,1950b). This is thought to be associated with natural adaption or selection of the most suitable parasite

population for the climatic and transmission conditions of the region (this topic is discussed further in Chapter 5, section 5.3 (iii)).

2.1 (ii) (b) The duration of infection

The natural life-span of a malarial infection varies widely according to the Plasmodium species and, to a certain degree, the strain or geographical origin of the infection. P.falciparum infections are generally the most short-lived, averaging 9-12 months (Pampana,1969). This is understandable since they are the only type of human malaria that have no secondary exo-erythrocytic cycle. Once the blood infection is eliminated, either due to the development of immunity or through drug treatment the infection is terminated both clinically and parasitologically. Infections with P.vivax and P.ovale usually last no longer than 2-3 years, P.malariae infections can last the life-time of an individual - periods of over 50 years have been recorded (Cohen & Lambert,1982). These differences in the duration of infection probably reflect, at least in part, the extent of parasite adaption to man. P.falciparum infections are clinically the most severe, frequently killing the host, and the infections are short-lived, indicating the least parasite adaption. P.malariae infections however are quite mild, exhibiting almost commensalistic features over many years, and parasite adaption is thought to be quite advanced.

The duration of a single malarial infection is most frequently estimated from subjects who have been artificially inoculated. Although estimates can be made from individuals infected naturally but who have moved away from any further source of infection. The average duration of infection for P.falciparum in most strains does not exceed 10 months (Bastianelli,1943 cited by Pampana,1969). Eyles & Young(1951) estimated the duration of infection for a North American strain to be 222 days +/- 25, the maximum duration was 16 months. For a Panamanian strain the range was 114-503 days (Jefferey & Eyles,1954). The best sets of data with which to estimate the duration of infection are by Ciuca(1955) using a Romanian strain and Earle et al(1939) using a strain from Puerto Rico. Earle et al(1939) estimated the average duration of infection to be 200 days. Further analysis of the duration of infection by Macdonald & Göckel(1964) demonstrated that the value of 200 days was a reliable one for many geographic strains of P.falciparum.

P.vivax infections normally last longer than those of P.falciparum. Infections contracted by Americans in Korea appeared to have all terminated after about 18 months (Arnold,1954), but Korean children removed to Rumania were recorded as having infections that lasted considerably longer

than 2 years (Ciuca et al,1958). Other records of infections lasting in excess of 2 years include Bastianelli(1943, cited by Pampana,1969), Nikolaev(1939 cited, by Pampana,1969) and Hill & Armuzio(1949, cited by Pampana,1969). Although infections by P.malariae and P.ovale are generally of considerable length if left untreated, it is thought that these infection are not capable of transmitting the disease to the vector except in the first few months of infection. In other words the infectious period is much shorter than the typical duration of infection.

The duration of naturally occurring malarial infection is however considerably complicated by the development of functional immunity which enhances the elimination of parasites. It is also further complicated by the existence of superinfections which are thought to considerably lengthen the patent period before all infections are eliminated. Molineaux & Gramiccia(1980) found that the duration of infection decreases from about 600 days among 1-4 year olds, where superinfections are frequent and the immune status is low, to about 50 days among adults in whom a high level of functional immunity both increases the rate of elimination of parasites and also reduces the occurrence of superinfections. The rate of development of this type of functional immunity and its effects on various aspects of infection are investigated and discussed in detail throughout the remainder of the thesis.

2.1 (ii) (c) Gametocyte production and infectivity

An epidemiologically important feature of P.falciparum infections is the relatively late appearance of gametocytes in naturally acquired infections. The first appearance of infectious gametocytes generally coincides with the remission of the first wave of asexual parasites, typically 10-15 days after the asexual forms (Vanderberg & Gwadz,1980; Wernsdorfer,1980). A further delay then occurs until gametocytes are mature enough to infect mosquitoes (Vanderberg & Gwadz,1980). The other human malarias follow a course of infectivity similar to the majority of other murine malarial species, in which the host becomes infective before the first peak of asexual parasitaemia.

Humans appear to be potentially infectious to mosquitoes for much of the time they are infected with malarial parasites. In P.falciparum infections in non-immune subjects, gametocytes continue to circulate in the blood for a year or longer following the first acute phase of infection (Jeffery & Eyles,1954,1955). Loss of infectivity does however appear to occur, (probably related to the development of an immune response), Carter & Gwadz(1980) found that 100 % of non-immunes are infectious for about one month after the onset of

infectiousness, 50 % for 3 months, and 20 % for 1 year or more. Individual gametocyte histories by Boyd(1949a), Earle et al(1939) and Miller(1958) could not determine the exact relationship between gametocytaemia and infectiousness, although several features of gametocyte production were elucidated. Miller(1958) showed that fever can act as a trigger for gametogenesis and that clinical relapses can induce a resurgence of gametocytes after an initial wave has disappeared from the peripheral blood. Smalley et al(1981) claim that longer-lasting infections enhance gametocyte production as the infection proceeds. Late gametocytes may however be considerably less infective (Vanderberg & Gwadz,1980). For P.vivax infections gametocytes also appear to be present and infectious for about a year after initial infection. In non-immunes early infectivity coincides with clinical symptoms. However, gametocytes appear to be produced and are infectious (similar to P.falciparum infections) even when parasitaemia is asymptomatic (Boyd et al,1936). Gametocyte production by P.malariae is highly variable (Young & Burgess,1961).

Diurnal rhythms of infectivity have been demonstrated for several malarial species (Hawking et al,1968; Hawking et al,1969; Hawking,1975), which have been correlated with the period of highest biting by vectors (Hawking et al,1971). It is thought that such synchronicity results from the periodic production of short lived gametocytes by successively synchronised generations of asexual parasites, and not by sequestration and release of gametocytes. P.falciparum infections are not however characterised by these patterns. Periodic infectivity however, is thought to be obscured by its poorly synchronised asexual cycles and by the production of gametocytes of relatively long functional life. The host being almost continuously in a state of infectiousness during the period when gametocytes are being actively produced.

Because of poorly understood immune processes infectivity cannot be equated with gametocytaemia. The factors that influence the infectivity of mature gametocytes in the blood to the vector have been grouped together by Boyd(1949a) under the terms "quantity" and "quality". One component of variation in infectivity is determined by the relationship between parasite and vector. Many vectors are refractory to or less susceptible to infection by certain parasite strains particularly ones originating from different geographical locations. Gametocyte density is also an important determinant of infectivity. There appears to be a trend of increasing infectivity for A.gambiae as gametocyte density increases for P.falciparum infections in native West Africans (Barber & Olinger, 1931; Draper,1953; Robertson,1945). In stable endemic areas, where gametocyte

densities for individuals are age-related (dependent on the immune status of the individual, see section 2.2 (i)), infectivity appears to be age-specific. However, the variability of infectivity at all gametocyte densities is quite striking and many studies fail to show any such relationship. The proportion of carriers with low densities (< 10 per mm^3) which infect more than 50 % of mosquitoes is also striking. Similar variability has been demonstrated for P.falciparum infections in A.balabacensis in Thailand (Rutledge et al,1969). A strong correlation is however seen between a given gametocyte density and the maximum oocyst load per mosquito fed at that density. Such a correlation indicates that under "optimal" conditions there is a direct relationship between gametocyte density and infectivity. Under controlled in vitro conditions good positive correlation between gametocyte density and infectivity has been found when a single sample of gametocyte carrying blood diluted by different amounts in uninfected blood was fed to mosquitoes through a membrane (Eyles,1952). The contrast between the results obtained under in vitro conditions and during natural infection emphasises the additional factors that operate under natural conditions.

In various species, including P.falciparum there appears to be an inverse relationship between asexual parasitaemia and infectiousness at any given density of gametocytes (Rutledge et al,1969; Carter & Gwadz,1980). The effects of asexual parasitaemia on infectivity could be due to the excretion of toxins by the asexual stages or some sort of modulation due to host immune reactions. Sera from humans naturally infected with, or largely immune to, P.falciparum has been seen to fail to agglutinate gametes or mediate significant suppression of infectivity of gametocytes to mosquitoes even though high titres of anti-parasite antibodies were present (Carter et al,1981).

Seasonal variations in the infectivity of malarious individuals has been noted by Young et al(1948) in S.Carolina, Rutledge et al(1969) in Thailand and by Muirhead-Thomson(1957) in Africa.

2.1 (iii) Human factors and susceptibility to infection

Several factors affect man's susceptibility to infection, these include both innate or physiological factors which are non-specific, (and requires no prior stimulation to appear) and also specific factors that are acquired, via experience of infection. These specific factors are not only dependent on the extent or rate of contact between man and vectors, but also depend on the ability of an individual to develop an acquired immune response. Innate and acquired characteristics are genetically controlled. It follows

therefore that susceptibility and resistance to disease may also be genetically determined.

Historically, inoculation rates have been computed in two ways. The entomological inoculation rate is determined by the biting-rate of sporozoite positive mosquitoes, and the parasitological inoculation rate is determined from cross-sectional prevalence rates or cumulative prevalence rates in infants. The entomologically based inoculation rate is always larger than that of the parasitologically based one. The ratio of the two is used to describe the proportion of sporozoite-positive bites which infect people (b), or alternatively the susceptibility of the human population to infection. The effects of age and season on the value of b are discussed in detail in Chapter 7. A brief analysis is made in this section of some of the major factors operational in endemic areas which associated with susceptibility to the various effects of malarial infection.

2.1 (iii) (a) Innate immunity

Complete immunity is a refractoriness to infection and is the basis for host-specificity frequently seen among malarial species. Innate immunity plays an important role in the distribution of P.vivax infection, which is virtually absent from West and Central Africa (Bray,1958; Young et al,1955) but is commonly found in East Africa. This apparent absence of disease is thought to be associated with the frequent absence of a red-cell-membrane antigen known as the Duffy antigen within the population. The absence of this antigen has been shown to coincide with the absence of the specific red cell receptor necessary for merozoite invasion. Invasion of the red-blood cells is thus completely prevented (Miller et al,1976).

Tolerance for P.falciparum infections also varies from population to population and, within populations, from individual to individual, as determined by various genetic factors. The innate mechanisms responsible for these effects such as haemoglobin types (sickle-cell haemoglobin,α - and ß-thalassaemia red cells, foetal haemoglobin and the minor blood groups C and E) and red cell enzymes such as glucose-6-phosphate dehydrogenase do not prevent parasite invasion. It is conditions within the abnormal red cell that retard but do not completely prevent the growth and development of P.falciparum. Innate mechanisms recognised so far all influence the development of asexual blood forms, and none have been shown to act on exo-erythrocytic forms or gametocytes (Miller & Carter,1976; Luzzatto,1979). This is not really surprising, since the main pathology associated with human malaria is associated with the rapidly multiplying parasite population in the erythrocytes.

Of the inherited blood disorders known to influence parasite development, the effects of the sickle-cell trait are by far the most obvious and best understood. The geographic distribution of the abnormal gene controlling the sickle-cell trait is well-defined and the trait is particularly prevalent in tropical Africa, where up to 45 % of the individuals in some tribes have the sickle-cell trait. The gene is also present at levels, often in excess of 20 %, in the negroid Veddoid aboriginals of India, the Achdam of southern Arabia and, to a lesser extent, in some of the Mediterranean peoples, particularly the Greeks (Wakelin,1978; Neel,1956; Ashworth & MacPherson,1968). It is recorded at very low levels in malaria free areas. There is intense selection against the sickling gene since homozygotes in malaria endemic areas invariably die during childhood and it has been shown that the trait is maintained at high frequencies in tropical Africa and other tropical and subtropical regions within the "malaria belt" because of the biological advantage they confer on heterozygotes through partial protection from P.falciparum. The specific advantage for heterozygotes in malarious areas appears to be able to more than outway the losses of the sickling genes through mortality among homozygotes (Allison,1954,1964; Livingstone,1971). Heterozygous children (AS) in malaria endemic areas often, but not always, have lower parasite rates than children with normal haemoglobin (Gilles et al,1967). Fatal/severe malaria infections are however considerably less frequent in heterozygous compared to normal children (Colbourne & Edington,1956; Fleming et al,1979). This protection is not present in homozygous children (SS) in whom infection is usually fatal (Adeloye et al,1971; Luzzatto,1974).

There is also good evidence from some parts of the world (the Near East, India, S.E Asia and Africa) that the thalassaemias may protect against malaria, by the persistence of foetal Hb in thalassaemic infants (see section (iii) below), which explains the persistance of these otherwise deleterious haemoglobinopathies (Wetherall & Clegg,1981; Flint et al,1986; Siniscalco et al,1966). The advantageous effects of the thalassaemias are however not as great as that of the sickle-cell trait. Similar, if less clear cut, correlations exist between the prevalence of thalassaemia in a country and the level of transmission of malaria. Haemoglobin C in West Africa (Ringelhann et al,1976) and haemoglobin E in S.E. Asia have also been associated on epidemiological grounds with resistance to P.falciparum.

The question of glucose-6-phosphate dehydrogenase deficiency (a sex-linked group of haemoglobin polymorphisms widely distributed in tropical and sub-tropical areas) protecting against malaria in children remains unsolved. Allison & Clyde(1961), Bienzle et al(1972) and Gilles et

al(1967) found significantly lower parasite counts in young enzyme-deficient African children. While Kruatrachue et al(1962) failed to find any difference in Thailand, however further work (Kruatrachue et al,1970) showed significantly lower parasite densities among enzyme-deficient children. It has been suggested that the ingestion of fava beans might act synergistically with G-6-PD deficiency to confer resistance to malaria (Huheey & Martin,1975). It is thought that the possible beneficial effects of G-6-PD deficiency are less clear cut because unlike sickle-cell haemoglobin, which results from the coding error of a single gene, G-6-PD deficiency seems to be under the control of at least 30 genes. Three hundred genetically different variants of G-6-PD deficiency have been identified, and these seem to provide differing levels of protection (Luzzatto et al,1985).

A number of non-specific mechanisms that reduce the number of sporozoites successfully invading hepatocytes are discussed later in Chapter 7.

2.1 (iii) (b) Acquired immunity

The development of acquired immunity among those living in malarious areas is a complex process and is also difficult to study under controlled conditions. The most important effects of acquired immunity on malaria epidemiology are outlined by the age-specific prevalence and intensity profiles, and the age-related mortality and morbidity rates discussed in section 2.2. Further discussion is made in Chapters 6 and 7 concerning acquired immune responses, its heterogeneous distribution both within and between populations, and the rate of development of naturally acquired immunity under field conditions.

2.1 (iii) (c) " Infant advantage "

The existence of maternally derived immunity has long been suggested to explain the low parasite rates and the mild, low grade parasitaemia infections experienced by infants aged 0-4 months in endemic areas (Garnham,1949). Maternally derived antibodies are thought to provide partial protection during the first few months of life, after which actively acquired immunity is slowly developed in response to repeated exposure to infection (Blacklock & Gordon,1925; Wilson,1936; Bruce-Chwatt,1952a). Antibodies to malaria blood-stages have been detected in the sera of neonatal Gambians by both precipitin (McGregor & Wilson,1971) and indirect immunoflorescence techniques (McGregor et al,1965). In addition, gamma globulin obtained from the cord blood of Nigerian infants has been shown to decrease parasitaemia when

passively transferred to a child with acute malaria infection (Edozien et al,1962). McGregor et al(1965) in a study in an endemic area showed that immunoflorescent titres rapidly declined during the first 16 weeks of life. During the remainder of the first year antibody levels appeared to remain low, and began increasing again during the second year as a result of exposure to infection. This increase was shown to continue throughout childhood. Similar results have been obtained with the gel diffusion test (McGregor & Wilson,1971) and the indirect hemagglutination test (Mathews et al,1976). The prevalence of precipitins to malaria showed a rapid decline after birth to its lowest level at 8 months, and rose thereafter until, by the age of 5 years it was at least 90 %. Mean titres continued to rise throughout childhood and adult life. It would appear that maternally derived antibodies may persist for 6-8 months after birth, any further persistance being masked by the development of active acquired immunity in infants in response to antigenic stimulus. A recent survey among Nigerian children born in the U.K. to immune mothers indicates protective passive immunity may last considerably longer than 8 months, the protective significance however is likely to be minimal (Udezue,1985). Immunoflorescent tests shows that serum positivity rates were 82 % in the first 6 months, 40 % in the second 6 months and 9 % in two years.

More recently, antibodies to sporozoites of P.falciparum have been found to occur in the sera of most babies born to mothers living in endemic areas of the Gambia (Nardin et al,1981). At 6 weeks anti-sporozoite antibody titres of infants correlated well with those of the mother. However, the antibodies were seen to gradually disappear from circulation and, in most cases were no longer detectable 6-7 months after birth. The exact nature of the protective effect of maternal antibodies to malaria is keenly debated, and is discussed in a later section (section 5.3 (iv)). Other innate protective mechanisms may also have an effect on the infection rates and severity of infections in young children, these include: (i) the exclusive milk diet of infants which would create a p-aminobenzoic acid deficiency in the blood, which in turn is unfavorable to the development of the parasite (Maegraith et al,1952; Hawking,1953,1954); (ii) the retarded growth of parasites in erythrocytes containing foetal haemoglobin (Hb F) as compared to normal haemoglobin (Pasvol et al,1976,1977). At birth 80 % of haemoglobin is Hb F, at 2 months 50 % and at 4 months 10 % ; (iii) the age of erythrocytes is another potential factor, since old red cells containing Hb F do not support P.falciparum (Pasvol et al,1980); (iv) selective biting by vectors (see section 2.1 (i) (b)).

2.1 (iv) The environment

Climatic and topographic features determine the ecology of both human and vector populations and also the rate of contact between the two populations. Malaria transmission requires that the source of infection (either an infected vector or human) be in close proximity to susceptible hosts. The normal flight range of the vector is only about 2km. from its breeding place (Charlwood et al,1986). The closer the proximity of human habitation to the breeding area, the higher the vector-man contact rate. Land vegetation, soil structure and hydrographic features determine, to a large degree the nature and stability of the breeding place.

The most important environmental factors influencing malaria transmission are meterological features, principally temperature, humidity and rainfall. For the first two factors there are extreme values that make transmission impossible. The mosquito is a heterothermal animal and consequently assumes the temperature of the surrounding environment. Mean temperature affects both the parasite developing within the vector and also the mosquito itself. Sporogony of P.vivax ceases below 15^0-16^0 C., that of P.falciparum below 20^0 C. If the mean temperature is 16^0 C. the extrinsic developmental period of P.vivax lasts 55 days; and at 28^0 C. it lasts only 7 days, while P.falciparum would still require 10 days (fig. 2.2). Optimum temperatures for the rapid completion of the extrinsic phase are around 25-27^0 C for both P.falciparum and P.vivax. Temperatures consistently higher than 32^0 C. are lethal for the parasite. This temperature dependence of sporogony explains why P.vivax is not found in areas outside the 16^0 C. isotherms and similarly why P.falciparum is restricted to areas within the 20^0 C. isotherm.

Relative humidity has not been shown to have any direct effect upon the parasite, but it does have a direct effect on mosquito longevity and thus upon the probability of the mosquito reaching an infective age. Wernsdorfer and Wernsdorfer(1967) estimated in the central Nile River basin a daily mortality for A.gambiae of 5% at relative humidities of 65 % or more, of 10 % at 55 % R.H. and of 15 % at 50 % R.H., at mean temperatures of 27-30^0 C. and day-night fluctuations of +/- 5^0 C. The longevity of A.gambiae at various levels of relative humidity is illustrated in fig. 2.3. It is seen that significant proportions of mosquitoes survive to a potentially infective age only when the relative humidity is 50 % or more. When conditions become very unfavorable to the life and activity of mosquitoes, the potential for malaria transmission decreases dramatically. In Western Sokoto, Nigeria Bruce-Chwatt(1953) found that during the rainy season the sporozoite rates for A.gambiae and A.funestus were 3.7 % and 2.3 % respectively. These values fell to approximately 0.007 % for both species during the dry season. Table 2.5

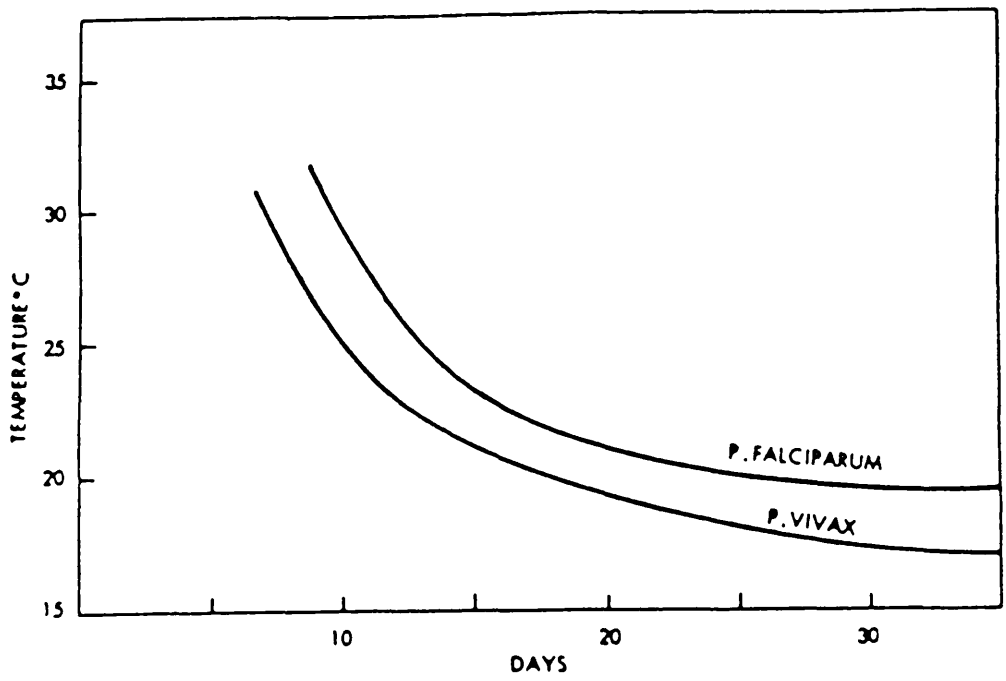


Fig. 2.2. The duration of sporogonic (extrinsic) development of malaria parasites in Anopheles in relation to environmental temperature (Macdonald,1957).

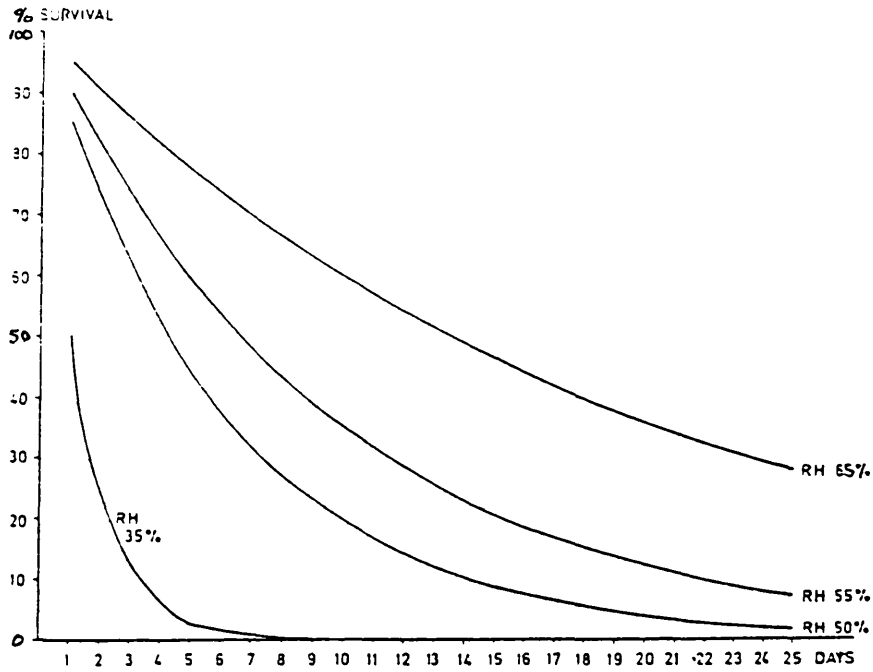
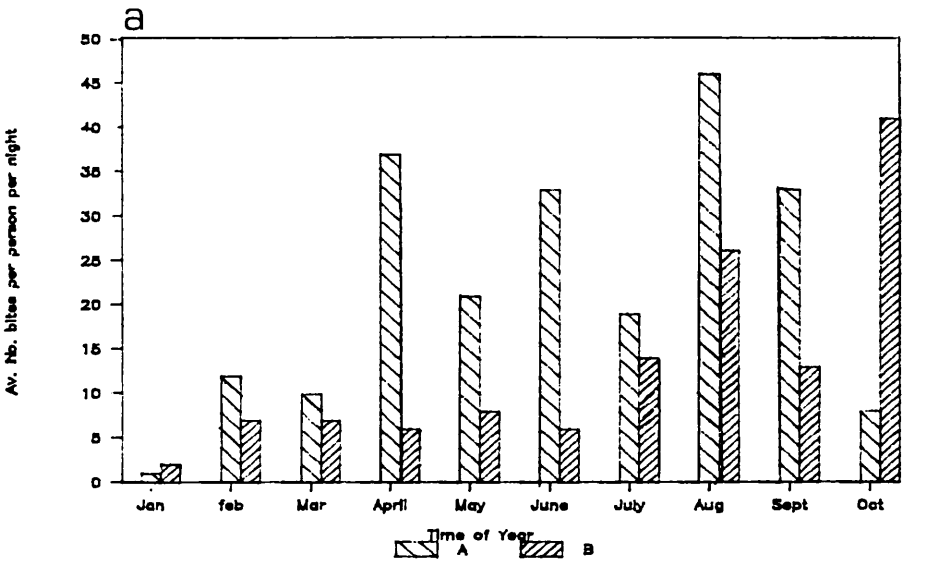


Fig. 2.3. The survival of A.gambiae in the Central Nile River Basin at various levels of relative humidity (Wernsdorfer & Wernsdorfer,1967).

further illustrates the effect of seasonal influences on the expected life-span and the average mortality rates of principle African vectors.

Temperature and rainfall also influences mosquito activity and regulates the rate of mosquito breeding. Temperature dependence of the aquatic stage of development is a feature common to all species of mosquitoes, although the minimum and maximum thresholds differ widely between the species according to the adaptive features of the particular species. Higher temperatures have been shown to shorten the duration of the gonotrophic cycle, thereby increasing the number of ovipositions made by the female and the potential for transmission of malaria through more frequent biting (Molineaux & Gramiccia,1980). Rainfall influences malaria transmission in many ways. For example, evaporation of water will tend to create higher relative humidities, thus prolonging the life span of the vector. The biggest effect however is on the breeding activity of the vectors. The effects are quite diverse and are critically dependent on the requirements of the aquatic larval stage and also the "degree of wetness". A.culicifacies, the major malaria vector of the Indian sub-continent, requires small open stretches of water to breed. Heavy monsoons raise the sub-soil water level and provide ideal breeding conditions, allowing the mosquito population to increase explosively (Christophers,1911). Conversely, deficient monsoon rains in Sri Lanka can transform rivers into pools and thereby create the ideal breeding conditions for A.culicifacies. Rainfall can also have a negative effect on malaria transmission. Excessive rainfall can wash away larvae or pupae of stream-breeding mosquitoes or leave them stranded on land when the water recedes. The spacing of rainfall is often more important than the quantity of rain. Heavy rains will damage most types of breeding-sites and be detrimental to the mosquito population. Lighter rains distributed over the month or year will usually tend to promote breeding. Figure 2.4 illustrates the massive "explosion" in the numbers of feeding mosquitoes, that is typical of areas with highly seasonal transmission. Temperature, humidity and rainfall are the three important determinants of the amount of transmission in a locality and also its periodicity and stability. If conditions are favourable during only part of the year transmission becomes seasonal. If they are favourable throughout the year transmission is perennial.


a
Av. No. bites per person per night
50
45
40
35
30
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20
15
10
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0
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feb
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June
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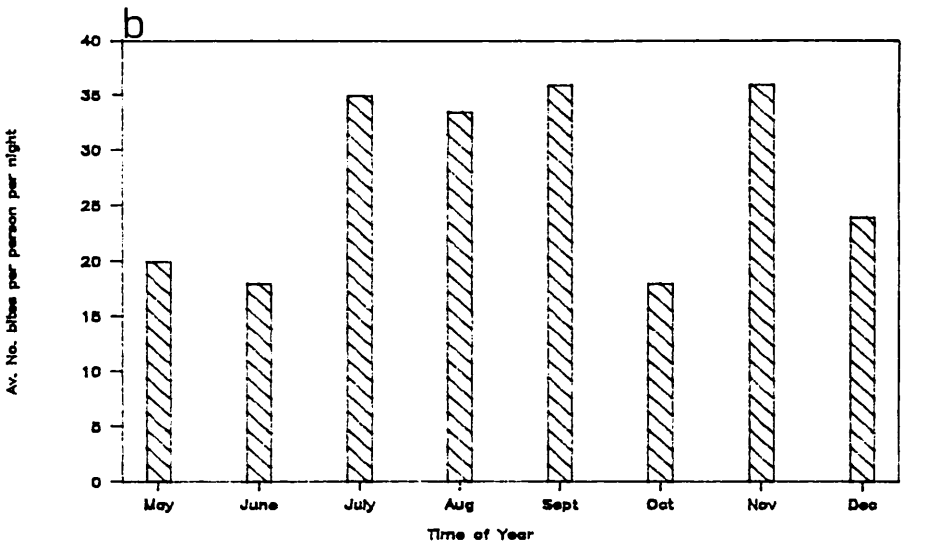
Time of Year
A
B


b
Av. No. bites per person per night
40
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30
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0
May
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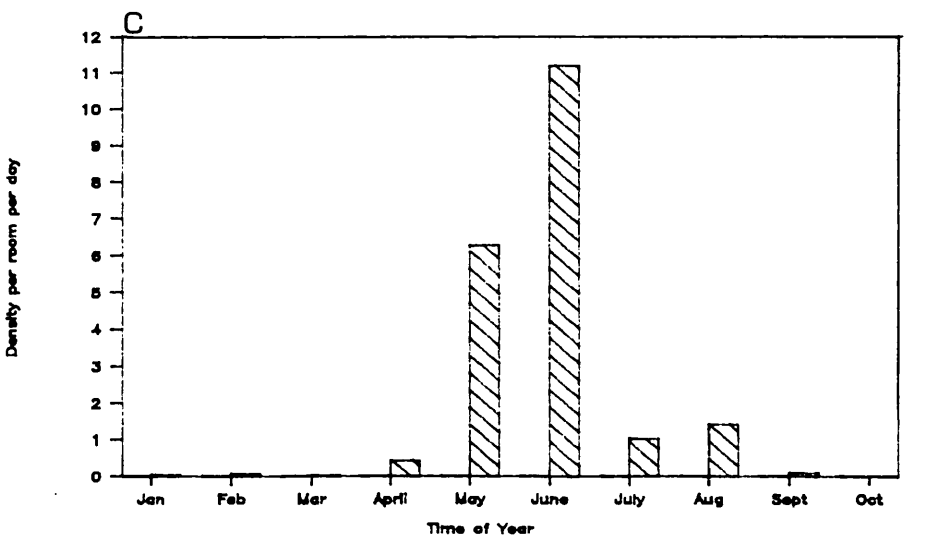
Time of Year


c
Density per room per day
12
11
10
9
8
7
6
5
4
3
2
1
0
Jan
Feb
Mar
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May
June
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Time of Year

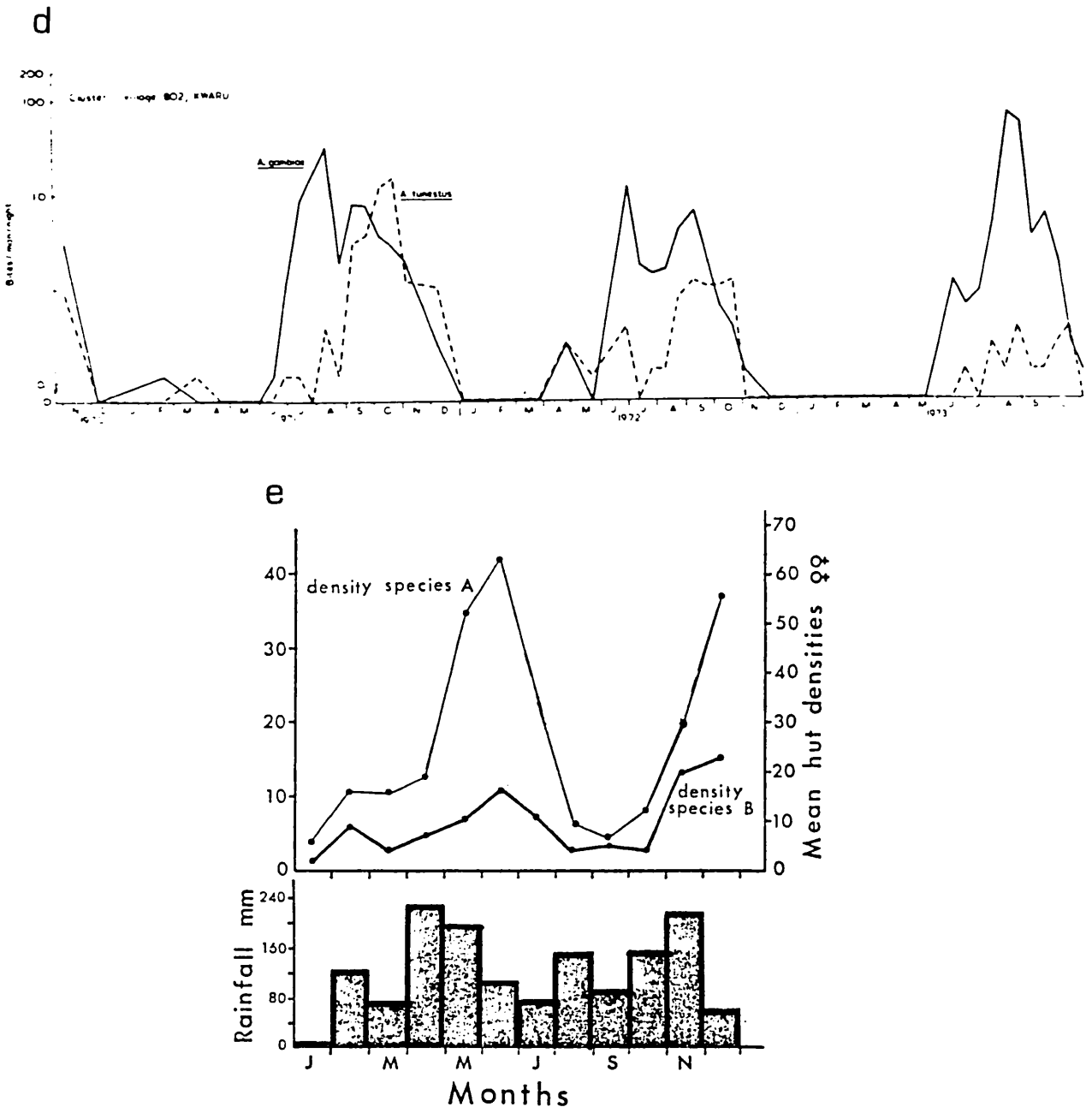


Fig. 2.4. Seasonal variations in vector densities (a) Estimated man-biting rates (A.gambiae and A.funestus) in degraded forest (A) normal forest (B), Togo, W.Africa (Bakri & Noguer,1977), (b) Estimated man-biting rates (A.gambiae (Sp.B)), Kankiya, N.Nigeria (Garrett-Jones & Shidrawi,1969), (c) Estimated frequency of A.gambiae according to room-densities, Brookfields - Freetown, Sierra Leone (Walton,1949), (d) Man-biting rates (A.gambiae and A.funestus) estimated from night-bite collections in Kwaru, N.Nigeria (Molineaux & Gramiccia,1980), (e) Mean hut densities of A.gambiae(Sp.A) and A.gambiae(Sp.B) in village huts, Kisuma, Kenya (Joshi, Service & Pradhan,1975) (Rainfall shown in the histogram recorded at nearby Kisumu airport).

Environmental factors, also include several human activities which may be conducive to the dispersal or increase of both vectors and/or gametocyte carriers in a locality, such as the building of canal, irrigation networks or dams or migration/movement of non-immune people or gametocyte carriers. Social and economic factors such as sanitation, housing, occupation, poverty etc., also have important effects since malaria is considerably more prevalent in underdeveloped countries.

2.2 Patterns of infection in human communities

Epidemiological studies of malarial infections in human populations are based on two quantitative measures of the abundance and occurrence of parasites, namely the prevalence and the intensity of infection. Both measures can be directly obtained by examination of bloodsmears. Other indirect measures of infection, such as immunological assays for the presence or absence of parasite antigens or specific parasite antibodies are of restricted use for diagnosis in endemic areas. The serological patterns produced in non-immune individuals are very consistent. A rapid rise in antibody within a few days of infection, constant high levels for maintained for a few weeks followed by a steady decline to lower levels which may persist for years (Kuvin et al,1962; Collins et al,1964a) are typical patterns. Reinoculation, relapses or recrudescences usually lead to rapid increases in antibody levels. In endemic areas serological patterns are less precise. Most infants are born with high antibody levels. These rapidly decrease to a trough at about 6 months after which seropositivity rises very steeply from birth, and antibody levels continue to increase steadily over many years (Voller & Bray,1962; McGregor et al,1965). The majority of people are found to be seropositive and high antibody levels do not appear to correlate with current parasitaemia. Parasite antibody persists after patent parasitaemia has terminated. Serological techniques can be used to establish malarial endemicity (indicated by the speed at which seroposivity and antibody levels rise with age), and age-related serological data has been interpreted mathematically to estimate malaria transmission rates (Draper et al,1972; Van der Kaay,1975). Serology can also be used to assess changes in transmission during or after control (Bruce-Chwatt et al,1975; Cornille-Brögger et al,1978), and identify malaria foci (Warren et al,1975). Ambroise-Thomas et al(1976) mention that serology is of particular use in areas where parasite rates fall to low levels and positive slides may well be missed. The "period prevalence" data generated by serological study also has the advantage that it is less

affected by small climatic fluctuations which can lead to over- or under-estimation of endemicity by parasitological prevalences taken at single time points. Examples of the longitudinal use of serology in malaria epidemiology (before, during and after control) are found in the studies by Cornille-Brögger et al(1978) and Molineaux et al(1978a). It must be stressed that at present serology is used to supplement parasitological data. The quantitative value and sensitivity of such techniques are uncertain at present.

Two further determinants of infection, mortality and morbidity are also of importance in assessing the pattern of infection in human communities. It has been recognised that measurement of the occurrence and abundance of parasites are of importance in determining the reservoir of infection in the human population. They are therefore a measure of transmission potential. However, patent parasitaemia is frequently asymptomatic, so that the diagnosis of "disease" in terms of mortality and morbidity is the only sure way to measure the disease incidence, prevalence or severity in any given community.

2.2 (i) Age-related changes in prevalence and intensity of infection

The majority of published studies have focussed on cross-sectional age-related trends, although a number of more detailed studies have involved investigating the changes in prevalence and intensity of infection within wet and dry seasons. The first pattern to note is that, (except when transmission is very low), age-related changes in both prevalence and intensity are convex in form. Prevalence and intensity tends to rise in childhood, reach a peak in children or adolescents and decline thereafter. This point is illustrated by figs. B.1 and B.2 in Appendix B, where prevalence and intensity respectively are recorded from a wide range of studies of predominantly P.falciparum infections. In addition to the convexity of the profiles a number of other quite striking general patterns also exist. The decline in intensity with age seems to occur at an earlier age and proceed much more rapidly than that of prevalence (fig. B.2). Convexity, especially that of prevalence also seems to be highly dependent on the intensity of transmission in a locality. In holoendemic areas, where transmission is intense, the prevalence rises sharply (figs. B.1 (a), (b) and (c)), peaks at an early age and then declines quite rapidly with increasing age. Seasonal changes to the level of transmission in hyperendemic areas cause the prevalences among children to remain high for longer, although the decline in prevalence among adults is still

pronounced (figs. B.1 (d), (e) and (f)). When transmission is less intense (mesoendemic) the rises and falls in prevalence are less marked, and the maximum prevalence is both lower and also occurs in an older age group (figs. B.1 (g), (h) and (i)), the parasite-intensity profiles however are still convex. For very low intensities of transmission the prevalence rate simply rises monotonically with age (fig. B.1 (j)), (the parasite-intensity profiles however are still convex).

Gametocyte rates, of both prevalence (Fig. B.1, lower profiles) and intensity (figs. B.2 (a), lower profile and (b)), show similar patterns to that of the total prevalence (fig. B.1, upper profiles) and intensity of infection (Figs. B.2 (a), upper profile, (c) and (d)). The general trend is that gametocyte indices decline much more rapidly with age than those of total parasite indices.

2.2 (ii) Age-related changes in morbidity and mortality rates

Although the debilitating and often fatal effects of malarial infection are well known, exact information concerning the contribution of malaria to mortality and morbidity is limited and frequently questionable. Records of date of birth are frequently poor in rural African areas and the exact cause of death is often difficult to diagnose, particularly among individuals "semi-immune" to malaria. Diagnosis is often subject to individual interpretation by medical officers. The majority of data concerning disease-related mortality and morbidity is obtained by selective sampling in hospitals and clinics. Information may also be confused by the inclusion of data from both indigenous and migrant populations.

A few examples of well recorded deaths by age and cause do however exist (Bruce-Chwatt,1952b; Colbourne & Edington,1954; Giglioli,1972; Marsh, unpublished). Mortality and morbidity varies considerably with the intensity of transmission, the time of year (see section 2.2 (iii)) and the age-group. A similar, if less clear cut, pattern to those described above for prevalence and intensity emerge for parasite-induced mortality and morbidity. Generally speaking, mortality rates in areas subject to stable and intense transmission decline much more rapidly with age than that of either prevalence or intensity of infection. Death caused by malarial infection is rare after the age of 5 years (figs. B.3 (a), (b) and (c)). When transmission is low or unstable, malaria-related mortality may still occur among adults, albeit at lower levels (fig. B.3 (d)).

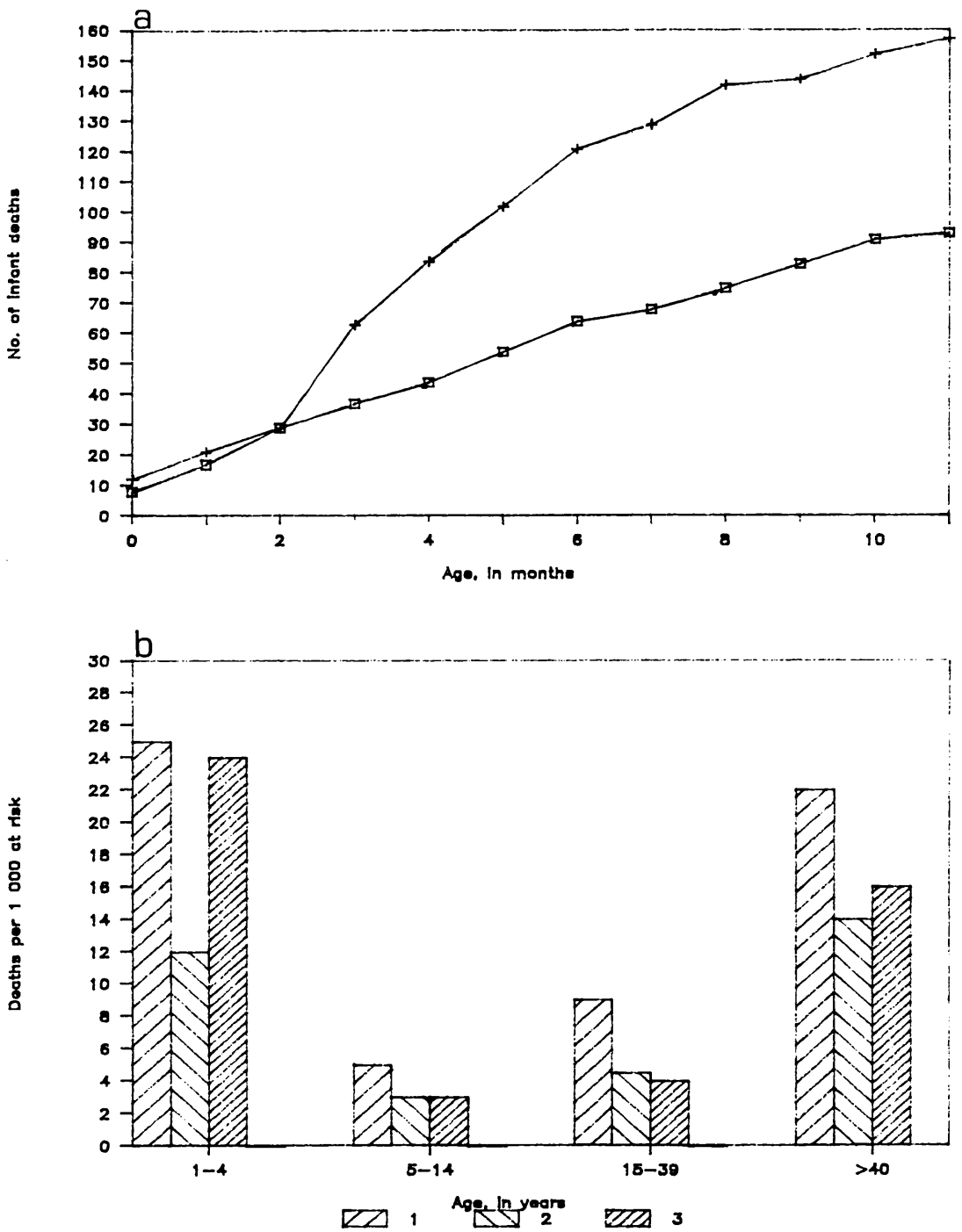


Fig. 2.5.
(a) Cumulative infant deaths (by age) in theoretical cohorts of 1 000 live births (+untreated area, □ treated area), exposed to intense transmission of P.falciparum (Payne et al,1976), (b) The changes in age-specific death rates per 1 000 at risk in Pare-Taveta, Tanzania during the three phases of a control programme (Pringle,1969). (1) At the beginning of intervention, (2) during intervention, (3) after intervention had ceased.

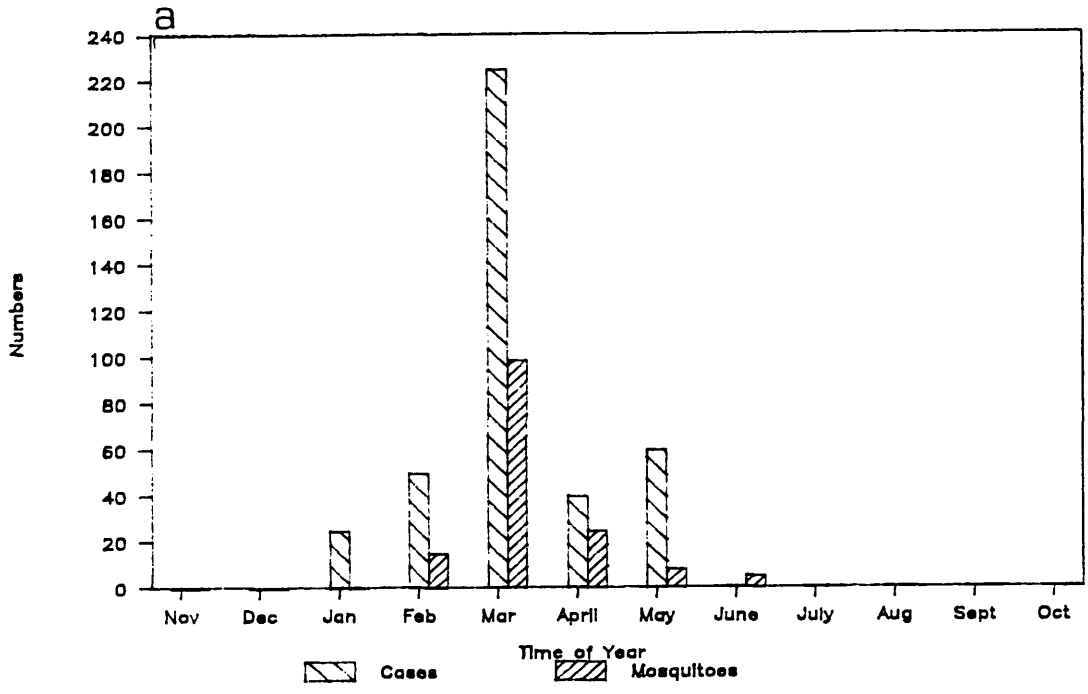
Further information concerning malaria-related mortality is available from other sources, (i) the pattern of mortality for unstable areas and during epidemics (Newman,1977; Giglioli,1972), (ii) the decrease in mortality during control (Fig. 2.5 (a) - Payne et al,1976; Fig. 2.5 (b) - Pringle,1969). The impact of malaria on mortality from malaria control programmes is however difficult to assess, since "indirect" effects on mortality of control may be 2-4 times greater than the "direct" effect on malaria-induced mortality. Giglioli(1972) recorded significant decreases in the number of deaths from acute and chronic respiratory disease following the implementation of malaria control. Removal of risk of malaria can however also allow deaths to occur from other causes. Infant mortality in the Garki region of Northern Nigeria caused by malaria was very high, but control appeared to result in a very minor reduction to the total number of deaths among infants (Molineaux & Gramiccia,1980).

Morbidity rates in highly endemic areas decline less rapidly than mortality rates, although still more rapidly than prevalence or intensity of infection (figs. B.4 (a) and (b)). It would appear that some sort of tolerance to parasite densities and the "anti-toxic" effects of parasite infestation develops quite rapidly with age under these conditions. It would appear that "anti-parasitic" effects, affecting parasite prevalence and intensity develop considerably more slowly. When transmission is less intense, morbidity rates still decline with age, although adults may suffer a significant number of febrile episodes during the course of the year (figs. B.4 (c) and (d)).

2.2 (iii) Additional infection patterns

Superimposed on the general patterns described above are the effects of seasonal epidemics. Epidemics often occur more or less annually, indicated by generally elevated parasite rates, case notifications and mortality rates (as illustrated by fig. 2.6). These changes correspond to climatic conditions which effect mosquito populations and their ability to transmit disease.

One further pattern of note is that of cumulative prevalence. Longitudinal surveys of malarial infection frequently reveal that individuals irrespective of age normally acquire at least one patent infection per year (fig. 2.7 (a)). This fact is particularly striking amongst adults where the prevalence at a single point in time may be as low as 20-30 %. By way of illustration fig. 2.7 (b) compares the total prevalences obtained from a single examination as compared to the rates when examinations are repeated at weekly intervals for various periods.


a
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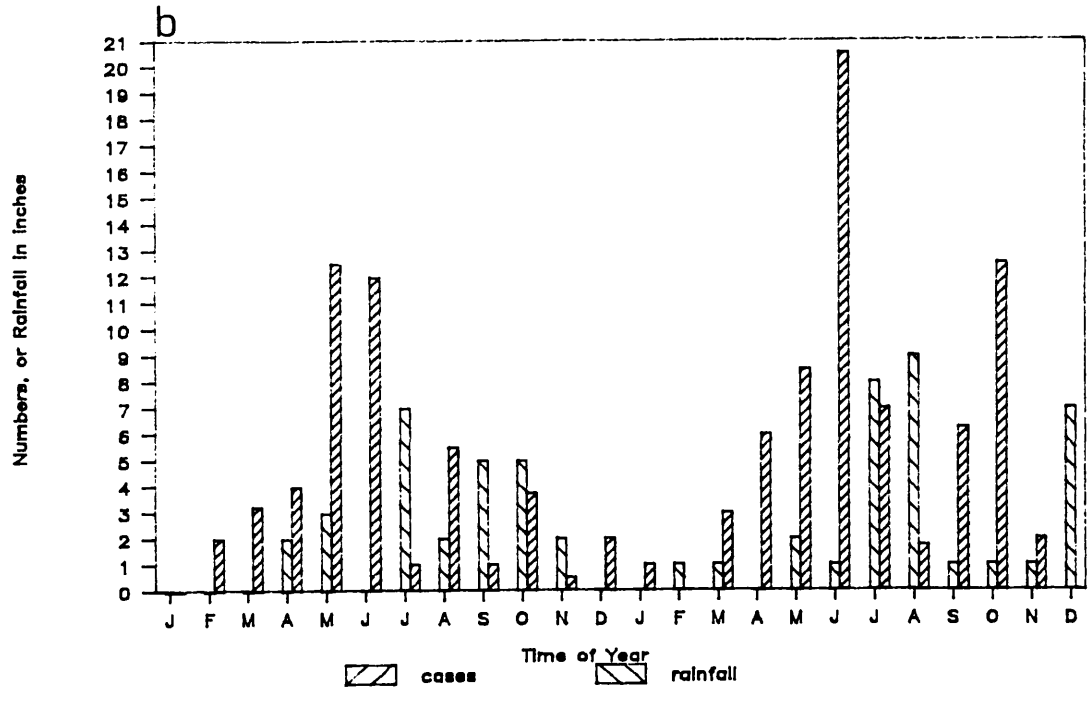
Time of Year
Cases
Mosquitoes


b
Numbers, or Rainfall in inches
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Time of Year
cases
rainfall

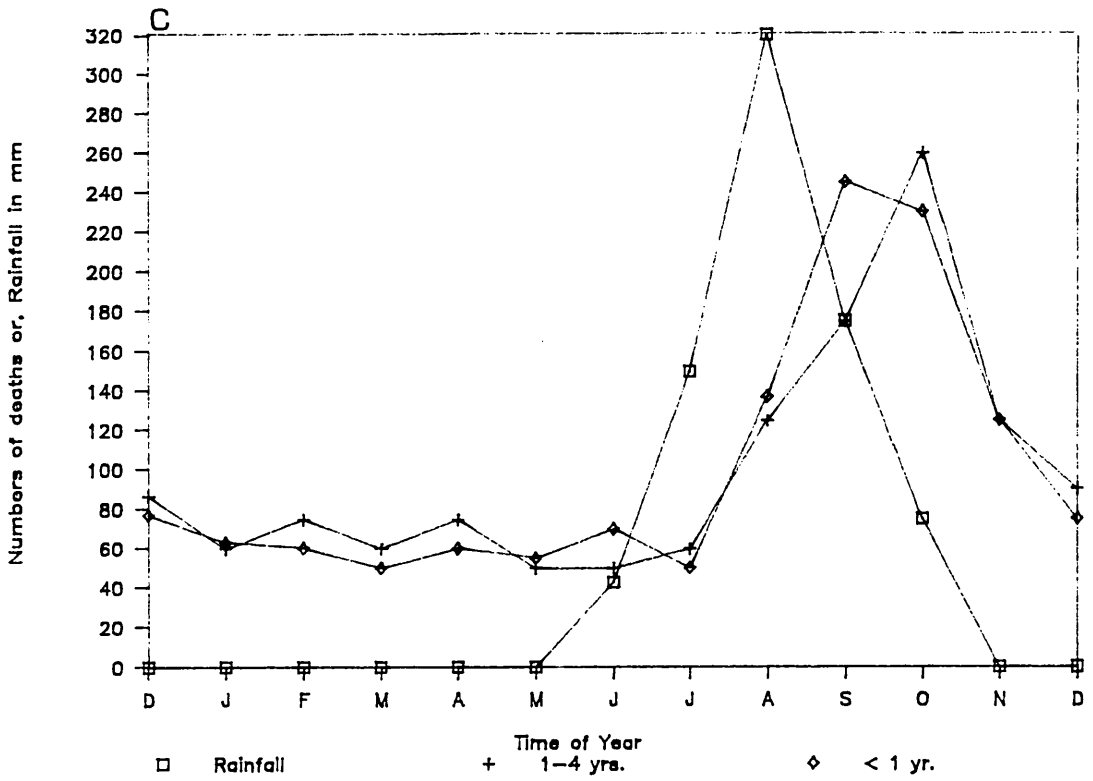


Fig. 2.6.
(a) Comparison of malaria notifications from rural clinics and hospitals in Zimbabwe, with mosquito captures of A.gambiae c.c. (Mpofu,1985), (b) Rainfall (in inches) compared to malaria case notification, Lagos, Nigeria (Smith,1943), (c) Seasonal rainfall (in mm) and mortality, Senegal-Niakhar (Carnevale & Vaugelade,1987).

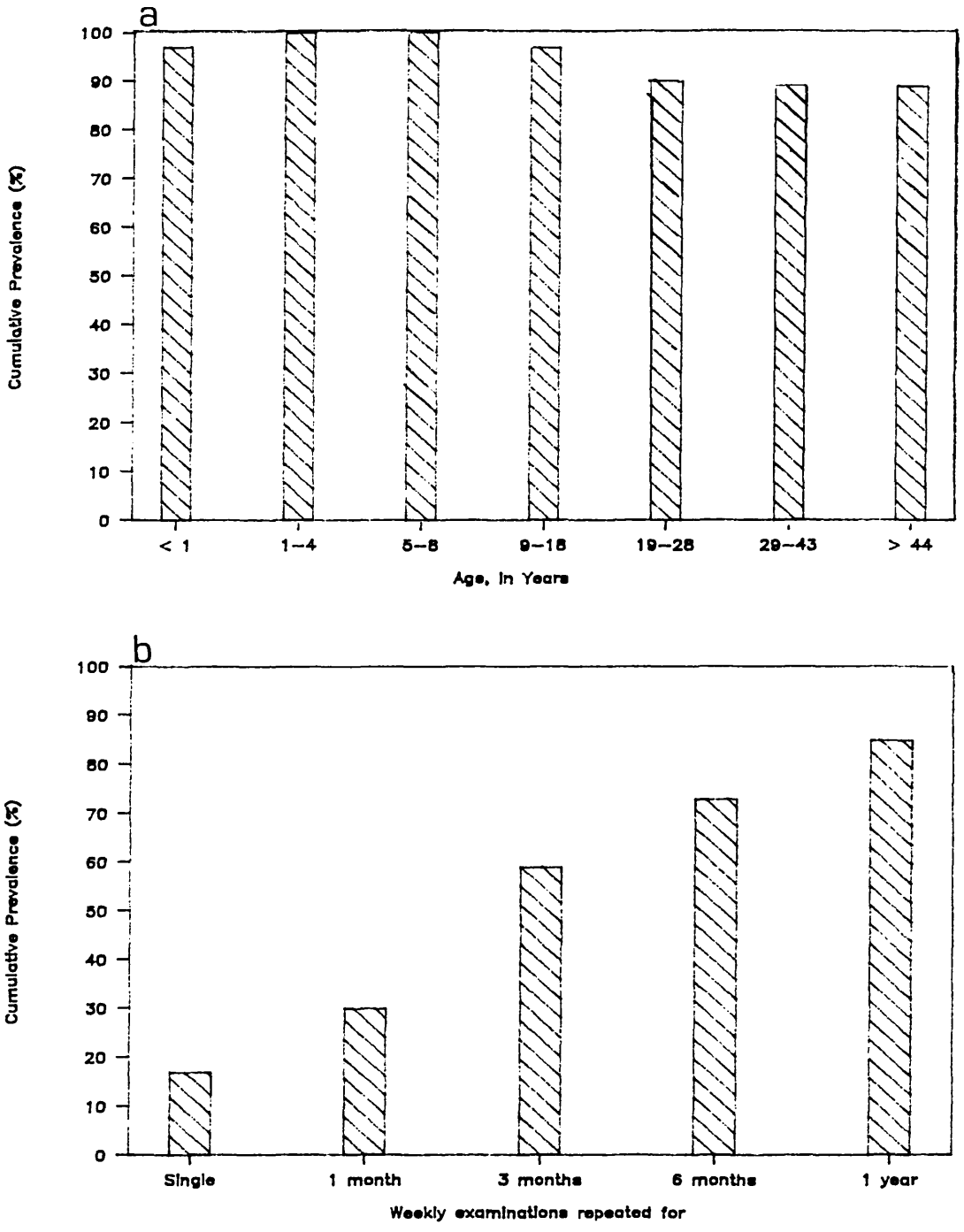


Fig. 2.7.
(a) Cumulative prevalence of P.falciparum over 8 surveys conducted at intervals of 10 weeks for 18 months (Molineaux & Gramiccia,1980), (b) Longitudinal survey of malaria infection in African adults. Parasite prevalence rates from a single examination, compared with cumulative rates when examinations are repeated weekly for periods of 1 month (4 examinations), 3 months (12 examinations), 6 months (24 examinations) and 1 year (52 examinations) (Bruce-Chwatt,1963a).

2.3 Infection in the vector population

2.3 (i) Vector "fitness"

Any adverse effect that vectors may suffer as a direct result of a malarial infection are usually considered to have a negligible effect on the overall dynamics of transmission. However, there is a growing body of literature and some convincing evidence (Molyneux & Jefferies,1986) that may well suggest that infection can have an adverse affect on the overall "fitness" of the vector. The inclusion of this effect can bring about quite major modifications to estimates of their survival, behaviour, flight performance and reproductive capacity. Considered in conjunction with the limited longevity of uninfected vectors in comparison with extrinsic development times and the observation that earlier crops of sporozoites are significantly less viable than older sporozoites (Nussenzweig & Chen,1974) a decrease in either the longevity or the ability to locate and feed on hosts, of infected mosquitoes may have a significant effect on transmission potential. Conversely, multiple feeding or increased probing by infected mosquitoes could enhance transmission.

Pathological effects have been demonstrated during several phases of parasite development in the vector. For example, penetration of midgut epithelial cells is thought to contribute significantly to the high mortality in C.quinquefasciatus infected with P.cathemerium (Maier (1973) cited in Maier et al(1987)), and in A.stephensi infected with P.berghei (Gad et al,1979; cited in Maier et al, 1987). Klein et al(1982) showed that the mean longevities of A.dirus infected with P.cynomolgi were significantly less ($p < 0.01$) than non-infected mosquitoes. The differences in mortality rates was most significant during the period 9-30 days post infection, the period 9-20 days post infection corresponds with the time when the oocysts rupture and the sporozoites migrate and penetrate the salivary glands. After day 20 it is thought that the difference in mortality is due to indirect effects such as increased susceptibility to bacterial and fungal infections. Maier et al(1987) also clearly shows that cumulative mortality is significantly increased in A.stephensi if the blood meal contains P.berghei (cumulative mortality at 20 days post blood meal: 10 % (uninfected), 15 % (infected)). The effect is considerably increased if the bacteria Serratia marcescens is also present, cumulative mortality rising to 37 %. Klein et al(1982) also reported that particularly heavily infected mosquitoes often possessed discoloured and partially decomposed salivary glands harboring numerous bacteria. A number of early workers (Buxton,1935; Sinton & Shute,1938;

DeBuck & Swellengrebel,1935) also suggested that Plasmodium parasites have a detrimental effect on the longevity of mosquitoes. However, other workers (Boyd,1940; Ragab,1958) concluded that there is no detrimental effect.

The developing parasites have also been shown to compete for nutrients, and several significant effects have been observed. Changes in mosquito metabolism may well influence ovarian development since reductions to the number of eggs in infected mosquitoes has been observed (Hacker,1971; Hacker & Kilama,1974; Freier & Freidman,1976). Mosquito fecundity is known to be related to the quality and quantity of blood in the blood meal (Woke,1937; Bennett,1970; Colless & Chellapah,1960), so that reduced fecundity may be related to changes in the quality of the vertebrate blood during infection. Conversely, the malaria parasite may exert its effect directly on the mosquito host by competition for nutrients. Hacker(1971) also demonstrated a differential effect among several genotypes of mosquito. This issue must be considered in terms of the potential impact of malaria on the genetic structure of the mosquito population. Further influences of infection are, reduced flight capabilites in terms of both distance and speed,a reduction in body weight was also observed (Schiefer et al,1977). Reductions in flight capabilities were found to be positively correlated to the severity of plasmodial infection. Reduction of flight ranges may have an important and significant effect on the spread of infection and in restricting infection to foci. Sporozoite migration has also been observed to effect mosquito mortality, penetration of the salivary glands by the sporozoites has been shown to deform and swell infected cells (Oelerich(1967) cited in Maier et al(1987)). An increase in mortality at this stage has been recorded in some but not all vector-parasite combinations (Rossignol et al,1986; Klein et al,1982). Sporozoites in the salivary glands of Ae.aegypti infected with P.gallinaceum have also been shown to reduce the amount of salivary apyrase - an enzyme secreted in the saliva during feeding which inhibits platelet aggregation in the hosts blood (Rossignol et al,1984). Mosquitoes deficient in salivary apyrase are seen to experience difficulty in locating host blood and engorging, probe more often and attempt to feed on several hosts in succession (Rossignol et al,1986). This type of effect could significantly increase the likelihood of sporozoite transmission to multiple hosts.

2.3 (ii) Observed sporozoite rates

The prevalence of vector infections (sporozoite rate) is characteristically low in areas where malaria is endemic. The epidemiological data presented in table 2.6 clearly illustrates this point. Sporozoite rates do show a great deal

Table 2.6

Estimated sporozoite rates recorded for several major vector species in various African and Asian countries

Location	Sporozoite Rate (%)	Species
EAST AFRICA		
Ethiopian Highlands (Rishikesh,1966)		
Awasa	0.11	A.gambiae
Adamitulu	0.31	A.gambiae
(yearly averages)		
Gambela, Ethiopia (Krafsur,1977)		
July-Feb	1.87	A.gambiae
Nov-Dec	2.4-2.6	A.gambiae
Feb-March	0.49-0.67	A.gambiae
July-feb	1.23	A.funestus
Busoga, Uganda (Onori & Benthein,1969)		
Feb-Nov	0.5-2.2	A.gambiae
Dec-Jan	0	A.gambiae
Yearly range	0.2-1.3	A.funestus
Bwamba County, Uganda (White,1973)		
May	1.5	A.gambiae
	2.3	A.funestus
Kisumu, Kenya (Joshi, Service & Pradhan,1975)		
May (i)	4.9+/-0.3	A.gambiae
(ii)	5.4+/-0.2	A.gambiae
Segera, Tanzania (White, Magayuka & Boreham,1972)		
March-April	4.23+/-0.71	A.gambiae (Sp.A)
	0.32+/-0.16	A.gambiae (Sp.B)
	1.62+/-0.28	A.funestus
EQUATORIAL AFRICA		
Djoumouona, Congo (Carnevale et al,1978a)		
Yearly average	5.33	A.gambiae

Location	Sporozoite Rate (%)	Species
EQUATORIAL AFRICA		
"PK Rouge", Congo (Carnevale et al,1982b)		
April-June	1.51	A.gambiae
Nov	1.16	A.gambiae
Jan	1.81	A.gambiae
Feb	0	A.gambiae
May-Oct	0	A.gambiae
Zaire (Vincke,1965)		
Sept-Nov	0.58	A.gambiae
WEST AFRICA		
Kankiya, Nigeria (Foll & Pant,1965)		
Dry Savanna (Wet)	3	A.gambiae
	0.48	A.funestus
(dry)	1.72	A.funestus
	1.58	A.funestus
Garki, Nigeria (Molineaux & Gramiccia,1980)		
Wet Season (indoor catch)	3.0	A.gambiae
	1.7	A.funestus
(outdoor catch)	1.9	A.gambiae
	0	A.funestus
Kpetoe, Ghana (Bakri,1968)		
Dry	4.5	A.gambiae
	11.5	A.funestus
Wet	3.0	A.gambiae
	1.86	A.funestus
ASIA		
India (Clark & Choudhury,1941)	3.4	A.balabacensis
W.Malaysia (Cheong,1968, cited in Slooff & Verdrager,1972)	3.0	A.balabacensis
Thailand (Scanlon & Sandhinand,1965)	6.7-8.7	A.balabacensis
Papua New Guinea (Burkot et al,1987)	0.1-1.62	A.farauti A.punctulatus

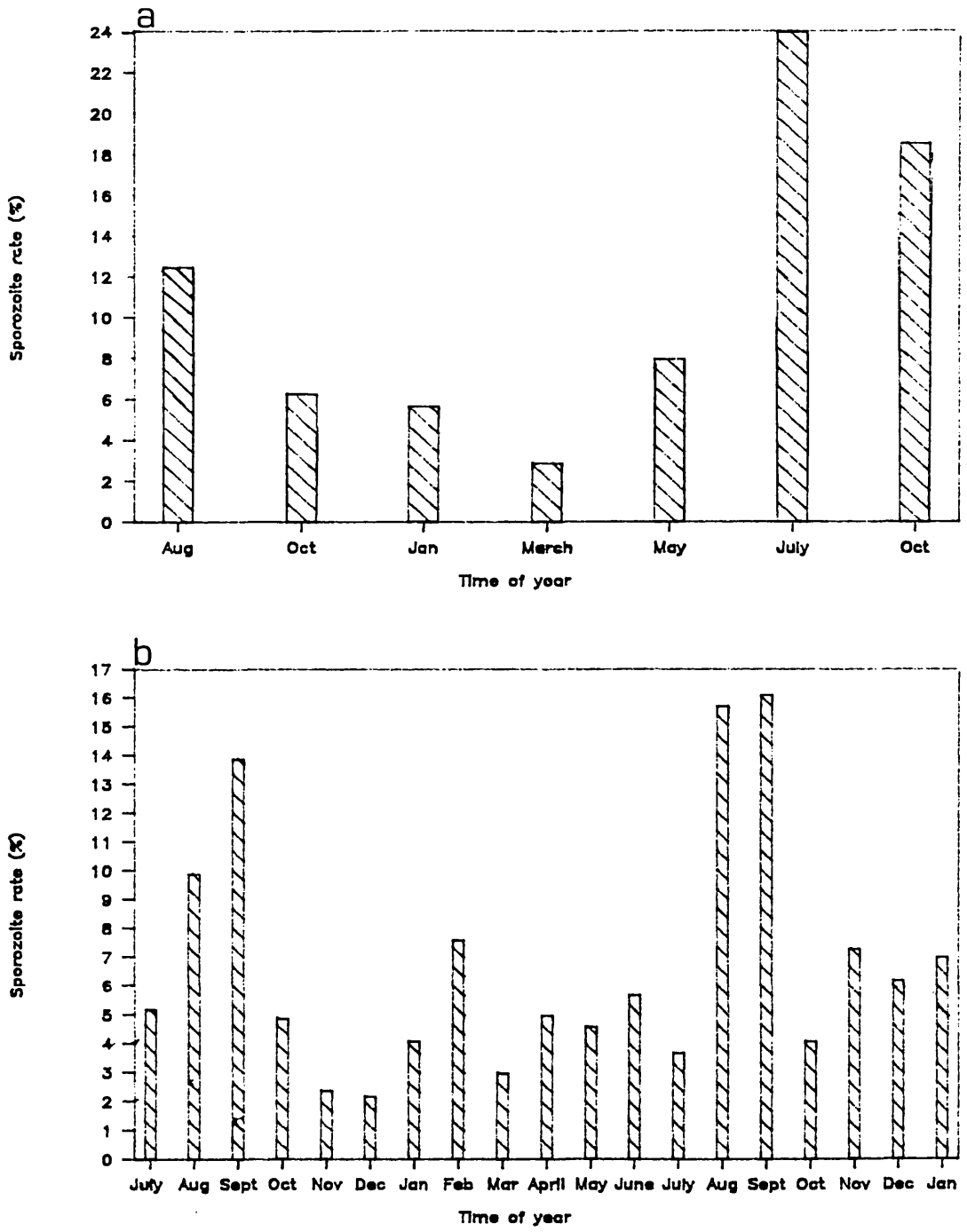


Fig. 2.8.
(a) Estimated sporozoite rates in A.gambiae for seven separate months in one locality (Wilson,1936 cited in Macdonald,1952a), (b) Estimated sporozoite rates in A.costalis in native houses in the coast region of Southern Nigeria by month (Barber & Olinger,1931).

of variation, the causes of which are many and varied. Variability in habitat suitability and the availability/magnitude of suitable sources of infection are likely to be of major importance. However, as already discussed climatic conditions can also have a marked effect. The climatic effects on both the extrinsic developmental of the parasite and the mortality of adult mosquitoes, sometimes produce quite significant variations to the sporozoite rates (fig. 2.8). Boyd (1949a, pp.634-637) summarizes data which shows that peak sporozoite rates occur approximately 2 months after the peak in mosquito numbers. Inter-specific variations in the HBI and mortality rates between vector species also will affect sporozoite rates and have a marked affect on the capability of a species to act as a vector. However it should be remembered that a low HBI index or high mortality rate does not necessarily produce a low sporozoite rate (table 2.7).

Table 2.7

Estimated HBI indices, mortality rates and sporozoite rates for two vector species in a survey of Cyprus (Barber et al,1936, cited in Macdonald,1952a).

Vector Sps.	HBI index	Mortality rate (/day)	Sporozoite rate (%)
A.sacharovi	0.826	0.223	1.8
A.elutus	0.187	0.005	7.8

Potent vectors generally have, in the transmission season, sporozoite rates higher that 1 %, however vector species with rates considerably lower than this but that exist at very high densities can also act as major vectors.

Studies by Gillies & Wilkes(1965) in Tanzania of sporozoite rates verses the physiological age of A.gambiae and A.funestus indicate that sporozoite rates rise more or less steadily with increasing age.

CHAPTER 3 A laboratory based animal model for superinfection

3.1 Introduction

Certain aspects of malarial infections in their vertebrate and mosquito hosts, and also inter-relationships between parasite and host populations, can be illuminated by the study of malarial infections in animal models. It is sometimes sensible and often practicable to extrapolate and apply knowledge gained from such laboratory systems to the problem of human malaria. However, many uncertainties surround the study of human malaria since a total of four separate species of malaria can infect man, of which the course of infection and the pathological and clinical effects of infection vary considerably between species. Additionally the course of infection may be affected by multiple-species infections. Despite the fact that no one animal model parallels exactly any of the human malarias some animal models do posses certain basic biological similarities which render them suitable for the study of some aspects of the human disease. A great deal of valuable information has been obtained from such models.

Epidemiological studies of human malaria suggest that in an endemic area a person might expect to be exposed to sporozoites through the bite of an infected mosquito many times during the course of a year. However, not every infected bite is capable of giving rise to a patent blood infection, since the inoculum may be too small, the sporozoites may be immature and therefore uninfective, or alternatively some sort of innate resistance may influence the development of exo-erythrocytic or asexual blood forms and prevent patency (Miller and Carter,1976). During peak transmission in highly endemic areas an individual may receive an infective bite, (i.e. a bite with the potential to produce a patent infection), every 7-15 days, depending on their age (Molineaux et al,1978a).

The absence of complete immunity following a single and even repeated infection in humans is well documented; the cumulative prevalence for an individual over a period of one year is often as high as 90-100 %, even among the relatively "immune" adults, (see section 2.2 (iii)), so that there is no real reason to suppose there should be a mechanism whereby the presence of an older, established infection should automatically prevent the development of new malaria infections. Cohen(1973) has shown that humans frequently

harbour simultaneous infections by different malaria species, and even different strains of the same species have been found in an individual (Thaithong et al,1984; Rosario,1981). Further evidence to suggest that people continually exposed to infected mosquitoes are not immune to superinfection is that despite the rapid recovery rate among partially immune adults exposed to a single inoculum (Bruce-Chwatt,1963a), naturally occurring malarial infections persist for months after the initial infection, years in the case of children where the recovery rate is very much slower (Molineaux and Gramiccia,1980; Bekessy et al,1976; Krafsur and Armstrong,1978); this indicates that individuals have to recover from several infections before they finally become sub-patent. Similarly recovery rates among children show a tendency to be lower in areas of higher transmission where the potential for superinfection is greater (Segal et al,1974; Gazin et al,1988).

Many of the early mathematical models for malaria, although they took no account of other aspects of immunity, were concerned with superinfection and its effect on the recovery rate (see section 4.1 (ii) for a fuller account). A wide range of models exist to describe the effect of a subsequent infection when one is already present, one extreme is a model in which infections form a "queue" and express themselves only when the previous infection has run its course (Macdonald,1950a). Other models assume that infections arrive and run their course entirely independent of each other (Dietz, pp317-322 in Bailey,1975), or that superinfections become successively easier to shed (Aron and May,1982). Although this last model is probably the most realistic, it is unlikely that what actually happens is as simple as the assumptions on which this model is based. A major problem associated with improving superinfection models however, is that existing data can in fact do little more than indicate that superinfection actually exists. Although it may be difficult to study the exact mechanisms involved it should be possible to study in more detail the effect of superinfection on the dynamics of individual infections and thereby elucidate the effects of competition between existing and new parasite populations, and whether the outcome is dependent on the phase of the existing infection and indeed whether certain phases of infection render an individual refractory to further infection.

There are obvious limitations to studying superinfection in human communities, in addition to the ethical issues surrounding controlled infections and the withholding of chemotherapy. In particular natural superinfections cannot easily be controlled or monitored. Genetic variability among both parasites and humans would serve to amplify the effects

of these and any additional variables, thus reducing the clarity of the study and making interpretation of results difficult. Model systems however, especially those of the rodent malarias using isogenic hosts, have characteristic and reproducible patterns of infection. Parameters such as the pre-patent period, the growth of the parasite population, the day and level of peak parasitaemia and length of patency are remarkably consistent; hence not only could superinfections be strictly controlled but their effects on the dynamics of the parasite populations could be easily identified.

3.2 Experimental design of a simple model system for superinfection in naturally occurring human malaria

To maximise the validity of information extrapolated from controlled laboratory experiments using animal models it is desirable to design a system that will reproduce as far as possible the conditions and therefore the results that might be expected from naturally occurring human malaria infections. Three important aspects are,

(i) the choice of host species,
(ii) the choice of plasmodium species,
and, (iii) the infection conditions.

There are of course several other factors that also have to be considered, such as practical and costing constraints involved in the number of animals to be used and, equally important, an understanding of external factors that can influence the course of infection and thereby effect the standardization and reproducibility of the experiment. Concomitant infections, diet and several factors associated with the selection and maintenance of test animals, e.g. the sex, age and weight of test animals, experimental stress and housing conditions, have all been found to have an effect on the outcome of experiments using malaria (Kretschmar,1972).

3.2 (i) The host species

The possibilities for experimental infections of primates with human or simian malaria are extremely limited due to both the scarcity of suitable hosts and also the high costs involved. Murine malaria systems are easily the most practical models for the study of human malaria, a large range being readily adaptable to laboratory rodent species and transmission through laboratory reared mosquitoes. A wide range of genetically defined mice are available to investigate host-parasite relationships, they are cheap and easy to maintain, and the isogenic nature of the hosts minimises variability of results.

3.2 (ii) The plasmodium species

Murine malarias are analogous to the malarias of man in most essential aspects of structure, physiology, range of preference of red cell types and life-cycle; there are however a number of fundamental differences in the dynamics of infection. Despite the fact that the number of exo-erythrocytic merozoites produced by each schizont among human malarias are an order of magnitude greater than any of the rodent malarias (Cohen and Lambert,1982; Carter and Diggs,1977), the course of infection is generally much more rapid, and the parasitaemias much greater than those of human malaria, the duration of both exo-erythrocytic and erythrocytic cycles being considerably shorter in murine species. One further major difference is in the synchronicity of blood infections; parasites of the Berghei group (P.berghei and P.yoelii) are highly asynchronous in laboratory animals as compared to the synchronized cycles of the Vinkei group (P.vinkei and P.chabaudi), and the human malaria species P.vivax, P.ovale and P.malariae. P.falciparum is less well synchronized particularly in the early stages of infection.

Generally rodent infections follow a course similar to that of human malaria with an acute phase followed by a tendency to chronicity, some, e.g. P.chabaudi, even show periodic recrudescence similar to those of P.malariae and in certain cases P.falciparum. The exact course and outcome of infection can however (to a certain extent) be manipulated by the choice of parasite and mouse strain. Depending on the host/parasite combination used infections can range from uniformly lethal to mild, non-lethal infections where solid immunity is produced. The availability of cloned lines of the various rodent malaria species negates the possibility of inadvertently studying mixed infections, and thereby minimises the possibility of variations between infections.

3.2 (iii) Infection conditions

In order to reproduce as far as possible the conditions under which superinfection would occur in humans under natural conditions, two important points relating to normal laboratory infection procedures need to be considered, since they could have an important bearing on the outcome of the infections.

3.2 (iii) (a) Method of inducing the infection

Except in exceptional circumstances, involving the transfer of parasite blood-stages, e.g. during a blood transfusion from an infected donor, re-use of an infected hyperdermic needle or very occasionally during pregnancy (Walton,1948; Garnham,1949; Bruce-Chwatt,1952a), human malaria is acquired only by the introduction of sporozoites into the blood-stream by an infected mosquito bite. However, most experimental infections are induced by intravenous or intraperitoneal inoculation of blood-stages.

Extensive amounts of literature exist on work involving the factors that influence the course of blood-induced infections in rodent animal models, including a small amount of work on the effect of introducing further blood-stages during a current infection (McLean et al,1982). In comparison there has been very little work done involving sporozoite-induced infections.

Until recently it was thought that there was no immune response by the vertebrate to the sporozoites, so that work was concentrated almost exclusively on the blood-stages, the infections being blood-induced due to the difficulties involved in maintaining cyclic transmission in the laboratory. However, Nussenzweig et al(1972), Spitalny and Nussenzweig(1973) and Golenser et al(1978) have all recorded antibodies in rodents bitten by infected mosquitoes, and anti-sporozoite antibodies have also been detected in the sera of people living in endemic areas (Nardin et al,1979). Cell-mediated immune factors that protect hepatocytes and animals against sporozoite challenge have also been found (Schofield et al,1987; Ferreira et al,1986).

Almost all the existing work using sporozoites has been directed at immune responses, particularly those associated with protective immunity and vaccine development (Nussenzweig et al,1969; Vanderberg et al,1969; Nussenzweig et al,1972; Nussenzweig and Nussenzweig,1986). Reinfection has been investigated, but the experiments were either carried out with massive unrealistic sized doses, or else the infections were not monitored past the pre-erythrocytic stage (Verhave,1975). These sorts of experiments provide very little real information as to the effect that naturally occurring superinfections might have on existing infections, or conversely the effect that current infections have on the establishment of new infections

Ideally animal models used to provide any information about human malaria should be by sporozoite-induced infections; for experiments involving superinfection it could be particularly important since the early stages of infection may have a critical effect on the outcome. Blood-induced

infections are generally initiated by between 10^4 and 10^8 blood parasites, this results in an almost instant parasitaemia, any larger amounts would invariably result in death within 1-2 days. Each sporozoite however has the capacity to produce 1-4 X 10^3 blood parasites; this massive sudden influx of parasites into the blood at the end of the pre-erythrocytic cycle could have important effects on the course of infection or superinfection.

3.2 (iii) (b) Size of inoculum

Another important consideration is the size of the inoculum, since it has been shown that for a number of malarial species the size of the inoculum can have a critical effect on the duration and course of infection. In P.vivax infections it is known that the number of sporozoites in the inoculating dose influences the length of the pre-patent period, the duration and severity of attacks, the tendency to relapse and also the length of latent time to relapse (Craige et al,1947; Shute and Maryon,1968)). Similar effects on the pre-patent period have been found in P.berghei (Nussenzweig et al,1966b; Vanderberg et al,1968; Gregory and Peters,1970) and P.yoelii (Fink,1970).

Experimental sporozoite-induced infections are normally induced by intravenous inoculations of large numbers of sporozoites, typically 1 X 10^3 viable sporozoites (Spitalny and Nussenzweig,1973) for P.berghei in A/J mice and 5 X 10^3 for P.vinkei in A/J mice (Nussenzweig et al,1969), although some experimental infections have been induced via the direct biting of infected mosquitoes (Verhave,1975). Comparatively little is known about the number of sporozoites that a malaria-infected mosquito actually introduces into the vertebrate host during feeding although the available evidence suggests that experimental inocula are far too large. Verderberg(1977) found that the number of P.berghei sporozoites inoculated by Anopheles stephensi into rodent hosts was probably no more than several hundred (and could be a lot less). Fink(1968) concluded that between as little as 25 and 1000 sporozoites were inoculated by Culex pipiens into canaries. Vanderberg(1977) also indicated that mosquitoes had a mean of about 1 000 salivary gland sporozoites per mosquito, and that it appeared that mosquitoes retain the majority of their sporozoites after a single feed. Shute(1945) showed that A.maculipennis infected with P.falciparum could go through more than 15 consecutive feeds on rabbits without any significant decrease in the number of sporozoites found in the salivary glands.

Of course, one must be cautious about extending results achieved under controlled laboratory conditions to what might be assumed to occur with a parasite of humans under natural

conditions; there is however reason to believe that similar principles do occur in the two situations. It is thought that the injection of small numbers of sporozoites by mosquitoes is the norm, the number transmitted being due largely to the general feeding behaviour of mosquitoes and the anatomy of mosquito salivary glands and the associated ducts and rather than the species of plasmodia being transmitted (Vanderberg,1977).

3.3 Experimental superinfection of mice with Plasmodium y.yoelii, by small sporozoite doses

3.3 (i) Introduction

For this brief study, superinfection was studied in BALB/c mice infected with Plasmodium yoelii yoelii(17x). This particular murine malaria produces a highly reproducible and self-limiting infection in BALB/c mice and evidence suggests that the initial acute phase of patent infection is followed by a chronic phase of sub-patent parasitaemia with possible sequestration in the kidneys (Jayawardena et al,1977; Freeman and Parish,1981); persisting parasites are only detectible by blood sub-inoculations. Patent recrudescence similar to those seen in P.chabaudi have not been recorded for either blood or sporozoite induced infections (Barker,1971; Garnham,1966; Freeman and Parish,1981). This does not however detract from its potential use as a model for human malaria since a single clinical episode is also the norm for P.falciparum infections. Additionally, P.y.yoelii sporozoites have the same high levels of infectivity as P.falciparum which may indicate that the anti-sporozoite responses and effects of superinfection may be similar for the two species.

Infective doses of 250 sporozoites were chosen so that the size of the inocula would not only be realistic but would also be large enough to produce parasitaemias in most of the mice. Lower doses of P.y.yoelii sporozoites were found to have a tendency to produce a greater amount of variation between the mice the characteristic infection being produced in some, but not all of the mice.

3.3 (ii) Materials and methods

3.3 (ii) (a) Hosts

Experimental groups of ten, 10 week old , male, BALB/c mice were used throughout the experiments. The mice weighed approximately 20 grammes at 7 weeks, and were bred under

specific pathogen-free conditions. All control mice were of the same stock as the experimental groups, and both controls and experimental groups were maintained in an air-conditioned animal unit at a temperature of $20^{o}C$ +/- $2^{o}C$, water and food ad libitum, and a 12/12 light regime.

3.3 (ii) (b) The parasite

Plasmodium yoelii yoelii (17x) was used throughout the experiments. This particular strain was derived from a cloned line provided by Dr. D. Walliker, Institute of Animal Genetics, Edinburgh. The original isolate was maintained for some time by cyclic transmission at Silwood Park, Ascot, Berks., but has subsequently been moved to Imperial College, London. P.y.yoelii produces generally self-limiting infections in laboratory mice; in BALB/c mice the infection is not severe, although a mortality rate of between 1-10 % is often observed.

3.3 (ii) (c) Initial infection of the mice

Sporozoites were obtained from the salivary glands of infected Anopheles stephensi. After dissection the infected glands were maintained in cold tissue culture medium. The infected salivary glands were triturated and the released sporozoites counted in a hemocytometer. Each mouse received an intravenous inoculation containing 250 sporozoites in 0.1 ml of media.

3.3 (ii) (d) Superinfections

On each of days 2 and 15 after initial infection (day 0), ten of the infected mice and two naive control mice were infected intravenously with 250 sporozoites in an identical way to that described above for the initial infections. The infections were then monitored by daily blood smears.

3.3 (ii) (e) Parasitological examination of blood slides

The course of infection in an animal was followed by determining the % parasitized erythrocytes. Thin blood smears were prepared from tail blood, these were then air dried rapidly to prevent damage to the erythrocyte membranes, fixed in pure methanol and stained in 20 % Giemsa's stain in Giemsa buffer (BDH Giemsa's stain, Gurr's improved R66, in a buffer of 3g sodium hydrogen phosphate, 0.6g potassium hydrogen phosphate in one litre of distilled water).

The smears were examined at X 1 000 magnification, the number of parasitized red blood cells was counted in a minimum of 15 microscopic fields and was expressed as a percentage of the total number of red blood cells scanned, (each field contained approximately 500 red blood cells). Estimates of mean percent parasitaemia based on all ten mice in each group were then calculated.

In order to investigate the distribution of parasites within the red blood cells and determine whether any changes in the spatial distribution of parasites within the host cells occurs either during the course of initial infection or following an experimental superinfection, the number of parasites within each infected erythrocyte was also recorded.

Thick blood smears were used to detect the first appearance of parasites in the peripheral blood when parasite levels are too low to be detected easily using the thin smear method. The smears were dried slowly in dry air, and then stained in the 20 % Giemsa's solution, without being fixed in methanol first.

3.3 (ii) (f) Presentation and analysis of results

Data is presented as % infected red blood cells, as determined by tail blood smears. The small group sizes, imposed by practical considerations, makes estimation of the variance within the group and the distribution of counts difficult. Thus statistical significance of differences in parasitaemia was analyzed by non-parametric methods, using the Sign Test to assess overall differences in mean parasitaemia values occurring during the rising and resolution phases (Brown et al,1976) and the Mann-Whitney test (Elliott,1979) to assess values on individual days. Probability values of 0.05 or less were taken as significant.

The effects of superinfection on patent parasitaemia were assessed by several key parameters. These were,

(i) the time taken to reach a pre-determined level of parasitaemia of 1 % (Warhurst and Folwell,1968),

(ii) the growth rate of the parasite.

(iii) the level and day on which peak parasitaemia occurred,

(iv) the length of patency,

(v) the occurrence of recrudescence,

3.3 (iii) Results

The % parasitaemias for the control and experimental groups recorded during the observation period are given in Appendix C.

3.3 (iii) (a) Course of initial infection - control group

The normal and characteristic pattern of infection by P.y.yoelii(17x) in BALB/c mice can be seen clearly in the control mice, (fig. 3.1). After intravenous inoculation of sporozoites, parasites could be detected in the peripheral blood, by thick blood film, by day 3, and the 1 % parasitaemia level was estimated to occur at 5.5 days post-infection. Thereafter the parasitaemia increased steadily, peaking at day 12 in all but one of the mice, at a value of between 27 % and 30 %, with a mean of 28.44 % . Immediately after this peak there was a rapid regression of parasitaemia in all the mice and parasites had disappeared from the peripheral blood by day 18 in all but one of the control mice, which eventually became aparasitaemic twenty days after initial infection. Similar patterns of infection for P.yoelii (17X) and BALB/c mice have been recorded by Freeman & Holder(1983) and Freeman & Parrish(1981).

All mice were monitored for a further 14 days but no recrudescence were seen. None of the control mice died as a result of infection with P.y.yoelii.

3.3 (iii) (b) Superinfection during pre-patency - group A_1

Group A_1 were superinfected on day 2 of the initial infection. The 1 % parasitaemia level was estimated to occur approximately 0.5 days earlier than in the control infection, which would indicate an approximate increase in exo-erythrocytic forms of 25 % compared to the controls. However, although the mean parasitaemias between days 5 and 12 were consistently higher than those of the control group, (fig. 3.2 (a)), the difference was found not to be significant at the $p = 0.05$ level. Peak parasitaemia on day 12, occurred on the same day as the control group, but the levels of parasitaemia among the experimental mice were significantly greater than the controls, with a mean of 31.04 % compared with 28.44 % . The patent period of parasitaemia was not significantly different to that of the control group, although it appears that in the early stages of resolution, parasite growth is not as effectively controlled as in the control mice, so that parasitaemias are significantly higher for days 12 and 13. No recrudescence were observed during the 14 days following recovery, and as with the control group none of the mice died as a result of the infection.

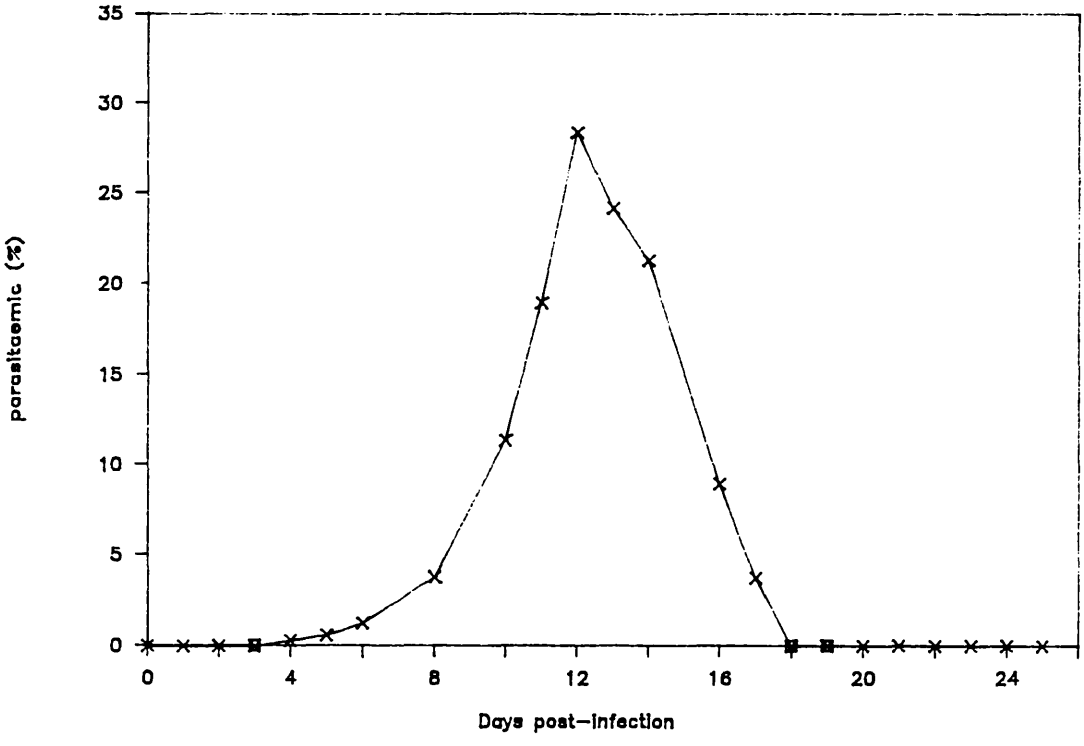


Fig. 3.1. The course of infection by Plasmodium yoelii yoelii in the control group of BALB/c mice (mean of ten mice) following inoculation with 250 sporozoites per mouse i.v., on day 0. (◻ - indicates blood positive by thick blood smear but negative by thin blood smear).

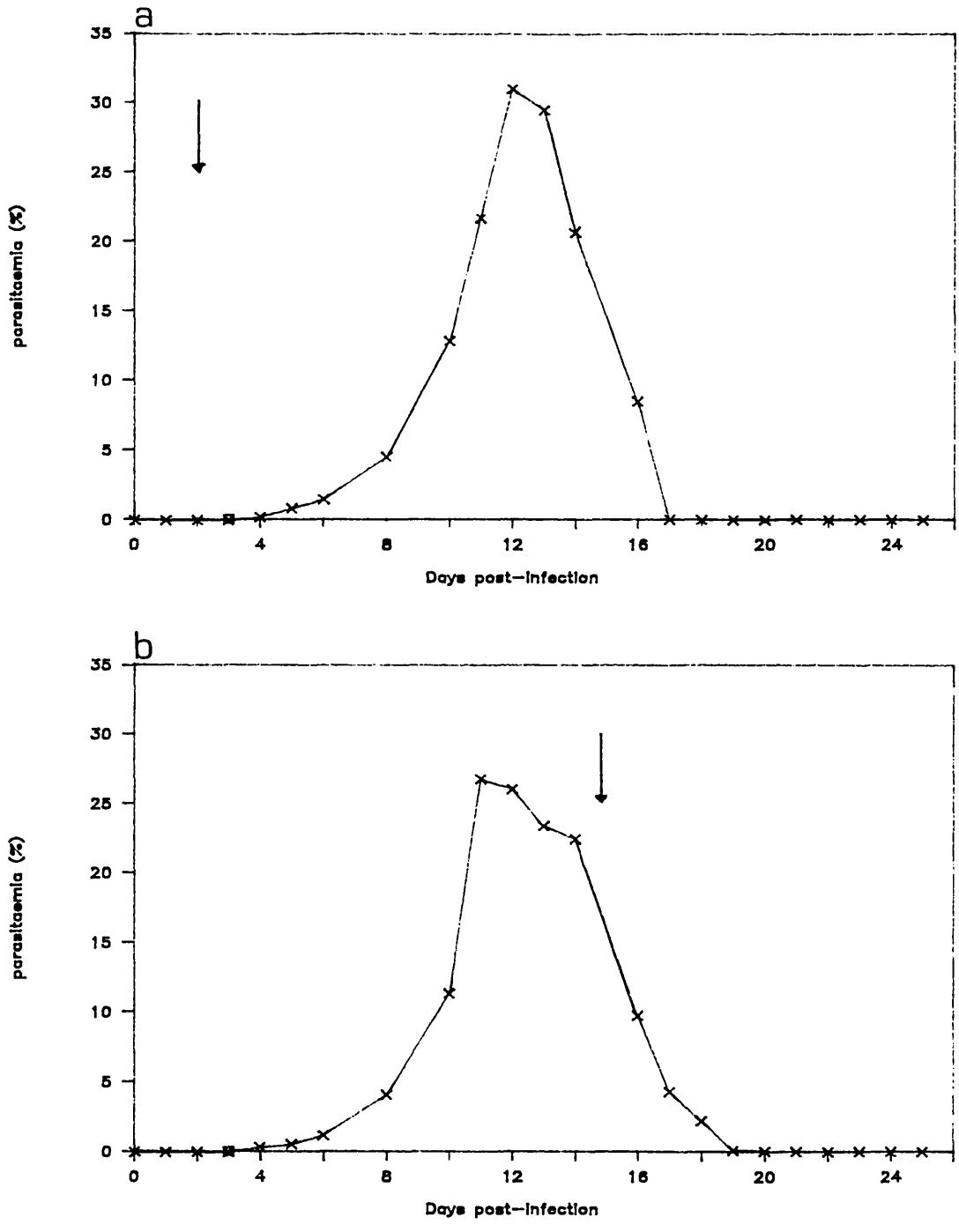


Fig. 3.2. The course of infection by P.y.yoelii (mean of ten mice) in BALB/c mice infected with 250 sporozoites per mouse i.v. on day 0, and re-inoculated with 250 sporozoites per mouse i.v. on, (a) day 2, group A_1, (b) day 15, group A_2. (□ - indicates blood positive by thick blood smear but negative by thin blood smear, ↓ - indicates time of second inoculation).

Although the parasitaemias of the mice in group A_1 were consistently higher than those of the control group during the log growth phase, (days 6-12), a least squares fit of the % parasitaemias against time, (fig.3.3), indicates that the rate of growth of the parasites in the two groups were identical to 2 d.pl. (0.4351 for the controls, 0.4327 for group A_1).

3.3 (iii) (c) Superinfection during resolution - group A_2

Group A_2 were superinfected on day 15 of the initial infection. The course of infection, prior to the superinfection, was not significantly different to that of the control group, (fig. 3.2 (b)). The 1 % parasitaemia period, mean parasitaemias and peak parasitaemias were all comparable although the mean peak parasitaemia occurred on day 11, one day earlier than the control group.

After superinfection the level of parasites in the peripheral blood continued to decline in all of the mice. The mice however maintained significantly higher parasitaemias between days 16 and 20, and patency among the individual mice in group A_2 appeared to be extended by between 1 and 3 days. Whereas on day 18 infections among the control group were all subpatent, 8/10 of the group A_2 mice were still patent with a group mean of 2.21 % . By day 19, 3/10 were still patent with a group mean of 0.095 % , and on day 20, 2/10 were patent with a group mean of 0.034 % ; infections finally became subpatent in all the mice on day 21.

None of the mice became patent again during the additional 14 day observation period and no mortalities occurred.

3.3 (iii) (d) Course of infection in naive control mice

No significant difference was found in the course of infection among any of the naive control mice of groups A_1, A_2 or A_3 when compared with the course of infection of the control group infected on day 0.

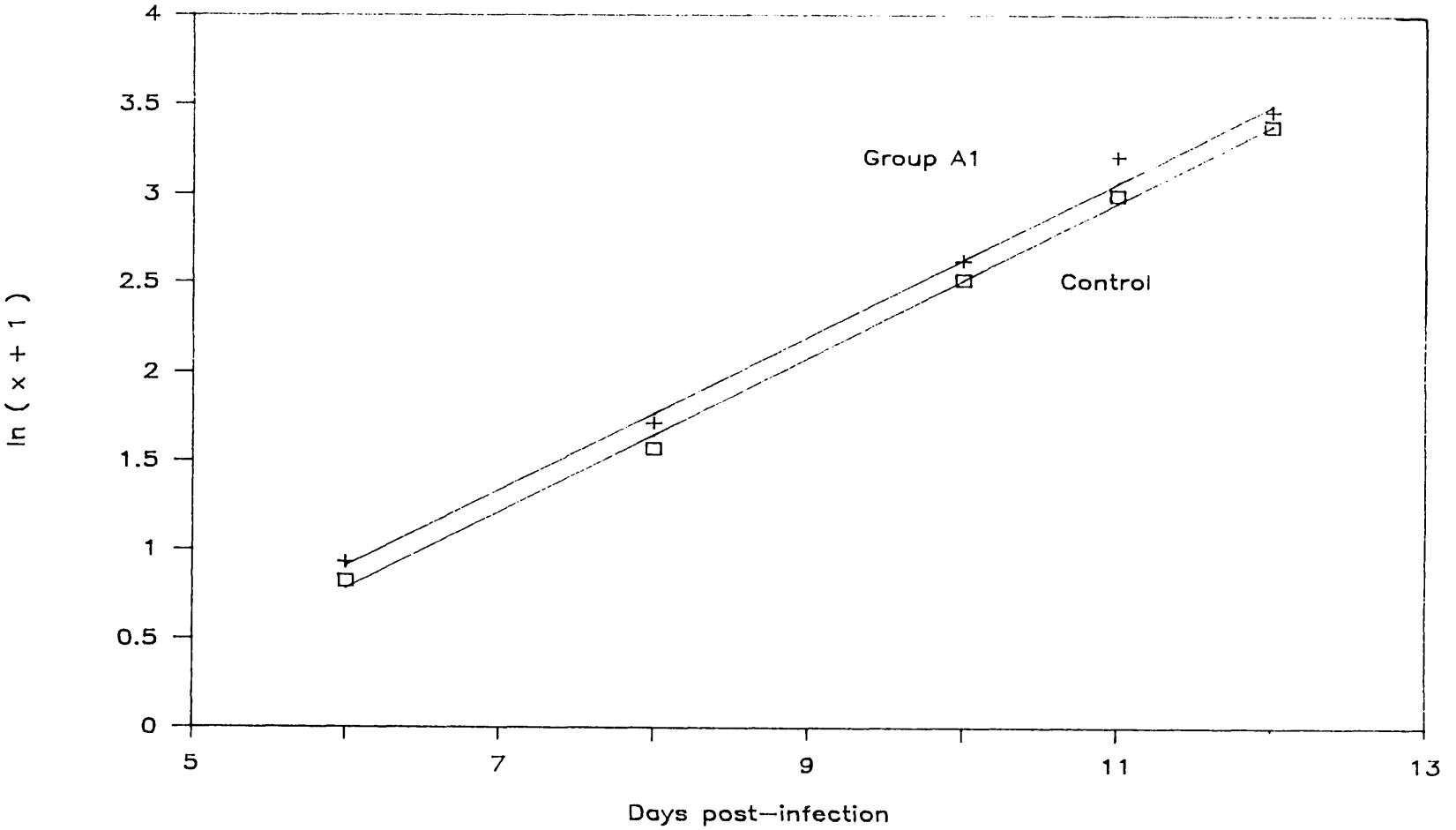


Fig. 3.3. Plot of In(x+1) for the control group and the group re-inoculated during pre-patency, group A_1, against days post infection, t, where x is the mean % parasitaemia of the individual mice, with the fitted least squares regression according to the equation: $In(x+1) = r_1 t + r_2$, (Control group, $r_1 = 0.4351$, $r_2 = -1.831$, Group A_1, $r_1 = 0.4327$, $r_2 = -1.690$).

3.3 (iii) (e) Distribution of parasites within the erythrocytes

It was found that the method used to investigate the spatial distribution of the parasites within the peripheral blood cells was not entirely accurate, since only some of the parasites in the cells being examined are in the viewing plane. This method can only be used as a crude guide to indicate gross changes in the distribution. Nevertheless the changes found could still be directly related to the parasite's intrinsic preference for reticulocytes and other young erythrocytes (Carter and Diggs,1977), and to the availability of these suitable host cells during infection.

During the earliest stages of infection in the control group, up to day 5/6, the parasitaemia is at a very low level and the infection is essentially randomly distributed throughout the reticulocyte population with no polyparasitism. However during the parasites log phase of growth when the parasitaemia is increasing, suitable cells appear to be in short supply. Healthy young adult mice of 8-14 weeks have only 1-2 % immature erythrocytes in the peripheral blood available for infection, (Kretchmar,1972). Almost all of the available reticulocytes become infected and polyparasitism becomes quite common, although none of the mature erythrocytes were invaded. Erythrocytic loss associated with malarial infections creates a compensatory increase in the production of immature erythrocytes, and so as the infection proceeds, the accelerated production of reticulocytes increases the availability of suitable host cells and polyparasitism appears to decrease again. During the resolution phase parasites again become randomly distributed in the reticulocyte population. There were no significant departures from this sequence of events among the test groups receiving superinfections.

One additional point of note is that when a reticulocyte contains more than one parasite, the parasites are always the same age. This observation cannot simply be explained by all of the parasites originating from the same parent schizont which may have been adjacent to the red cell when the merozoites were released (thus multiple entry of merozoites facilitated), because polyparasitism is only seen during the parasites log phase of growth. Since polyparasitism occurs only when reticulocytes are in short supply, it is probable that when the merozoites are released they try to rapidly invade any available host cell, after which a physiological mechanism of some sort prevents further entry. During log phase growth when there are huge numbers of merozoites seeking suitable host cells multiple entry into the cell will become inevitable. A similar observation has been made for P.falciparum (H. Carter, pers. comm.).

3.4 Discussion

The results of the experiment seem to indicate quite clearly that a current infection does interfere in some way with the establishment and/or erythrocytic multiplication of parasites in a new infection, the most striking observation being that two separate inoculations of sporozoites do not produce an infection with two peaks of parasitaemia. This observation does not however indicate that the mice are refractory to superinfection since a second inoculum appears to modify key population parameters as compared to controls with no superinfection.

The theory that superinfections will tend to lengthen patency is only partially borne out and seems to be dependent on the phase of the established infection at the time of superinfection. A superinfection during pre-patency appears to have no significant effect on patency, although it does effect other aspects of infection such as the time taken to reach a predetermined level of parasitaemia and, more interestingly the levels of parasitaemia during the early stages of resolution. Superinfection during the resolution phase however significantly delays recovery so that patency in individual mice is extended by between one and three days.

It is difficult to assess how significant this effect might be in naturally occurring human malaria since the superinfection only increased patency by a few days. However it is possible that the effect is amplified if more than one superinfection occurs during the resolution phase. Since the course of infection in humans is less rapid and resolution takes longer, there is a high probability that multiple superinfections will occur during resolution enabling superinfections to have a very significant effect on recovery rates in human communities, especially in high transmission areas. The observation that patency is not extended when superinfection occurs during the pre-patent period is particularly important, and needs to be considered in future mathematical models for superinfection. One further point concerning the assumptions on which previous superinfection models have been based concerns Macdonald's assumption that infections form a queue and express themselves only when the previous infection is over (Macdonald,1950a). The experiments suggest that this is incorrect. They seem to indicate that Aron and May's assumption (Aron and May,1982) that infections are successively easier to shed is basically correct, since the infection resulting from the second inoculum was always considerably less severe. A second wave of parasitaemia was not seen in any of the mice and the time elapsing between the second infection and eventual recovery was considerably shorter than in the control infections.

It appears likely from the experiment that a superinfection during the early stages of pre-patency may not recognised as a separate infection. Results indicate that the changes in population parameters may simply reflect the larger total dose of sporozoites received by each mouse, and that they are not as a result of an accelerated growth rate due to superinfection (fig. 3.3). It has already been mentioned in section 3.2 (iii) (b), that dose size is known to affect latency and the course of malarial infections. It is likely therefore that the decrease in the time taken to reach the predetermined level of 1 % parasitaemia and the consistently higher parasitaemias during the phase of rising parasitaemia is due to the reduction in the time to patency, (produced by the "double dose"), which caused a 0.5 day shift in the course of infection.

Another interesting effect that may also be related to total dose is that mice which have been superinfected during early pre-patency are less effective at controlling parasitaemia during the early stages of resolution. It would appear that although superinfection during pre-patency has no effect on the length of patency it does have several other effects that could have important consequences to human infections.

Although this type of investigation into the effects of superinfection experiment has never been done before, a series of experiments by Verhave(1975) concerned with the interference exerted by a current infection on the establishment of a new infection is closely related to the topic. Verhave found that a second inoculation of sporozoites during the 48 hour period of exo-erythrocytic maturation of the first infection resulted in a considerable reduction in the establishment of sporozoites from a second inoculum, as compared to controls. A reverse effect on the development of the exo-erythrocytic forms from established infection was not apparent, and the magnitude of the effect on the new infection was found to be dependent on the size of the first inoculum. Verhave concluded that the effect must be the result of some sort of non-specific immune activity since the time period of 48 hours is too short for the induction and implementation of a specific cellular or humoral response.

A current patent infection was also found to reduce establishment of a new parasite population, the greatest reduction being when the parasitaemia was at its peak or during the resolution phase. Since these infections had been blood-induced, Verhave reasoned that the effect could not be a specific anti-sporozoite response but must be some sort of interference caused by the patent infection. He concluded that since the greatest effect was seen at a time when anti-parasitic activity against the blood-stages was maximum, the effect must be produced by mechanisms such as cross-reacting antibody, or a general enhancement of cellular immune

function. Since parasite establishment was assessed by the density of exo-erythrocytic forms in the liver at autopsy 45 hours after infection, the possible further effects on patency and the course of infection of such superinfections is not known.

To understand more fully why the phase of the established infection is so critical to the outcome of the superinfection it is necessary to consider both immunity and red cell availability during infection. This is a brief view of these two factors concentrating on their effects on the dynamics of infection rather than the exact mechanisms involved. In an infection such as P.y.yoelii in BALB/c mice the rise and fall of parasitaemia can be correlated directly with anti-parasitic activity. The fact that P.y.yoelii is maintained at sub-patent levels for up to seven weeks (Freeman and Parish,1981) is however indicative that the immune response is not entirely effective and that the parasite must be able to evade the response for some time before it is finally wiped out. Although the development of immunity in P.y.yoelii has been found to be both T and B cell dependent, it is thought that antibody plays the major role in the control of P.y.yoelii infections (Weinbaum et al,1976; Jayawardena et al,1977). The predominant antibody response to the blood-stages is thought to be that of IgG (Langhorne et al,1984). P.y.yoelii induces a rapid and high antibody response to asexual parasites that is detectable by day 6 and reaches a maximum 11-12 days post-infection (Langhorne et al,1984). This coincides directly with the turnover in the parasite population and high levels are maintained for at least 3-6 weeks post-infection (Freeman and Parish,1981). This also coincides with the reduction of parasitaemia and maintenance at sub-patent levels. Sera from donors in the sub-patent phase will hold the parasitaemia of recipients with a primary infection at sub-patent levels (Freeman and Parish,1981). It therefore appears that the course of infection is modified by immune responses and not by some intrinsic property of the parasite population. Similar antibody effects are seen in other rodent malarias although there appear to be variations as to whether the infections are controlled predominantly by antibody-independent or antibody-dependent mechanisms (P.chabaudi, Langhorne et al,1984; P.berghei, Phillips and Jones,1972). Mclean et al(1982) have also shown that the onset of recrudesce in P.chabaudi can be correlated with a reduction protective activity of sera. Additionally, Verhave(1975) suggests that there are non-specific mechanisms that inhibit the exo-erythrocytic development of superinfections as little as 8 hours after initial infection, and specific mechanisms which develop later in the initial infection.

Parasite multiplication and the outcome of infection has also been linked with the availability of red blood cells in the host so that the multiplication of parasites in the blood of such species as P.y.yoelii which have marked preferences for immature erythrocytes is inhibited during the rising phase due to the lack of suitable cells. Erythrocyte loss associated with the infection induces the production of erythrocytes and the rise in parasite numbers is directly correlated with the availability of suitable cells (Roth and Herman,1979; Oster et al,1980). Experimental infections can be selectively controlled by manipulating the ratio of mature to immature erythrocytes with irradiation, drugs or blood letting (Fahey and Spitalny,1984).

Since the course of malarial infections are so clearly determined by the development and maintenance of protective immunity and the availability of suitable red cell types it is probable that the outcome of superinfection is also determined by both the erythropoietic and immune "status" of the host at the time of superinfection. There is much evidence to suggest that parasites can, in most cases, establish themselves and undergo exo-erythrocytic maturation in hosts that are apparently "immune" to infection. The barrier to patent infection being the penetration of erythrocytes by the merozoites (Verhave,1975; Yoeli,1966). It would appear therefore that the major influence on the outcome of superinfections will occur in the blood-stages. If superinfection occurs during pre-patency, competition between the two parasite populations for the available erythrocytes might be expected, with antibody levels in the blood not yet at a level to have a significant effect. Superinfection later in patency may become increasingly less affected by the availability of suitable cells (the reticulocyte population will be rising rapidly) and more by the rise in both specific immunity against parasite establishment and also antibody directed at blood-stages. Superinfections during resolution enter a very "hostile" environment with anti-parasitic activity at its maximum, as a result one might expect parasite establishment to be low and population growth to be negligible. This is of course a over simplistic view of what might occur since it has been shown that in addition to the type of events mentioned above, superinfection can itself modify the effectiveness of the immune response particularly its effectiveness at resolving infection however it does help to explain the dependency on the phase of the established infection.

Work by McLean et al (1982) has examined the changing in vivo levels of anti-parasitic activity during P.chabaudi infections (induced by 10^6 parasitized red blood cells) by monitoring the rate of clearance of large i.v. challenges given at various points throughout a normal infection.

Although the experiment involved administering a second infection during a current infection, the challenge cannot really be considered as representative of naturally occurring superinfections. The infections were induced by large numbers of parasitized blood cells (5-7 X 10^8) which results in an immediate 5-8 % parasitaemia and death within 3 days in naive mice. What it does illustrate, however, is that the longer the interval between the time when the last patent parasitaemia was controlled (P.chabaudi infections typically have one or more recrudescence) and the challenge infection the less able the immune response is at controlling the challenge infection. This gives us a further indication that competition between established and new parasite populations will be at a maximum when the superinfection occurs before patency or between recrudescences.

3.5 Suggestions for further work

There is a great deal of scope to examine further features of naturally occurring superinfections using model systems, in addition to the investigation of the possible immunological and erythropoietic mechanisms involved in determining the outcome of a superinfection and the possibility of the development of a sporozoite vaccine, many much more basic aspects of superinfection remain unknown.

3.5 (i) The individual effects that established and new infections have on each other

Although the outcome of superinfection can be identified, the exact effects that the established and new infections have on each other cannot be assessed since the growth of the two separate parasite populations is not known, only the combined course of infection is seen. To identify these effects one needs to be able to recognise the two separate infections. This could be done by selecting infections with different courses of infection or growth rates (Thaithong et al,1984), differences in morphological characteristics, or infections that are antigenically distinct at the blood stage.

For superinfections using the same strain as the original infection separation by differences in growth rate or morphology are unlikely to be possible, however separation of blood-stages by a diagnostic monoclonal is possible (Holder and Freeman,1984). Fluorescent methods for counting malaria infections have proved remarkably accurate (Rodwell and Howard,1981), so if one infection was positive for the monoclonal and the other negative a simple fluorescent marker

attached to the monoclonal would enable one to distinguish the two populations in a blood-smear. A number of pairs of rodent malarias clones and diagnostic monoclonals exist, one for P.chabaudi (D.Walliker, pers.comm.) several for P.yoelii (R.Freeman, pers.comm.). These pairs are different cloned lines of the same strain which are identified as antigenically different by a single monoclonal at the blood-stage only. The vertebrate immune system views the two clones as identical, and would respond to them as two separate parasites only if they were antigenically different at multiple sites at more than one stage of the life-cycle.

3.5 (ii) More detailed investigation on the effects of the phase of the existing infection

The experiments described above examined superinfection at only two points during infection, however, to assess more fully the importance of the phase of the infection and its effect on the outcome of naturally occurring superinfections further work needs to be done, possibly by using the technique mentioned in the previous section.

Although both Verhave(1975) and McLean et al(1982) have already carried out extensive investigations on the effects of re-infection at various points during infection, the experiments were carried out using massive numbers of either blood-stage parasites (McLean et al,1982) or sporozoites (Verhave,1975) to induce infections, which may have an important effect on the outcome. The responses might be quite different from those expressed by the small inocula of natural human malaria. Verhave himself points out that the effects that he found were critically dependent on the size of the dose. The experiment outlined in section 3.3 has also emphasised the importance of monitoring throughout the whole course of infection so that all the possible effects of superinfection can be identified.

3.5 (iii) The immunity problem and genetic variability in the parasite population

Among rodent malarias, prior infection with P.chabaudi, P.vinckei, P.yoelii or P.berghei induces solid immunity against homologous challenge (Cox and Voller,1966; Nussenzweig et al,1966a; Cox,1978). Barker(1971) indicates that protective immunity to P.yoelii induced by infection with 2×10^6 parasitized erythrocytes was strongest two months after infection and waned somewhat by twelve and seventeen months. An attempt was in fact made to investigate whether small sporozoite doses might be less effective at inducing solid immunity. Protective immunity, however, was

still solid at nine months post infection. This type of solid immunity has rarely been reported in humans, and it may be that for some reason immunity is much slower to develop in human malaria. Even individuals living in endemic areas show only partial immunity to challenge with P.falciparum (Bray et al,1962). This could either be due to an intrinsic property of the host-parasite relationship, or to the fact that infections are generally abbreviated by chemotherapy (the duration of the patent infection is thought to be a major component in the development of effective immunity to malaria). However one additional factor that could be important is that variations in immunizing and challenge strains may occur. Strains of a single species are known to behave quite independently with homologous immunity to single strains only.

Over a wide geographic area individuals have been shown to harbour multiple genetic strains of P.falciparum (Thaithong et al,1984) which indicates that inoculations do indeed show a great deal of genetic variation. Although at present it is not known whether the antigens recognised in these studies have any role in the protection against infection. Thaithong et al(1984) consider that the amount of genetic variability found is significant and that it is highly likely that antigens involved in immune protection would also vary between isolates.

It would be relatively easy to investigate the effect of genetic variations between infecting and superinfecting inocula using an animal model, however an important feature of such an experiment would be to determine whether inocula should contain numerous different strains or that inocula contain single strains which are varied between inocula.

Collins et al(1984) has shown that among mosquitoes infected with human malaria the majority of infections originate from a single oocyst, which might indicate that inocula of single strains only should be used. However, Rutledge and Ward(1973) and Pringle(1966) both found that malarial oocysts were distributed according to the negative binomial distribution with up to 350 oocysts per mosquito being possible. Further to this Pringle(1966) commented that it was possible to see oocysts in various stages of development originating from different feeds in single mosquitoes which obviously increases the chances of mosquitoes with mixed sporozoite populations and thus the need to carry out experiments using inocula of mixed infections.

3.5 (iv) Multiple superinfections

Since the possibility of multiple superinfection in natural human malaria is so great, especially in high transmission areas, it is important to have some idea of the effect that more than one superinfection may have on the course of infection. Evidence from other parasite systems suggest that immune responses to "trickle" infections i.e. multiple infections, may be different to those of single infections (Crombie & Anderson,1985).

CHAPTER 4 Mathematical models for transmission and control of malaria - a review

Mathematical models can be used not only to further an understanding of the transmission dynamics of disease, but also to provide guidelines as to the best way or ways to control and/or eradicate disease. However, the value of models are dependent on a quantitative understanding of the epidemiology of the disease. For acute infections which produce "sterile" immunity, the theory is well developed (Anderson & May,1985). In contrast, the dynamics of malaria are not well understood. Malaria models help to define the limits of knowledge and have suggested what information is critical for improved understanding. Developments in the field of malaria modelling have frequently paralleled those of laboratory and field studies. Several malaria models will be reviewed in this chapter, a few of which will be discussed in greater detail in the following chapters.

4.1 Basic models

4.1 (i) Ross's malaria models

Having elucidated in 1897 the crucial importance of the mosquito in the life-cycle of the malaria parasite, Sir Ronald Ross developed the first mathematical models concerned with the transmission and control of malaria (Ross, 1909,1911,1916). Ross's models although simple in structure provided some important basic epidemiological insights and were used to offer practical solutions for the prevention of malaria in Mauritius (Ross,1909). Ross's models have since been used as the basis for the majority of the malaria models to date, and many of the conclusions drawn from this early model are still used today in control theory (Dietz,1975).

Ross's two models differ only slightly, one involves a single first-order differential equation describing the course of malaria in an infected community (Ross,1911, section 27), which is an approximation of the second more complex model. The second model involves two first-order differential equations which describes the behaviour of both host and vector (Ross,1911, section 66). These models assume, in epidemiological terms, the simplest sort of endemic situation in which the infection rate is constant and mosquitoes and individuals can be in one of only two states, namely infected or uninfected.

Ross assumed a proportion x, of the human population was infected and a proportion (1-x) uninfected, and equivalently

for the mosquito population, he assumed a portion y infected and a proportion (1-y) uninfected. The infection rate per unit time, h, and the recovery rate, r, for the human population were both assumed to be constant. For the mosquito population, the infection rate h" and the death rate μ" were also assumed to be constant. Recovery among mosquitoes and the death rate amongst humans were assumed to be negligible. On the basis of these constant rates, and the further assumptions, that disease-induced mortalities were negligible and that the human population size and the density of mosquitoes remained constant, Ross formulated his basic models. The single equation model, describing the rate of change, with time, of the infected portion of the human population, takes the form;

$$\frac{d}{dt} x = h\,(1 - x) - rx \qquad (4.1)$$

This equation can be integrated directly to define the prevalence rate of infection x(t), at time, t:

$$x(t) = x_0 \exp(-(h + r)t) + \frac{h}{h + r}\,[\,1 - \exp(-(h + r)t)\,] \qquad (4.2)$$

where, x(0) is the initial prevalence .

If time is equated to age, an age-prevalence curve for a population living under the defined endemic conditions is obtained. Figure 4.1, shows that the prevalence rate in the human population rises monotonically with age toward a limiting value, defined by h/(h+r) in the older age-groups, giving a typical "catalytic" curve of the type later described by Muench(1959), (x(0) is assumed to be zero).

The most important practical conclusions from the model are summarized by Ross (1911, section 28) as:

> "(1) That the amount of malaria in a locality tends toward a fixed limit determined by the number of malaria-bearing mosquitoes and by other factors.
>
> (2) That if the number of malaria-bearing Anophelines is below a certain figure, that limit will be zero."

These two points are relevant for both the planning and evaluation of malaria control programmes, and are discussed further in Chapter 5.

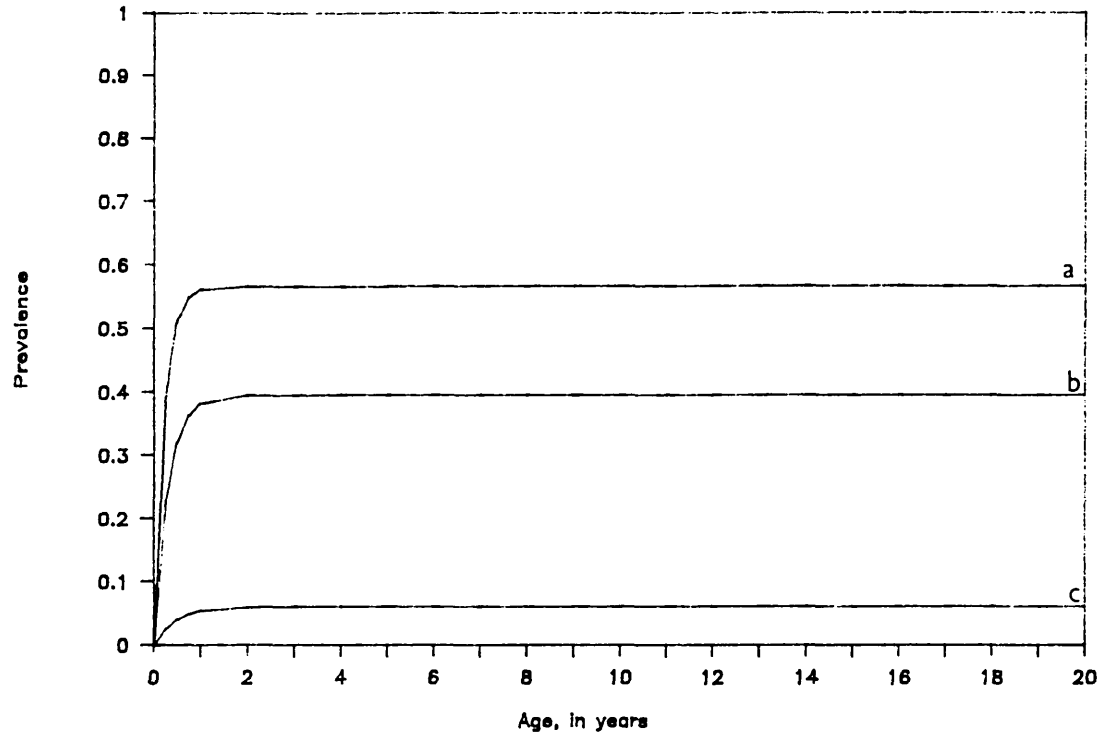


Fig. 4.1. Age-prevalence predictions for the basic Ross Model (eq.(4.2) in the text), (a) h = 0.05 /week, (b) h = 0.025 /week and (c) h = 0.0025 /week.
(Additional parameter values, r = 0.038/week, x(0) = 0).

The more complex two equation model, (which has been used as the basis for the models developed in Chapters 5 and 6), defines the rate of change, with time, of infected portions of both human and mosquito populations as follows:

$$\frac{d}{dt} x = h\,(1 - x) - rx \qquad (4.3)$$

$$\frac{d}{dt} y = h''\,(1 - y) - \mu'' y \qquad (4.4)$$

Where h and h" are defined as,

$$h = \frac{(a\,b\,M)}{N} y \quad \text{and} \quad h'' = a\,x \qquad (4.5a \text{ and } 4.5b)$$

The parameter a is the rate of biting on man by a single mosquito per unit time, b is the proportion of infected bites that produce an infection and M/N is the number of female mosquitoes per human host.

These simple deterministic models, although capturing the basic features of the transmission process and the interaction between the infected portions of the human and vector populations, contain major over-simplifications and omissions which are reflected in the fact that model predictions are strikingly different from reality. Some of the more important omissions include the failure to account for the existence of superinfections or immunity in the human population, and the pre-patent period in the mosquito.

4.1 (ii) Infection and superinfection in the human host

Attempts to account for the magnitude of the rate of recovery from infection make up a large part of the history of mathematical modelling of malaria, (Fine(1975)). Ross's early models (Ross,1911,1916), assumed that the presence of an infection prevented the establishment of other infections. The recovery rate was thus independent of any infectious contacts made during the course of infection. However, work by Macdonald(1950a,1950b) examined the applicability of Ross's one equation model (eq.(4.2)) to field data on the prevalence of malaria in successive age-groups of children living in endemic areas. The Ross model could be made to fit the data. However, the resultant estimates for the recovery

rate were often found to be very low, typically 0.001 - 0.0005 per day. These estimates imply a duration of parasitaemia from a single infection lasting for a period of 3-6 years, which was regarded as unacceptably long. Focussing upon Ross's assumption of a constant simple recovery rate and its implied assumption of a simple infection rate, Macdonald (1950a) initiated a departure from the classical theory of malaria epidemiology formulated by Ross(1911,1916). He assumed that individuals could harbour multiple "broods" of parasites and argued that -

> "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 others." (Macdonald,1950a).

(Earlier models for this type of superinfection, divided the human population into a potentially infinite number of states according to the number of infections they carry, (Mckendrick,1926; Kostitzin,1934)).

Briefly, Macdonald(1950a) concluded that,

$$r = (r_0 - h) \qquad (r_0 > h) \qquad (4.6a)$$

$$r = 0 \qquad (r_0 < h) \qquad (4.6b)$$

where r_0 is the rate of recovery from a single infection.

When this new formula for the recovery rate was used with eq.(4.1), and applied to prevalence data (Macdonald,1950b), (using an estimate of $r_0 = 0.005$ per day, derived from Earle et al(1939)), good agreement was found. However, despite the fact that this approach is undoubtedly a more realistic one than that of Ross, the mathematical formulae that were eventually devised probably do not reflect Macdonald's original verbal concept of superinfection.

It is now generally accepted that eqs.(4.6a) and (4.6b) do not accurately represent the implications of superinfection. The model implies that individual infections have to "queue" to be terminated. Hence in areas of very high transmission once infected, an individual would never recover, since new infections would accrue faster than any infection could be eliminated. The recovery rate from infection thus becomes zero. Recognition of the error is attributed to Dietz although the paper was never published. Further interpretations of the problem have also been made by Aron & May(1982), Fine(1975), Nåsell(1980a,1980b,1980c cited in Bailey,1982), and Bailey(1957) who used the same approach

as Kostitzin(1934). Dietz (described in Bailey,1975, pp317-322) published a new formula for the recovery rate based on a queuing system. Infections were assumed to arrive according to a poisson process with rate h, the human body was assumed to recover from each infection simultaneously and independently with a constant rate r_0. In such a queue, when h is constant, the equilibrium transition rate from a state of one or more infections to that of no infections is:

$$r = \frac{h}{\exp(h/r_0) - 1} \qquad (4.7)$$

Papers by Fine(1975), Aron & May(1982) and Dietz(1988a,1988b) discuss in detail the implications of the three different interpretations of the recovery rate in terms of the equilibrium prevalence rates for human and vector populations. In brief, all three theories predict an exponential rise in human prevalence with age, or time, under constant values for h and r_0. For low infection rates, the age-prevalence curves generated by the three models are very similar, since there are few superinfected individuals, and thus both interpretations of superinfection resemble the basic Ross model. However for higher infection rates, although the qualitative dynamics are similar for the three models, the predicted equilibrium prevalences diverge considerably. Macdonald's model predicts the highest equilibrium prevalences, whereas the Ross model predicts the lowest. Aron & May(1982) mention the existence of other superinfection models, nested between those of Ross(1911,1916) and Dietz et al(1974), in which superinfections are successively easier to shed, rather than being totally independent of each other.

4.1. (iii) Malaria infection and the vector population

Ross's original models have experienced amplifications related to the vector population in two principal directions; namely, in accounting for the extrinsic period of development by the parasite in the mosquito, and in incorporating temporal variation in the mosquito density.

4.1 (iii) (a) Pre-patency in the vector

Although Macdonald attempted to re-formulate the assumptions associated with human recovery rates based on the existence of superinfections, he was primarily concerned with the entomological aspects of transmission. His work included

several important contributions which are fortunately independent of the Macdonald superinfection model. Macdonald(1952a) considered the effects of numerous entomological factors, including the duration of the extrinsic development of the parasite (which had previously not been accounted for), on the magnitude of the sporozoite rate. A formula was given for the sporozoite rate in terms of the various factors, which formally explained why mosquito populations in highly endemic areas have such low sporozoite rates (see table 2.6). Briefly explained; the pre-patent period (n) of between 10 and 30 days (fig. 2.2), during which a vector is infected but not infectious (sporozoite positive), coupled with an expectation of life ($1/\mu''$) of only 2-30 days (table 2.5) means that a significant proportion of the infected mosquitoes die before the infection becomes patent. Hence sporozoite rates remain low despite high human prevalences. The effect of pre-patency in the vector is discussed further in Chapter 5.

4.1 (iii) (b) Variable mosquito density

Although mosquito densities vary seasonally in many parts of the world, modelers have frequently ignored this complication. Early work by Martini(1921,1936 cited by Bruce-Chwatt(1976)) related quantitative studies on malaria to the behavioral characteristics of mosquitoes and the seasonal changes in their density. The work, however, has received little attention.

Aron(1981) and Aron & May(1982) include work on the differential effects of variable mosquito density in areas of high and low transmission, and illustrate how seasonal epidemiological patterns can all be explained as a consequence of variable mosquito populations densities. It appears that fluctuations in the density of vectors may determine the timing of not only epidemics among the human population but also peak sporozoite rate among vectors.

The Garki model, developed by Dietz, Molineaux & Thomas(1974), (which is to be described in greater detail in section 4.4 (i)), also simulates seasonal changes in vector density in an endemic situation, based on observed man-biting rates.

4.2 Basic Reproductive Rate and the Vectorial Capacity

4.2 (i) The Basic Reproductive Rate

The keystone of Macdonald's analysis of the transmission of infection is the concept of the Basic reproductive rate, R, (Macdonald,1952b,1957). This parameter is defined as the average number of secondary cases of malaria arising from a single primary case in a very large population of susceptibles. Algebraically, R is expressed as:

$$R = - \frac{M a^2 b p^n}{N r \log_e p} \qquad (4.8)$$

(refer to table 4.1 for parameter definitions).

Macdonald's heuristic arguments, are as follows: if the human recovery rate is r, the primary human case would be infective for an average of 1/r days. On each day of infection, the case would be bitten by an average of Ma/N mosquitoes. The probability that any one of these vectors survives the n days of the extrinsic cycle is p^n, after which, the expectation of infective life is $1/(- \log_e p)$. On each day the vector takes an average of a bites, of which only a proportion b are actually infective. The resultant number of secondary cases is thus simply all these factors multiplied together. The eq.(4.8) has been derived algebraically, by the analysis of the stability properties of eqs.(4.3) and (4.4) (Macdonald,1957; Lotka,1923).

The condition $R \leqslant 1$, means that any initial malarial infection will die out, while if $R \geqslant 1$ an endemic level of disease will be reached and maintained. In general the larger the value of R the greater the resistance of the disease to control and eradication, as indicated by the phase-plane analysis of the dynamic behaviour of the model (Aron & May,1982).

The structural significance of the expression is well understood, most importantly:

(i) Transmission depends on the number of vectors per host, rather than on the product of vector and host densities, since the vector takes a fixed number of blood meals per unit time, irrespective of host and vector abundance. Therefore transmission is facilitated by large numbers of mosquitoes per host.

(ii) Transmission is hindered by high mosquito death rates or by fast recovery.

Table 4.1

Parameter notation used in malaria models described in chapter 4

Parameter	Definition
x	Proportion of human population infected.
y	Proportion of female mosquito population infected.
h	The per capita infection rate for humans.
h''	The per capita infection rate for mosquitoes.
r	The per capita rate of recovery for humans.
μ''	The per capita mortality rate for mosquitoes.
$x(t)$	The prevalence rate of humans, at time t.
$x(0)$	Prevalence rate of humans at time 0 (or age 0).
a	The rate of biting on man by a single mosquito.
b	The proportion of infected bites on man that produce infection.
(M/N)	The number of female mosquitoes per human host.
r_0	The rate of recovery in man from a single infection.
R	The Basic Reproductive Rate
p	The probability that a mosquito will survive through one day.
n	Time for the completion of the extrinsic cycle, in days (= pre-patent period in mosquito).
C	The Vectorial Capacity.
X_1, X_3	Proportion of population non-patent (1 = non-immunes, 3 = immunes).
X_2, X_4	Proportion of population incubating, parasites in the liver only (2 = non-immune, 4 = immune).
Y_1	Proportion of population patent individuals who are also infectious.
Y_2, Y_3	Proportion of population patent, but non-infectious individuals (2 = recovering slowly, 3 = recovering fast).
R_2, R_3	Recovery rate from patent infection, corresponding to the states Y_2 and Y_3 above.
q_i	The probability of detecting parasites in the blood, corresponding to the state Y_i, i = 1, 2, 3.
α_1	The rate of loss of infectivity.
α_2	The rate at which a state of increased in recovery rate and decreased in detectibility is acquired.
W	The rate of transfer from an "incubating" class to a patent class.
τ	The duration of immunity in the absence of re-exposure.

(iii) Because the mosquito has to take two blood meals to be able to transmit the disease, transmission is sensitive to changes in the biting-rate which appears twice in the equation.

(vi) Because the extrinsic cycle of the parasite takes up a significant proportion of the vectors expected lifespan, and for many species actually exceeds it, transmission is especially sensitive to changes in the life-expectancy of the mosquito.

Macdonald(1957) concluded from eq.(4.8) the important qualitative conclusion that imagocides are more effective at controlling malaria than larvicides. The larval survivorship enters the expression for R, linearly, through the mosquito population density (M/N). In contrast adult survivorship enters in a highly non-linear way, through the factor, (p^n / Log_e p). Reduction of larval recruitment by a half, would result in only a halving of the value of R, whereas doubling the adult mortality rate (- $\log_e$ p), decreases the value of R in an exponential manner. The policy change made to the planning of control measures as a result of these conclusions, is described by Harrison(1978).

Macdonald(1957) also used this model to categorize regions of stable and unstable malaria. He singles out as an index of sensitivity the number of potentially infective blood meals on man by one vector throughout its life, (a / (-Log_e p)). This result was obtained by calculating the derivative of the prevalence in man with respect to the Basic Reproductive Rate for R = 1. Since this derivative yields 1/ (a / (-Log_e p)), in his model he concludes that for small values of (a / (-Log_e p)), small changes in R lead to large changes in prevalence. Conversely, if (a / (-Log_e p)) is large, then the changes in prevalence are small. Therefore in areas where (a / (-Log_e p)) is small, seasonal variations to vector longevity etc. will produce fluctuations in transmission (and thus the value of R), which might easily move the human prevalence toward zero or toward large values, thus creating epidemic patterns of disease. When (a / (-Log_e p)) is large, however, seasonal fluctuations in transmission lead to only minor changes in prevalence. Thus the typical endemic pattern of disease is produced.

4.2 (ii) The Vectorial Capacity

An important modification of Macdonald's Basic Reproductive Rate, (eq.(4.8)) has been made by Garrett-Jones(1964). Macdonald's index refers to the potential of the disease to spread in the context of the dynamic interaction between both the human and the vector population. Garrett-

Jones's approach was to quantify all the entomological components of transmission, in isolation from the parasitological components, and then calculate the maximum daily reproductive rate of the disease, which he called the Vectorial Capacity. Algebraically the Vectorial Capacity, C, can be given by the formula:

$$C = - \frac{M a^2 p^n}{N \log p} \qquad (4.9)$$

(refer to table 4.1 for the relevant definitions of parameters)

The omission of the parameter b, compared with Macdonald's index, means that the Vectorial Capacity assumes that all bites by infective mosquitoes are capable of transmitting infection. Estimates from the formula are therefore a measure of the potential of transmission, since the formula characterizes the mosquitoes as vectors irrespective of the status of the parasite population. However when the disease is at an equilibrium the Vectorial Capacity will vary in proportion to the inoculation rate. Dye(1986) argues that the real value of this index is that it should be able to make long-term parasitological fore-casts from the immediate results of vector control. A reduction in the vector population will have an immediate effect on the value of the Vectorial Capacity but the parasite population will take much longer to reach a stable lower level.

4.3 Heterogeneous contact models

Malaria models are generally based on the assumption that individuals are randomly exposed to infection, however there is much empirical evidence to suggest that exposure is in fact overdispersed. Chapter 2.1 (i) (b) outlines some of the evidence for variability of biting rates between age-groups and between individuals of similar age. The significance of heterogeneous contact rates for the population dynamics of schistosomiasis has been investigated by Barbour(1978) and Dietz(1980). Their results indicate that the Basic Reproductive Rate of the parasite is at a minimum when contact is homogeneous. Additional work by Dye and Hasibeder(1986) on non-homogeneous mixing between biting flies and hosts have supported these findings. The work also suggests that Basic Reproductive Rates calculated under

certain conditions of heterogeneous mixing may be more than two and a half times as large as it would be under homogeneous mixing. The significance of these findings are important when considering predictions made from population models.

Age-dependent contact rates, which probably account for the majority of the variability in rural areas, have never been incorporated into transmission models for malaria. However, a model by Dutertre(1976), to be described in section 4.4 (i), includes a negative binomial distribution for the infection rate on the assumption that there is a natural innate immunity to malarial infection distributed heterogeneously throughout the human population.

4.4 Immunity models

All of the basic malaria models described above fail to account for any development of immunity in man. The age-specific prevalence predicted by these models increase monotonically with age, which is in direct contrast with observations in endemic communities (refer to Appendix B.1). A number of these models can, and have been, used as useful approximations to situations where immunity does not play a major role (i.e where the basic reproductive rate approaches the critical value of one). The inclusion of immunity into transmission models is however of primary importance when attempting to construct models for endemic situations (Dietz et al,1974; Dutertre,1976) where the basic reproductive rate is far above one, or when designing a more general model capable of describing the age-prevalence profiles for a range of different transmission intensities.

Mathematical models of the epidemiology of other infectious diseases, have for sometime incorporated the idea of immunity to re-infection (Bailey(1975)). It is however relatively easy to formulate models of infections with either no immunity or "sterile" and permanent immunity. Malaria fits neither of these descriptions. The difficulties involved in mathematically modelling an immune process which not only confers only partial immunity but is also dependent on continued exposure to infection are much more complex. Attempts to model the generation and maintenance of partial immunity to malaria in order to simulate age-specific prevalence profiles more realistically include several complex compartmental and continuum models.

4.4 (i) Compartmental immunity models

4.4 (i) (a) The Garki Model

This particular model was constructed as part of the WHO Project in the African Savannah, Kano State, Northern Nigeria, and was developed to investigate the effects of alternative methods of control (Dietz et al,1974; Molineaux and Gramiccia,1980). The model describes the temporal changes in P.falciparum infection rate and also the immunity level of the population as a function of the dynamics and characteristics of the vector population. Dietz et al(1974) incorporated the idea that the decline in prevalence observed in adults was primarily caused by, (i) a decline in the intensity of infections leading to "false negatives", (ii) a greater ability of adults, via experience of past infections, to suppress the number of parasites acquired through infectious bites.

The most important feature of the model is that it takes into account the effect of three aspects of immunity on transmission: loss of infectivity, increase in recovery rate and a decrease in detectibility of the infection. The model also takes into account superinfections, using the modified Macdonald-Dietz formula (Dietz, described in Bailey,1975, p317-322). Superinfections are assumed to slow down the recovery rate, but not to reduce them to zero as suggested by Macdonald(1950a). They are, however, assumed to have no effect on infectivity. The model does make the implicit assumption that all superinfections are non-infectious.

The model is basically deterministic, (although some assumptions are based on stochastic models that provide estimates of average values), and is constructed as a set of non-linear difference equations. The human population is divided into seven epidemiological classes, as shown in Fig. 4.2, (the symbols X_i and Y_i defined below refer to the proportions of the human population in the relevant epidemiological classes). Individuals with no parasites in the blood are negatives (X_1 and X_2), those with parasites in the liver only are incubating (X_3 and X_4), and individuals are positive if they have parasites in the blood, (Y_1, Y_2 and Y_3). Only Y_1 positives are capable of infecting mosquitoes, Y_2 and Y_3 positives are assumed to have insufficient sexual parasites circulating in the blood to be capable of producing a patent infection in the vector. Non-infectious positives in Y_2 have a low recovery rate, R_1, while those in Y_3 have a higher recovery rate, R_2. Positives Y_i have a probability q_i of being detected, (i = 1, 2, 3) and it is assumed that $q_1 = q_2 > q_3$. The incubation period is assumed to be a constant value for all individuals.

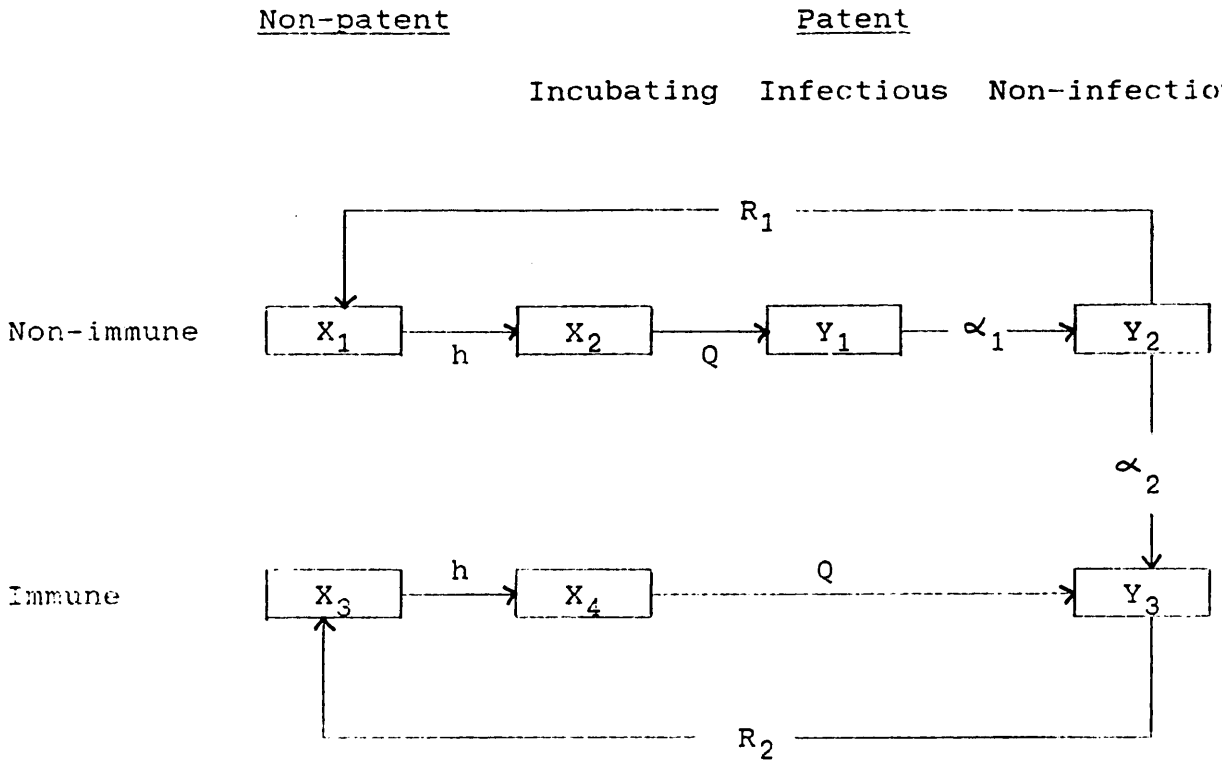


Fig. 4.2. Diagrammatic flow chart illustrating the transition between classes for the malaria model of Dietz, Molineaux & Thomas (1974). Arrows show direction of transfer. Associated symbols are transfer-rates (see table 4.1 for notation and main text for explanations), and α defines the rate of transfer from the latent class to the patent class. Symbols in boxes indicate population proportions. All classes also have a death rate.

In this model all individuals are assumed equally susceptible to infection and are repeatedly exposed, (at a rate, h), become infected and recover from infection. Detectibility of parasiteamia, recovery and infectivity are, however, dependent on the immune status of the individual. These aspects of immunity are assumed to be acquired at different rates; newborn infants enter the "non-immune" susceptible state, X_1, loss of infectivity is assumed to occur first, at a rate, α_1, (this type of immunity is however temporary if the individual recovers to the non-immune susceptible state, X_1), increased recovery and decreased detectibility, (from 100% to 70%), are acquired later, through the transfer from Y_2 to Y_3, at a rate, α_2. The acquisition of a higher recovery rate and lower detectability is associated with a general raising of "immune status" and the model assumes that once this status is acquired it cannot be lost. For simplicity no account is taken of any maternally derived immunity or congenital malaria.

All the information concerning the vector population is summarized in one time-dependent variable, the Vectorial Capacity C, (Garrett-Jones,1964). The infection rate, h, is calculated from this value by simply allowing for incubation in the vector, a random distribution of contacts and a fixed probability that infected bites produce patent infections. This form of infection rate implies strong density-dependent regulation of transmission.

The model gives a good fit to the observed variation in malaria prevalence by age and season for the one year of baseline data in the project and also predicts patterns comparable to those observed in a similar study of another hyperendemic area in Kenya. (Molineaux et al,1978b).

4.4 (i) (b) Accumulated immunity models

Aron(1983), (also described in Aron & May(1982)), and Dutertre(1976) have developed what have become known as, accumulated immunity models. Both these models incorporate periods of temporary immunity when individuals cannot acquire infection. Furthermore, superinfection in immunes is assumed to slow down the rate of loss of immunity. The two models differ in their assumptions concerning the loss of immunity. Aron and May(1982) assume that immunity lasts for a fixed period of time, τ, in the absence of any further re-infections. If a re-infection occurs, immunity is maintained until an interval of τ occurs without any re-infection. If infections arrive at a constant rate in an independent and random way the result is that the rate at which immunity is lost decreases as the transmission rate increases (i.e. repeated infections helps maintain immunity). The rate of loss of immunity is therefore explicitly related to the

intensity of transmission. These assumptions lead to a model in which the age-prevalence curves change with transmission in a way qualitatively similar to observed age-prevalence profiles. Aron(1983) indicates that the underlying assumption is that acquired immunity gradually weakens in the absence of exposure to infection but quickly strengthens if additional exposure occurs before the "immune period", (Υ) of the previous infection has elapsed. A more detailed examination of this model is made in Chapter 5.

The model by Dutertre(1976) is more complicated, involving two forms of immunity and describes the loss of acquired immunity by two parameters. In addition to immunity acquired through past experience of infection it is assumed that there is a natural (innate) immunity to infection, distributed heterogeneously throughout the population. This natural immunity is accounted for by a negative binomial distribution of new infections per person. In the absence of re-infections, acquired immunity is lost at a constant rate, which is assumed to be about 10 years. If infection occurs within an interval of one year, then immunity is prolonged. This model assumes two separate levels of immunity, one long-term and a second which occurs on a much shorter time scale. After recovery from an infection long-term immunity is acquired and re-infections induce additional short-term immunity. Dutertre(1976) makes similar assumptions to the Garki model concerning the non-infectiousness of superinfections. However, in Dutertre's model positive individuals transfer between the infectious and non-infectious state several times before either becoming immune or returning to the susceptible state. The age-specific prevalences for this model are qualitatively similar to the Aron and May model. Among older age groups however, the prevalence is lower due to the effects of the long term immunity assumption.

4.4 (ii) Continuum immunity models

Many of the age-related factors mentioned in Chapter 2 seem to indicate that malaria epidemiology depends on quantitative aspects of infection rather than the presence or absence of the disease. For example, children gradually acquire the ability to control the pathological effects of infection but only after repeated infections. These types of gradual changes cannot accurately be described by models which divide the human population into discrete categories such as uninfected and infected. One further problem with the compartmental approach is that parasites can exist in widely varying densities within the human population, the levels of which can fluctuate in an individual quite widely from day-to-day. The estimation of prevalences from individuals with

low parasite densities will tend to produce "false negatives", so that any model based on such data will also reflect the error (Aron,1982). Aron and May(1982) suggest that an accurate model for the transmission dynamics of malaria will need to move away from the traditional approach of dividing people into discrete classes, toward a more detailed model in which the clinical symptoms and the dynamics of the immune response depend on the magnitude of the parasite burden of the individual or the accumulated past experience of infection, (similar to the "macroparasitic" continuum models described by Anderson and May,1978; 1979). Dietz(1980) proposed such a model, where the recovery rate and the production of gametocytes were assumed to be dynamic variables which responded continuously to the rate of infection, this idea was subsequently formulated into a continuum model by Elderkin et al(1977).

In brief, the model assumes that asexual stages originate indirectly from the reservoir of gametocytes by way of bites from sporozoite positive mosquitoes, the gametocytes are themselves recruited from the asexual stages. Gametocyte levels decline through natural mortality and only the asexual stages are destroyed by immune processes. Resistance depends on levels of parasites in the blood and is boosted by stimulation from the asexual stages but decays in the absence of stimulation. The model demonstrates a similar type of immune response boosted by exposure to infection as the Aron and May model(1982). The conclusion is also qualitatively similar. Both models show that the "gametocyte reservoir" in the population is at a low level when transmission is low but increases as transmission rises, and hence the asexual population increases, (Aron and May (1982) state that the adult prevalence in their model may be used as an indication of the "gametocyte reservoir"). The gametocyte levels start to decline in the presence of higher transmission levels as the immune processes act more and more strongly against the asexual stages.

CHAPTER 5 An age-structured Ross/Macdonald model

5.1 Introduction

Compartmental models are widely used to study the dynamics of viral, bacterial and some directly transmitted protozoan infections, and in the past they have been used extensively to describe the population dynamics of malaria (Ross,1916; Dietz et al,1974; Aron and May,1982). Compartmental models are based on the approach of dividing a population of hosts into discrete classes according to their epidemiological status, for example susceptibles, those incubating, infectious individuals, carriers and immunes. The types of classes selected and the rate of transition between the classes being determined by the individual epidemiological properties of the disease and the biology of the life cycle of the infectious agent.

The simple deterministic model introduced in this chapter is based on what has become known as the Ross/Macdonald model. This model combines the basic structure of Ross's two equation model (Ross,1911), (section 4.1 (i), eqs.(4.3) and (4.4)), with the Macdonald(1952a) notation and ideas concerning the mosquito population, (section 4.1 (iii), and eqs.(4.5a) and (4.5b)). The Ross/Macdonald model defines the rates of change with time of the infected portions of the human (x) and vector populations (y):

$$\frac{dx}{dt} = \frac{(a\ b\ M)}{N}\ y\ (1-x) - rx \qquad (5.1)$$

$$\frac{dy}{dx} = a\ x\ (1-y) - \mu'' y \qquad (5.2)$$

where, r is the human recovery rate, μ'' is the mosquito mortality rate, a is the rate of biting on man by a single mosquito per unit time, b the proportion of infected bites that produce an infection in man and M/N is the number of female hosts per human host.

The Ross/Macdonald model captures most of the basic features of the man-vector interaction and is universally recognised as the basis for mathematical models for malaria epidemiology. The structure and behaviour of the model and also the classic work by Ross and Macdonald from which it was

derived have been comprehensively described by Aron and May(1982), Nedelman(1985), Bailey(1982), Molineaux(1985a).

This chapter outlines the structure, assumptions and essential features of the model but also includes several modifications. These include a more detailed division of the human population with respect to epidemiological status and age, and also detailed equations which describe the incubation and transmission of disease in the vector population. The model is extended and developed in the chapter to investigate the effects that age-dependent human death rates, parasite-induced death rates and immunity in the human population have on the predicted prevalence of malaria. The models are used to look at the consequences of a variety of different assumptions concerning the exact nature of immunity and their impact on the accuracy of the model as assessed by suitable epidemiological data. Comparison of observed and model results is also used to highlight such parts of the model structure that generate a reasonable mirror of observed patterns.

The following chapter focuses on the acquisition and maintenance of effective immunity in different portions of the human population and also explores other aspects of the interaction between the parasite and host populations which may be important in determining the prevalence of malaria in the human population. The patterns generated by the models of both this chapter and those in the following chapter, are used to discuss the shortcomings of the basic Ross/Macdonald type model.

5.2 Model structure and parameters

5.2 (i) The basic model for the human population

The basic model is a deterministic compartmental model, in which the human population is divided into four epidemiological classes; susceptibles (X), latents (H), infectious individuals (Y) and Immunes (Z). Latent individuals are identified as those who have been infected but in whom infection is not yet patent, such that transmission to mosquitoes is not possible if an individual is bitten by a susceptible mosquito. Infected individuals are defined as being both patent and capable of transmitting the disease to susceptible vectors. The number of individuals of age a, at time t, who are susceptible, latent, infectious and immune are given by the variables X(a,t), H(a,t), Y(a,t) and Z(a,t) respectively. The numbers in each epidemiological class vary with age and time as individuals move into, out of and between classes according to defined rates of flow, such

as the birth rate, death rate, the rate of disease transmission and the rate of acquisition of immunity (see fig. 5.1).

The total number of susceptible, latent, infectious and immune individuals at time t, are X(t), Y(t), H(t) and Z(t) respectively; these aggregated values are derived by summing or integrating over all age classes, e.g.

$$X(t) = \int_0^{\infty} X(a,t)\ da \qquad (5.3)$$

The total population size, N(t) is the sum of X(t), Y(t), H(t) and Z(t).

A very simple demographic model is included in the basic structure. It is assumed that the size of the human population remains constant throughout time, so that at each time interval, new born susceptible individuals enter the population in equal numbers to the deaths in all age classes that have occurred in the previous time interval. Initially the death rates are assumed to be independent of both age and parasite status, so that the population is stable and has an exponential age distribution.

All susceptible (X) individuals become infected at a rate L (where L is the transmission rate from mosquito to man), and leave the latent class (H) to join the infectious class (Y) at a constant age-independent rate σ, where $1/\sigma$ is the average duration of the pre-patent period. Similarly infectious individuals recover at a constant rate r, where $1/r$ is the average duration of infectiousness, to become immune (Z). Immunity is assumed to be lost at a rate π. The death rate, μ, for all individuals is assumed to be constant up to age, a^{max} = 40 years, irrespective of epidemiological status, and infinite thereafter. An additional disease-induced death rate α, for individuals in the infectious class (Y), is considered later in the chapter. A flow diagram summarising the various epidemiological classes and parameters of the human population is shown in fig. 5.1.

This simple sequence of events provides the framework for the part of model which describes the dynamics of malarial infection in the human population. A series of first-order non-linear partial differential equations can be used to describe the rates of change of X, H, Y and Z with respect to both age and time:

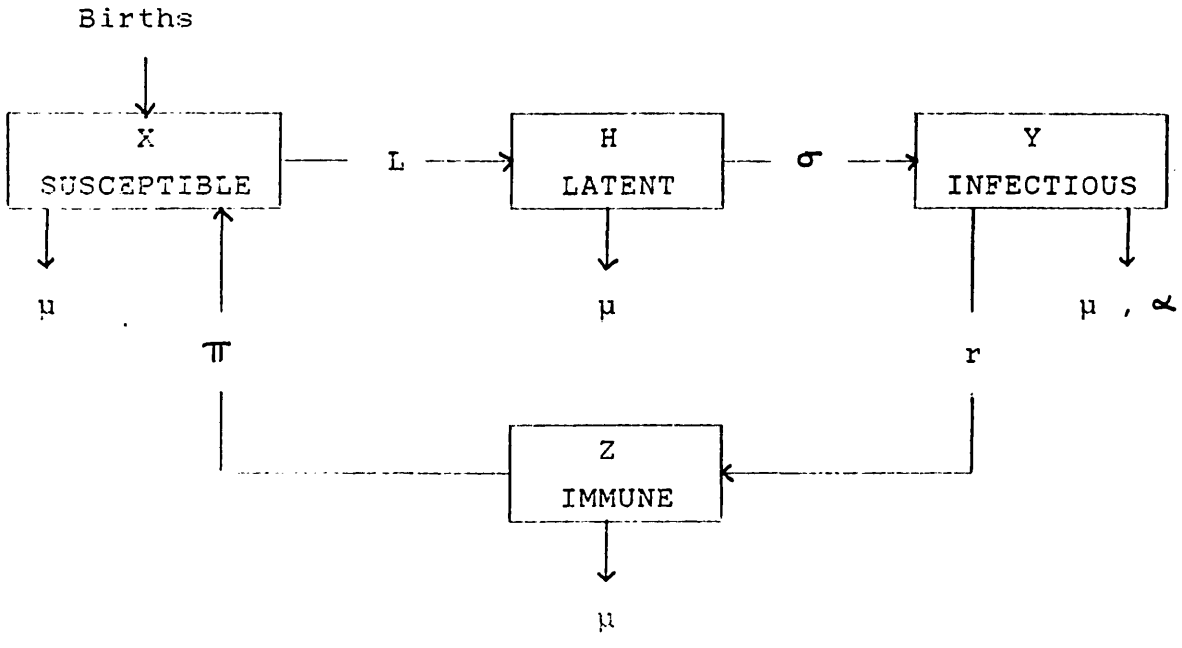


Fig. 5.1. Diagrammatic flow chart illustrating transitions between classes of the human population for the model in eqs.(5.4) - (5.7).

The human population is divided into susceptibles (X), latent individuals (H), infectious individuals (Y) and immune individuals (Z). Rate parameters determine the flow of individuals between these classes: the per capita transmission rate L, the natural death rate μ, the disease induced death rate α, the recovery rate r, the rate at which individuals become infectious σ, and the rate of loss of immunity π (see table 5.1).

$$\frac{\delta X(a,t)}{\delta t} + \frac{\delta X(a,t)}{\delta a} = -(L + \mu)\, X(a,t) + \pi\, Z(a,t) \qquad (5.4)$$

$$\frac{\delta H(a,t)}{\delta t} + \frac{\delta H(a,t)}{\delta a} = L\, X(a,t) - (\sigma + \mu)\, H(a,t) \qquad (5.5)$$

$$\frac{\delta Y(a,t)}{\delta t} + \frac{\delta Y(a,t)}{\delta a} = \sigma H(a,t) - (r + \mu + \alpha)\, Y(a,t) \qquad (5.6)$$

$$\frac{\delta Z(a,t)}{\delta t} + \frac{\delta Z(a,t)}{\delta a} = r\, Y(a,t) - (u + \pi)\, Z(a,t) \qquad (5.7)$$

The total population size is given by the sum of eqs.(5.4) - (5.7),

$$\frac{\delta N(a,t)}{\delta t} + \frac{\delta N(a,t)}{\delta a} = -\mu\, N(a,t) - \alpha\, Y(a,t) \qquad (5.8)$$

These partial differential equations describe the system, provided that initial and boundary conditions are specified. One such condition is determined by specifying the age distributions at time t = 0 (i.e. by specifying X(a,0), H(a,0), Y(a,0), Z(a,0), for all values of a). The other boundary condition follows from the assumption that all individuals are assumed to be born susceptible to infection, so that X(0,t) = N(0,t) and H(0,t) = Y(0,t) = Z(0,t) = 0, for all values of t. The validity of this second assumption with regard to maternally derived antibodies is discussed later in the chapter.

The estimates for the rate parameters are made from available data reported in the literature. Data sources include both field studies and experimentally controlled laboratory studies. Table 5.1 summarises the values used for the model parameters in the human population, as estimated from the data presented in Chapter 2. Although it may be biologically more accurate to describe the movement between the various epidemiological compartments as occurring only after a certain duration of time, rather than at a constant rate (this is particularly true of latency/incubation), Aron(1983) indicates that the simpler constant rates do not have a significant effect on the outcome of this type of model. Additionally, the recovery rate is assumed to be a constant age-independent value which is also independent of the transmission rate. However, this does not mean that the

model follows Ross's assumptions concerning recovery and superinfections (Ross,1916). The estimate for the recovery rate is based on the average time taken for an individual to revert to an uninfected state, and not on the average duration of a single infection. In this way some allowance is made for the existence of superinfections without overcomplicating the model at this stage by the use of the Dietz's expression (in Bailey, 1975, pp317-322) for superinfection, (see also Chapter 4, section 4.1 (ii)).

Table 5.1

Parameter values used in the basic model for humans

L	the per capita infection rate	eq.(5.20)
b	the probability that an infected bite produces an infection in the human host	0.02
σ	the per capita rate of transfer from the latent to infectious state	0.46/week
r	the per capita rate of becoming negative	0.038/week
π	the per capita rate at which immunity is lost	0
μ	the per capita natural mortality rate	0.0005/week
α	the per capita disease induced mortality rate	0 0.00384/week

(unless otherwise stated, these values have been used in all simulations)

5.2 (ii) Transmission rate and basic model structure for the vector population

For the purposes of the model, transmission is assumed to take place in an isolated homogeneous community where no migration in either the human or the mosquito population occurs. A constant female mosquito population of size, $N''(t)$ is also assumed; a similar set of parameter definitions using primes ("), is used for the mosquito population, although the parameters $N''(t)$, $X''(t)$, $H''(t)$ and $Y''(t)$ all refer to the female mosquito population only. Deaths occur in the mosquito population at a constant rate of μ'', per mosquito, and each death is compensated for by the emergence of one mosquito.

All newly emerged mosquitoes are assumed to be susceptible to infection.

The rate of transmission of the disease from vector to humans L, has two major components,

(i) the proportion of the female mosquito population that is sporozoite positive, $\frac{Y''(t)}{N''(t)}$,

and (ii) the transmission coefficient ß, which is a measure of the rate of contact between humans and mosquitoes.

The transmission coefficient may be divided into two further components; the probability of contact between infected mosquitoes and susceptible humans MBR, (known as the man-biting rate, or number of bites per person per unit time), and the probability that contact results in infection in the human, (b).

Assuming that both mosquito and human populations are unchanging, the simplest expression for the transmission rate L, is the product of the sporozoite rate and the transmission coefficient:

$$L = ß \frac{Y''(t)}{N''(t)} \qquad (5.9)$$

where, ß is the product of MBR and b.

MBR is a measure of both the number of female mosquitoes per human host (M/N), and the biting behaviour of the individual vectors, IMBR. IMBR is essentially a behavioral parameter dependent on the product of the feeding frequency U, (calculated as the inverse of the duration of the gonotrophic cycle), and the probability that a human is chosen for the blood meal (P). Thus:

$$IMBR = U\ P \qquad (5.10)$$

A refinement to the rather simplistic expression for the transmission rate given in eq.(5.9) is possible if all the rates involved in the mosquito population i.e. infection rate, deaths and births are interpreted as continuous rates. It is then possible to produce a pair of differential equations representing the dynamics of disease in the mosquito population.

$$\frac{d}{dt} X''(t) = \mu''(X''(t) + Y''(t)) - (L''+ \mu'') X''(t) \qquad (5.11)$$

$$\frac{d}{dt} Y''(t) = L'' X''(t) - \mu'' Y''(t) \qquad (5.12)$$

Recovery from infection is assumed to be negligible in the vector population and therefore omitted, as is any changes in behaviour or mortality due to infection. All female mosquitoes are assumed to feed only once during the gonotrophic cycle. The expression for the transmission rate from man to mosquito, L", is similar to that of mosquito to man (eq.(5.9)) and is expressed as:

$$L'' = ß'' \frac{Y(t)}{N(t)} \qquad (5.13)$$

where ß" is the product of IMBR and b". IMBR is described as the number of bites by a single mosquito per person per unit time. The definitions for the two transmission coefficients ß and ß" are not exactly symmetrical, since the transmission of disease is controlled in both cases by the biting behaviour of mosquitoes. Uninfected mosquitoes can acquire infection only by their own bites (IMBR) whereas uninfected humans may acquire infection from the bite of any infected mosquito (MBR). Appendix E.1 summarises the relationship between the various parameters involved in the calculation of the transmission coefficients, ß and ß".

If the derivatives $\frac{d}{dt} X''(t)$ and $\frac{d}{dt} Y''(t)$ of eqs.(5.11) and (5.12) are set to zero, the equations can be solved simultaneously to give an expression for the equilibrium sporozoite rate (proportion of infectious vectors) in terms of the equilibrium number of infectious humans, $\overline{Y}(t)$.

$$\frac{\overline{Y}''(t)}{\overline{N}''(t)} = \frac{ß'' \overline{Y}(t)}{ß'' \overline{Y}(t) + \mu'' \overline{N}(t)} \qquad (5.14)$$

Using eqs.(5.14) and (5.9) it is possible to produce a expression for the mosquito to man transmission rate at equilibrium $L(\overline{Y})$, in terms of the number of infective people:

$$L(\overline{Y}) = \frac{ß\ ß"\ \overline{Y}(t)}{ß"\ \overline{Y}(t) + \mu"\ \overline{N}(t)} \qquad (5.15)$$

This quite simple refinement in the calculation of the transmission rate describes most of the important features of the dynamic interaction between the infectious parts of the vector and human populations. However the relationship between Y"(t) and Y(t) described in eq.(5.14) will inevitably lead to a situation whereby if a large proportion of the human population is infectious, a correspondingly high proportion of the vector population must also be infectious. This does not accurately reflect observed sporozoite rates. The epidemiological data presented in Chapter 2, table 2.6, clearly illustrates that the prevalence of mosquito infections in endemic areas is characteristically low. Higher prevalences have rarely been recorded, and the trend is for the prevalences to lie in the region of 1-8%, irrespective of geographic location or species of mosquito. Spatial or other heterogeneities might account for some of the difference. However, by simply taking into account the extrinsic development time of the parasite in the mosquito, more realistic estimates for the sporozoite rates can be achieved.

To include latency in the parasite population the structure of the mosquito model must be expanded to include latent vectors (H"). If the extrinsic cycle has duration $\tau"$, eqs.(5.11) and (5.12) can be replaced by three first-order differential-delay equations:

$$\frac{d}{dt} X"(t) = -(L" + \mu")\ X"(t) \qquad (5.16)$$

$$\frac{d}{dt} H"(t) = L"X"(t) - \mu"H"(t) - L"\ X"(t-\tau")\ e^{-\mu"\tau"}\ \theta(t-\tau") \qquad (5.17)$$

$$\frac{d}{dt} Y"(t) = L"X"(t-\tau")\ e^{-\mu"\tau"}\ \theta(t-\tau") - \mu"Y"(t)\ ) \qquad (5.18)$$

where, $t < \tau"$, $\theta(t-\tau") = 0$ and $t > \tau,"$ $\theta(t-\tau") = 1$.

$\theta(t-\tau")$ is a step-function which ensures that latents only transfer to the infectious class Y"(t) after a time $\tau"$. The variables X"(t), H"(t) and Y"(t) have initial values of X(0)=N(0) (where N(0)= $\mu"N"$), and H(0)=Y(0)=0. The model for infection in mosquitoes defined by the above eqs.(5.16) - (5.18) possesses an exact analytical solution which is set out in Appendix E.2 (i). The new expression for the equilibrium sporozoite rate calculated from these solutions becomes:

$$\frac{\overline{Y"}(t)}{\overline{N"}(t)} = \frac{ß" \overline{Y}(t)\, e^{-\mu"\tau"}}{ß" \overline{Y}(t) + \mu" \overline{N}(t)} \qquad (5.19)$$

(see Appendix E.2 (ii)).

The expression for $L(\overline{Y})$ as a function of $\overline{Y}(t)$ may now be given as:

$$L(\overline{Y}) = \frac{ß\, ß" \overline{Y}(t)\, e^{-\mu"\tau"}}{ß" \overline{Y}(t) + \mu" \overline{N}(t)} \qquad (5.20)$$

This simple refinement means that low prevalences in the vector population can occur despite high prevalences in man. The explanation is that a significant proportion of infected mosquitoes die before the infection becomes patent.

Many of the parameters used to describe transmission between the vector and host populations can be estimated from both field studies (quantitative measures of vector population biology and behaviour) and laboratory studies (incubation periods and death rates). Table 5.2 indicates the values used in the model which are estimated from data presented in Chapter 2. These parameter values are based on the major vector species in Africa, A.gambiae and A.funestus and are typical of a tropical continental region of W.Africa, with savanna climate. U and P are used as simple representations of complex sequences of biological processes which thus avoids the need to describe IM3R as a complex dynamic function of numerous human and mosquito factors. These two parameters can be estimated quite easily since analysis of blood-meals allows estimation of the value of P for a particular vector species (Garrett-Jones, Boreham and Pant,1980), (section 2.1 (i) (a)), and if it is assumed that a female mosquito feeds only once during the gonotrophic

cycle, U is simply the inverse of the duration of the gonotrophic cycle which can also be quite easily estimated (section 2.1 (i) (c)). Accurate estimates for b and b" are almost impossible since they depend on an infinite number of human and vectorial factors about which very little is known. Sections 2.1 (i) (d) and 2.1 (ii) (c) summarise the qualitative evidence available for the estimation of b and b"; in the absence of any conclusive evidence on which a more exact estimate may be based and for simplicity, b" is assumed to have a value of one.

Table 5.2

Parameter values used in the basic model for the vectors

L"	the per capita mosquito infection rate	eq.(5.13)
γ"	the extrinsic developmental period of the parasite in the mosquito	1.714 weeks
μ"	the per capita mortality rate	0.595/week
b"	the probability that a bite on an infected human host results in an infection in the vector	1.0
U	the feeding frequency	0.4/week
P	the probability that a human host is chosen for the blood-meal	0.815
M/N (m)	the number of female mosquitoes per human host	225

(unless otherwise stated, these values have been used in all simulations)

5.3 The "sterile" immunity model

The original Ross/Macdonald model assumed that a malarial infection did not induce any immunity to further infection, i.e. $\pi = 1$. Individuals were assumed to be either susceptible (X) or infected (Y). The "sterile" immunity model represents the other extreme concerning the nature of immunity to human infections i.e. $\pi = 0$. A single infection is assumed to induce life-long immunity to all further malarial infections.

5.3 (i) Equilibrium solutions for the human model

By assuming that the size of the human community is constant, i.e. that births are exactly balanced by deaths, and $\pi = 0$, it is relatively simple to obtain the equilibrium versions of eqs.(5.4) - (5.8) for infection with "sterile" immunity. By dropping all dependence on t and equating all the partial derivatives to zero, $\frac{\delta}{\delta t} = 0$ the equilibrium equations that result are a set of ordinary linear differential equations for X(a), H(a), Y(a) and Z(a):

$$\frac{d}{da} X(a) = -(L + \mu) X(a) \qquad (5.21)$$

$$\frac{d}{da} H(a) = L X(a) - (\sigma + \mu) H(a) \qquad (5.22)$$

$$\frac{d}{da} Y(a) = H(a) - (r + \alpha + \mu) Y(a) \qquad (5.23)$$

$$\frac{d}{da} Z(a) = r Y(a) - \mu Z(a) \qquad (5.24)$$

$$\frac{d}{da} N(a) = -\mu N(a) - \alpha Y(a) \qquad (5.25)$$

On integration these equations give explicit expressions for the equilibrium age-distributions for the four epidemiological classes and also for the total population. These expressions are set out in Appendix E.3 (i).

Most mathematical models for protozoan infections in human populations assume that the death rate of the individuals within the population is constant and independent of age (Bailey,1975). Although this type of mortality is unrealistic, particularly for populations in developing countries, it is mathematically more convenient to assume that mortality is constant throughout a life time. When the parameter μ in eqs.(5.21) - (5.25) is replaced by $\mu(a)$, the dependence on age has to be factored out before the equations can be solved. This can be done by introducing a new set of "starred" variables (Anderson and May,1983), e.g.

$$X(a) = X^{*}(a)\ D(a) \qquad (5.26)$$

where,

$$D(a) = \exp\ \left(- \int_{0}^{a^{max}} \mu(g)\ dg \right) \qquad (5.27)$$

and a^{max} is the maximum age of the population.

The expressions for the equilibrium age-distributions for populations with age-dependent mortality are outlined in Appendix E.3 (ii).

5.3 (ii) Calculation of the human birth rate

The simple demographic model included in the structure of the model for humans (whereby the size of the population remains constant through time), allows the birth rate N(0), to be calculated very simply. The calculations involved in estimating the birth rate are outlined in Appendix E.4. This appendix also shows, how the addition of parasite-induced mortality and age-dependent mortality modifies these calculations.

5.3 (iii) Qualitative effects of mosquito parameters

Simple mathematical models are often helpful in exposing qualitative features of the dynamics of infectious disease. Re-arrangement of eq.(5.20) and the integration of eq.(E.13) over all age classes,

$$\bar{\bar{Y}}(t) = \int_{0}^{a^{max}} Y(a)\ da \qquad (5.28)$$

(where a^{max} is the maximum age limit of 40 years), gives two independent expressions for the equilibrium number of infectious individuals, $\bar{Y}(t)$. By varying the value of the transmission rate L, until the difference between the estimates from the two expressions is at a minimum (e.g. by iteration) the values of the equilibrium transmission rate and equilibrium mosquito and human prevalences can be obtained for a given set of parameters.

5.3 (iii) (a) Sporozoite rate

The model for the vector population (eqs.(5.16) - (5.18)) which takes into consideration the pre-patent period of infection, predicts equilibrium sporozoite rates which are in rough accordance with observed patterns. This point is illustrated in fig. 5.2, where model predictions for equilibrium sporozoite levels are portrayed for various values of Υ" and μ".

The results also indicate very clearly how dependent the sporozoite rate is on seasonal factors. During periods of adverse environmental conditions, such as low temperatures (longer latent periods) or drought/low relative humidities (high mosquito death rates), the model predicts a rapid drop in sporozoite rates (fig. 5.2 (a)). Similar changes in prevalence created by seasonal factors have been recorded in many African regions (Zahar,1985a; 1985b; 1985c). However, observed levels are generally lower than the model predicts. The tendency for the predictions to be slightly higher than observed patterns may be accounted for, at least in part, by the underestimation of mortality rates measured in the field. Additionally overestimation of the degree of anthropophily would also lead to an overestimation of prevalences.

Work by Krafsur (1970), Carnevale (1982a), Hamon(1964) and others strongly suggests that during periods of low rainfall the death rates may fall significantly. For A.funestus in the Gambela region of Ethiopia, estimates for the death rates in the dry and wet seasons have been calculated as 0.534/week and 0.7945/week respectively (Krafsur,1970); and for A.gambiae in the Congo rates of 0.243/week (dry) and 0.4094/week (wet) have been recorded (Carnevale,1982). These results seem to indicate that much of the observed seasonal patterns of infection in mosquitoes could be accounted for by the daily survival probabilities and their effects on the proportion of the vector population that survives long enough to become infective, (eq.(5.19)). Changes in mortality, however, are not the only cause, since seasonal fluctuations in prevalence may also be generated by the influence of temperature on the period of extrinsic development of the parasite. For example, much of West Africa experiences the wide annual (and diurnal) ranges of temperatures typical of tropical continental regions; average daily temperatures vary between about 30^0C in the hot season to 20^0C in the cool season (Molineaux and Gramiccia,1980). Such decreases in temperature increase the latent period of P.falciparum from 10 days to approximately 30 days (Macdonald,1957). With a mortality rate of μ"=0.595, eq.(5.19) predicts that the prevalence of infection in the mosquito would drop from 3% to less than 1% .

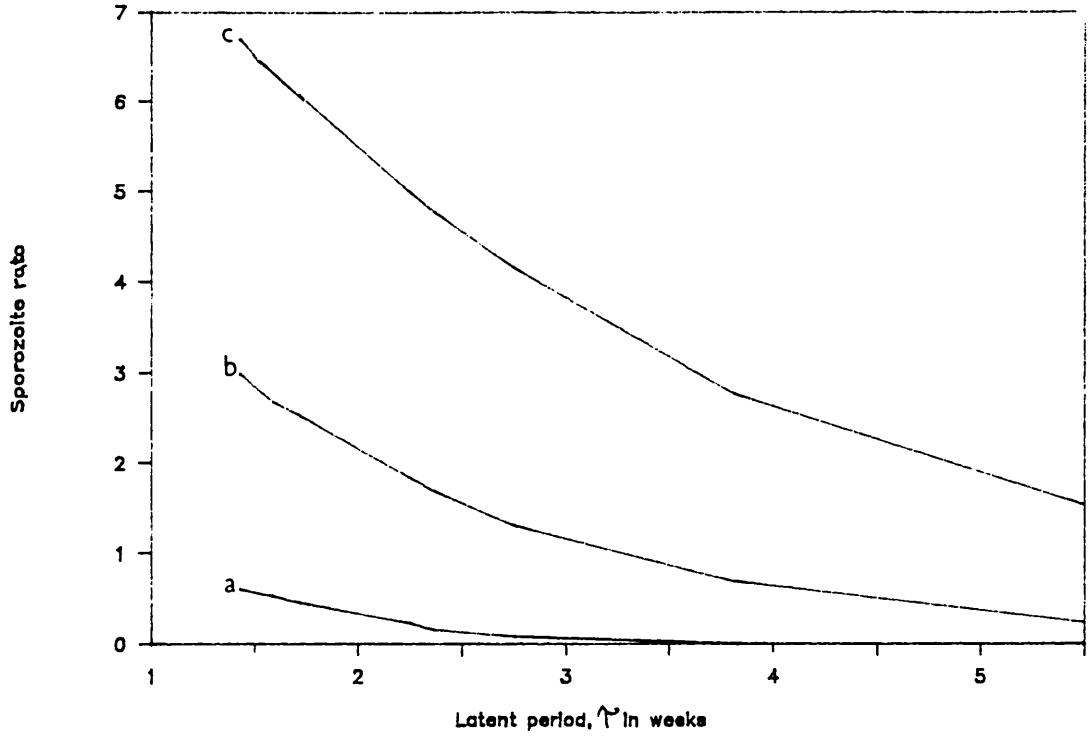


Fig. 5.2. The relationship between the prevalence of mosquito infection at equilibrium and the parameter τ'' (the extrinsic developmental period of the parasite within the vector) for three different values of μ'' (the death rate of uninfected and infected mosquitoes), (a) $\mu'' = 1.2$/week; (b) $\mu'' = 0.595$/week and (c) $\mu'' = 0.359$/week, as predicted by eq.(5.19) in the text.
(Parameter estimates as in table 5.1 and table 5.2, $\alpha = 0$)

If the additional effect of relative humidity (which can be as low as 8% in the dry season) on mortality rates is considered, this level drops below zero (fig. 5.2).

The inter-relationship of the factors which produce the sporozoite rate have been demonstrated elsewhere using alternative models and methods by Bailey(1982) and Macdonald(1952a); many of the assumptions about the vector population were however similar to those stated for the above model. Additionally, Aron and May(1982) has shown that the timing of the peak sporozoite rate within the year and between years is primarily affected by fluctuations in the numbers of vectors, a factor that is not considered in this model, where vector populations are assumed constant.

Although it is clear from the model that changes in prevalence of infection within the mosquito population may be closely correlated with the impact of rainfall, humidity and temperature on latency, mortality and the rate of emergence of adults, what is not clear, is the possible effects that seasonal changes in both the susceptibility of humans to infection and also the availability of infective gametocytes may have on mosquito prevalences.

5.3 (iii) (b) Implications for transmission

These climatic effects also have certain implications for the transmission dynamics of malaria, fig. 5.3 (a) shows how changes in the latent period and death rates affect the transmission capabilities of the mosquito population. The general point made is that latent periods and mortalities can considerably reduce the transmission from mosquito to man. One particular point of note is that long latent periods or high mortality rates may place the level of transmission in the region of the "threshold" level, below which the parasite is unable to maintain itself within the human population. Using the example of the Garki project made above, estimates of death rates and extrinsic development periods for the dry season place transmission of P.falciparum well below the threshold level. This prediction agrees very well with the observation from the Garki project that transmission of P.falciparum ceases during the cool, dry season (Molineaux and Gramiccia,1980). Figure 5.3 (a) also helps explain the inability of vectors to sustain malaria transmission at high altitudes, where a 1^0 drop in temperature per 1 000 ft. is normal, and why, in the Punjab region of India and in Pakistan, where vector species such as A.culicifacies generally have high mortality rates with low expectation of life (see table 2.5), malaria is not endemic.

Peaks in transmission during the wet season however do not occur simply because lower values for both the period of extrinsic development and death rates allow a greater

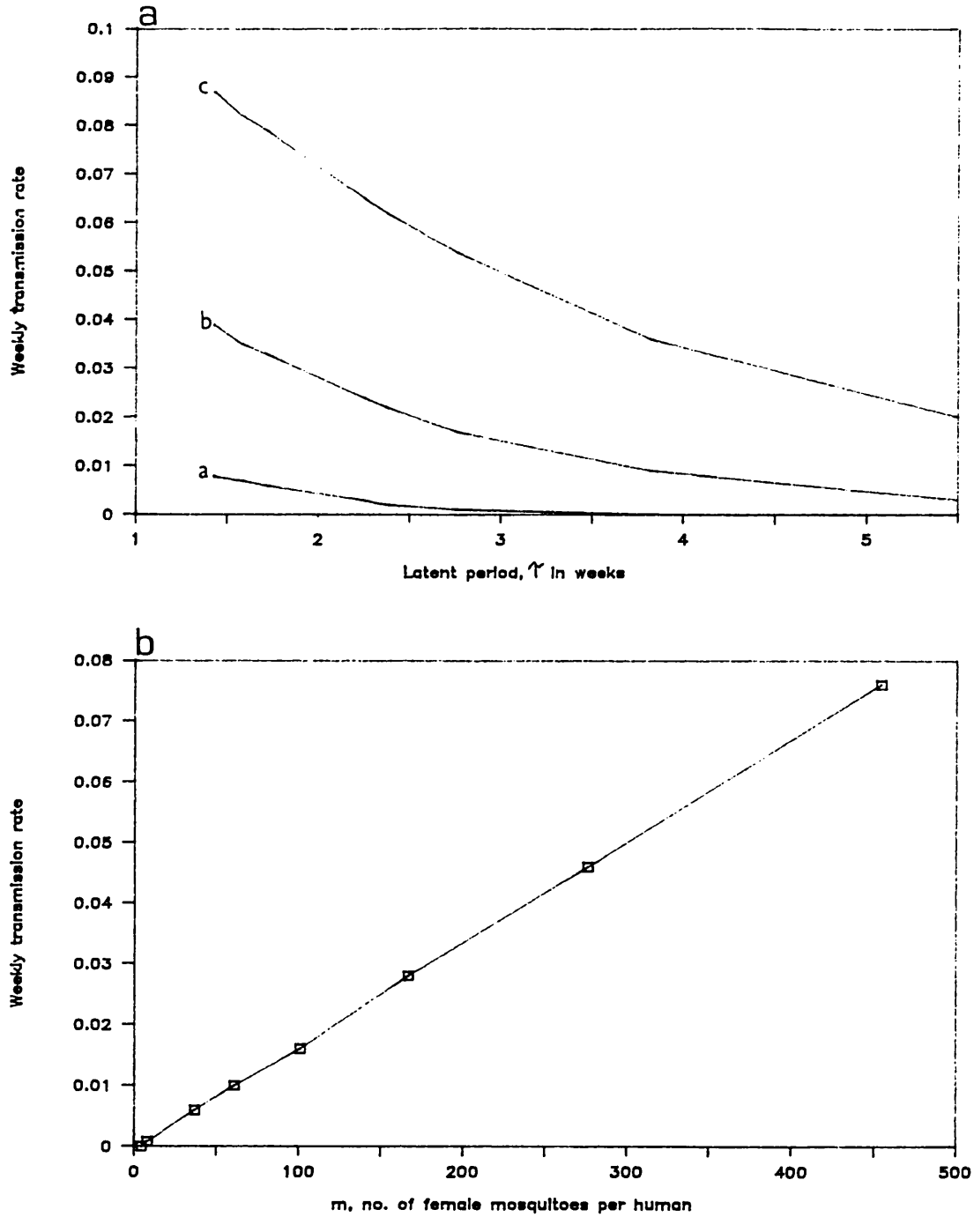


Fig. 5.3.
(a) The impact of the extrinsic developmental period (τ'') on the transmission rate from mosquito to man L, for different mosquito death rates: (a) μ'' = 1.2/week; (b) μ'' = 0.595/week and (c) μ'' = 0.359/week as predicted by eq.(5.20) in the text. (Parameter values as in table 5.1 and table 5.2, α = 0), (b) The influence of an increase in mosquito density (m) on the transmission rate from mosquito to man L, as predicted by eq.(5.20). (Parameter values as for Fig. 5.3 (a)).

proportion of mosquitoes to survive to transmit the disease. The more favorable conditions of the wet season also increase breeding success so that the density of mosquitoes also increases. Figure 5.3 (b) illustrates the rapid rise in transmission which might be expected merely allowing for increased mosquito densities during the wet season.

5.3 (iii) (c) Infection within human populations

Figure 5.4 (a) shows the human prevalence as a function of the mosquito man-biting rate (MBR). Although the predicted human prevalences are far too low compared with observed values (see Appendix B, fig. B.1) due to the oversimplified assumption of "sterile" immunity (this will be discussed further in the next section), the results are of qualitative relevance and illustrate the non-zero nature of the critical threshold in a way almost identical to Ross's original model with no immunity. The relationship is highly non-linear; close to the threshold, small changes in the abundance of mosquitoes produce large changes in the prevalence in humans. At higher abundances even large changes in the mosquito biting-rate produce little or no change in the prevalence of malaria. This relationship has an important bearing on the interpretation of the success of control programmes.

It should also be noted that the critical mosquito level is lower for longer lasting infections, fig. 5.4 (b); this is particularly interesting when considering P.vivax which is found only at the southern and northern limits of malaria distribution. Studies of this parasite in tertiary syphilitics and in prisoner volunteers have shown the infections to be long-lived with distinct relapse patterns (Coatney et al,1950a,1950b). Strains from New Guinea have frequent random relapses; sub-tropical infections generally relapse 9 months after the primary infection and strains in temperate regions frequently have primary infections which are delayed by 9 months. It is possible that seasonal conditions have modified parasite behaviour. Selection for these strains may be due to the pressure of climate in non-tropical zones where mosquitoes suitable for transmission are present in sufficient numbers only for six weeks of every year. Delayed primary or relapse infections coincide with the time when mosquito numbers are increasing, and the lower recovery rate allows transmission to continue at lower mosquito levels.

Despite the low human prevalences generated by the model, the predicted human prevalences for a range of Υ" and μ" values (fig. 5.5) seem to indicate, that when mosquito death rates are low, the infection in humans is remarkably stable and robust, even to quite large changes in the extrinsic development period.

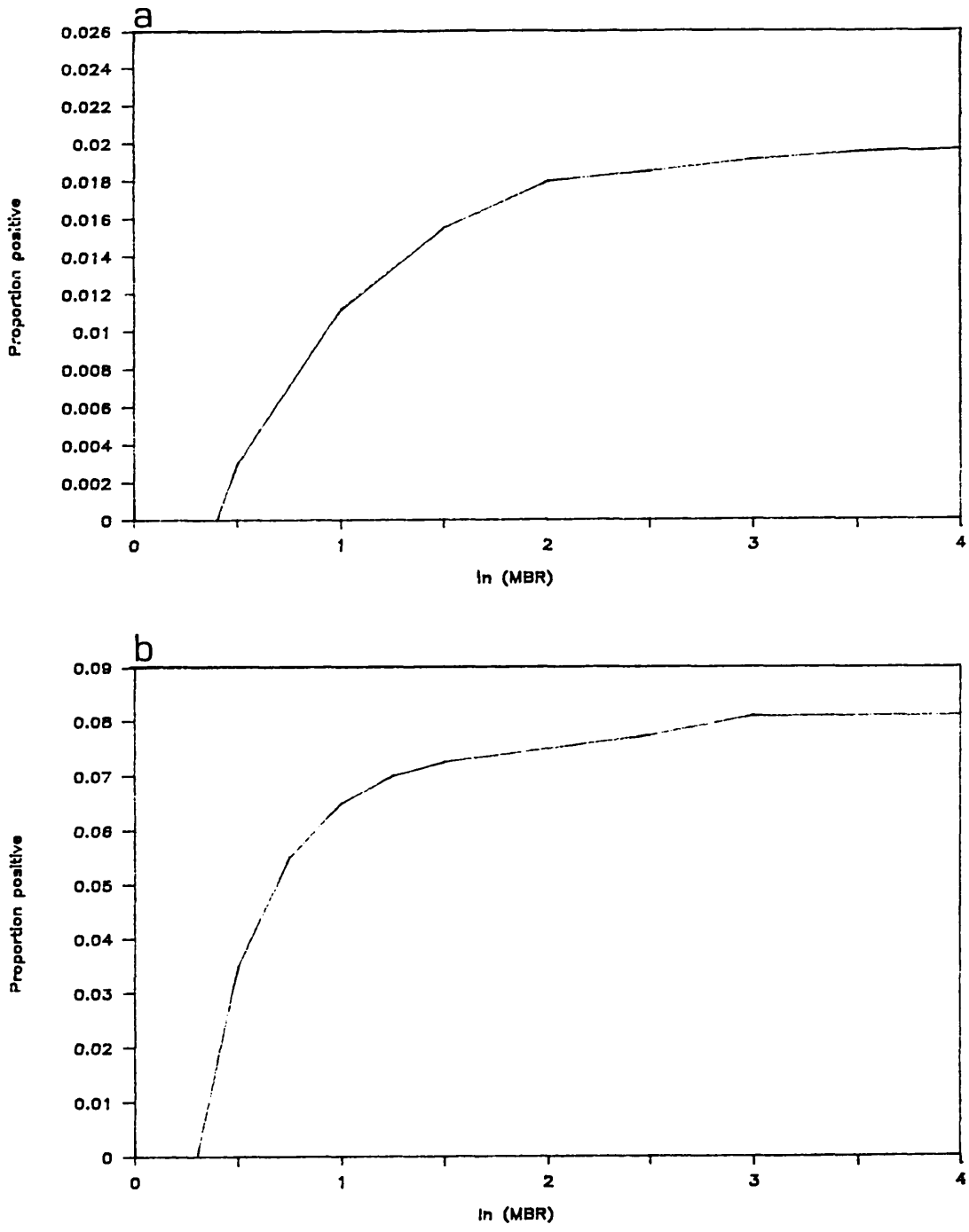


Fig. 5.4. The relationship between the prevalence of infection in the human population and In (MBR) (where MBR is the man-biting rate), as predicted by eqs.(5.28) and (5.20), when the rate of recovery in humans is assumed to be, (a) r = 0.038/week and (b) r = 0.009/week.
(Parameter values as in table 5.1 and table 5.2, α = 0)

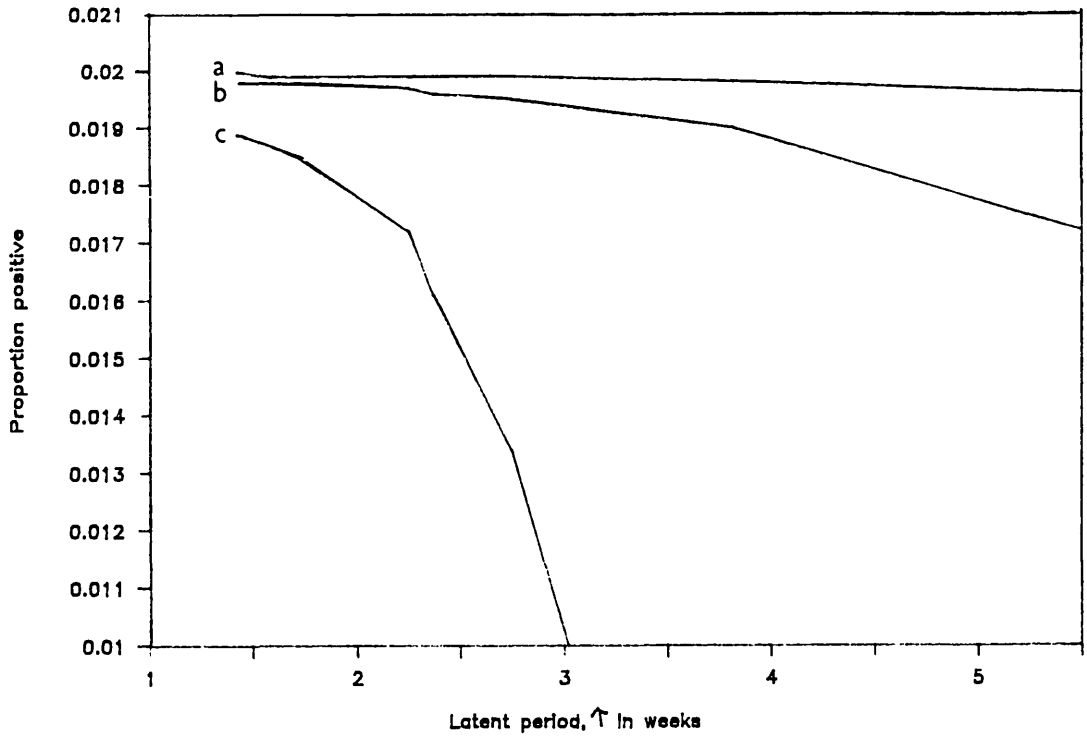


Fig. 5.5. The influence of The mosquito death rate (μ'') on the prevalence and stability of infection in the human population for three values of μ'': (a) μ'' = 1.2/week; (b) μ'' = 0.595/week and (c) μ'' = 0.359/week.
as predicted by eqs.(5.28) and (5.20).
(Parameter values as in table 5.2 and table 5.2, α = 0)

As the mortality rates among the vectors increase, the infection in humans becomes more sensitive to changes in Υ". These predictions agree with the observations by Macdonald(1952b) and Aron and May(1982) that μ" is a critical determinant of the stability of malarial infections.

5.3 (iv) Age-prevalence of human infection

5.3 (iv) (a) Equilibrium profiles

Epidemiological studies show clearly that the prevalence of malarial infection varies among different age classes within the human population (fig. 5.6 (a)). Since the model presented here is an age-structured model, it is possible to define an explicit expression for the equilibrium age-prevalence (eq.(E.13), obtained by direct integration of the equilibrium equation for Y(a) (eq.(5.23)). The dynamical predictions of the model, which assumes life-long immunity is illustrated in fig. 5.6 (b), (lower profile). The prevalences are seen to rise with age and then fall away to zero in older children, (the maximum prevalence never reaches higher than 35%). This is in sharp contrast to the predictions of the original Ross(1916) model which assumes that there is no immunity to infection (upper profile, fig. 5.6 (b)); the prevalence of infection monotonically approaches a saturation level, defined by $\frac{h}{(h + r)}$, where h is the infection rate. (For high rates of infection this level will be 100%).

The recovery rate r, and infection rate L, (equivalent to the transmission rate), have a marked influence on the shape of the age-prevalence curve generated by the "sterile" immunity model. Slower recovery times aid the "accumulation" of infection within the population, so that higher prevalences are sustained over wider age ranges (fig. 5.7 (a)); whereas high infection rates result in a higher peak prevalence and exaggerate the decline in prevalence with age, so that both maximum prevalence and zero prevalence occur at a younger age (fig. 5.7 (b)).

The models assume mortality to be independent of age and disease status; observed data however indicates that mortality is at a minimum in adults and older children, and maximum in young children (where malaria is most prevalent), with mortality starting to rise again in ageing adults. Chapter 2, section 2.2 (ii), outlines the available data on malaria related mortality.

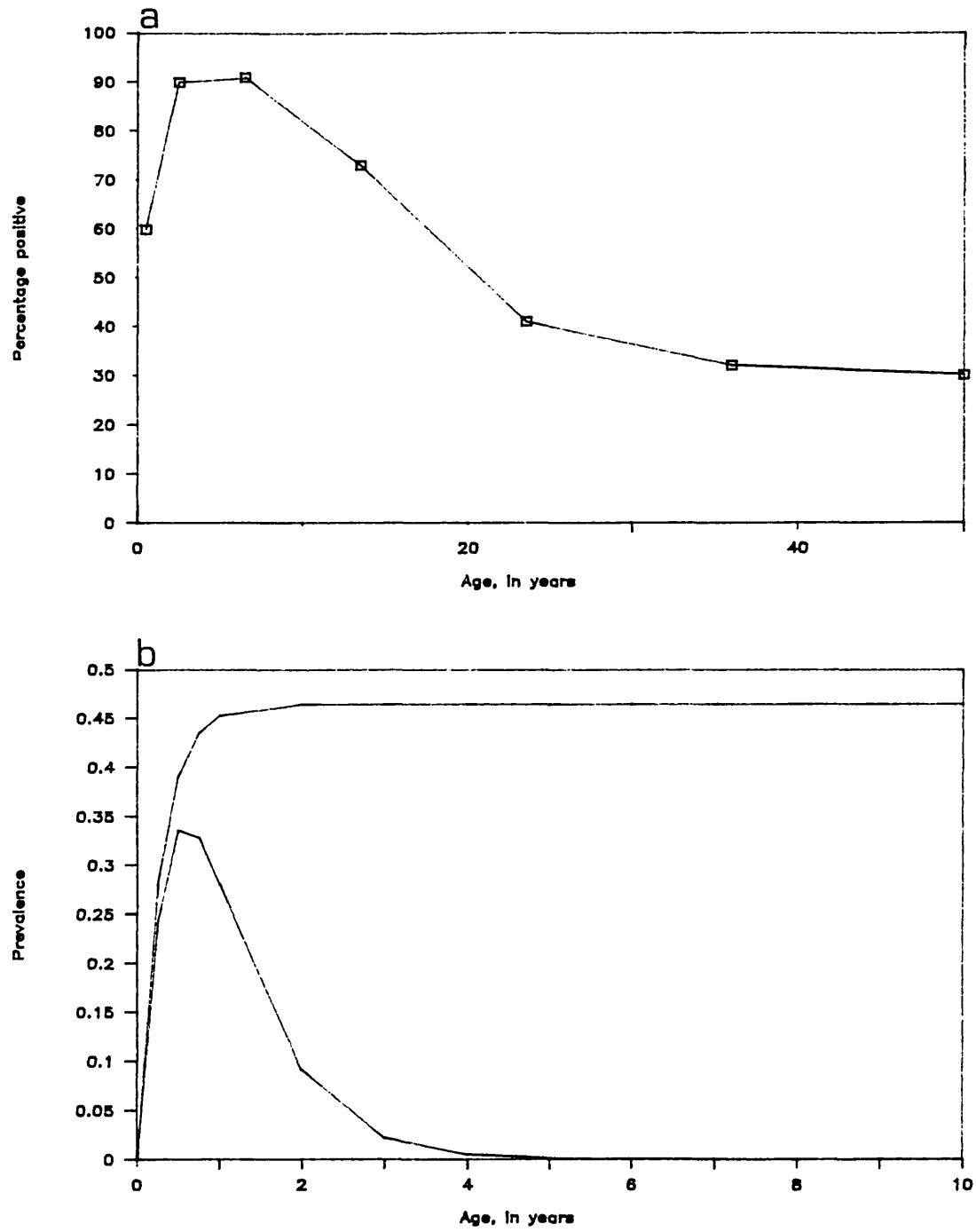


Fig. 5.6.
(a) The age-specific prevalence for P.falciparum in the hyperendemic Garki region of Northern Nigeria (Molineaux and Gramiccia,1980).
(b) Age-prevalence predictions, lower profile - the "sterile" immunity model (eqs.(5.21)-(5.25)), (Parameters as for table 5.1 and table 5.2, $\alpha = 0$); upper profile - the Ross model (Ross,1916) with no immunity, eq.(4.2) (Parameter values equivalent to above; r = 0.038/week and h = 0.0331/week).

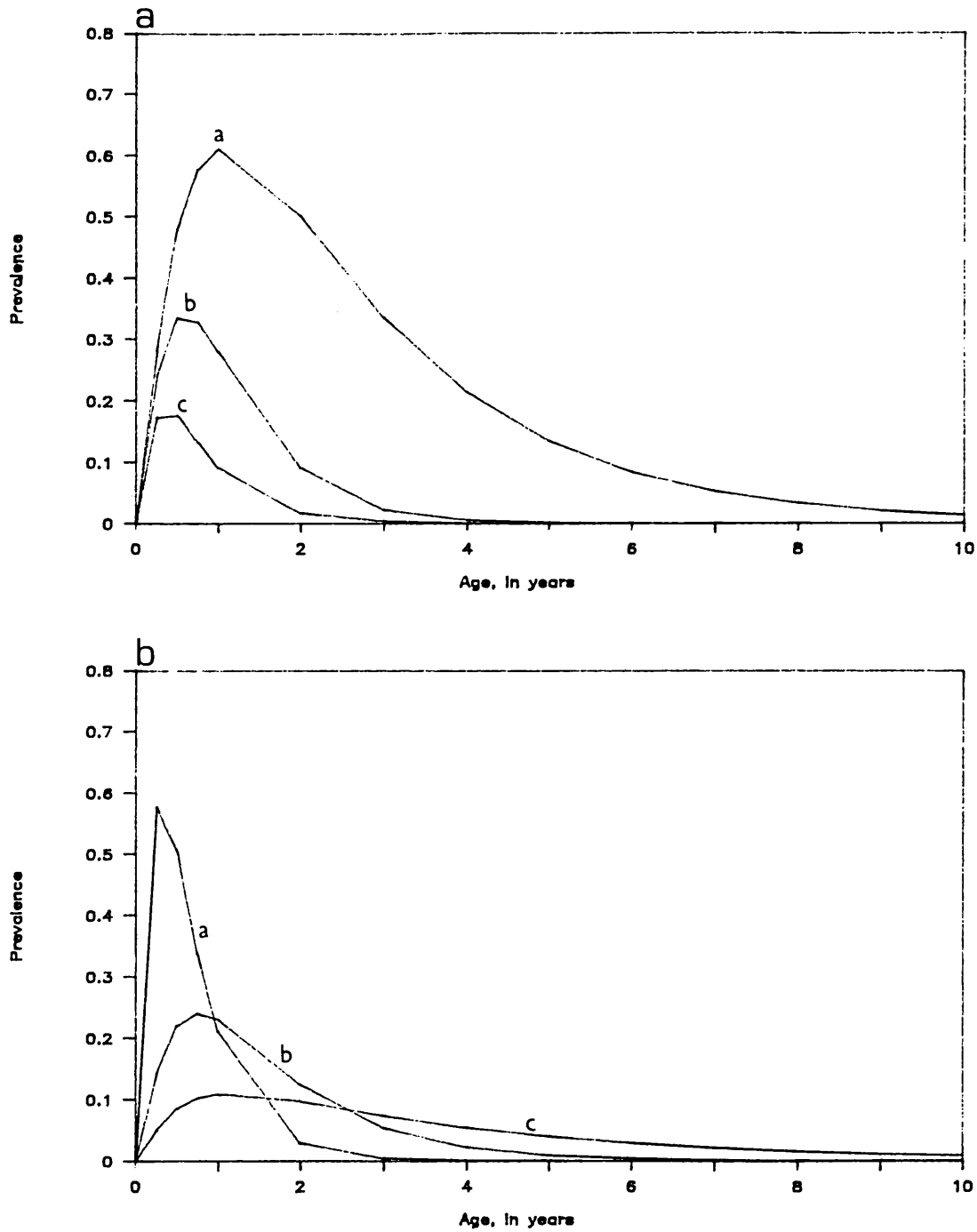


Fig 5.7.
(a) The impact of the recovery rate (r) on the age-prevalence profiles generated by eqs.(5.21)-(5.25), a: r = 0.009/week, b: r = 0.038/week and c: r = 0.1/week, (Parameters as in table 5.1 and table 5.2, α = 0).
(b) The impact of the infection rate (L) on the age-prevalence profiles generated by eqs.(5.21)-(5.25): a: L = 0.13/week, b: L = 0.018/week and c: L = 0.006/week. (Parameter values as in table 5.1 and table 5.2, α = 0)

The addition of quite high rates of disease-induced mortality e.g. 200 per 1000 per year, was found to have only a marginal effect on the value of the equilibrium transmission rate, (e.g. 0.03276/week as compared with 0.03310/week without the additional mortality). Figure 5.8 shows that the equilibrium age-prevalence profiles are also not significantly changed by the inclusion of disease-induced mortality, the prevalence is only slightly lowered. Modification of the death rate parameter μ, to take into account high rates of mortality in young children and low rates among adults also has very little effect, it changes details but not the overall pattern of change in prevalence with age. The lack of any infection in individuals over the age of eight of course minimises much of the effect that $\mu(a)$ might have on the profiles.

Although this age-prevalence model can generate a quite wide variety of dynamical patterns, neither it nor the Ross/Macdonald model (which represent the two extremes forms for immunity to infection), are able to mirror the observed patterns of the change in prevalence with age. However, the model serves as a point of departure.

5.3 (iv) (b) Maternal antibodies

An important issue that can be investigated with the model, despite the fact it fails to accurately describe immunity to infection in man, is the possible effect that maternally derived antibodies may have on the prevalence of malarial infection among infants. Chapter 2, section 2.1 (iii) (c) briefly described the available evidence for the existence of maternal antibodies. However unlike viral infections, these antibodies do not prevent infection and infants are commonly found infected with malaria. Some reduction in the severity of malarial infections among infants is attributed to these antibodies, though whether they cause a lessened prevalence is still debated.

Acquired immunity is thought to play a very small role in protection against infection well past the age of 1 year (Fleming et al,1979). Comparison of the results generated by the model for ages 0 - 36 weeks, irrespective of the assumptions concerning acquired immunity, can therefore still give valid predictions which may be compared with observed cumulative prevalence data. An overestimation by the model will indicate that maternal antibodies do make infants less susceptible to infection. In the framework of the model the cumulative prevalence (CP(a)) is represented by:

$$CP(a) = 1 - \frac{X(a) + H(a)}{N(a)} \qquad (5.29a)$$

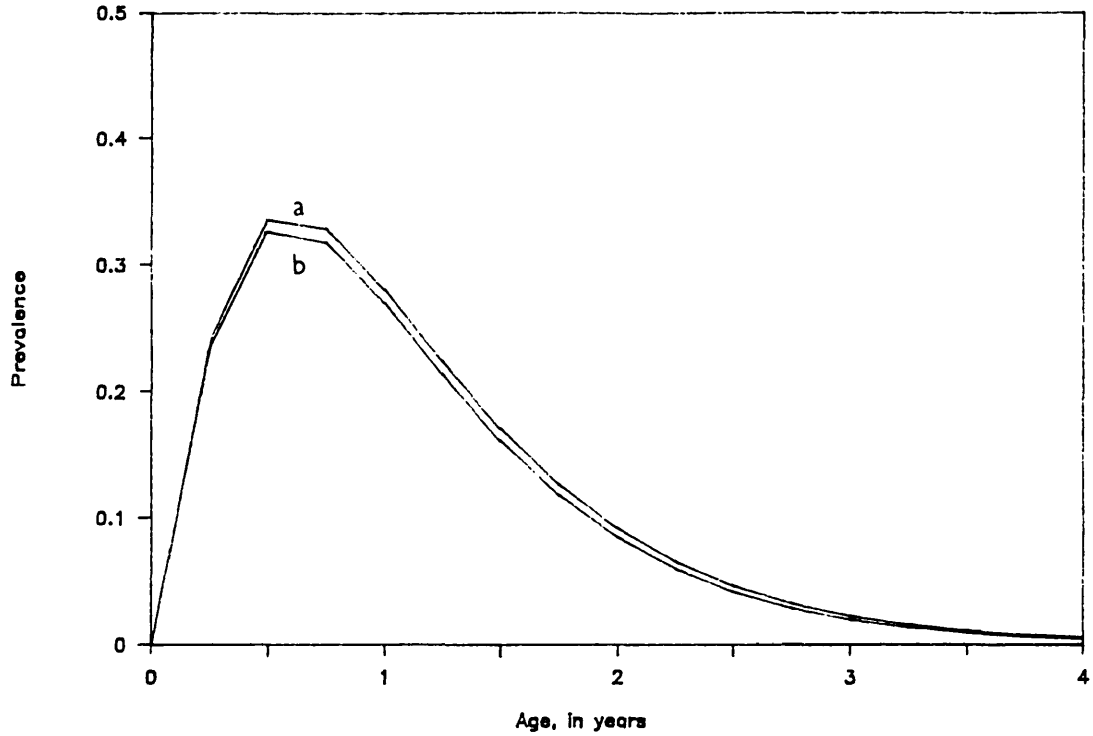


Fig. 5.8. The influence of an additional, parasite-induced mortality rate (α), on the shape of the age-prevalence profile generated by eqs.(5.21)-(5.25): (a) α = 0.00384/week and (b) α = 0.
(Parameter values as in table 5.1 and table 5.2)

Using the equilibrium expressions for X(a), H(a) and N(a) (eqs.(E.11), (E.12) and (E.20)), this prevalence may be written as:

$$CP(a) = \frac{(1 - \exp(-La)) + L(\exp(-\sigma a) - 1)}{(\sigma - L)} \quad (5.29b)$$

Due to the practical difficulties and the expense involved in the follow-up of a large number of susceptible newborns and the estimation of infant infection rates, only a very limited number of these cumulative prevalence surveys have been carried out successfully. Figure 5.9 compares the results of two such surveys with predictions made by the model. There seems to be a fairly good agreement between observed and estimated prevalences over the ages 0 - 36 weeks, particularly for the survey shown in fig. 5.9 (a). In fig. 5.9 (b) the fraction positive does however appear to increase more slowly than the observed data after the age of 12 weeks; this may well indicate that the older infants are exposed to slightly higher infection rates, although seasonal influences in the data could also create such results. On the whole, the small deviation does not seem to indicate that the generated curve departs significantly from the observed, and it is reasonable to assume that maternal antibodies do not significantly affect the acquisition of first infection and that the framework of the model accurately reflects the acquisition and latency of first infections.

It should however be noted that the two surveys mentioned above are based in the dry tropics, and evidence from more humid tropical areas seems to suggest that maternal antibodies may be important in these areas. Additionally, observed seasonal variations in the level of maternal antibody are thought to have a significant effect on the prevalence of malaria in infants (R.S. Bray, pers. comm.). If new additional evidence confirms that this factor does operate in the humid tropics, then the existing model can be easily modified to take into account the fact that for the first 6 -9 months infants may be partially protected from infection and thus acquire infections at a lower rate (L1). Protection provided by maternal antibodies may be described by the addition of a further class of individuals MA(a), of partially protected infants with maternal antibodies being lost at a constant per capita rate C.

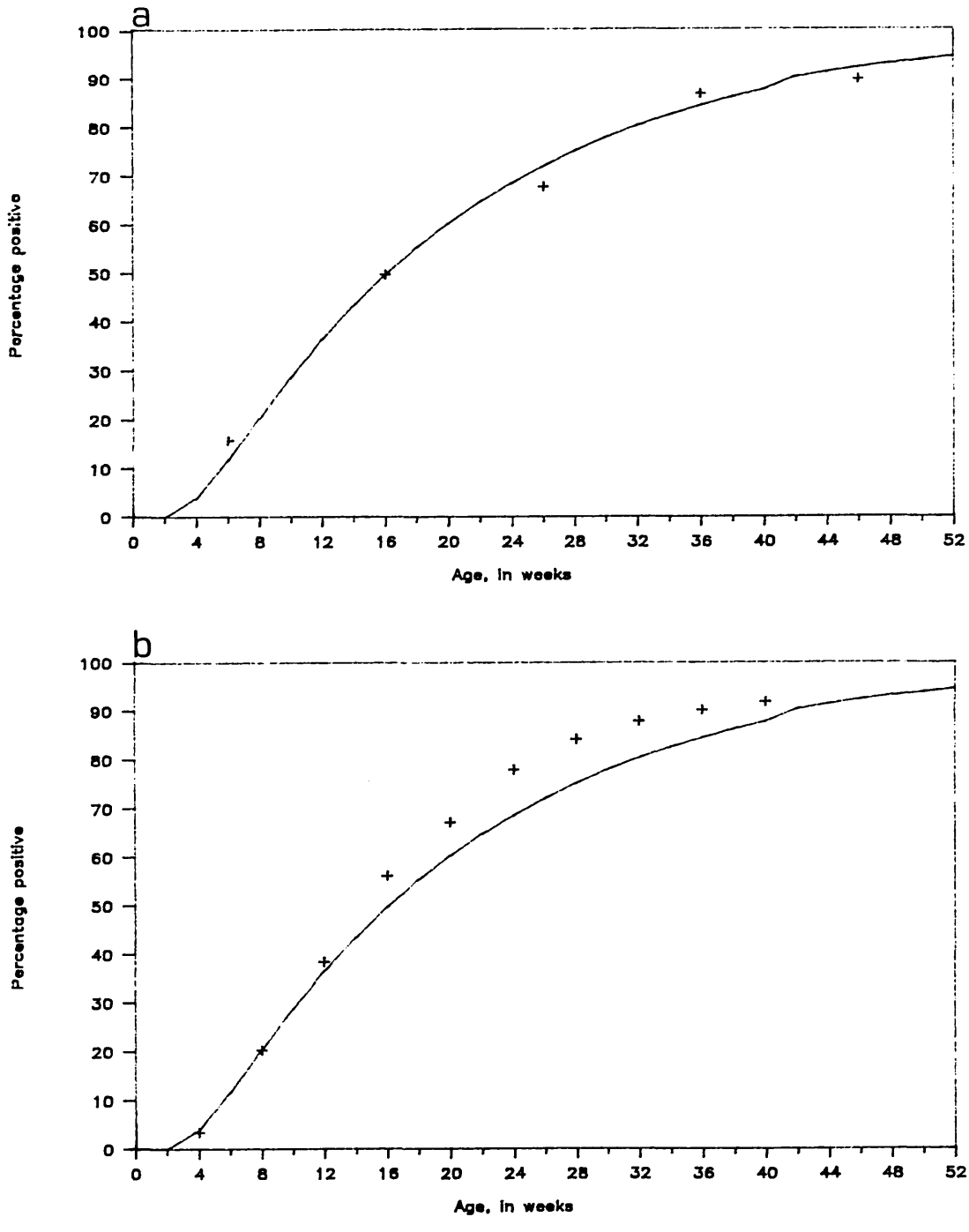


Fig. 5.9. Observed cumulative prevalence of P.falciparum by age for infants regularly tested since birth (symbols), from surveys in, (a) the Garki region of N.Nigeria, (Molineaux and Gramiccia,1980) and (b) the Nyanza Province, Kenya, (Pull and Grab,1974) compared with the cumulative prevalence generated by the model (solid line), (Parameter values as in table 5.1, α = 0 with estimated infection rates of L=0.0462/week for (a) and L=0.0588 for (b)).

Thus the equation for susceptibles (X) (eq.(5.21)) becomes replaced by:

$$\frac{d}{da} MA(a) = -(C + Ll + \mu)\, MA(a) \qquad (5.30)$$

$$\frac{d}{da} X(a) = C\, MA(a) - (L + \mu)\, X(a) \qquad (5.31)$$

and the latents (H) (eq.(5.22)) by,

$$\frac{d}{da} H(a) = L\, X(a) + Ll\, MA(a) - (\sigma + \mu)\, H(a) \qquad (5.32)$$

where, $Ll < L$, $1/C$ is the average duration of the maternal antibody effect, and the initial conditions $MA(0)$ = births, $X(0) = H(0) = Y(0) = Z(0) = 0$ exist. (Mathematical models of viral infections with maternally derived protection exist, although in the case of viruses protection is assumed to be total and of 6 months duration (Anderson and May,1983)).

5.3 (iv) (c) The impact of reduction in prevalence

The "sterile" immunity model also can provide qualitative insight into the impact of both temporary control, such as a single nation-wide administration of drugs to a portion of the population, and/or permanent control. A reduction in the prevalence of reservoirs of infection within the human population without alteration of the capacity of the vector population to transmit malaria results in an immediate reduction in transmission followed by an oscillatory return to the original level, (fig. 5.10 (a)). The total prevalence (fig. 5.10 (b)), and total number of cases per quarter (fig. 5.10 (c)), follows the same type of oscillations, with the peaks and troughs occurring at identical time periods as those exhibited by the transmission rate. The impact of permanent reduction results in similar oscillatory behaviour before the disease reaches a new lower equilibrium level (fig. 5.11 (a), (b) and (c)). In both cases the initial reduction in prevalence is not maintained and prevalences quickly rise again to equilibrium levels (but below pre-control levels for permenant control) which are stable through time; the speed with which this return to equilibrium occurs is an indication of the stability of the disease to changes in the parasite reservoir.

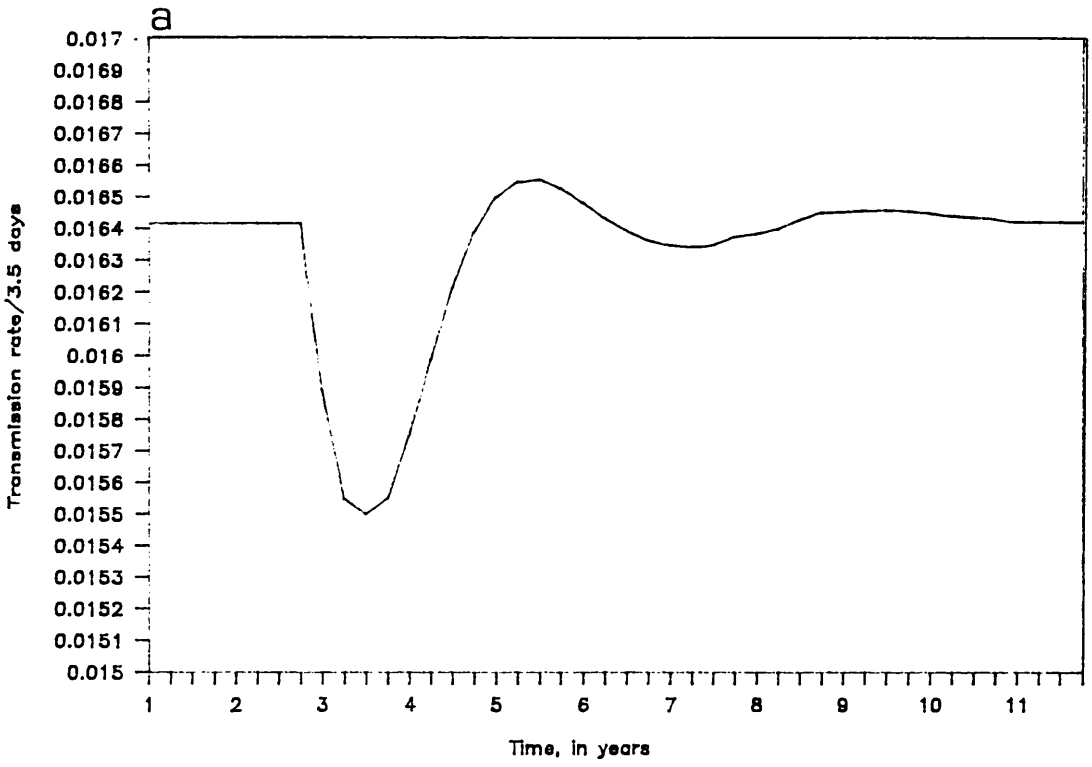
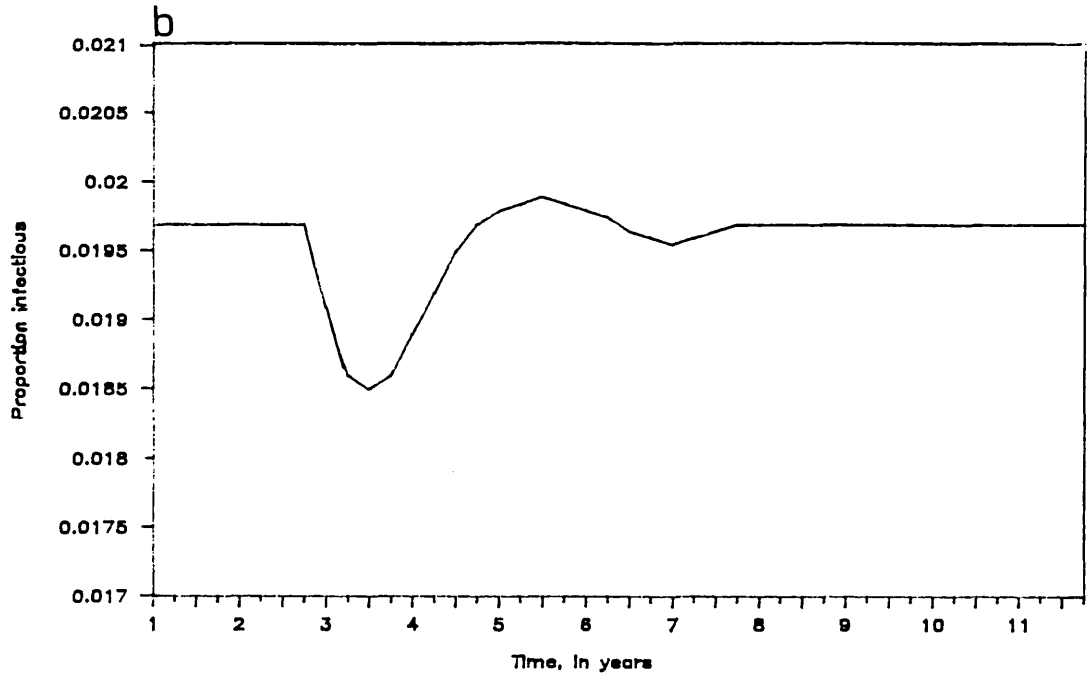
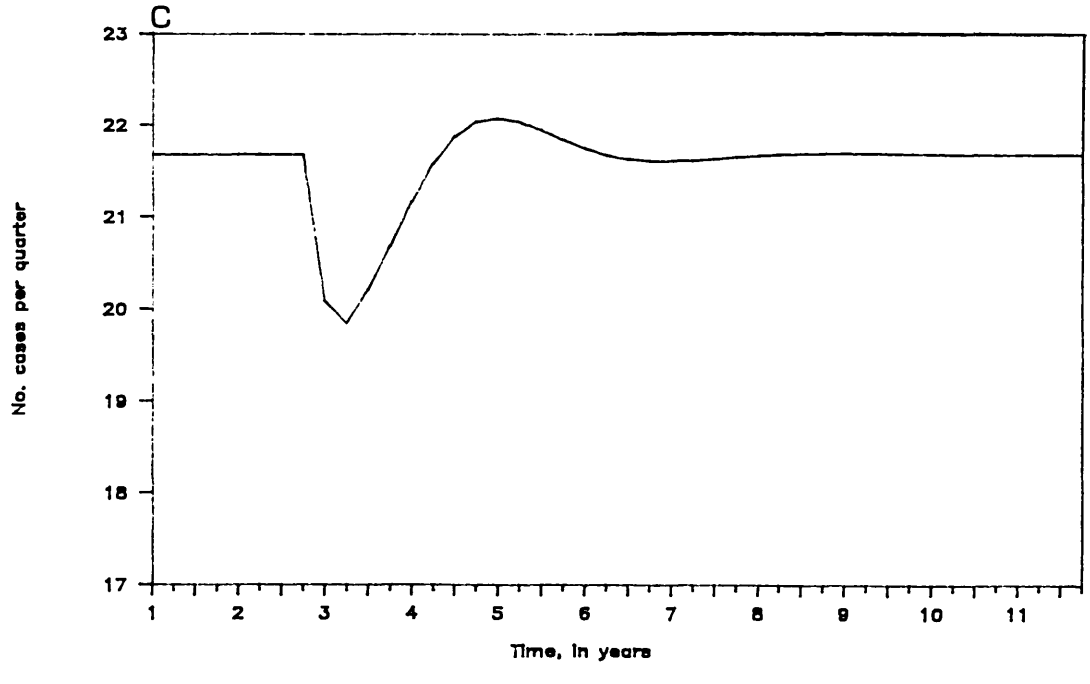


Fig. 5.10 (a)-(c)
The predicted impact on (a) the transmission rate, (b) the total prevalence of infection in the human population and (c) the total number of cases per quarter; of a single 10% reduction to the reservoir of infection in an isolated human population of 2 000, at time = 3 years, without any alteration to the capacity of the vector population to transmit malaria, (Parameter values as in table 5.1 and table 5.2, $\propto$ = 0.00384/week)


b
0.021
0.0205
0.02
0.0195
0.019
0.0185
0.018
0.0175
0.017
Proportion infectious
1
2
3
4
5
6
7
8
9
10
11
Time, in years


c
23
22
21
20
19
18
17
No. cases per quarter
1
2
3
4
5
6
7
8
9
10
11
Time, in years

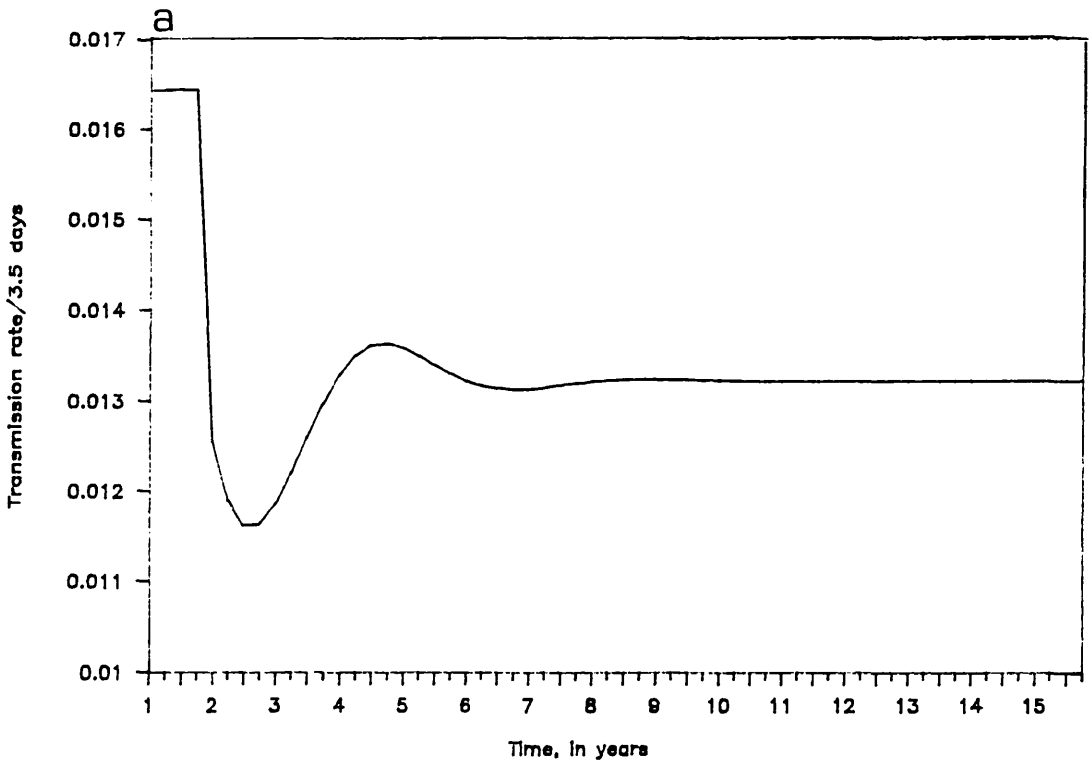
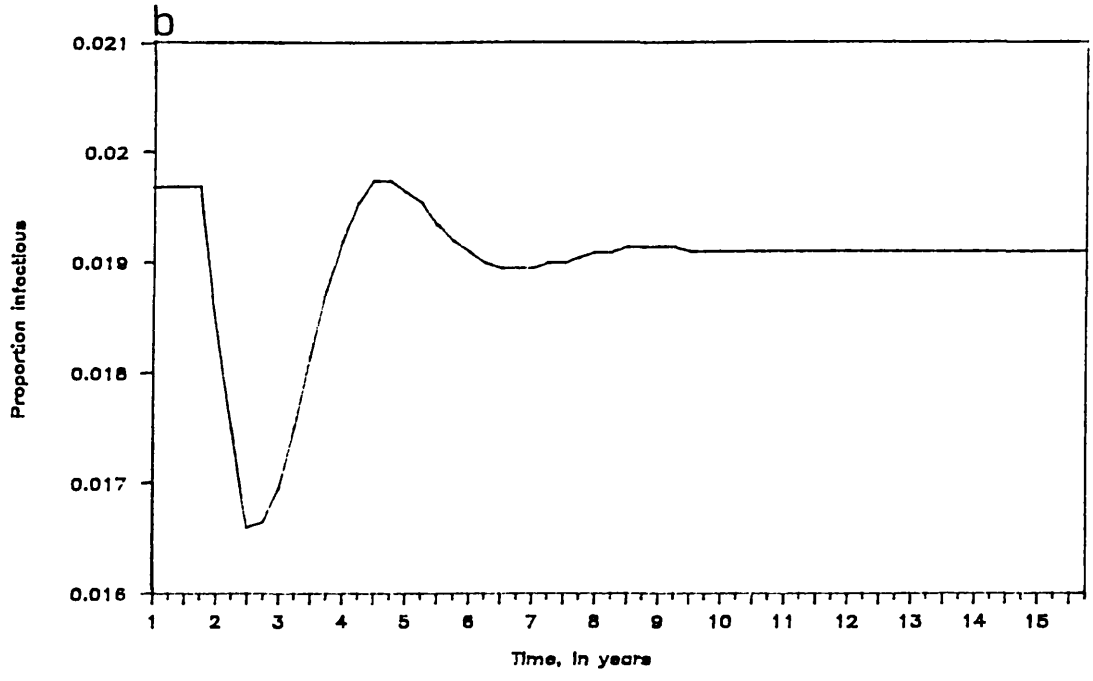
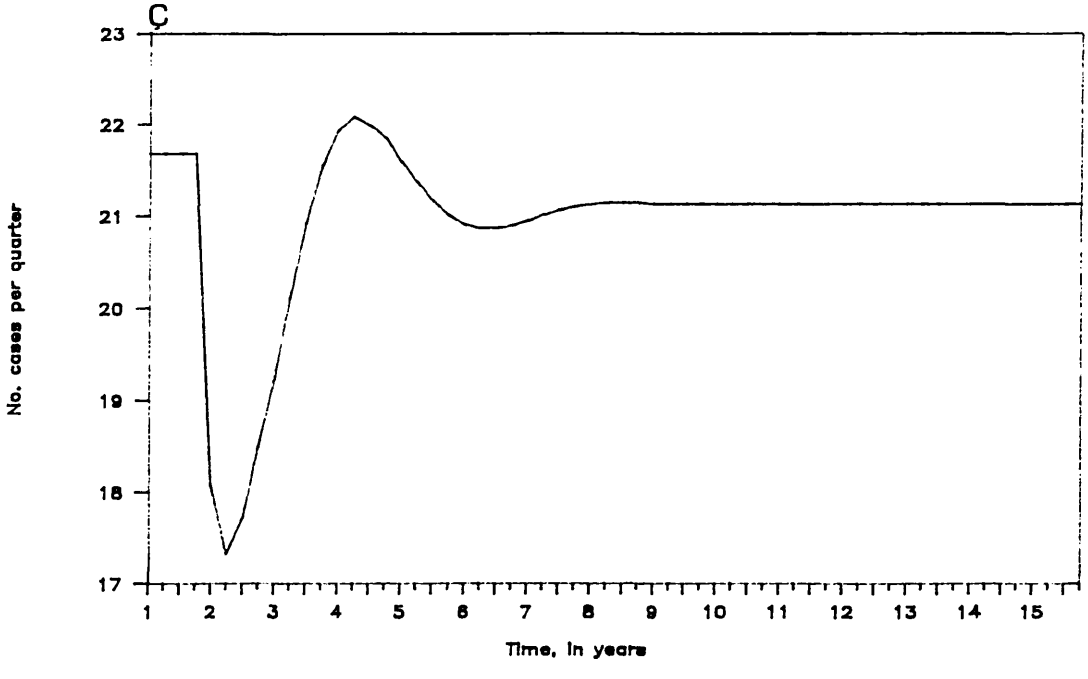


Fig. 5.11 (a)-(c)
The predicted impact on (a) the transmission rate, (b) the total prevalence of infection in the human population and (c) the total number of cases per quarter; of a continuous 20% reduction to the reservoir of infection in an isolated human population of 2 000, without any alteration to the capacity of the vector population to transmit malaria. Control implemented at time = 2 years.(Parameter values as in table 5.1 and table 5.2, α = 0.00384/week).


b
0.021
0.02
0.019
0.018
0.017
0.016
Proportion infectious
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Time, in years


c
23
22
21
20
19
18
17
No. cases per quarter
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Time, in years

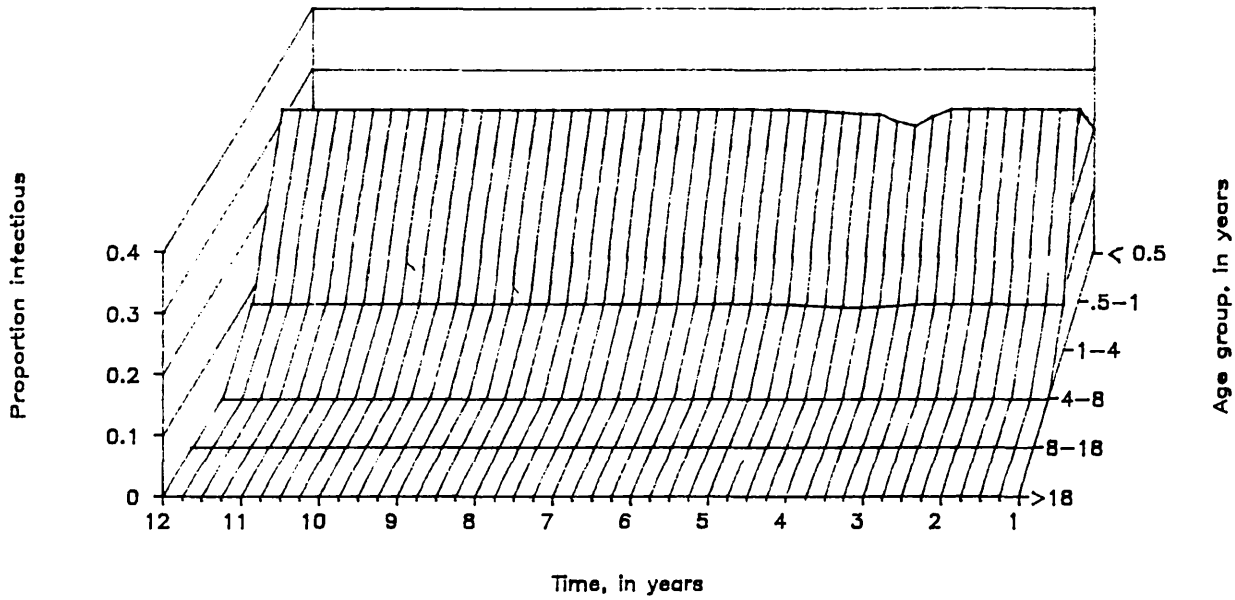


Fig. 5.12. The predicted impact on the age-specific prevalence of a single 10% reduction to the reservoir of infection in an isolated human population of 2 000, at time = 3 years, without any alteration to the capacity of the vector population to transmit malaria.(Parameter values as in table 5.1 and table 5.2, α = 0.00384/week)

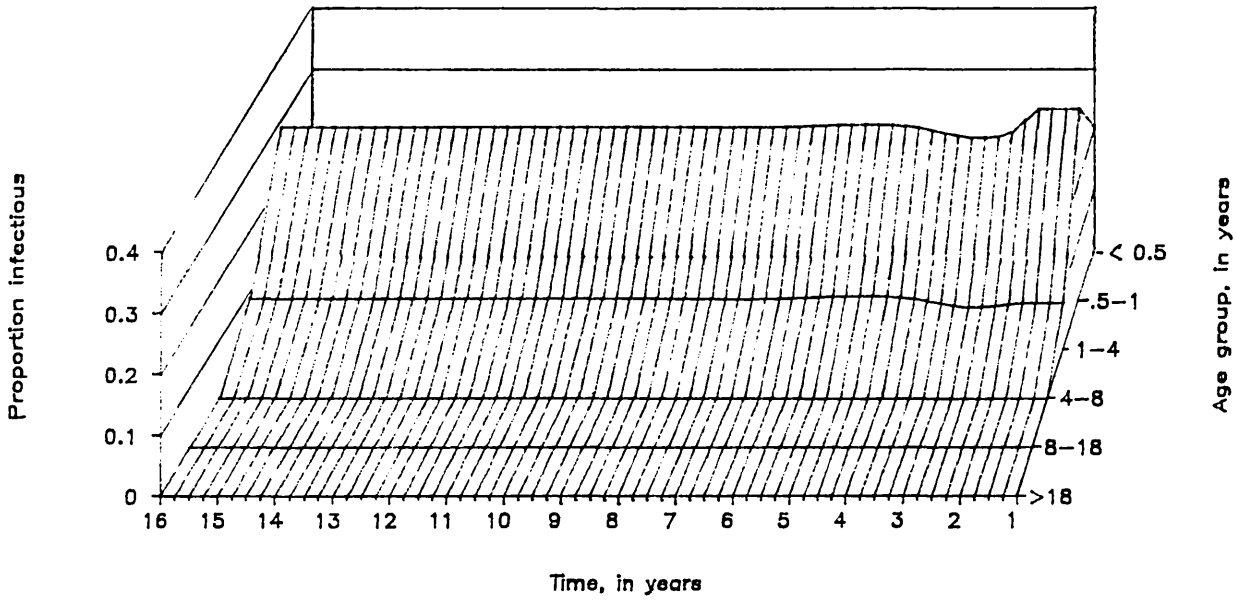


Fig.5.13. The predicted impact on the age-specific prevalence of a continuous 20% reduction to the reservoir of infection in an isolated human population of 2 000, without any alteration to the capacity of the vector population to transmit malaria. Control implemented at time = 2 years. (Parameter values as in table 5.1 and table 5.2, α = 0.00384/week)

The age-related changes in prevalence, resulting from temporary and permanent control are shown in figs. 5.12 and 5.13. An important conclusion, with significant implications for public health and control programmes is illustrated in fig. 5.13. The figure shows that a reduction in the intensity of transmission leads to an increase in prevalence among older age classes, the 20 % reduction to the transmission rate resulted in a doubling of the prevalence of infection in the 4-8 year age-class. Similar conclusions have been made using more complex models, where immunity is assumed to be maintained by re-infection (Aron & May(1982) and Elderkin et al(1977)). The effects on prevalence were however, more pronounced since the reduction in transmission also affected the duration of acquired immunity.

The changes to the total prevalence generated by the model crudely resemble the patterns observed during the intervention and post-spraying phases of several of the control regimes studied in the Garki project, Northern Nigeria (Molineaux and Gramiccia,1980). In particular, those recorded for the control regime which involved the spraying of carbamate insecticide (propoxur) at intervals of two months combined with high-frequency mass administration of the anti-malarial drug sulfalene-pyrimethamine over a period of 18 months. The results were also comparable to those observed in a similar control programme in a holoendemic area of Kenya. Intervention involved the spraying of an organophosphorus insecticide (fenitrothion) at three monthly intervals for a period of two years (Fontaine et al,1976).

5.4 The temporary immunity model

It is obvious that the assumptions of either no immunity or "sterile" immunity are far too simplistic to be able to mirror the complex observed age-prevalence curves. However, the results generated by these models can be helpful in furthering our understanding of the nature of immunity to malarial infection in man. The observed pattern of a rise in prevalence with age followed by a fall to much lower prevalences is roughly reproduced by the "sterile" immunity model, albeit that the model predicts a fall to zero prevalence and over a much narrower age range. This may indicate that some sort of temporary or partial immunity must play a role in creating the overall prevalence pattern. Temporary or partial immunity to infection would enable individuals to pass through the infected class more than once during a lifetime. Thus, higher more realistic prevalences which decline with age (but without falling to zero) would result. The effect of recovery rates on the prevalences seen in the previous section indicates that the effect of

superinfections (re-infections occurring during a current infection) needs to be considered in more detail.

The patterns of age-prevalence profiles are known to vary widely depending on the intensity of transmission (fig. B.1). Figure 5.14 summarizes schematically the profiles typical of the three basic levels of transmission. For all levels of transmission the highest prevalences are seen amongst children, the highest levels occurring in children exposed to the most intense transmission. Adult prevalences are much lower, the highest levels being recorded at intermediate levels of transmission. The decline in prevalence from high childhood levels to the stable lower adult levels is most pronounced when transmission is very high. At low levels of transmission prevalences rise monotonically with age and there is no decline in prevalence with age. To be of real use a model must be capable of describing the dynamics of immunity in such a way as to be able to reproduce the correct patterns for a wide variety of different transmission rates - immunity both depends on and affects the level of transmission.

A knowledge of the relationship between immunity and transmission is central to an understanding of the epidemiology of malaria, especially for control purposes where reductions in the transmission may have unexpected effects on population prevalence. However, information on the topic is at present limited. For the purposes of this model an estimation for the duration of immunity $\uparrow$, is based on the time interval between successive patent infections. Age-specific cumulative prevalences, (i.e. the proportion of persons found patent at least once during a given period of time), from the WHO study in northern Nigeria (Molineaux and Gramiccia,1980) suggests that the average duration of immunity is less than one year. Cumulative prevalences over an 18 month period were found to between 90 and 100% irrespective of age or sex, with the highest numbers of positive results per person found in the youngest individuals, despite the fact that children are bitten less frequently than adults (Carnevale et al,1978).

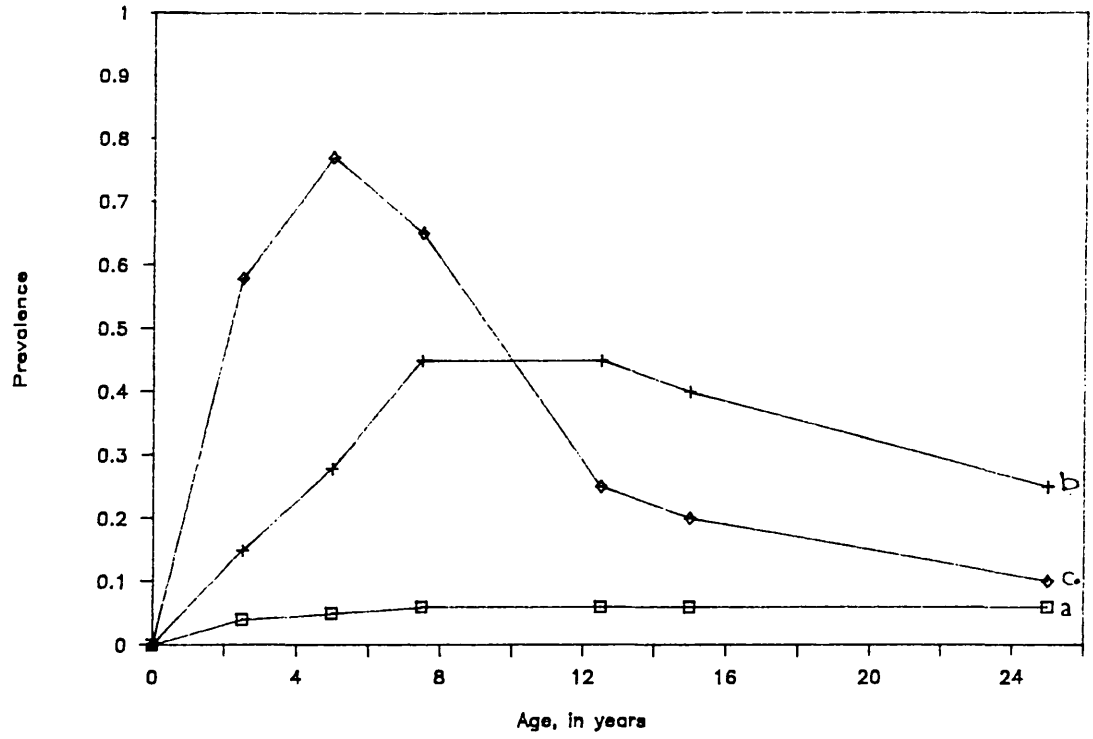


Fig. 5.14. A schematic illustration of observed age-prevalence patterns for acute malaria in stable indigenous populations in Africa for differing levels of endemicity: (a) Low endemicity, (b) Moderate endemicity and (c) High endemicity (Boyd,1949).

5.4 (i) Constant rate of loss of immunity

5.4 (i) (a) Model structure and equilibrium solutions

The simplest assumption that mirrors the notion of temporary immunity is to assume that, following infection, individuals are immune to infection for a short finite period before they become susceptible again (i.e. π is a instantaneous rate, which can adopt values, $0 < \pi < 1$). Thus the equilibrium equations for the susceptibles (X) (eq.(5.21)) becomes replaced by:

$$\frac{d}{da} X(a) = -(L + \mu) X(a) + \pi Z(a) \qquad (5.33)$$

and the immunes (Z) (eq.(5.24)) by,

$$\frac{d}{da} Z(a) = r Y(a) - (\mu + \pi) Z(a) \qquad (5.34)$$

where immunity lasts for a fixed period of time τ and $\pi = 1/\tau$.

In addition to these changes associated with immunity, the recovery rate r, is now calculated according to Dietz's model for superinfection (eq.(4.7)). The rate of recovery from a single infection r_0 was estimated as 0.0152/week, which is approximately the value estimated for the model based on the Garki project (Dietz et al,1974), and the average duration of immunity was taken to be 1 year so that π has a value of 0.0192/week.

Unlike the model with "sterile" immunity ($\pi = 0$), the equilibrium equations for this finite immunity model (eqs.(5.33), (5.22), (5.23) and (5.34))) have no exact analytical solution. However, by estimating the equilibrium number of infectious individuals, $\bar{Y}(t)$, and calculating the parameter values dependent on this value i.e. the transmission rate (eq.(5.15)) and the birth rate N(0),

$$N(0) = \mu \bar{N}(t) + \alpha \bar{Y}(t) \qquad (5.35)$$

(which maintains a constant population size), these equations may be solved by numerical methods. Numerical methods can then be used to integrate the numerical function for Y(a)

over the age range of the population to find the calculated value of Y(t) for given parameter values. By varying the estimated Y(t) value the actual equilibrium number of infectious people can be obtained. This will occur when the difference between the value calculated by the numerical integration and the estimated value is approximately equal to zero (e.g. by iteration). The numerical procedures used to solve the equilibrium equations were designed for the integration of a stiff system of first-order ordinary differential equations, and employed a variable-order variable- step Gear method (Hall and Watt,1976). This method allows integration of the system despite the widely differing parameter values. A step length of 3.5 days was found to meet the accuracy requirements. The numerical methods used to integrate the numerical function for Y(a) was based on a Gill-Miller method (Gill and Miller,1972) of third-order finite-difference formulae.

5.4 (i) (b) Age-prevalence of infection: model predictions and observed patterns

The model is able to generate a wide range of different patterns dependent on the transmission rate L, fig. 5.15 (a), and for π, fig. 5.15(b). The age-prevalence curve tends to be convex, rising to a peak and then declining at older ages, if immunity is long-lived i.e. π small, and/or transmission is intense. Monotonic growth to a stable plateau in older age classes arises if immunity is short-lived and/or transmission is low. Infection rates are seen to exaggerate the decline in prevalence in a similar way to that of the "sterile" immunity model shown in fig. 5.7 (b), although decline is to a non-zero plateau. Zero prevalence is attained only if $\pi = 0$ (fig. 5.15 (b)).

As with observed trends the peak prevalence increases with the rate of infection. However, the high prevalences fall too rapidly and the stable plateau to which the prevalence declines is reached much more quickly than in observed profiles. Additionally, although this level is determined in part by the intensity of transmission, the highest stable prevalences among adults are seen to be generated not by intermediate levels of transmission, (as they are in observed age-prevalence curves), but by the highest transmission levels. The generated curves therefore deviate from observed trends in four major ways: (i) the decline from peak prevalence occurs at too early an age, (ii) the decline occurs too rapidly, (iii) the final equilibrium level attained in the older classes is too high, and (iv) the "crossing-over" of prevalences, whereby the highest prevalences among adults are recorded for intermediate transmission intensities is not seen.

Slower declines in prevalence and lower stable levels may arise from age-related changes in the recovery rate and/or the rate of loss of immunity. For malarial infections of man, both recovery rates and infection rates (a reflection of both the rate of contact with vectors and also immunity), are seen to change with age (this topic is discussed in detail in Chapter 7), and it is probable that observed patterns of change in prevalence with age are modified by such age-related factors. Figure 5.16 (a) shows the age-prevalence profiles for two different recovery rates; the fall in prevalence and also the level of both the peak prevalence and the stable plateau level are affected by the parameter r. The simple loss of immunity model can be adapted to take into consideration, for example, an age-related change in recovery rate, by replacing the r parameter in eqs.(5.23) and (5.34) with a simple age-dependent recovery rate, r(a).

The equilibrium age-prevalence distribution of infection may adopt a wide variety of patterns, depending on the functional form of r(a) and the values of the parameter controlling the loss of immunity to infection. A power function describing the increase in recovery rates between the ages of 2.5 years and 30 years, with a constant fixed rate for children under 2.5, was found to be the best fit for observed data. Figure 5.16 (b) illustrates how a realistic age-dependent recovery rate can act to slow down the decline in prevalence and also lower the stable prevalence level.

At present, due to the obvious practical difficulties associated with estimating the duration of immunity, no attempt has been made to estimate possible age-related changes to the rate at which immunity is lost. The level of protective immunity to many aspects of infection is however known to vary with age (Section 2.2). Very few quantitative studies have been carried out to determine the nature of a recovery rate which appears dependent on age; the available evidence seems to suggest that recovery is not dependent on the age of the individual but on exposure levels, both current and past. Recovery rates are seen to change throughout the year according to periods of high and low malaria transmission (Segal et al,1974; Krafsur and Armstrong,1978; Bekessy et al,1976), although the limits between which they fluctuate are dependent on total past exposure to infection. The effect of current and past exposure levels on infection and recovery rates will be discussed in detail in the final chapter.

If one considers the combined effect that $\bar{\pi}$ and r have on the shape of age-prevalence curves (figs. 5.15 (b) and 5.16 (a)), it is possible to see how "age-related" changes in both r and $\bar{\pi}$ may be important factors determining the overall shape of observed patterns. Recovery rates are high in adults and low in children, and it is possible that the duration of

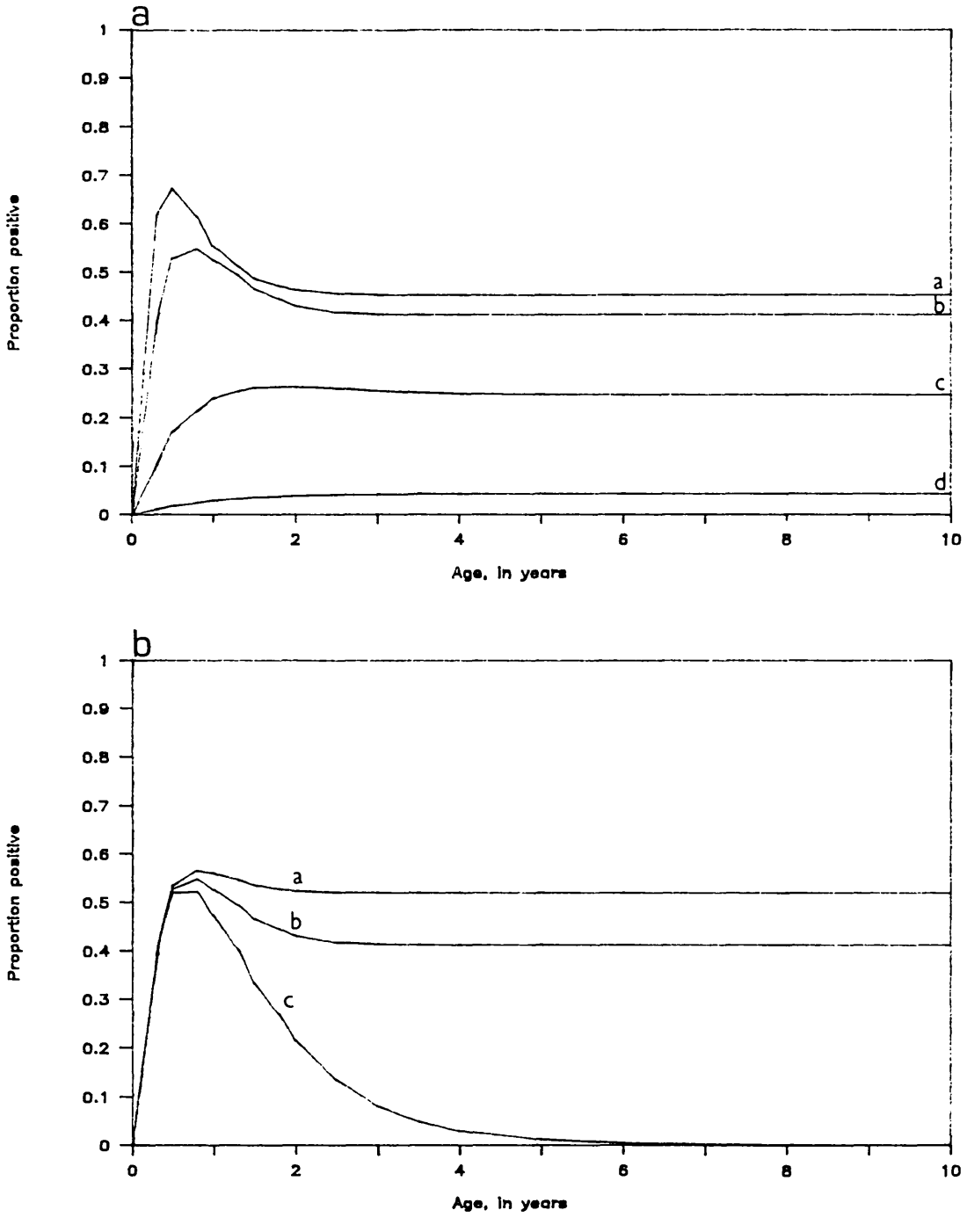


Fig. 5.15. The age-prevalence predictions for the model defined by eqs.(5.33), (5.22), (5.23) and (5.34). (a) The impact of transmission (L), where L = 0.096/week (a), 0.0462/week (b), 0.0096/week (c) and 0.00096/week (d), (Parameters as in table 5.1, with π = 0.0192/week, α = 0.00384/week and r_1 = 0.0152/week). (b) The impact of the rate of loss of immunity (π), where π = 0.00384/week (a), 0.00192/week (b) and 0 (c), (Parameters as in table 5.1, with L = 0.0462/week, α = 0.00384/week and r_1 = 0.0152/week).

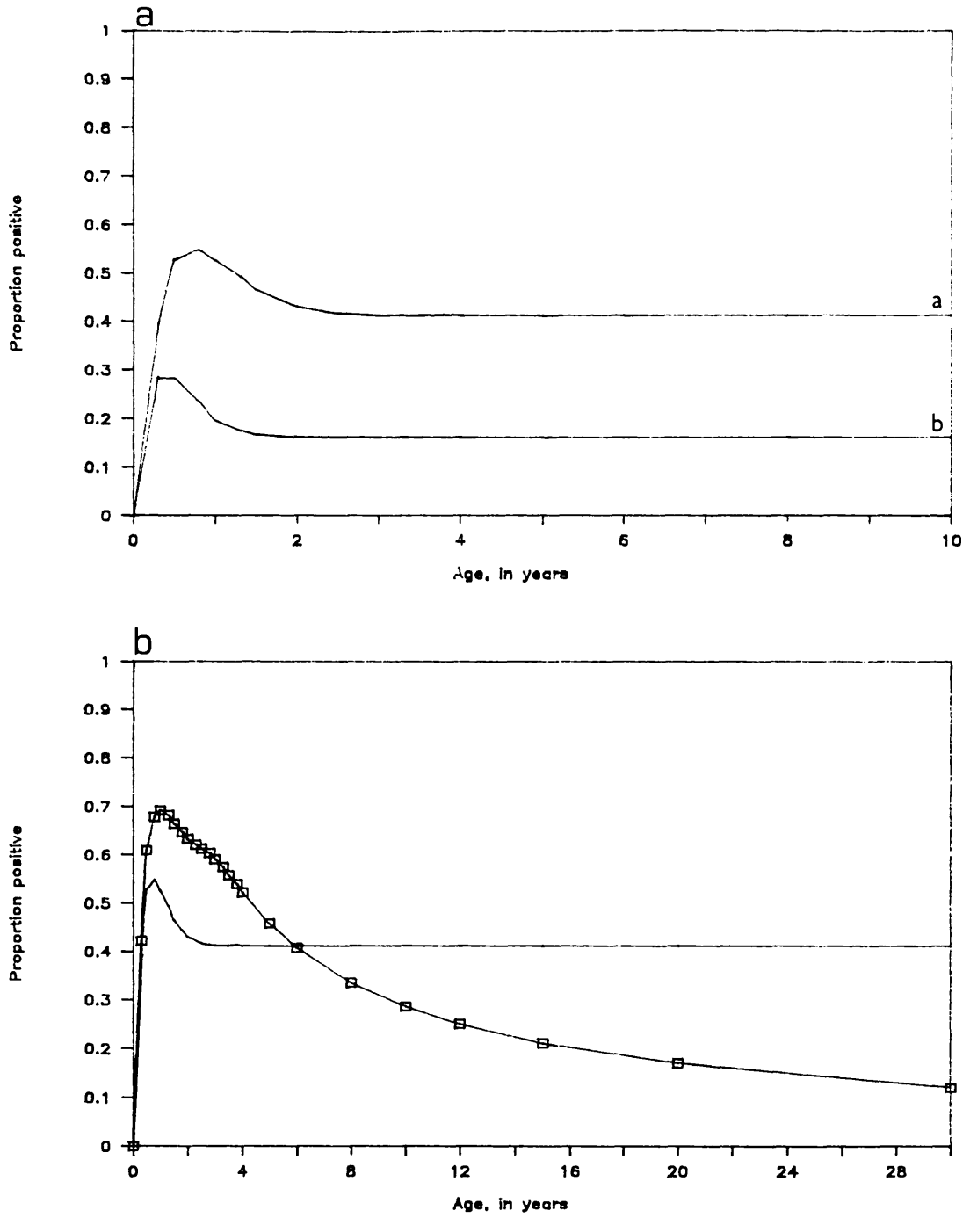


Fig. 5.16. The age-prevalence predictions for the model defined by eqs.(5.33), (5.22), (5.23) and (5.34), the influence of the recovery rate.
(a) Constant rate, r = 0.0192/week (a) and 0.0384/week (b), (b) Age-related rate (symbols), $r(a) = P\,a^{k}$ /week, (a>130 weeks) and r(a) = 0.0009/week (a<130 weeks) compared to a constant rate (solid line), (r = 0.0192/week).(Parameters as in table 5.1, with π = 0.0192/week, α = 0.00384/week, L = 0.0462/week, P = 8.2×10^{-5} and k = 0.967).

immunity is longer in adults so that π is high in children and lower in adults. Molineaux and Gramiccia(1980) certainly showed that the highest numbers of patent infections during a given period were found among children. In fig. 5.17, line a represents the curve generated when recovery is slow and immunity is short-lived, and line b represents the curve when recovery is fast and immunity much longer-lived. The prevalences generated in a and b are fairly good representations of the prevalences found in the young child and adult portions of the population respectively. Intermediate values for r and π among older children and young adults could produce the steady decline in prevalence with age reminiscent of observed profiles.

5.4 (ii) Transmission dependent loss of immunity

5.4 (ii) (a) Formulation of immunity loss

Although "age-related" changes in r and π may well explain much of the observed pattern of age-prevalence, evidence suggests that in malaria the duration of immunity is extended by continued exposure to infection. Antibodies and cell-mediated responses to malaria are slowly lost in the absence of exposure to malaria (Ambroise-Thomas et al,1976; Ballet et al,1985). The relationship between antibody titres in blood from infected patients and protection is however unclear (Ambroise-Thomas et al,1976). A WHO study in northern Nigeria shows that artificial suppression of malarial parasites by chemotherapy for two wet seasons caused a slight loss in immunity and increased prevalences (Molineaux and Gramiccia,1980). A study in Tanzania also showed that drug protection for as little as two months reduced immunity levels (Pringle and Avery-Jones,1966).

The striking "cross-over" feature illustrated in fig. 5.14 has been linked to acquired immunity boosted by re-exposure. The lowest adult prevalences being generated by a high "immune status" where immunity is continually boosted and therefore maintained for longer periods, and the highest adult prevalences result from immunity of minimal duration where transmission is too low to maintain immunity. Using the expression for the duration of immunity dependent on re-exposure (eq.E.29b) derived by Aron(1981), the rate of loss of immunity boosted by re-exposure can be represented by,

$$\pi (L) = \frac{(L + \mu)}{(\exp (L + \mu)\Upsilon - 1)} \qquad (5.36)$$

(see Appendix E.5). The value of Υ was set at 39 weeks.

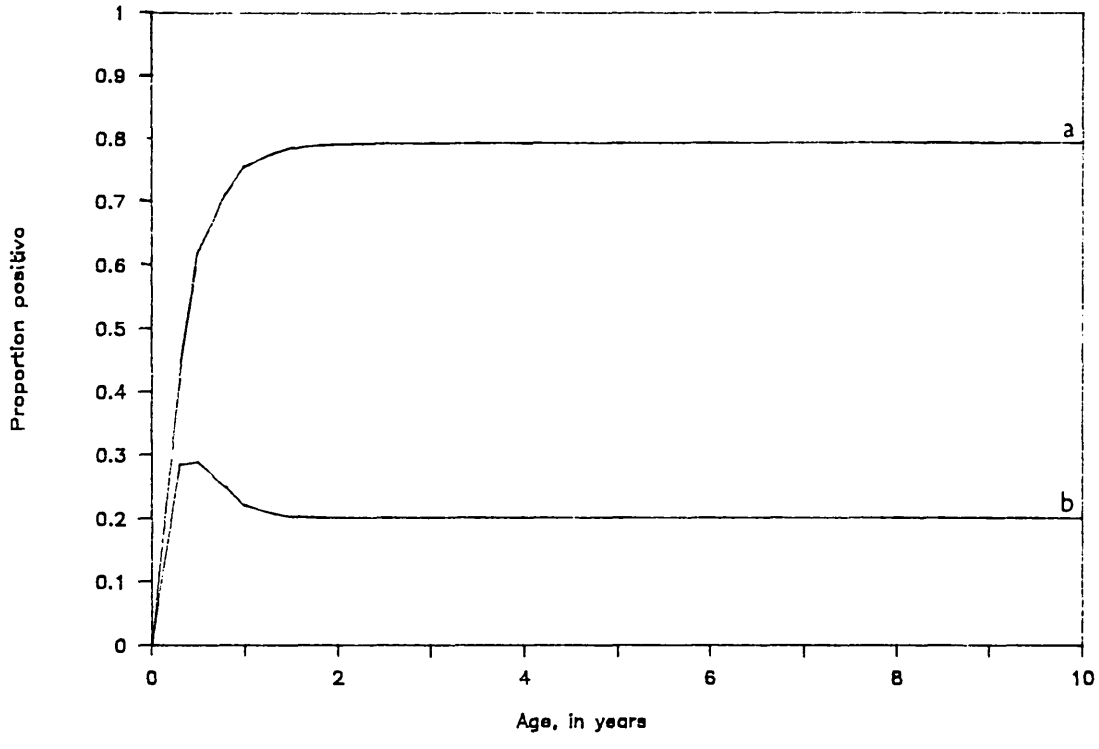


Fig. 5.17. A comparison of the prevalences predicted by eqs.(5.33), (5.22), (5.23) and (5.34) when recovery is slow (r = 0.01/week) and immunity short-lived ($\overline{\pi}$ =0.25/week), (profile a) and when recovery is fast (r =0.07/week) and immunity longer lasting ($\overline{\pi}$ =0.0288/week), (profile b). (Parameter values as in table 5.1, α = 0.00384/week and L = 0.0462/week).

The equilibrium solutions for this model can be calculated by the same methods used previously, for the constant rate of loss of immunity. However, $\overline{\Pi}$ is now dependent on the value of L and must be calculated along with L and N(0) from the estimated value for Y(t).

5.4 (ii) (b) Age-prevalence predictions

Figure 5.18 illustrates the equilibrium age-prevalence profiles for a range of infection rates, L: L = 0.096/week, L = 0.0096/week and L = 0.00096/week, which roughly correspond to low, medium and high levels of endemicity respectively. The highest L value illustrated here is comparable to the rates of infection observed in the Garki project (Molineaux and Gramiccia,1980); the age-prevalence profile observed for this level of infection is illustrated in fig. 5.6 (a). The profiles generated by the model appear to reproduce the "cross-over" of prevalences with increasing endemicity described by Boyd(1949) for tropical Africa (fig. 5.14). The decline in prevalence with age however still deviates from observed profiles by occurring at too early an age and too rapidly. The stable plateau being achieved by 5 years of age.

The pattern of cross-over is not dependent on the absolute values of r or Υ , and replacing the rate of recovery with superinfection by a constant simply reduces the prevalences slightly. The effect of replacing α = 0.00384 by α = 0 is also negligible. The model can produce a wide variety of different profiles dependent on the exact values for the rate parameters; however the choice of parameter values does little to improve the realism of the generated curves and the curves illustrated in fig. 5.18 represent those generated by the most realistic estimates for r, σ and Υ.

The basic cross-over features of the generated profiles are identical to those of the model developed by Aron(1981,1983). The finer details of the patterns are however different. The most obvious feature being that the Aron model requires a much longer time to reach a stable equilibrium in the older ages. The differences can however be attributed directly to the slightly different assumptions concerning the development of immunity (reflected in a different model structure), and to the estimate made for the duration of immunity in the absence of re-exposure.

The structure of the Aron model is similar to that of the Ross/Macdonald model of no immunity, but with an additional slowly developing acquired immunity superimposed.

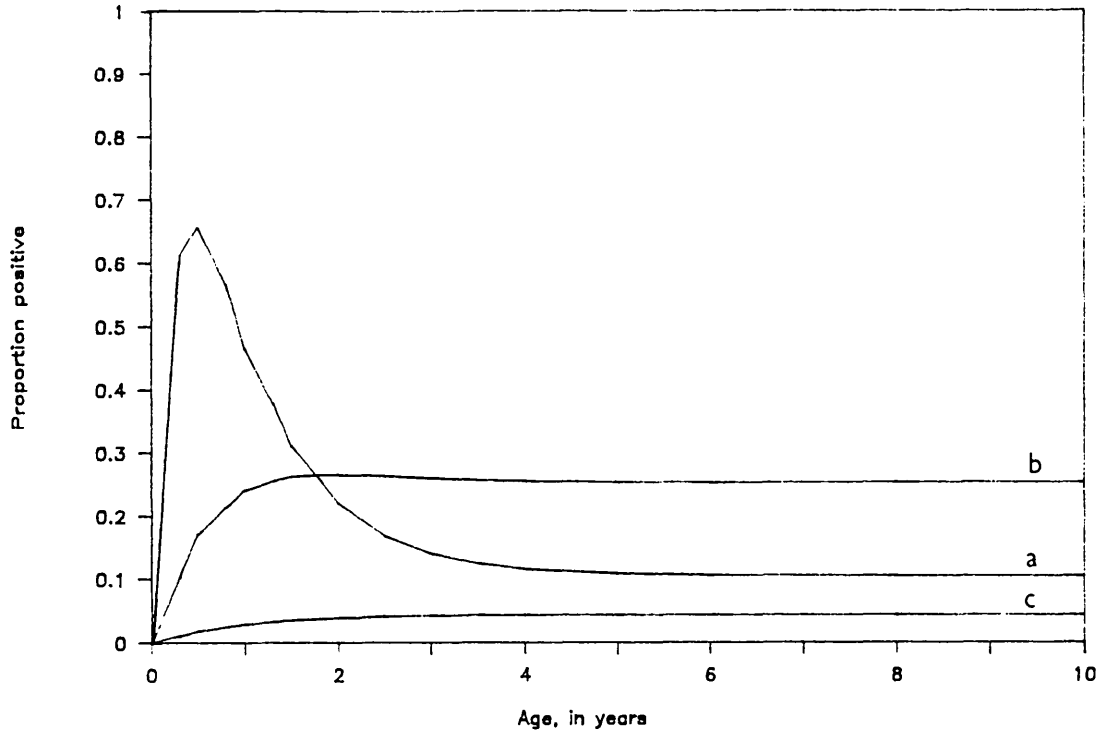


Fig. 5.18. The age-prevalence predictions for the model where loss of immunity (π) is dependent on the transmission rate, as defined by eqs.(5.36), (5.33), (5.22), (5.23) and (5.34), for three levels of transmission: (a) L = 0.096/week; (b) L = 0.0096/week and (c) L = 0.00096/week. (Parameter values as in table 5.1, with r_1 = 0.0152/week, α = 0.00384/week and τ = 39 weeks).

Using current notation the structure of the model is represented by:

$$\frac{d\,X(t)}{dt} = \pi Z(t) + r\,Y(t) - L\,X(t) \qquad (5.37)$$

$$\frac{d\,Y(t)}{dt} = L\,X(t) - (r + \delta)\,Y(t) \qquad (5.38)$$

$$\frac{d\,Z(t)}{dt} = \delta\,Y(t) - \pi Z(t) \qquad (5.39)$$

δ represents the slow development of immunity (where δ = 0.2/year), the estimated value of τ is taken as 5 years. Additionally, the immunes (Z), are described as those with mild, chronic malaria.

The differences in the generated profiles for identical transmission rates may be summarized as follows; a slower decline in prevalence with age (prevalences continue to decline until the age of 25 in all except the lowest infection rate), adult prevalence levels that are appreciably lower than those of fig. 5.18, child prevalences that are slightly higher, and for the highest level of transmission levels, prevalences decline to a level below that of the lowest so that by age 25 years prevalences are zero.

Although the profiles generated by the Aron model show a slower more realistic decline in prevalence with age, the tendency for age prevalences to fall to zero in adults when transmission is high does not appear to correspond with observed prevalences for areas with similar levels of transmission. These low levels are generated mainly as a result of the large value of τ. Smaller values, however, would cause a more rapid decline in prevalence. The inclusion of recovery to the susceptible state however prevents the decline being as rapid as those of fig. 5.18 and also allows higher child prevalences.

In contrast to the Aron model which has no age-structure, the model presented above can be used to analyze changes in prevalence with age where the transmission rate varies through time. This is of particular interest since aspects of the disease are very different in the adult and child portions of the population.

5.5 Conclusions

The latent period that elapses between the time when a mosquito becomes infected and the time when it becomes infective to humans is clearly an important factor in determining the prevalence of infection within the vector population. Also of importance, however, is the length of the latent period in relation to the expected life-span of infected mosquitoes. It is clear that observed seasonal changes in the sporozoite rate within endemic regions are largely determined by the impact of fluctuations in temperature and relative humidity. However, since transmission of malaria is controlled via the vector biting, it must be remembered that the sporozoite rate in a locality is also affected by factors determined by the vector species present, (e.g. death rates, susceptibility to infection and the biting behaviour of individual mosquitoes vary considerably between species).

It is important to note that additional factors may contribute to observed levels of vectorial infection within a particular locality. For example, the use of insecticides (which affects mosquito densities and death rates), chemotherapy, and the availability of human hosts (determined by both the use of bed nets and the by the actual numbers of inhabitants) all have an effect on malaria prevalence. Another important factor is heterogeneity, due either to genetic variability in susceptibility to infection or to spatial effects. Spatial heterogeneity is thought to lead to effects such as "pockets" of highly infected areas within regions with observed low sporozoite rates. Small area variations exist wherever detailed data is available. For example in Madang Province, Papua New Guinea (Charlwood et al,1986) it is thought that individual villages act as "islands" with little or no vectorial contact between villages as close as 2 km apart.

Decreased latent periods, decreased mortality and accelerated breeding success during the wet season all act to enhance transmission from mosquito to man. Long latent periods or high vector mortality may place the level of transmission in an area close to the "threshold" level. Within areas of high endemicity, parasite populations appear to be fairly robust to changes in environmental conditions or reductions created by control measures. It would appear that the rate parameters controlling the disease in both human and vector populations combine to maximise the selective advantage of the parasite in terms of the stability of its life-cycle.

It is obvious that entomological factors have a significant effect on the transmission and prevalence of malaria, particularly in areas subject to epidemics or very low levels of malaria. The models presented in this chapter do, however, highlight the importance of acquired immunity in regulating transmission and age-prevalence profiles in endemic areas. During epidemics age-prevalence profiles are generated almost solely by the age-dependent biting preferences of the vectors (i.e. prevalence is highest among adults), although human behavioral factors which influence the rate of contact between vectors and humans modify this effect. For areas where the basic reproductive rate is close to unity, models which assume no acquired immunity generate profiles close to those of observed profiles. However in areas of higher transmission, immunity generated by continual exposure plays an increasingly important role in regulating transmission and shaping age-prevalence curves. The higher the transmission rate the more the profiles generated by the models deviate from observed patterns.

The acquired immunity models described in this chapter highlight some of the possible effects of immunity at the population level. However, they are clearly very crude mimics of the complexities of immunity to infection. They do, however, suggest that a critical feature of the epidemiology of malaria is the relationship between acquired immunity and the intensity/duration of exposure. The exact form of age-prevalence profiles appear to depend on more complex interactions than simply immunity maintained by re-exposure. A model assuming that the duration of immunity is dependent on the level of transmission while other rate parameters are constant and independent of age can do little more than capture the most basic features of observed age-prevalence profiles.

Many of the other rate parameters must also be modified in some way by features of the immune response and some of the possible effects of total exposure(age)-related changes in recovery and the duration of immunity have been outlined. However, a detailed understanding of the influence of immune mechanisms on the various population rate parameters involved in the dynamics of malarial infections remains unclear. It is interesting to note, that simply accounting for the observed age-related biting rates so that infection rates vary with age would have the effect of producing an age-related change in the duration of immunity.

CHAPTER 6 Age-prevalence models for malaria

6.1 Introduction

The construction of age-prevalence profiles, via the screening of blood films for the presence of parasites, is a standard method of measuring the transmission of malaria in a given locality. In endemic areas these profiles show highly characteristic patterns, the dimensions of which are thought to depend on complex interactions between the transmission rate, the rate of acquisition of immunity in the population, (i.e. herd immunity) and various environmental and demographic factors.

Although the basic shapes of age-prevalence profiles for areas of similar transmission are comparable, a certain amount of variation is to be expected. Prevalence is dependent on both age (especially in the youngest age groups) and transmission rate, so that the exact dimensions of the profiles will fluctuate according to seasonal variations and the age-grouping chosen. To achieve as precise an evaluation as possible of the amount of malaria in a locality, the WHO recommends that parasite rates be determined for relatively narrow age ranges: infants (0-11 months), toddlers (12-23 months), small children (2-4 years), school children (5-9 years), adolescents (10-14 years) and adults (15 years and over), (WHO,1963). Even so, the relative proportions of younger and older children in the age groups will affect the shape of the profile. The reliability of the age-prevalence profiles also depends on accurate techniques for the collection and examination of blood slides. A bias in the data may occur if, for example, sample sizes are small or individuals are not randomly selected but are selected on the basis of attendance at a hospital clinic. Although these may be critical factors in determining the finer details of the observed patterns, in order to investigate a number of what are thought to be the major determinants of the patterns, this chapter concerns itself simply with reproducing the right basic shape and not with reproducing the exact quantitative dimensions of observed profiles.

Age-prevalence surveys on their own cannot ascertain what factors are responsible for determining the changes in prevalence with age. When considered in isolation from other epidemiological and environmental data it is possible to suggest several ways in which the highly characteristic patterns of fig. 5.14 can arise. By way of illustration, there are two simple assumptions which can create the basic patterns but which we know from additional epidemiological data, are not in fact responsible: (i) age-dependent exposure

to infection - children are assumed to be exposed more frequently than adults (fig. 6.1 (b)); and (ii) infections are very long-lived and result in permanent immunity to further infection (fig. 6.1 (a)). A comprehensive knowledge of the environmental and epidemiological features of malaria (relating to both human host and vector) is essential for investigation into the possible factors responsible for shaping observed age-prevalence curves (see Chapter 2).

Towards the end of the previous chapter, some simple ideas concerning the possible factors responsible for the shaping of age-prevalence profiles were explored. These included temporary states of immunity and partial immunity dependent on the magnitude of the transmission rate. In the following sections these and other ideas are investigated further. Various simple compartmental models are introduced and developed, and these are used to explore aspects of the interaction between parasite and host populations, such as genetic heterogeneity in the human community with regard to susceptibility and acquisition of immunity and the existence of multiple "strains" of parasites. These types of factors may be important in determining the overall prevalence of malaria in the human population. More complex ideas concerning the acquisition and maintenance of immunity in different portions of the human population are also considered. Factors including the effects of relapses, and the combined effects that superinfections and immunity have on the recovery rate are considered in the next chapter.

The models developed in this chapter are also used to examine the question, " How far is it possible to reproduce observed age-prevalence profiles using a combination of simple assumptions concerning the epidemiology of the parasite ? "

Fig. 6.1.

(a) The age-prevalence profiles obtained from a simple model with life-long immunity,

$$\frac{d}{da} PX(a) = - L\, PX(a)$$

$$\frac{d}{da} PY(a) = L\, PX(a) - r\, PY(a)$$

$$\frac{d}{da} PZ(a) = r\, PY(a)$$

where, the prevalence of infection is calculated as,

$$PY(a) = \frac{(\exp(-ra) - \exp(- La))\, L}{(L - r)}$$

the recovery rate, r= 0.035/year, and L is the transmission rate, (A) L = 1/year, (B) L = 0.075/year and (C) L = 0.005/year.

(b) The age-prevalence profile obtained by numerical methods from a simple model where there is no immunity and the vector biting rate depends on the hosts' age,

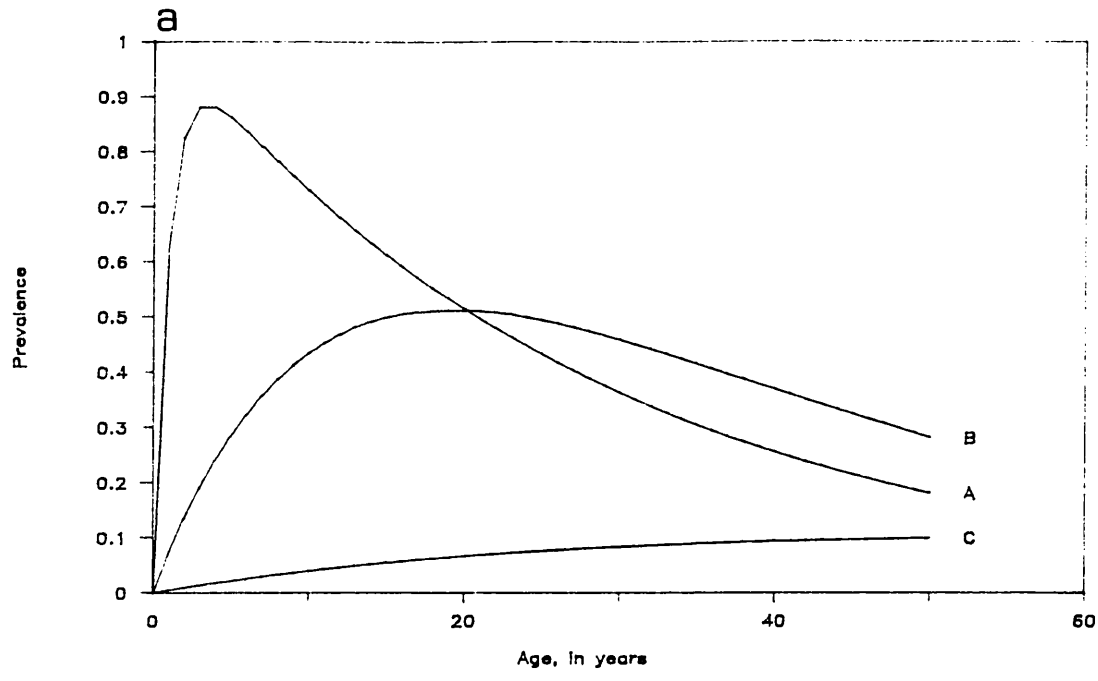
$$\frac{d}{da} PX(a) = r\, PY(a) - L(a)\, PX(a)$$

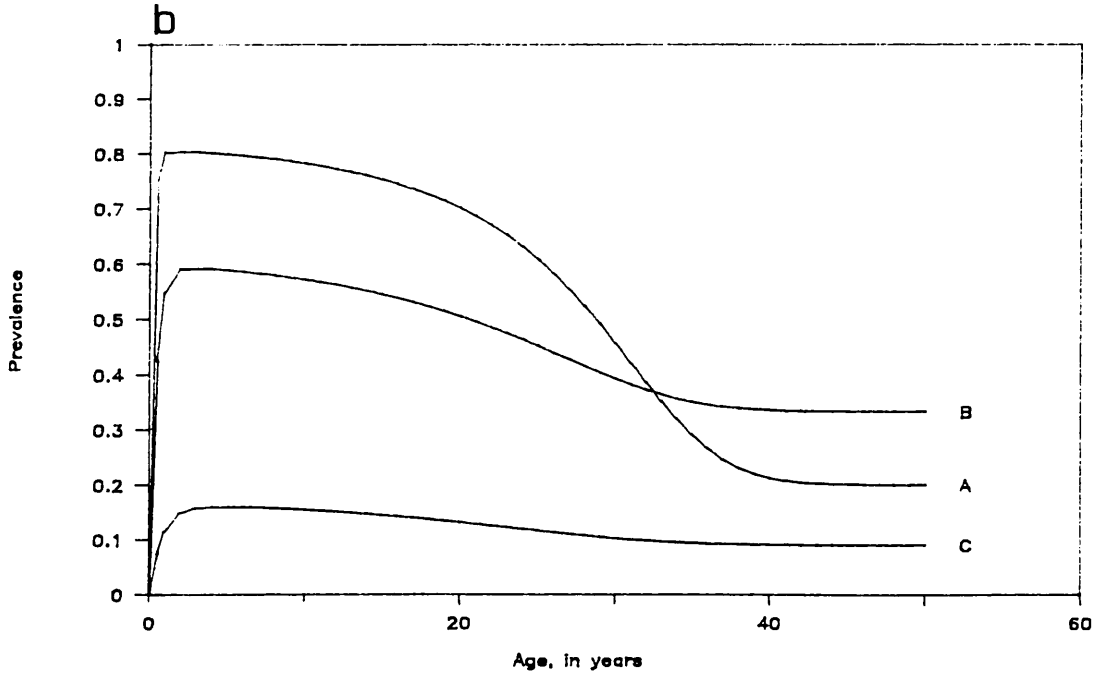
$$\frac{d}{da} PY(a) = L(a)\, PX(a) - r\, PY(a)$$

the recovery rate, r= 0.035/year, L(a) is the age dependent transmission rate according to the equation,

$$L(a) = k_1 + k_2 \exp\left(\frac{a}{b}\left(1 - \exp ba \right)\right).$$

where, a= 0.01, b= 0.1 and (A) k_1= 0.25, k_2= 5, (B) k_1= 0.5, k_2= 1 and (C) k_1= 0.1, k_2= 0.1.


a
Prevalence
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
Age, in years
B
A
C


b
Prevalence
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
Age, in years
B
A
C

6.2 Genetic variability of immunological responsiveness in the human population

6.2 (i) Introduction

The occurrence of intraspecific variation in the response to malarial infections is well documented, both from laboratory studies of host-parasite systems and also in naturally occurring infections in man. The variations in response can have a physiological basis, as is evident in strata of the population. Variations according to factors such as age (Foetal Hb - Allison,1954; Pasvol et al,1976,1977), diet (Fava bean - Huheey & Martin,1975; Golenser et al,1983: PABA deficient milk diet - Hawking,1954; Macraith et al,1952: general nutritional status - McGregor,1982), reproductive status (Fleming et al,1968; McGregor,1984; McGregor et al,1983) or presence of concomitant infections (Omondi-Odhiambo et al,1984; Molineaux,1985b; Smith,1943) have been recorded among human populations. Alternatively heterogeneity can be genetically based so that the variations occur throughout the population. Genetically based variations, however, will be different among populations of different genetic or racial origin.

As shown in section 2.1 (iii), intraspecific differences in susceptibility or innate resistance to the disease are thought to have had important evolutionary consequences for the human host-parasite relationship. The apparently slow acquisition of functional immunity to malaria, and its concomitant cost in infant life, mean that the selective forces imposed by malaria are intense. Genotypes which confer some protection and enable the individual to survive until acquired immunity has had a chance to develop are highly polymorphic in many human populations living in malaria-endemic areas.

Malaria has probably also been a major selective force for genes which influence and control specific immunological responses. Laboratory studies of animal models suggest that host genetics can be an important determinant of immunological responsiveness to malarial infection (Wakelin,1978). Comparison of the responses to *P.knowlesi* of the natural simian host *Macaca fascicularis*, and the closely related but unnatural host *Macaca mulatta*, shows that the natural host produces protective antibody of broad serological specificity within 2 weeks of infection and develops mild chronic parasitaemia, while the latter shows little or no protective immunity and suffers rapid and fatal infection (Cohen et al,1977). This suggests that the natural host, through antecedent selection, has developed the capacity to recognize and respond effectively to the

functionally important malarial antigens, whereas in the unnatural host the capacity to mount an effective response is not present. Examples from experimental rodent-malaria systems also show similar variations in immune responsiveness. The genetic constitution of the H-2 region of inbred mice has been shown to influence not only the course of infection and extent of mortality, but also the acquisition of functional immunity (Kretschmar,1962; Greenburg et al, 1953,1954). In mice, the ability to respond to the immunodominant epitope of P.falciparum circumsporozoite (CS) protein is under the control of immune response genes (Del Giudice et al,1986; Good et al,1986).

Among human populations, similar if less clear cut, examples are available. Among rural East Africans a strong correlation exists between HLA antigens A_2 and Aw 30 and high titres of protective antibodies to P.falciparum (Osoba et al,1979). The potential for HLA linked variations within native populations is apparent from evidence of the divergence of HLA-A types between malarious and non-malarious populations. Work by Piazza et al(1972) in four Sardinian villages, two located in lowlands, which until recently were malarious, and two others in nearby historically malarious-free highlands, found several genetic differences between the two populations. In addition to the expected absence in the highland villages of red cell abnormalities associated with innate resistance to malaria, divergent HLA-A types, possibly linked to control of immunity against plasmodial infection, were also evident. Analysis of 22 other neutral genetic polymorphisms, however, indicates that apart from the adaptive variations in haemopathies and HLA-A types the four populations are genetically closely related.

Further evidence that individuals in the human population do not all respond to malarial infection in the same way can be found among malariometric data. The density of parasitaemia, the severity of clinical malaria and the degree of antibody production have all been shown to vary widely between individuals of genetically distinct origin. The quantity and rate of production of antibody to experimental infection with P.vivax is significantly higher in previously unexposed American Negroes than in Caucasians (Contacos,1970). East Indians in Guyana (these immigrants settled in the area prior to 1917) are very much more susceptible to malaria in comparison with the local Negroes (Giglioli(1972)). In spite of continued and active transmission, the East Indians were found to rarely develop effective tolerance to infection. They suffered much higher parasitaemias and more frequent and severe clinical attacks than the native Negroes. Among populations which are less genetically distinct consistent, if less dramatic, variations

are found (Oomen et al,1979; Molineaux and Gramiccia,1980; Jensen et al,1983; Cox,1984). Members of the Falani tribe in Northern Nigeria are seen to suffer extended periods of parasitaemia with clinical symptoms and appear to acquire effective immunity at a much older age than individuals of the Hausa-Maguzawa tribe, in spite of both tribes living in the same villages (Oomen et al,1979). An extensive study (the Garki project - Molineaux and Gramiccia,1980), carried out in the same area, showed similar parasitological heterogeneity notably in the number of positive results per person in a given time period, Ig M levels and IHA results (indirect haemagglutination). Moreover, it was found that there was a positive correlation between the results in the same person at different surveys. The position within a person's age-group was found to be relatively stable over a three year period, including periods of decrease and increase in average patterns. Variability between people of the same age, however, was found to decrease with age. This suggests that the level of immune responsiveness and its parasitological and serological effects are quite stable and are determined early in life. As such they are probably under genetic control. Marsh et al(1988) also recorded wide variations in the anti-sporozoite responses of people living in rural Gambia. Both inter- and intra-household variations were observed, and subjects of the same age living in the same household frequently varied markedly in antibody levels. Heterogeneity in the hosts' capacity to respond to sporozoite epitopes was suggested as an important factor. Wide variations in the rate at which effective immunity is developed by individuals have also been recorded in Tanzanian children. Again significant differences were recorded not only between households in the same village but also between members of the same family (Del Giudice et al,1987).

In the Sudan, Jensen et al(1983) found that the ability of serum samples collected from adults living in holo-, hyper- and hypoendemic areas to inhibit intra-erythrocytic development could be correlated with clinical status. The greatest inhibition was induced by sera from individuals who had suffered only mild occasional malaria as children, and who therefore would appear to have a greater ability to control infection. A similar survey carried out in Indonesia is reported to have produced similar results (Cox,1984). What is particularly interesting, is that whereas in the Sudan clinical immunity was correlated with non-antibody serum factors, in Indonesia the correlation was with merozoite-blocking antibody. This indicates that genetic differences may also be important in determining whether antibody or non-antibody factors predominate in immunity to human malaria.

6.2 (ii) The model structure

The acquired immunity model introduced in section 5.4 (ii), (eqs.(5.33), (5.36), (5.22), (5.23) and (5.34)) can be extended to include variability in the way in which different groups of individuals within the population respond to parasitic infection. If there are n different groups of individuals, with parameters defined as L_i, r_i, π_i and μ_i (for simplicity the latent class (H) and the parameter have been omitted), and the fraction in group i, aged a, that are susceptible is given by $PX_i(a)$, the model equations can be transformed so that they reflect the rate of change of the population fractions and not simply the rate of change of numbers of individuals. i.e.

$$\frac{d}{da} PX_i(a) = \frac{d/da \; X_i(a)\, N(0)\, e^{-\mu a} + X_i(a)\, \mu\, N(0) e^{-\mu a}}{(\, N(0)\, e^{-\mu a}\,)^2} \qquad (6.1)$$

where $PX_i(a) = X_i(a)/N_i(a)$. Rearranging eq.(5.33),

$$\frac{d}{da} X_i(a) + \mu\, X_i(a) = -\, L\, X_i(a) + \pi_i\, Z_i(a) \qquad (6.2)$$

therefore,

$$\frac{d}{da} PX_i(a) = -\, L\, PX_i(a) + \pi_i\, PZ_i(a) \qquad (6.3)$$

Similarly,

$$\frac{d}{da} PY_i(a) = L\, PX_i(a) - r_i\, PY_i(a) \qquad (6.4)$$

$$\frac{d}{da} PZ_i(a) = r_i\, PY_i(a) - \pi_i\, PZ_i(a) \qquad (6.5)$$

and for the total population prevalence of malaria PY(a):

$$\frac{d}{da} PY(a) = L \sum_{i=1}^{n} PX_i(a) - \sum_{i=1}^{n} r_i PY_i(a) \qquad (6.6)$$

The age-prevalence distributions by age for each of the groups (Y_i, Y_j) and for the total population (Y) can be obtained with the use of a computer programme which enables the system of differential equations to be solved numerically. A wide range of different patterns can be generated depending on the values of the parameters of the groups and the fraction of the total population found in each group.

6.2 (iii) Age-prevalence predictions

By way of a simple illustration of the effect that heterogeneity in immunological responsiveness can have on the shape of an age-prevalence profiles, consider two very basic cases in which the population consists of two fractions, K1 and K2 (where K1 + K2 = 1). Assuming that the loss of immunity rates differ in the two groups with values π_1, π_2 and that the recovery rates and infection rates for both groups are identical.

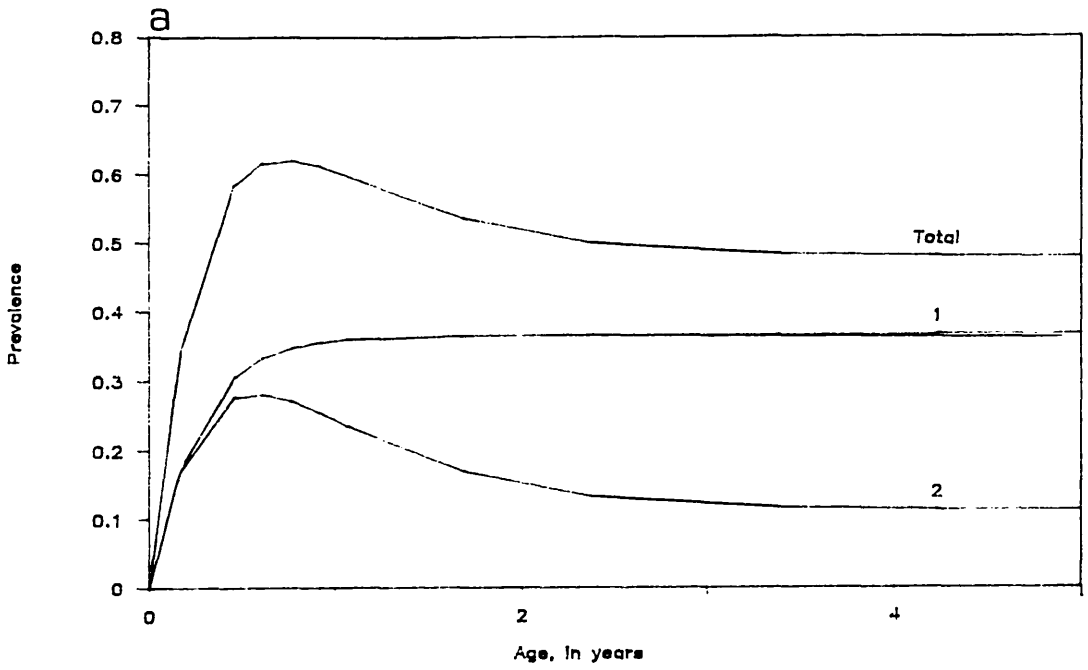
For the first case, a fraction K1, of the human population is assumed to be totally unable to develop any sort of acquired immunity following infection; recovery from infection results in these individuals immediately becoming susceptible to further infection, i.e. $\pi_1=1$. The other fraction K2, is able to develop functional immunity in the normal way, so that recovery is followed by a finite period of immunity, such that $0 < \pi_2 < 1$, the exact value being determined by the magnitude of the transmission rate and the duration of immunity (eq.(5.36)). An example of the change in prevalence by age for each of the two groups and for the total population is shown in fig. 6.2 (a). Although the model still reflects the problems discussed in the previous chapter (fall in prevalence occurs at too early an age and too rapidly by comparison with observations), it is interesting to see how the overall population prevalence can mask the much lower observed prevalences seen in the fraction K2 of the population.

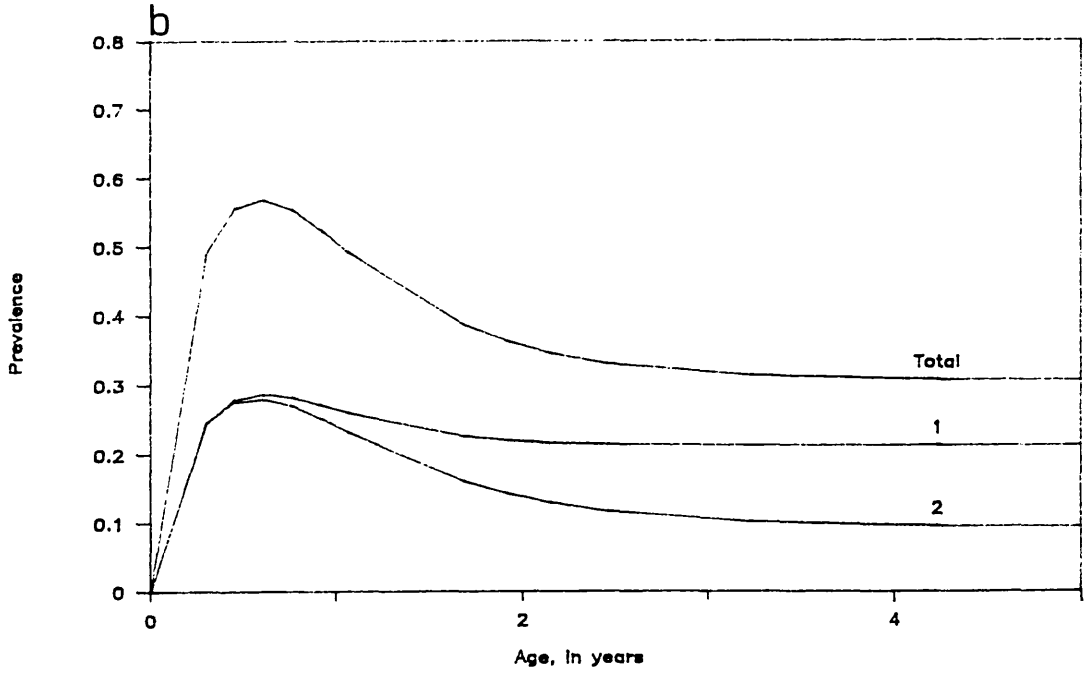
Similarly, if one assumes that all members of the population can acquire effective immunity, but that the fraction K2 is able to produce a much stronger and more effective response so that $\pi_1 < \pi_2$ and $\pi_1 \neq 1$, age-prevalence profiles similar to those in fig. 6.2 (b) can be obtained.

Fig. 6.2. Heterogeneity in immunological competence.

(a) Changes in prevalence with age predicted by eq.(6.4) within two groups of people. Group 1 constitutes 50% of the population (K1=0.5) and are unable to develop functional immunity to infection (π_1=1). Group 2 (k2=0.5), and are able to develop functional immunity in the normal way (π_2=0.0064/week). The top profile shows the total prevalence by age. (Parameter values L=0.055/week, r=0.02/week).

(b) Changes in prevalence with age predicted by eq.(6.4) within two groups of people. Group 1 constitutes 50% of the population (K1=0.5) and are able to develop functional immunity to infection in the normal way (π_1=0.02). Group 2 (k2=0.5), and are able to develop a much more effective response (π_2=0.005/week). The top profile shows the total prevalence by age. (Parameter values L=0.055/week, r=0.02/week).


a
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Prevalence
Total
1
2
0
2
4
Age, in years


b
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Prevalence
Total
1
2
0
2
4
Age, in years

By changing the assumptions concerning the acquisition of immunity between the groups, we have simulated genetic heterogeneity in immunological responsiveness, albeit in a very simple manner. Similar patterns would be produced if π was assumed to be constant throughout the population and infection rates varied between groups. Different infection rates could result from different exposure rates, either due to age or other factors (section 2.1 (i) (b)), or from aspects of innate immunity which affect susceptibility to infection (section 2.1 (iii)). These types of model can of course be extended to n groups of people, whose exposure/susceptibility to infection or ability to acquire functional immunity are distributed non-randomly through the population. Infection rates or ability to acquire functional immunity could, for example, be represented by a negative binomial distribution.

6.3 Multiple "strains" of parasite

6.3 (i) Introduction

The concept of genetic variability in natural parasite populations and the possible significance that different infecting and re-infecting or superinfecting strains may have on the acquisition of immunity in humans was introduced in Chapter 3, (section 3.5 (iii)). The use of the term "strain" in connection with natural malaria parasite populations is however somewhat erroneous, since individual strains or isolates similar to those defined for use in the laboratory do not exist. Genetic recombination has been observed to occur between genetically distinct clones of P.falciparum during the mosquito phase of the life cycle, (Walliker et al,1987). Furthermore, two other types of mechanisms for genetic recombination have been recorded, which lead either to novel repeat sequences (the primary target of human antibodies) for polypeptides encoded by genes (Saint et al,1987) or which generate novel molecules (Tanabe et al,1987; Peterson et al,1988). Individual "strains" as such cannot therefore be preserved. A blood meal often contains mature gametocytes from several different infections and not only are there few barriers to the fusing of the various gametocytes to produce novel genotypes, but the possibility of mixed infections cannot be ignored. However, individual sub-populations, identifiable by specific genetic markers (such as single polymorphic antigens) which include the major merozoite surface antigen (PMMSA) and several S-antigens, do exist (McBride et al,1985; Saint et al,1987). Such sub-populations will form a heterogeneous group, since homogeneity for the single genetic marker does not ensure

homogeneity for other polymorphic antigens. Molecular studies with P.falciparum have shown that this parasite has several polymorphic antigens, and the genes encoding these antigens can be quite widely spaced. Independent genetic recombination could be expected to occur quite frequently (Kemp et al,1987).

The existence of antigenic heterogeneity as well as many other types of phenotypic variations within natural malaria populations is widely recorded. Phenotypic variability includes: the dynamics of gametocyte production (Burkot et al,1984; Graves et al,1984a), infectivity to mosquitoes (Graves et al,1984a), drug resistance (Thaithong,1983; Graves et al,1984b) and patterns of proteins on 2-dimensional electrophoresis (Fenton, Walker & Walliker,1985). Antigenic heterogeneity in P.falciparum isolated from a number of different geographical areas has been demonstrated both by cross-immunity studies (Sadun et al,1966) and serological analyses. The antigenic differences between isolates ranged from minor ones, detected by only one monoclonal to multiple differences detected by all the monoclonals tested. (McBride et al,1982,1985; Wilson et al,1969; Marsh and Howard,1987; Howard et al,1986; Graves et al,1985; Schofield et al, 1982).

It has been suggested that natural antigenic diversity in parasite populations might be a major factor contributing to the slow development of acquired immunity to malaria in humans, compared to that of laboratory animal models. Additionally if this diversity is quite wide and/or unstable through time it may also explain the reason why the cumulative prevalence in individuals over a period of one year might be as high as 100%, even among what are considered to be the relatively immune adults. Other factors contributing to this slow development of immunity may be the abbreviation of immune stimulating parasitaemias by chemotherapy, and the fact that children are exposed to infective bites much less frequently than adults due to the host age-dependent biting rate of the vectors.

It seems possible therefore that although laboratory animal models for malaria often display complete and life-long immunity after infection, with varying degrees of cross-immunity between strains and to a lesser extent between species, this type of sterile immunity is not seen in human communities where infections may be made up of numerous parasite sub-populations and re-infections may contain antigenically different parasite populations. As a result, functional immunity against malarial infection appears to develop very slowly, and only begins to become effective when an individual has experienced infection by a wide range of parasite sub-populations found in the locality. Some sort of cross-immunity between the various parasite sub-populations

may explain the decrease in the severity of many aspects of infection with age. It is well documented that adults with sufficient functional immunity to maintain most infections received in their own locality at sub-clinical levels, may suffer quite severe clinical illness when exposed to infections originating from other localities (Cohen et al,1961).

Forsyth et al (1988a,1988c) remark on the fact that despite the demonstration of considerable antigenic heterogeneity in natural parasite populations and a comprehensive knowledge of the genetic mechanisms that cause some of the antigenic diversity in P.falciparum, comparatively little is known about the epidemiology of serotypes of variant antigens in endemic areas. Although these studies describe the use of serotypic analysis in epidemiology, and more specifically the small-area variation in prevalence of a serotype of P.falciparum in Papua New Guinea, to date no work has ever been carried out to investigate the importance of serotype-specific immunity in the generation of functional immunity, the relative virulence or transmission efficiency of serotypes, or the effect of infections by mixed serotypes.

6.3 (ii) Multiple parasite sub-population models

Simple models, derived from the basic model introduced at the beginning of Chapter 5 but where infection can be by any of i different parasite sub-populations, can be used to investigate whether observed age-prevalence profiles could be generated by assuming that the "parasite pool" is made up of a number of sub-populations or serotypes. For two parasite sub-populations i and j, the human population is now divided into susceptibles (X), those infected with parasite i only (Y_i), those infected with parasite j only (Y_j), those infected with both parasites i and j (Y_{ij}) and those immune to further infection by sub-population i (Z_i), sub-population j (Z_j) or both sub-populations (Z). Additionally, there are two further classes possible W_i and W_j; these are individuals who are infected by parasite i but immune to parasite j and infected by parasite j but immune to parasite i respectively. The latent class (H) is omitted for simplicity. For the parasite populations i and j the population parameters are defined as L_i, L_j, r_i, r_j and μ with additional parameters for the recovery rate from infections by both types of parasite (r_{ij}), the infection rate by parasite j in an individual already with a parasite i infection ($_iL_j$), and the infection rate by parasite i in an individual already with a parasite j infection ($_jL_i$).

6.3 (iii) Life-long homologous immunity model

6.3 (iii) (a) Model structure

Based on the available experimental and epidemiological data, the simplest assumption concerning the specificity of immunity would be that recovery from infection by a particular sub-population results in the induction of life-long homologous immunity to reinfections by that particular sub-population, but that heterologous immunity to any other parasite sub-populations is not produced. From serological analyses we know that simultaneous infection by more than one serotype is possible, so it is also assumed that cross-infection by different serotypes can occur. However, for simplicity we shall assume that there is simultaneous recovery from all infections. Figure 6.3 is a schematic illustration of the model which will be referred to as the life-long homologous immunity model.

Assuming that there is no parasite-induced mortality, that constant human population size is maintained, and that the two parasite sub-populations i and j, have constant rates of transmission L_i and L_j, the rate of change of the various population fractions can be calculated by the method shown in eqs.(6.1)-(6.3). The rates of change of the fractions in the population who are susceptible (PX), infected (PY) and immune (PZ) can thus be given as:

Susceptible

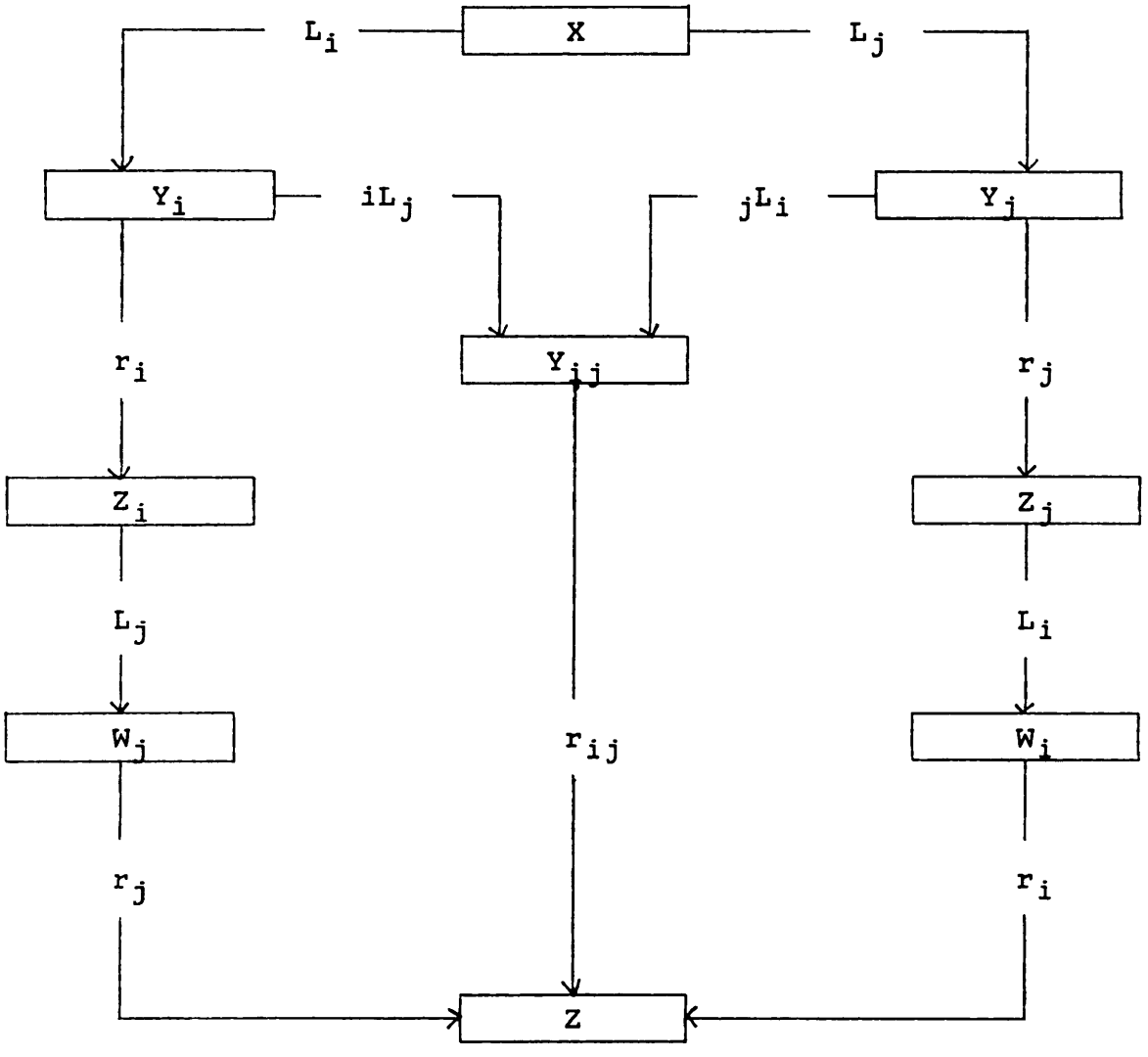
$$\frac{d}{da} PX(a) = - \sum_{i=1}^{j} L_i \; PX(a) \qquad (6.7)$$

Infected

$$\frac{d}{da} PY(a) = \sum_{i=0}^{j} L_i \; PX(a) + L_i \; PZ_j(a) + L_j \; PZ_i(a)$$

$$- (r_j \; PY_j(a) + r_i \; PY_i(a) + r_{ij} \; PY_{ij} + r_i \; PW_i(a) + r_j \; PW_j(a)) \qquad (6.8)$$

Fig. 6.3. Diagrammatic flow chart illustrating transitions between classes of the human population when there are two parasite sub-populations.
The human population is divided into susceptibles (X), those infected by parasite population i (Y_i) or j (Y_j) only (but still susceptible to infection by the other parasite type), those infected by both types of parasites (Y_{ij}), those immune to infection by parasite i (Z_i) or parasite j (Z_j) or both (Z) and individuals infected by parasites i (W_i) or j (W_j) who are immune to infection by the other parasite type. Rate parameters determine the flow of individuals between the classes: the transmission rate for parasite i (L_i) and parasite j (L_j), the recovery rate for single parasite type infections (r_i and r_j) and infections by both types of parasite (r_{ij}). ${}_iL_j$ and ${}_jL_i$ are the transmission rates for parasite populations j and i respectively for individuals already infected by the other parasite type.


L_i
X
L_j
Y_i
iL_j
jL_i
Y_j
r_i
Y_{ij}
r_j
Z_i
Z_j
L_j
L_i
W_j
r_{ij}
W_i
r_j
r_i
Z

(the equations for the individual fractions PY_i, PY_j, PY_{ij}, PW_i and PW_j are shown in Appendix F)

Immune

$$\frac{d}{da} PZ(a) = r_{ij} PY_i(a) + r_i PW_i(a) + r_j PW_j \qquad (6.9)$$

and immune to single sub-populations,

$$\frac{d}{da} PZ_i(a) = r_i PY_i(a) - L_j PZ_i(a) \qquad (6.10)$$

$$\frac{d}{da} PZ_j(a) = r_j PY_j(a) - L_i PZ_j(a) \qquad (6.11)$$

Homologous immunity to a particular sub-population is assumed to have no effect on the transmission efficiency, or on the duration of patency of the subsequent infection by the other sub-population.

The total age-prevalence distributions by age and the prevalences for each sub-population can be obtained with the use of a computer program which enables the above system of differential equations to be solved numerically.

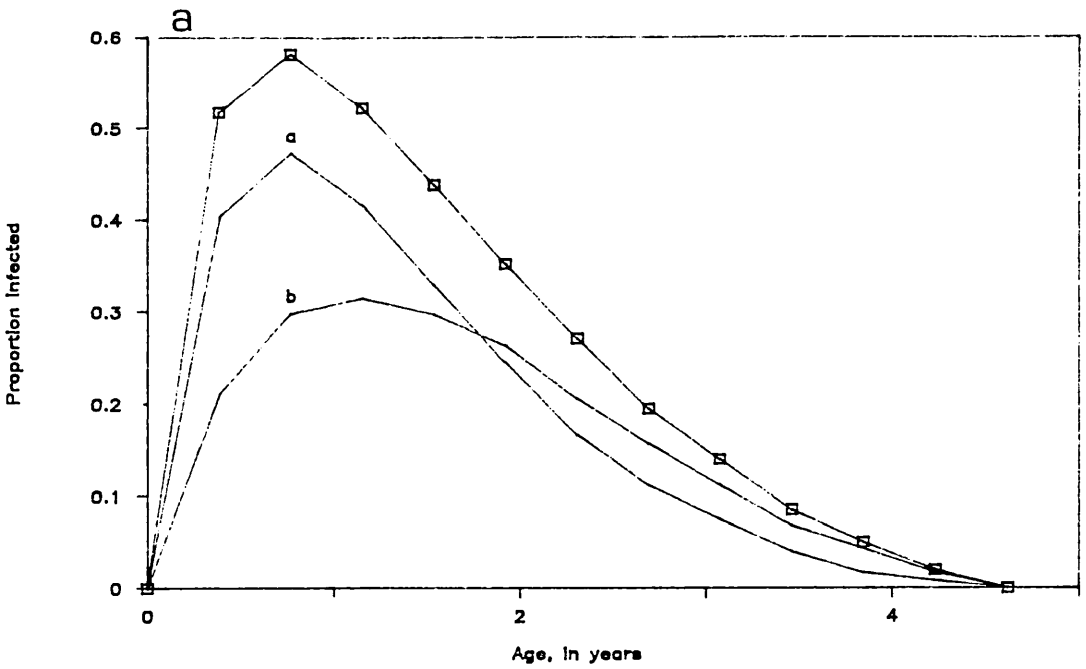
6.3 (iii) (b) Age-prevalence predictions

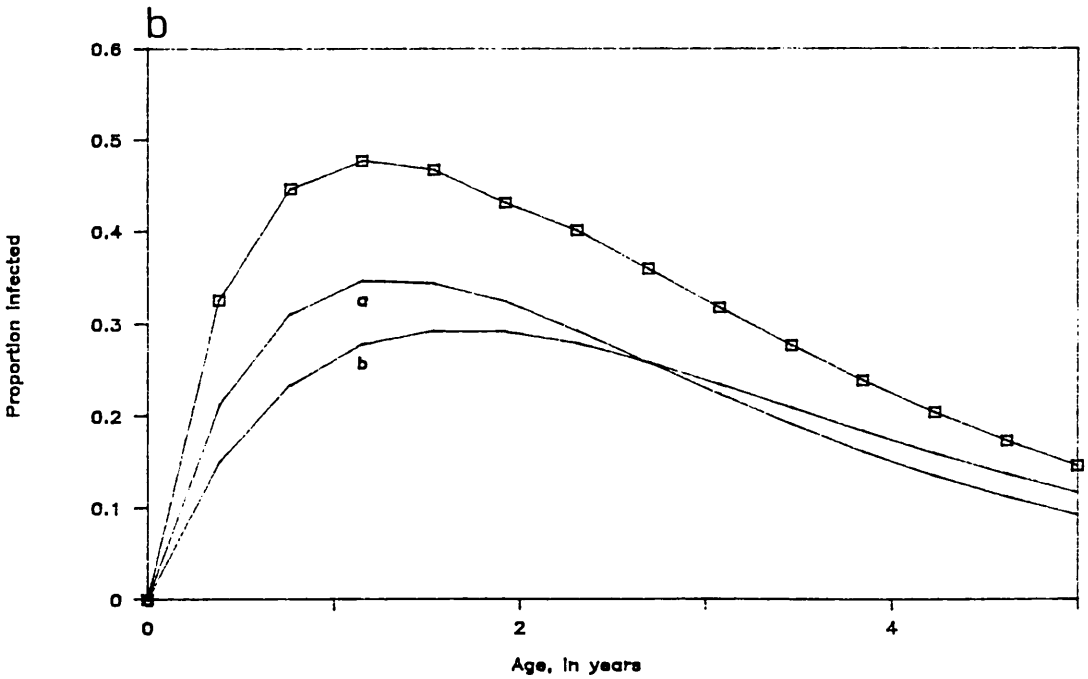
The simple population model defined above, with infection by only two separate sub-populations, is obviously a gross oversimplification of any natural parasite population which undoubtedly would be made up of many more separate sub-populations. However, the addition of just one more sub-population would increase the number of infectious classes from 5 to 19 and the immune classes from 3 to 7, thus making the model very complicated and unwieldy. Despite the simplicity of the two sub-populations model, it still allows us to examine the basic patterns generated when the parasite population is not homogeneous with respect to the induction of immunity.

A variety of different patterns can be generated dependent on the choice of parameter values. Figures 6.4 (a) and (b) illustrate the total prevalences and the prevalence of sub-population i ($PY_i+PY_{ij}+PW_i$) and sub-population j

Fig. 6.4. Heterogeneity in the parasite population

The change in total prevalence of infection with age (symbolled line), where the parasite population consists of two sub-populations. Cross-infection by either strain is possible and each strain is capable of producing life-long homologous immunity. Line a represents the total prevalence of strain i ($PY_i+PY_{ij}+PW_i$) and line b represents the total prevalence of strain j ($PY_j+PY_{ji}+PW_j$), as predicted by eqs.(6.7)-(6.11) and (F.1)-(F.5), for total infection rates of: (a) L = 0.05/week,
(Parameters L_i= 0.035/week (=$_jL_i$) and L_j= 0.015/week (=$_iL_j$))
(b) L = 0.025/week
(Parameters L_i= 0.015/week (=$_jL_i$) and L_j= 0.01/week (=$_iL_j$))
(Additional parameters r_{ij}= r_i= r_j= 0.02/week).


a
0.6
0.5
0.4
0.3
0.2
0.1
0
Proportion Infected
a
b
0
2
4
Age, in years


b
0.6
0.5
0.4
0.3
0.2
0.1
0
Proportion Infected
a
b
0
2
4
Age, in years

(PY_j+PY_{ij}+PW_j) in human communities subjected to two very different total transmission rates (L_i+L_j). The transmission rate in fig. 6.4 (a) is twice that of fig. 6.4 (b). The recovery rate for both sub-populations is, however, assumed to be the same. It is obvious that the distinct differences in the age-prevalence patterns observed in areas of widely different transmission rates is not seen, the patterns being similar when transmission is both high and low. Supplementary to this observation is that the existence of two separate sub-populations allows individuals to become infected twice during a life-time (this may of course occur simultaneously as is the case for Y_{ij}), thus slightly raising prevalence levels for all age-classes and extending prevalence levels into older age-classes. (Compare with the complete immunity model in section 5.3 where only one infection is possible, eq.(5.23) and fig. 5.6 (a)).

The addition of further sub-populations would undoubtedly raise prevalences still higher. However, no matter how many sub-populations there are, if the parasite population is stable with regard to both the presence and transmission efficiency of the various sub-populations and within the constraints of realistic total transmission rates, prevalence levels could never remain at observed levels across all age-classes. Children rapidly become infected by all available strains and become immune to further infection. Thus the prevalence rate becomes zero in older age classes. For high non-zero prevalences in older age-groups to exist the age of maximum prevalence of infection by some of the sub-populations must be placed among the older age classes. Equations (6.7)-(6.11) and (F.1)-(F.5) cannot be solved by analytical means to give an expression for the age of maximum infection by a particular sub-population. However from fig. 6.4 it is obvious that the age of average infection is increased if L_i is small. Alternatively, a decrease in the value of r_i would allow infection to accumulate in the population and thus also have the effect of increasing the age of maximum infection (fig. 6.5). For a sub-population with a very low transmission efficiency (L_i small), infection levels would be very low in all age-groups (not comparable with those of observed profiles). High prevalence levels in older age-classes would only be possible if recovery from infection was exceptionally slow, so that infection could accumulate in the population. Although quite wide variations in recovery rates from infections in different geographic areas have been reported (Ciuca,1955; Earle,1962), the magnitude of the recovery rates required to generate realistic age-prevalence profiles within the model framework above are probably prohibitively large for stable multiple parasite populations with life-long homologous immunity to be a viable explanation for observed age-prevalence patterns.

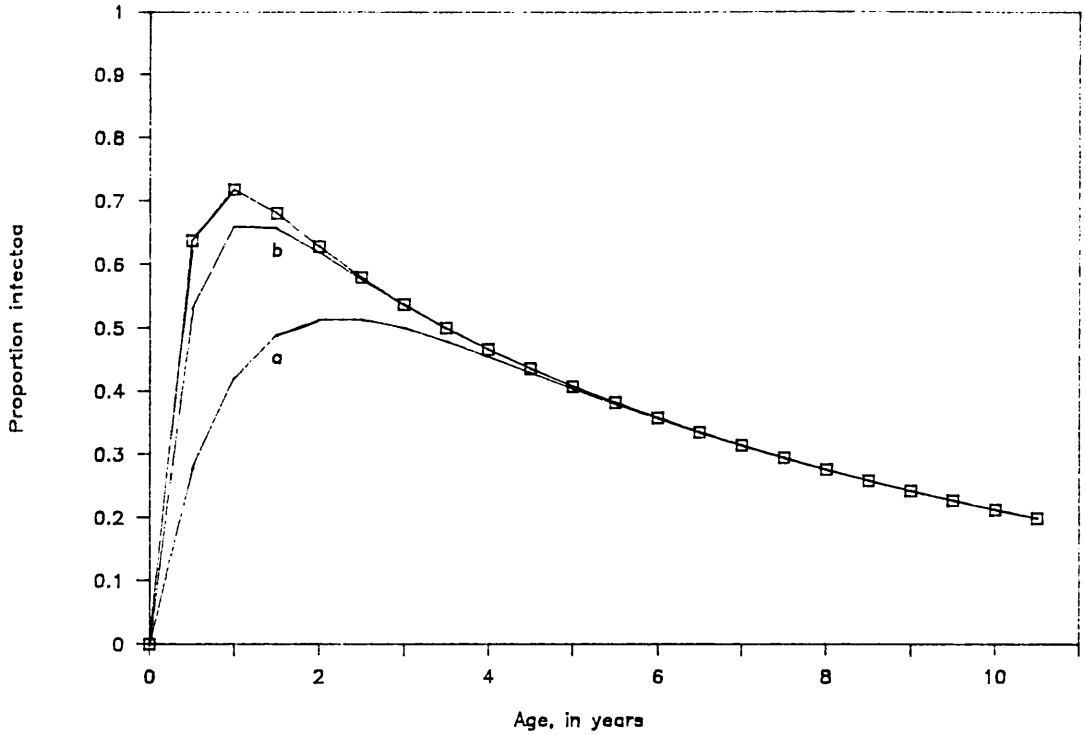


Fig. 6.5. The change in total prevalence of infection with age (symbolled line), where the parasite population consists of two sub-populations. Cross-infection by either strain is possible and each strain is capable of producing life-long homologous immunity. The recovery rate is related to the magnitude of the transmission rate such that, $r_n = (r_0 - bL_n)$ for n = i, j or ij.

Line a represents the total prevalence of strain i ($PY_i+PY_{ij}+PW_i$) and line b represents the total prevalence of strain j ($PY_j+PY_j+PW_j$), as predicted by eqs.(6.7)-(6.11) and (F.1)-(F.5). (Parameters L_i= 0.035/week ($=_jL_i$), L_j= 0.015/week ($=_iL_j$), L_{ij}= (L_i+_Lj), r_0= 0.02 and b= 0.35)

However, a pattern of infection involving small sub-sections of the parasite population (L_i small) that produce chronic infections which tend to fluctuate between patency and sub-patency over an extended period of time without true recovery (r_i small) may be worth further investigation, (especially in view of the observed tendency for this type of infection to increase with age).

6.3 (iv) Temporary homologous immunity model

The life-long immunity model can easily be adapted to take into account the possibility that immunity may be of finite length, the exact duration of immunity being dependent on re-exposure. The equation for the rate of change in the proportion susceptible (eq.(6.7)) becomes replaced by:

$$\frac{d}{da} PX(a) = - \sum_{i=1}^{j} L_i PX(a) + {}_i PZ_i(a) + {}_j PZ_j(a) + PZ(a) \qquad (6.12)$$

and for the immunes (eqs.(6.9)-(6.11)),

$$\frac{d}{da} PZ(a) = r_{ij} PY_i(a) + r_i PW_i(a) + r_j PW_j - \pi PZ(a) \qquad (6.13)$$

$$\frac{d}{da} PZ_i(a) = r_i PY_i(a) - PZ_i(a) (L_j + \pi_i) \qquad (6.14)$$

$$\frac{d}{da} PZ_j(a) = r_j PY_j(a) - PZ_j(a) (L_i + \pi_j) \qquad (6.15)$$

The rates of loss of immunity for the individual sub-population (π_i or π_j) and for both parasite populations (π) are calculated using the equations:

$$\pi_n = \frac{L_n}{(\exp (L_n \tau_n) - 1)} \qquad (6.16a)$$

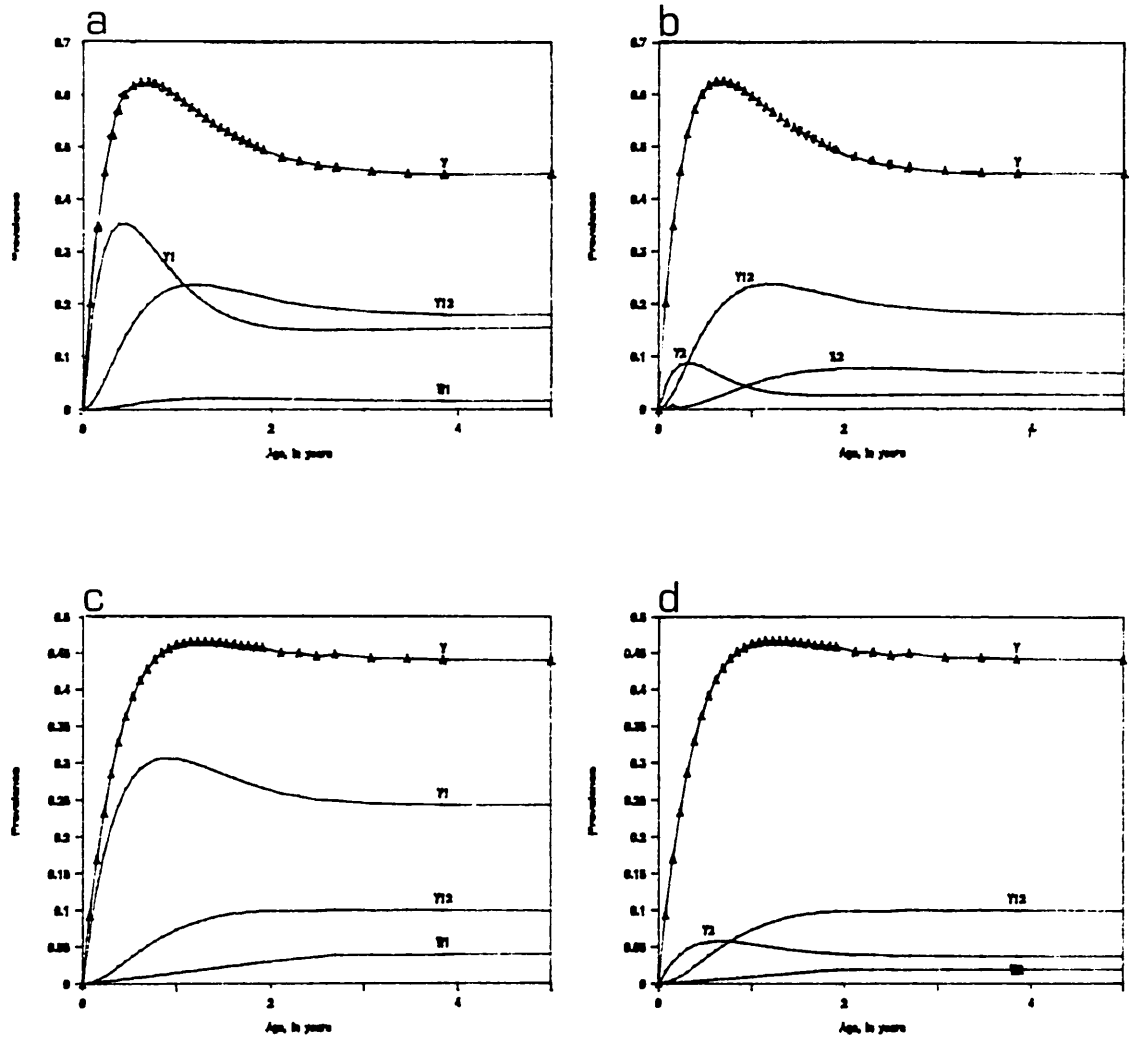


Fig. 6.6. The change in total prevalence of infection with age (symbolled line), for two parasite sub-populations, with temporary homologous immunity. (a) and (c) prevalence of strain i ($PY_i+PY_{ij}+PW_i$), (b) and (d) prevalence of strain j ($PY_j+PY_{ji}+PW_j$), as predicted by eqs.(6.8), (6.12)-(6.16b) and (F.1)-(F.5). Total infection rate, (a)-(b) L = 0.06/week, (L_i= 0.045/week ($=_jL_i$), L_j= 0.015/week ($=_iL_j$)); (c)-(d) L = 0.025/week, (L_i= 0.02/week ($=_jL_i$), L_j= 0.005/week ($=_iL_j$)), ($r_i = r_j = r_{ij}$ = 0.02/week, $\tau = \tau_i = \tau_j$= 26 weeks)

$$\pi = \frac{(L_i + L_j)}{(\exp((L_i+L_j)\Upsilon) - 1)} \qquad (6.16b)$$

Υ and Υ_i are the durations of immunity in the absence of re-exposure (see section 5.4 (ii)).

Figure 6.6 illustrates the total prevalence and the prevalence of sub-population i ($PY_i+PY_{ij}+PW_i$) and sub-population j ($PY_j+PY_{ij}+PW_j$). The duration of immunity for the two sub-populations is assumed to be the same, the actual loss of immunity is however quite different since re-exposure by the two populations does not occur at the same rate.

6.3 (v) Non-stable parasite sub-populations

The age-prevalence curves generated by both the life-long and temporary homologous immunity models above would appear to indicate that observed profiles cannot be produced by stable multiple parasite sub-populations where transmission of a particular sub-population is constant through time. Members of the human population rapidly experience infection by all available parasite sub-populations. Infection is therefore confined to the youngest age-classes and, in the case of immunity being temporary, a small portion of the adult population. In reality of course, transmission does not occur at a constant rate but depends on the proportion of infected people (eq.(5.15)). Hence transmission will vary with, and depend on, the availability of infected humans. Transmission rates will only be constant if conditions affecting the transmission and maintenance of disease remain constant and these conditions allow all sub-populations to co-exist. The presence of individual parasite sub-populations therefore may not be constant through time but may be transitory with new sub-populations constantly being introduced and others dying out. Anderson(1979) shows how the establishment of infectious disease in a particular population depends on the ability of the parasite population to grow (i.e. $R_0 > 1$). Persistance through time depends critically on the availability of infected individuals as a source of infection and the availability of susceptibles to continue the cycle of transmission. The size of the population and the birth rate, as they affect the net rate of input of susceptibles, and the disease parameters of the individual parasite sub-populations, as they affect the ratio of losses and gains to those individuals infected by a particular sub-population, will therefore be important in determining whether sub-populations persist through time and

display endemic patterns of infection or whether the parasite sub-populations move through communities in waves or epidemics.

Analysis of the stability properties of eqs.(6.7)-(6.11) and (F.1)-(F.5) is extremely complicated. However data by Forsyth et al(1988a,1988b) concerning the stability and persistence of a distinct parasite sub-population, the FC27 serotype (identifiable by the presence of FC27 S-antigen) in two very different sized communities in Papua New Guinea may be used as an indicator of possible patterns of infection. The distribution of the FC27 serotype in the town Madang was not found to vary significantly in the population in the 2 year study period. The serotype is known to have been present in the parasite population in Madang for at least 8 years (Forsyth et al,1988a). Village-based studies in the areas surrounding Madang, however, show a quite different pattern of disease. The prevalence of the FC27 serotype was seen not only to vary quite substantially in individual villages during the 2 year study period, but also to vary between villages (Forsyth et al,1988b). The fact that the majority of anopheline vectors were found to return to the same village for blood meals (Charlwood et al,1986) is obviously an important factor in restricting infection to foci. The patterns of infection observed included both significant increases or decreases (including fade-out) in the prevalence of the FC27 serotype, and also undetectable or very low levels of FC27 serotype. Assuming that the transmission capabilities and induction of immunity to the serotype are identical in rural and urban areas, it is possible that the differences in the patterns of infection between these two areas are due to variations in the availability of susceptibles. If, after the introduction of a serotype, the number of people infected rapidly rises to a peak and then slowly decays as the majority of people join the immune class (either temporarily or permanently) the serotype can only persist if there is a constant influx of susceptibles. In the small villages around Madang it might be expected that serotypes " fade-out " once a high proportion of the population becomes immune, or if the reservoir of infection in the infected individuals becomes low. Whereas in Madang, movement of people within the town and the larger influx of susceptibles, due to the higher number of births, may allow the serotype to persist. The reservoir of infection within the villages was found to be low and, particularly in the villages with higher FC27 serotype prevalences, antibody to the serotype was high. It should be noted, however, that in Madang a second serotype K1 was also found to be stable in the population over the same time period, whereas a third serotype NF7 was found to have died out. Whereas it is possible that different disease parameters for this serotype may have effected its ability to persist in the population,

it is also possible that fade-out is an intrinsic property of malarial transmission in communities of people of moderate or small size.

The factors controlling the patterns of prevalence are clearly very complex. However, the evidence described above does seem to support the idea that transmission of sub-populations of P.falciparum can be periodic. If the persistance of sub-populations vary so much between rural and urban areas, one might expect these differences to be reflected in the age-specific prevalence profiles found in rural and urban areas. There do not appear to be any obvious differences between profiles from urban and rural areas.

Assuming that serotype-specific immunity exists, and that epidemics of serotypes enable stable prevalence levels to be maintained in the population, a question that must be addressed is: " Can the observed 90-100% cumulative prevalence rates amongst all people irrespective of age be explained ? " If life-long serotypic immunity exists, the classical pattern of periodic epidemics of a number of different serotypes (which can occur concurrently) would not give rise to the level of infection observed amongst adults, since by adulthood they would be immune to all serotypes. Additionally, reservoirs of infection for the re-introduction of old serotypes may be limited in isolated human communities. The spontaneous appearance of new and novel serotypes could of course produce infection in all age-groups. However, it is improbable that the malarial parasites have an antigenic repertoire wide enough for serotypes to be produced over the full life-time of an individual and still be recognised as antigenically distinct.

6.3 (vi) Evidence for and against homologous immunity

The literature contains evidence both for and against the existence of sterile or "true" homologous immunity to re-infection with "strains" of P.falciparum in humans. However, the lack of sterile heterologous immunity toward both heterologous "strains" and heterologous species appears to be universal (James & Shute,1926; Jeffery,1966). The majority of controlled reinfection experiments (on human neurosyphilitics and prison volunteers with no prior history of malarial infections) show that although prior homologous infection (and to a lesser extent heterologous infection) affects qualitative features of infection such as parasitaemias, the frequency of recrudescences and clinical attacks and pre-patent periods, very few individuals showed signs of "true" immunity after homologous re-infection (James & Shute,1926; Jeffery,1966; Ciuca,1955). It should be noted, however, that

these infections were not identified as originating from genetically distinct parasite populations, but were merely isolates from known geographical locations. So although re-infections were by identical parasite populations complications to the outcome of re-infection due to infections being of more than one antigenic type cannot be ruled out. Furthermore, primary infections were abbreviated by the use of drugs and were invariably eliminated prior to re-infection. Covell & Nicol(1951) and Collins et al (1964a,1964b) both show that in the absence of drug treatment, "true" homologous immunity appears to exist for at least some individuals, in each case the presence of sub-patent parasitaemias at the time of challenge was suspected to be an important factor.

Studies on the persistance of specific antibody following experimental infections (Collins et al,1964a,1964b; Collins et al,1971) have shown that high antibody levels persist during terminal periods of low and sub-patent parasitaemia in a similar way to the premunition type immunity seen among many experimental animal models (Jayawardena et al,1977; Freeman & Parish, 1981). The existence of such responses may be operative in the prevention of reinfection by a homologous parasite population. In most cases this antibody level was seen to decrease sharply to a low level following the termination of infections. However, antibody responses have been seen to persist for months and even years after the termination of infection (Collins et al,1968). This low level of antibody would appear sufficient to modify the course of the re-infection but is unable to prevent patency. Ciuca(1955) and others have found that the partial protection afforded by the initial infection decreases significantly if the interval between the two inoculations is greater than one year. No mention is made in the literature of the effect of repeated infections on the development and maintenance of homologous immunity.

Although these types of controlled experimental infections may be indicative of the response to infection of naive infants born in endemic areas, they may differ significantly from infections in older individuals in the same area where infection patterns may be complicated by the presence of pre-existing infections or partial immunity. Bray et al(1962) in Liberia and Bruce-Chwatt(1963a,1963b) in Nigeria have reported results of inoculations to adults in endemic areas with P.falciparum. In these studies over half of the individuals inoculated (parasites isolated either from children or from naturally infected mosquitoes in the same area) developed patent parasitaemias with between 15% and 25% showing signs of clinical symptoms. It is surprising that infection should be so easy in African adults, who having been exposed to malaria since childhood must have

considerable "multi-strain" experience of infection, and assuming homologous immunity exists, considerable "multi-strain" resistance to infection. Although drugs were not administered during the experiments (those requiring treatment were excluded from the final assessment of results), the adults tested had been in the habit of taking chloroquine to treat clinical symptoms of infections received prior to the experimental period. Hence impairments to the development of immunity during this period cannot be ruled out.

As indicated in the introduction to this section, the immunological means of distinguishing between two genetically defined sub-populations of the same species of malaria has recently become available. Controlled homologous re-infection studies, however, have not been done and indeed modern moral and ethical objections to such experiments may prevent such work in the future. These genetic markers can however be used to identify the occurrence of natural re-infections; Forsyth et al (1988b) indicates that reinfection by the FC27 S-antigen serotype was not recorded in any of the 31 individuals tested at a time 7 months after detection of the original FC27 S-antigen type infection. This seems to indicate that there may be serotype-specific immunity toward parasites bearing the FC27 S-antigen. The small number of people recorded as having FC27 S-antigen infections (and therefore a potential source of infection), and the fact that actual active transmission of this particular serotype was seen to vary considerably during the time period, does make it difficult to ascertain just how much opportunity there was for homologous reinfection and therefore whether sero-type specific immunity exists. Another explanation could be that repeated re-infection, or superinfection by the FC27 S-antigen serotype, may well be able to generate sero-type specific immunity. The draw back of this explanation is that one would have expected some re-infection among those individuals that had not yet developed full immunity.

At present, due to the lack of work carried out on homologous re-infections by very defined parasite populations, it seems impossible to determine whether homologous immunity to small defined sub-populations of parasites does exist. Analysis of such re-infections and the development of multi-parasite population models may be quite unrealistic in the context of natural malaria infections which undoubtedly contain mixed populations of parasites. The majority of evidence at present would seem to indicate that if "true" homologous immunity to reinfections by mixed parasite populations does exist, it is undoubtedly of short duration. However, the existence of temporary partial homologous and heterologous immunity to re-infection is in less doubt. The chronic nature of malaria infections in man

and the slow development of immunity (dependent on the repeated exposure to infection, Howard,1986) could well be an intrinsic feature of the host-parasite relationship (probably modified by the presence of antigenically variable parasite populations). Features such as these are typical of malarial infections in their natural hosts. The observation that immunological memory may well be of finite length is of particular interest and is pursued further in the next chapter.

6.4 Gradual acquisition of functional immunity into the human population

6.4 (i) Introduction

In the preceding sections it was suggested that although heterogeneity within both human and parasite populations can modify both the amount of malaria in the human population and also the age-prevalence patterns, they do not appear to be one of the major factors associated with creating the characteristic variations in observed age-prevalence curves between areas subject to different transmission levels. Although the modifying effects that these heterogeneities have should be noted, models indicate that they may not be instrumental in producing the observed gradual decline in prevalence with age. The models based on simple examples of such heterogeneity do little to improve on the general patterns generated by the simple transmission dependent loss of immunity model introduced in section 5.4 (ii). The importance of the transmission rate, in both modifying recovery and loss of immunity and thus in shaping age-prevalence profiles was demonstrated in that section.

A major problem with the structure of the transmission dependent loss of immunity model (section 5.4 (ii)) is that the response to infection among children and adults is assumed to be the same. Epidemiological data, however, indicates that many aspects of malarial infection among children are quite different (section 2.2), and adults subject to high transmission rates have an intrinsic capability to recover more quickly and remain uninfected for longer periods between infections than children. To summarise, children are seen to suffer longer, more severe and repeated bouts of infection, with many of the qualitative features of infection also being more severe. Children experience higher parasitaemias, more frequent and distinct pathological and clinical expressions of the disease, and have significantly higher malaria-related mortality. These features of childhood malaria are seen to gradually decrease in intensity with age in all human communities, except those

which experience very low levels of infection. It is evident that these observations are in keeping with the hypothesis that functional immunity against malarial infection is gradually acquired.

Further indication that acquired immunity is not at a level high enough to have a significant effect on infection in early childhood, and only becomes an important modifying influence later in life, is the rise in the proportion of the population who are heterozygous for the sickle-cell gene (Hb AS) with age throughout early childhood but not beyond. Individuals who are Hb AS posses an innate ability to partially control the severity of infection by P.falciparum. As discussed in section 2.1 (iii) children who are Hb AS have lower average frequency and density of P.falciparum asexual stages, higher probabilities of surviving the severe malarial infections of early childhood. In a few studies the prevalence of malaria was found to be significantly lower in Hb AS children than in normal individuals (Gilles et al,1967; Walters and Bruce-Chwatt,1956). In comparison to acquired immunity, innate immunity to infection has only a small effect on the severity of infection. However, among children, where acquired immunity has not yet developed, and during pregnancy (particularly in the lower transmission areas) when acquired immunity is often impaired (Fleming et al,1968; Allison,1964), the sickle cell trait protects against severe malaria. The rise in the proportion of Hb AS children during early childhood is a reflection of the survival advantage of Hb AS. The age at which the proportion who are Hb AS stops rising is probably the age at which being Hb AS is no longer a significant advantage and where functional immunity starts to become effective. Several studies have indicated the survival advantage of Hb AS individuals (Edington,1967; Livingstone,1971; Raper,1960; Fleming et al,1979). They illustrate how the prevalence of Hb AS in the population rises until the age of about 5-8 years, after which the level is maintained throughout all the older age-groups. The study by Fleming et al(1979) among a large population in Kano State, Northern Nigeria found the prevalence of Hb AS to be 0.24 among newborns, rising to 0.29 in those aged over five years. One point of note however, is that from malariometric data it is evident that functional immunity to the various aspects of infection are not acquired at the same rate, so that the age at which the functional immunity that reduces the severity of infection becomes effective (the prevalence of Hb AS is seen to reach a plateau), does not correspond with the age at which acquired immunity begins to increase recovery rates or increase the periods between infections. A rough comparison of the ages at which malaria-induced mortality and morbidity and the density of parasitaemia and average prevalence rates start to decline seems to indicate that functional immunity against the various aspects of

infection follow the following sequence: (i) mortality, (ii) morbidity, (iii) density of parasitaemia, (iv) prevalence levels.

6.4 (ii) Basic structure of the model

From the available evidence, a more realistic framework to describe the acquisition of functional immunity would be the one shown in fig. 6.7. The sequence of events may be summarised as follows. All individuals are assumed to be born into the susceptible non-immune group (X_1), where they rapidly become infected at a rate L, to enter the infected non-immune class (Y_1). The recovery rate among the non-immunes r_1, is assumed to be slow, and there is no period of temporary immunity following infection. Low recovery rates, and a rate of loss of immunity equivalent to one means, that for high values of L, high prevalence rates similar to those observed will be generated. Functional immunity is assumed to be acquired at a rate δ, which results in a much more rapid recovery rate r_2 , from the infected state (Y_2), and in periods of temporary immunity (Z_2). These factors effectively reduce the duration of time which individuals are infected, and also increases the time between infections. Functional immunity among adults will therefore generate much lower prevalences among the oldest age groups. The infection rate amongst the functionally immune is assumed to be identical to that of the non-immune. Although the structure of the flow diagram in fig. 6.7 describes the gradual acquisition of functional immunity by the population (determined by the value of δ), immunity is not gradually acquired by individuals, for them functional immunity will still be either "all or nothing".

The sequence of events illustrated in fig. 6.7 can be used to form a model framework which describes the dynamics of malarial infection in the human population where functional immunity is gradually acquired by the population. A series of first-order nonlinear differential equations can be used to describe the rates of change of the proportions of $X_1(t)$, $Y_1(t)$, $X_2(t)$, $Y_2(t)$ and $Z_2(t)$ individuals through time.

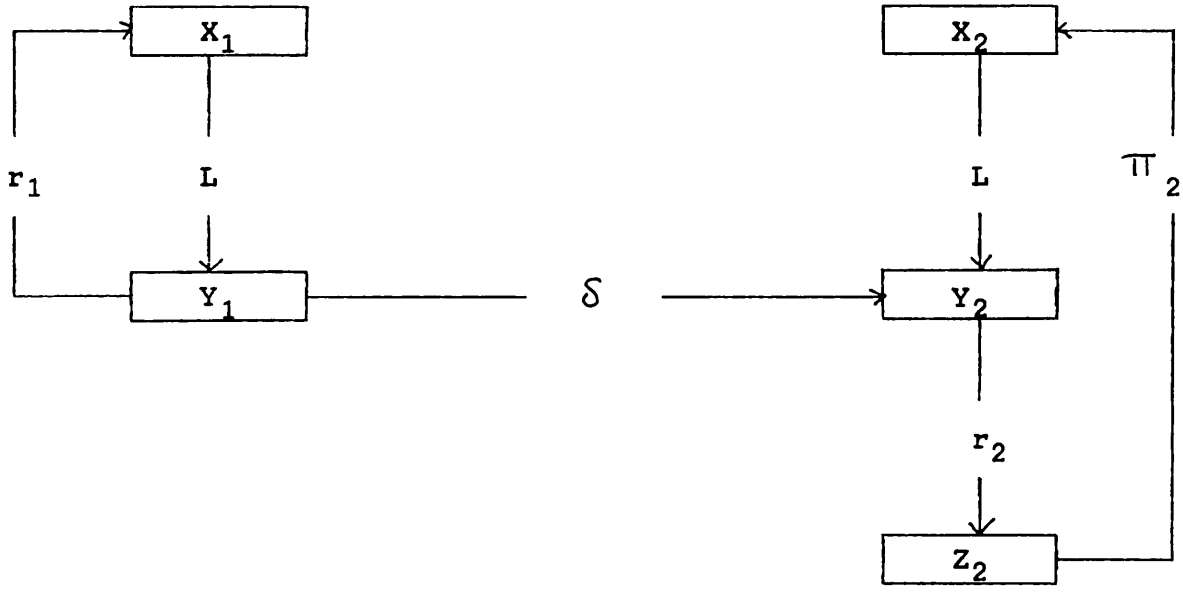


Fig. 6.7. Diagrammatic flow chart illustrating transitions between classes of the human population for the model in eqs.(6.17)-(6.22).
The population is divided into non-immunes (X_1 and Y_1) and functionally immunes (X_2, Y_2 and Z_2): where X_1 and X_2 are susceptible to infection, Y_1 and Y_2 are those infected and Z_2 are immune to infection. Rate parameters determine the flow of individuals between these classes: the per capita transmission rate (L), the recovery rates for the non-immunes (r_1) and the functionally immunes (r_2), the rate of loss of immune status (π_2) and the rate of acquisition of functional immunity (δ).

For the non-immunes,

$$\frac{d}{dt} PX_1(t) = - L\, PX_1(t) + r_1\, PY_1(t) \qquad (6.17)$$

$$\frac{d}{dt} PY_1(t) = L\, PX_1(t) - r_1\, PY_1(t) - \delta\, PY_1(t) \qquad (6.18)$$

For the Functionally immunes,

$$\frac{d}{dt} PX_2(t) = - L\, PX_2(t) + \pi_2\, PZ_2(t) \qquad (6.19)$$

$$\frac{d}{dt} PY_2(t) = L\, PX_2(t) - r_2\, PY_2(t) + \delta\, PY_1(t) \qquad (6.20)$$

$$\frac{d}{dt} PZ_2(t) = r_2\, PY_2(t) - \pi_2\, PZ_2(t) \qquad (6.21)$$

and the equation for the change in total population prevalence PY(t), where $PY(t) = (PY_1(t) + PY_2(t))$, through time is given by:

$$\frac{d}{dt} PY(t) = L\, PY(t) - r_1\, PY_1(t) - r_2\, PY_2(t) \qquad (6.22)$$

At time $t = 0$, $PX_1(t) = 1.0$, and $PY_1 = PX_2 = PY_2 = PZ_2$. The structure of the model assumes that all individuals are inherently capable of acquiring functional immunity.

6.4 (iii) Age-prevalence predictions and observed patterns

If conditions for transmission of infection are assumed not to have changed dramatically over the life-span of the oldest individuals in the population, then trends with increasing age can be considered equivalent to changes occurring in a cohort of newborns through time. Consequently, equations formulated for $X_1(t)$ etc. may be used in an age-prevalence model where time is equated to age.

The equations (6.17)-(6.22) do not have an exact analytical solution. The equilibrium age-prevalence profiles

may be obtained with the use of a computer programme which enables the above system of differential equations to be solved numerically.

6.4 (iii) (a) Functional immunity acquired at a constant rate

Initially all the rate parameters controlling the movement within and between the non-immune and the functionally immune are assumed to be constants, independent of both the transmission rate and the age of the individual.

Figures 6.8 (a), (b) and (c) show the total age-specific prevalence of infection generated by different values of δ, for a transmission rate of 0.05/week. It is apparent that not only is the decline in prevalence with age much more gradual than for the model which assumed a homogeneous response to infection by both children and adults (figs. 5.15 (a) and 5.18), but also that total prevalence levels among both the very young and also the adult section of the population closely resemble those expected for an infection rate of about 0.05/week.

Looking in detail at the profiles, we can see that even small changes to δ can have quite a marked influence on the shape of the total age-prevalence profiles generated by the model. It is evident that a small δ value aids the "accumulation " of infection within the population, so that higher prevalences are sustained over wider age ranges. Larger values for δ however tend to exaggerate the decline in total prevalence with age, so that maximum prevalence occurs at a younger age and also prevalences are lower among adults aged 30. Figures 6.8 (a), (b) and (c) also shows the relative contributions to the total prevalence by the non-immune and functionally immune sections of the population. An interesting feature of these individual prevalence rates is the "cross-over" point, or the age at which the prevalence rate amongst the functionally immune contributes more to the total prevalence than the non-immunes, is also seen to modified by the value of δ . For δ values of 0.0027, 0.00192 and 0.00128 the "cross over" occurs at the age of 13, 18.5 and 26 years respectively.

Although the total prevalence profiles generated by the model do resemble observed age-prevalence profiles much more closely, there is still one major difference between observed profiles and those of fig. 6.8. Whereas high prevalences (0.8-0.7) are maintained between the ages 4-8 years in observed profiles, in fig. 6.8 maximum prevalences are reached by the age of 1-2 years after which the prevalences start to decline. Therefore, although the predicted prevalences among the very young and those over 16 years are

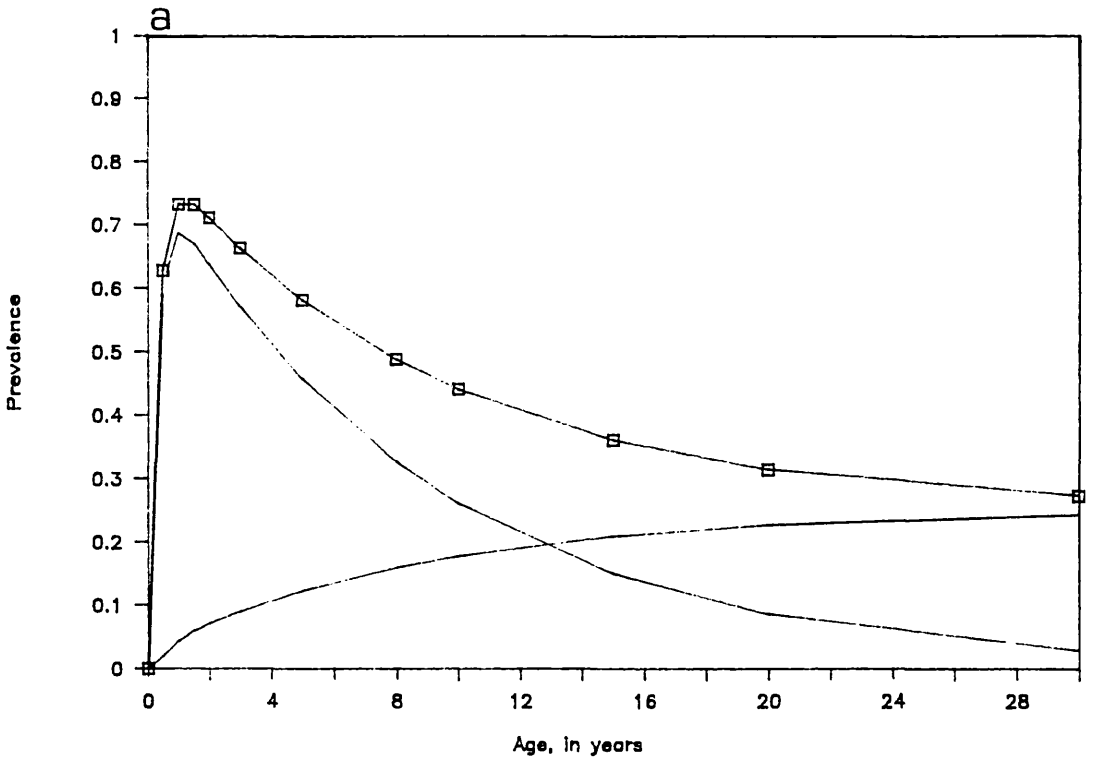
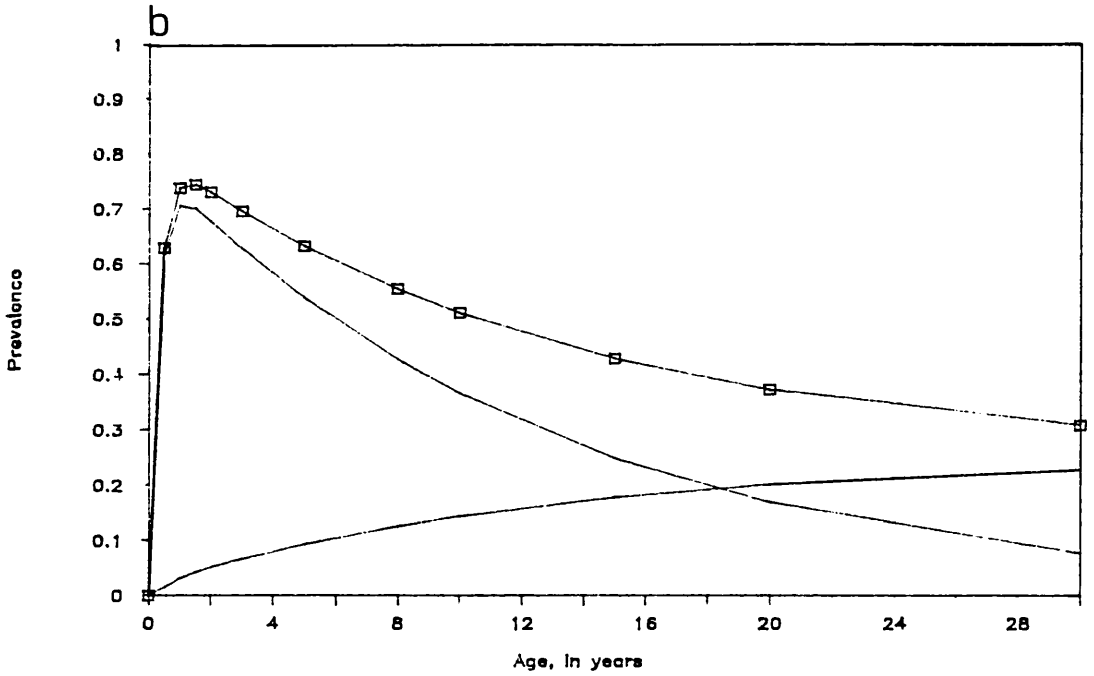
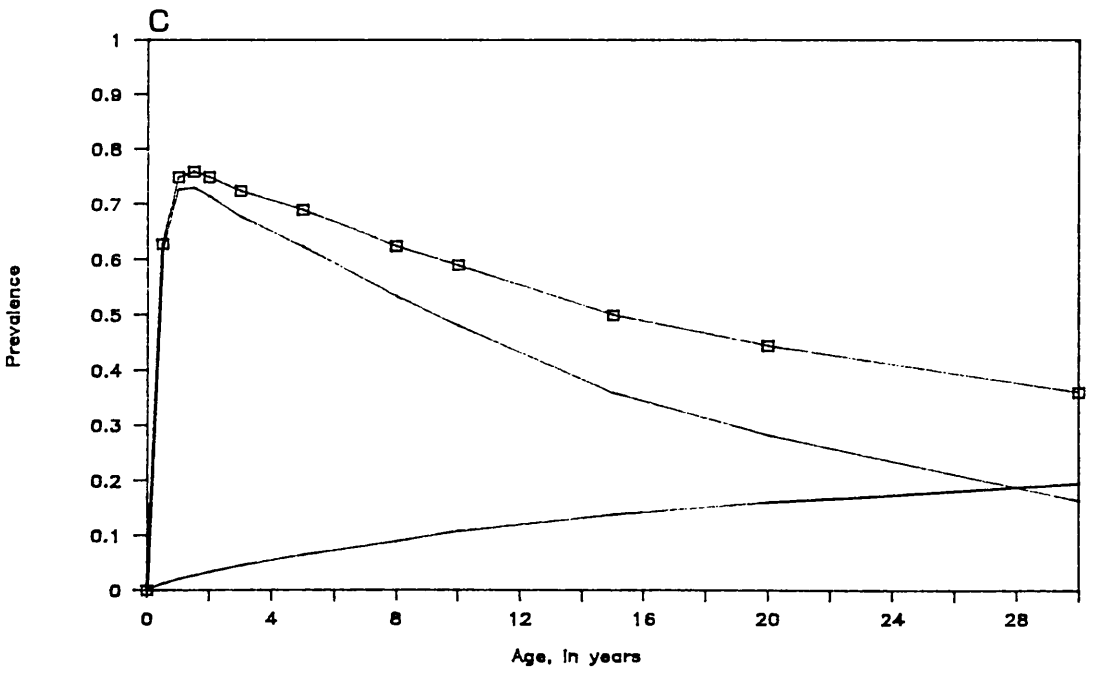


Fig. 6.8. The change in total prevalence with age (symbolled line), showing the rise in the contribution of the functionally immunes (Y_2) and the decline in that of the non-immune (Y_1) with increasing age, as given by eqs.(6.18), (6.20) and (6.22). Where, (a) δ = 0.0027/week, (b) δ = 0.00192/week and (c) δ = 0.00128/week (Parameter values L=0.05/week, r_1=0.014/week, r_2=0.03/week and π_2=0.0128/week)


b
Prevalence
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
4
8
12
16
20
24
28
Age, in years


c
Prevalence
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
4
8
12
16
20
24
28
Age, in years

a reasonable estimate of observed prevalences, predicted prevalences between the ages 3-15 years are much too low, being in the order of 0.65-0.48. A reduction in the value of δ would not produce a differential increase in prevalence amongst these 3-15 year olds, it would simply increase prevalences in all age groups. It is evident therefore that the rate of transfer from the non-immune class to the functionally immune cannot be a constant, but must be less rapid in these young age-groups and then increase with age amongst the older children.

Figure 6.9 illustrates the rise in functionally immune individuals ($X_2 + Y_2 + Z_2$) with age, for a constant rate of transfer, $\delta = 0.0027$. By the age of 5 years, 40% of the children are assumed to be functionally immune, which rises to 57.5% amongst those aged 8 years. This rise would appear to be far too rapid, particularly since it was mentioned in the introduction that epidemiological data seems to suggest that functional immunity directed at increasing both the recovery rate and also the length of time between infections (and thereby reducing the prevalence of infection) is slow to develop in comparison with the ability to control other aspects of infection.

6.4 (iii) (b) Functional immunity acquired at a rate which is a function of age

More realistic profiles can be produced if the rate at which functional immunity is acquired (δ) is replaced by an age-dependent rate, $\delta(a)$. A wide variety of patterns can be generated depending on the functional form of $\delta(a)$. The decline in prevalence among the 3-15 year olds is of course extremely sensitive to the functional form of $\delta(a)$, and to the estimated values for the parameters controlling the function. However, it is important to note that the prevalence of infection among both infants and adults is also critically dependent on the estimates for the parameters, and on the values for the function when $a \approx 0$ and $a \approx a^{max}$ (where a^{max} is the maximum age limit). A linear increase in the value of $\delta(a)$ with age or an exponential increase in $\delta(a)$ with age, are two simple functions which might fit the assumption discussed above, whereby functional immunity is acquired less rapidly by infants but much more quickly by older children.

Figure 6.10 shows the pattern for the age-prevalence profile (fig. 6.10 (a)) and the rise in functional immunity with age (fig. 6.10 (b)) when $\delta(a)$ is an exponential function of age. Although the high observed childhood prevalences are now maintained over realistic age limits, the exponential relationship of $\delta(a)$ makes the change-over from the high prevalences among children and the low prevalences among

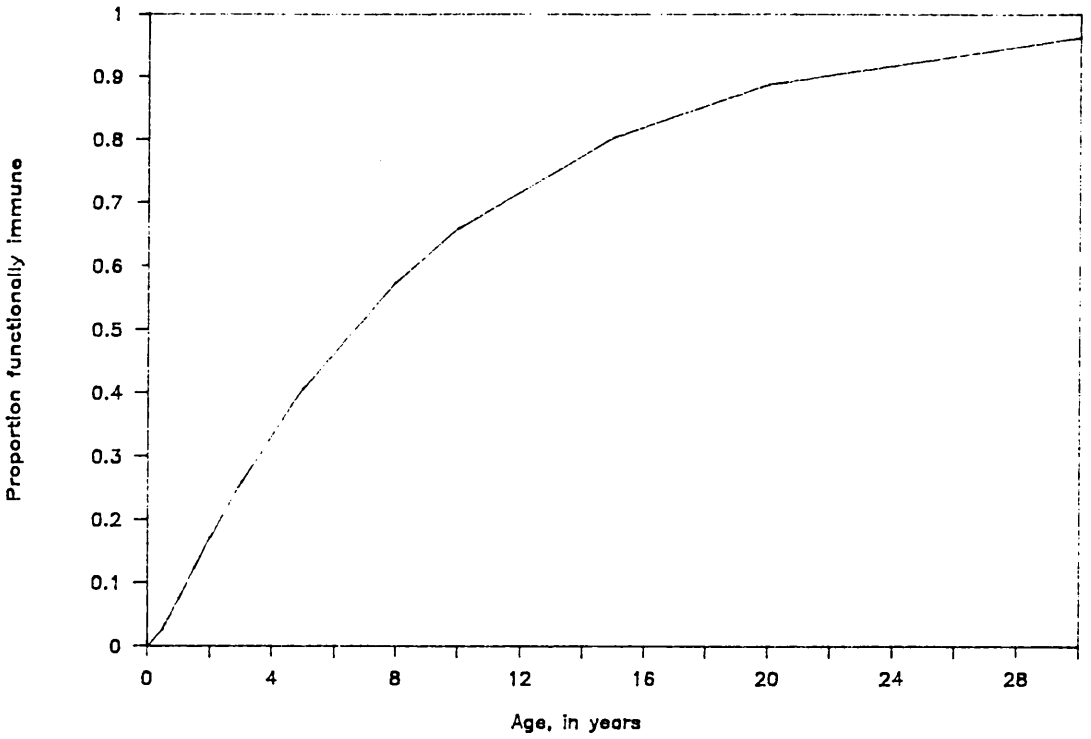


Fig. 6.9. The rise in the proportion functional immune (PX_2 + PY_2 + PZ_2) with age given by eqs.(6.19)-(6.21). (Parameter values L=0.05/week, r_1=0.014/week, r_2=0.03/week, π_2=0.0128/week and δ =0.00277).

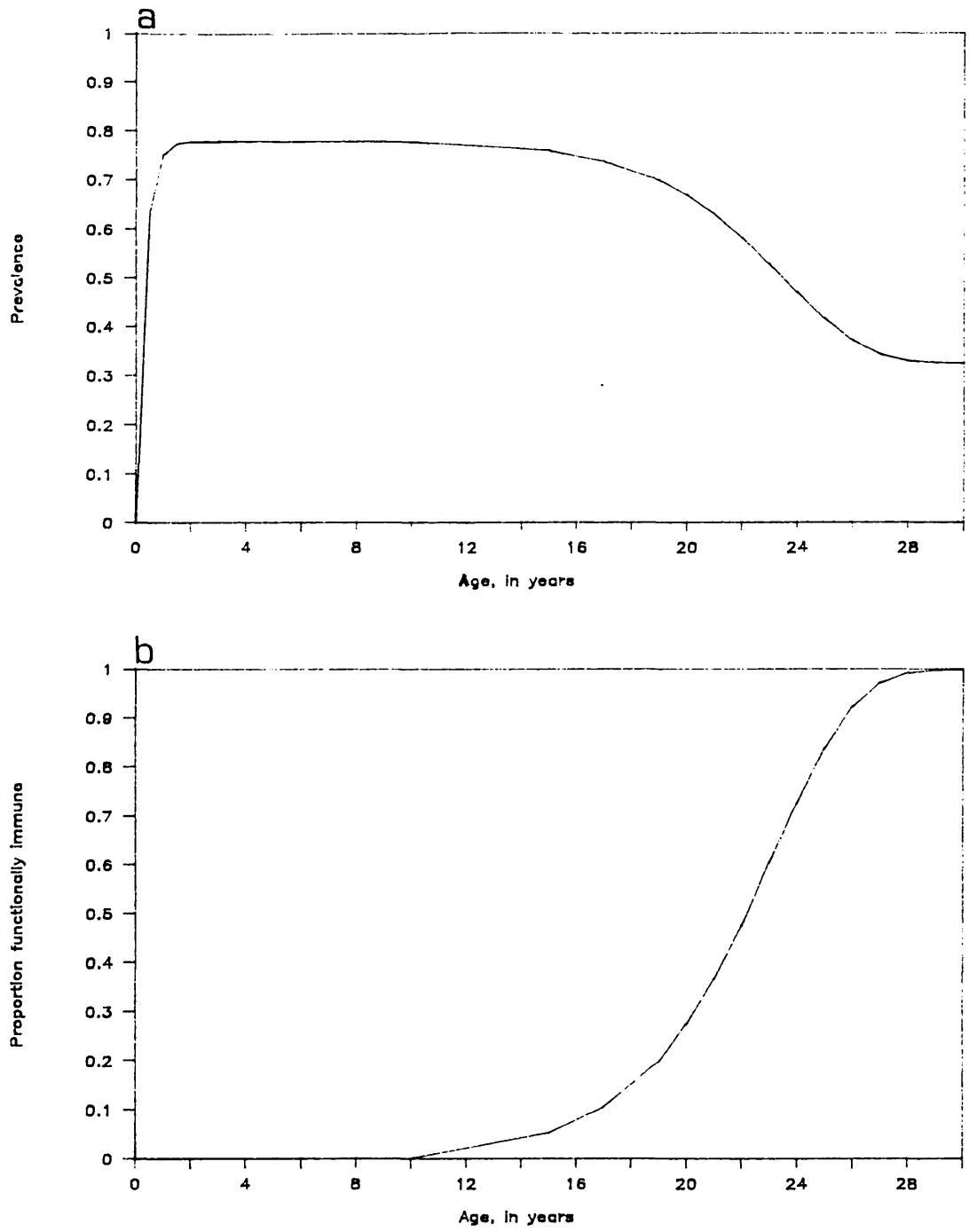


Fig. 6.10.
(a) The predicted age-prevalence profile given by eq.(6.22),
(b) The rise in the proportion functional immune (PX_2 + PY_2 + PZ_2) with age given by eqs.(6.19)-(6.21),
where the rate at which functional immunity is acquired ($\delta(a)$) is related exponentially to age:

$$\delta(a) = \alpha \, \mathrm{Exp}\,(\beta\, a)$$

(Parameter values L=0.05/week, r_1=0.014/week, r_2=0.03/week, π_2=0.0128/week, α=0.00384 and β=0.00004)

adults occur rather swiftly. Additionally the shape of the profile is exceptionally sensitive to very minor changes in the other rate parameters.

As can be seen in fig. 6.11 (a), age-prevalence profiles generated when $\delta(a)$ is a linear function of age produce profiles much more reminiscent of observed trend. The linear function for $\delta(a)$ appears to maintain high child prevalences and also create the necessary slow decline in prevalence with age. Similarly the pattern for the rise in the proportion functionally immune ($X_2+Y_2+Z_2$) with age shown in fig. 6.11 (b) also appears more realistic. By the age of 5 years the model predicts 7% of children will be functionally immune, rising to 17% amongst 8 year olds (compared to 40% and 57.5% respectively in the example above where δ was constant). Functional immunity is seen to be acquired by 50% of an age-cohort by the age of 16 years. It is interesting to compare the rise in functional immunity illustrated in fig. 6.11 (b) with the rise in seropositivity to the immunodominant epitope of the circumsporozoite protein (CSP -a single polypeptide which covers the surface of the sporozoite) of P.falciparum recorded in a rural Gambian community exposed to stable malaria of moderate endemicity (fig. 6.12 (a)). At the population level it can be seen that the humoral response is slow to develop, with a progressive rise after the age of about 11 years and stable levels of 90-100% achieved among adults. These findings are in very good agreement with the rise in functional immunity generated by the model. Although the exact role of anti-sporozoite antibodies in protective immunity to naturally occurring malaria is unclear, at the population level a striking temporal relationship between the fall in parasite levels (fig. 6.12 (b)) and the rise in anti-sporozoite antibodies can be seen. In the individual the relationship with parasitaemia also indicates that the response may well be protective, that is, the initial positive relationship changes to a negative one with continued exposure. The pattern is however quite distinct from that shown by antibodies to asexual stages, which rises much more rapidly and can be correlated with the development of immunity to clinical malaria. Such patterns must however be interpreted with caution due to the close inter-relationship of so many age-related responses to malarial infections.

Similar anti-sporozoite effects have been recorded in other areas using a variety of assays to detect anti-sporozoite antibody (Nardin et al,1979; Druilhe et al,1986; Hoffman et al,1986; Del Giudice et al,1987). It appears that in addition to the striking feature involving the long period of exposure to malaria required before the anti-sporozoite response becomes significant, the rate of development of the anti-sporozoite response in a population appears to vary with, and reflect, the intensity of malarial transmission.

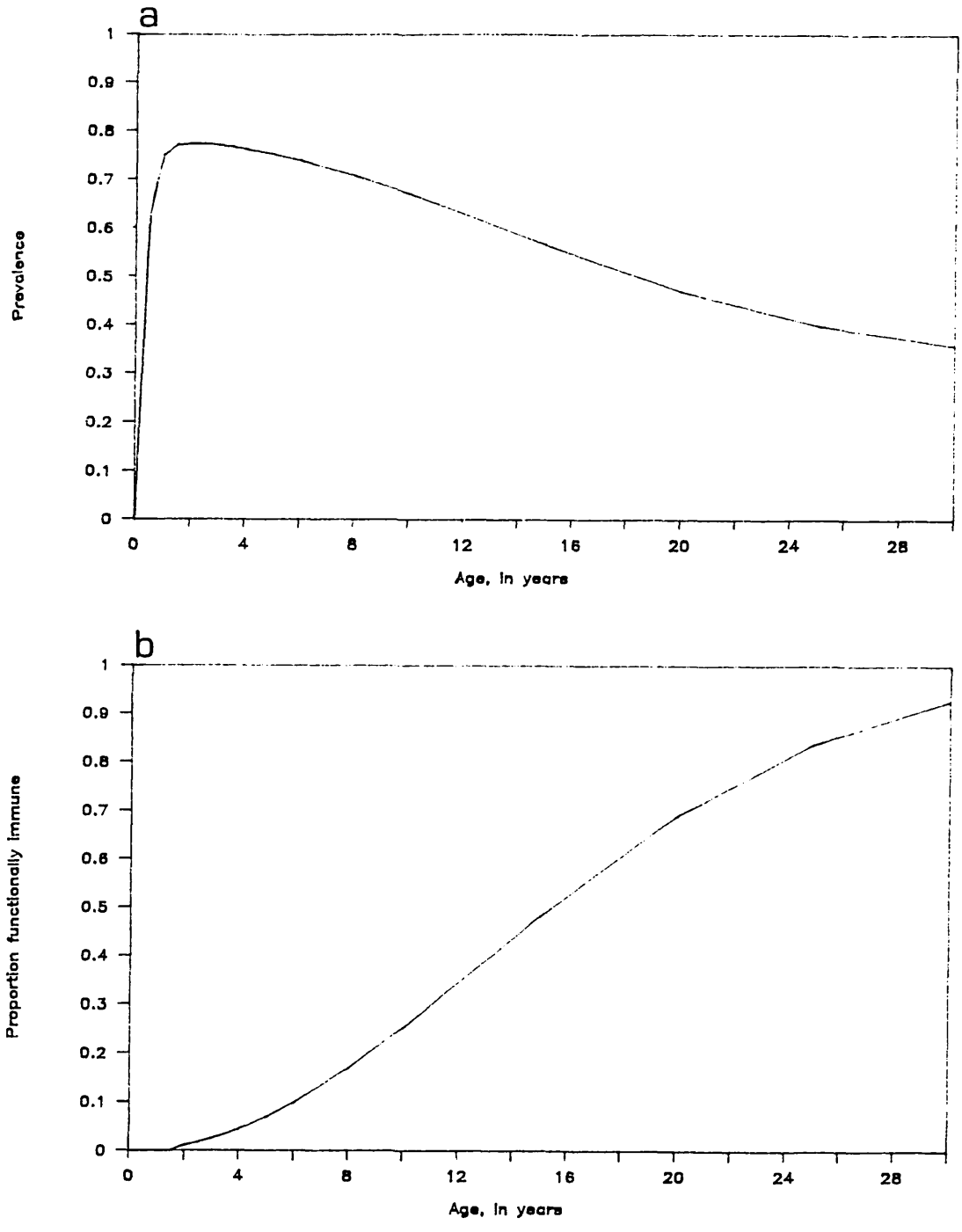


Fig. 6.11.
(a) The predicted age-prevalence profile given by eq.(6.22),
(b) The rise in the proportion functional immune (PX_2 + PY_2 + PZ_2) with age given by eqs.(6.19)-(6.21).
Where the rate at which functional immunity is acquired ($\delta(a)$) is related linearly to age:

$$\delta(a) = Ma + c$$

(Parameter values L=0.05/week, r_1=0.014/week, r_2=0.03/week, π_2=0.0128/week, M=2.8 X 10^{-6} and c=0)

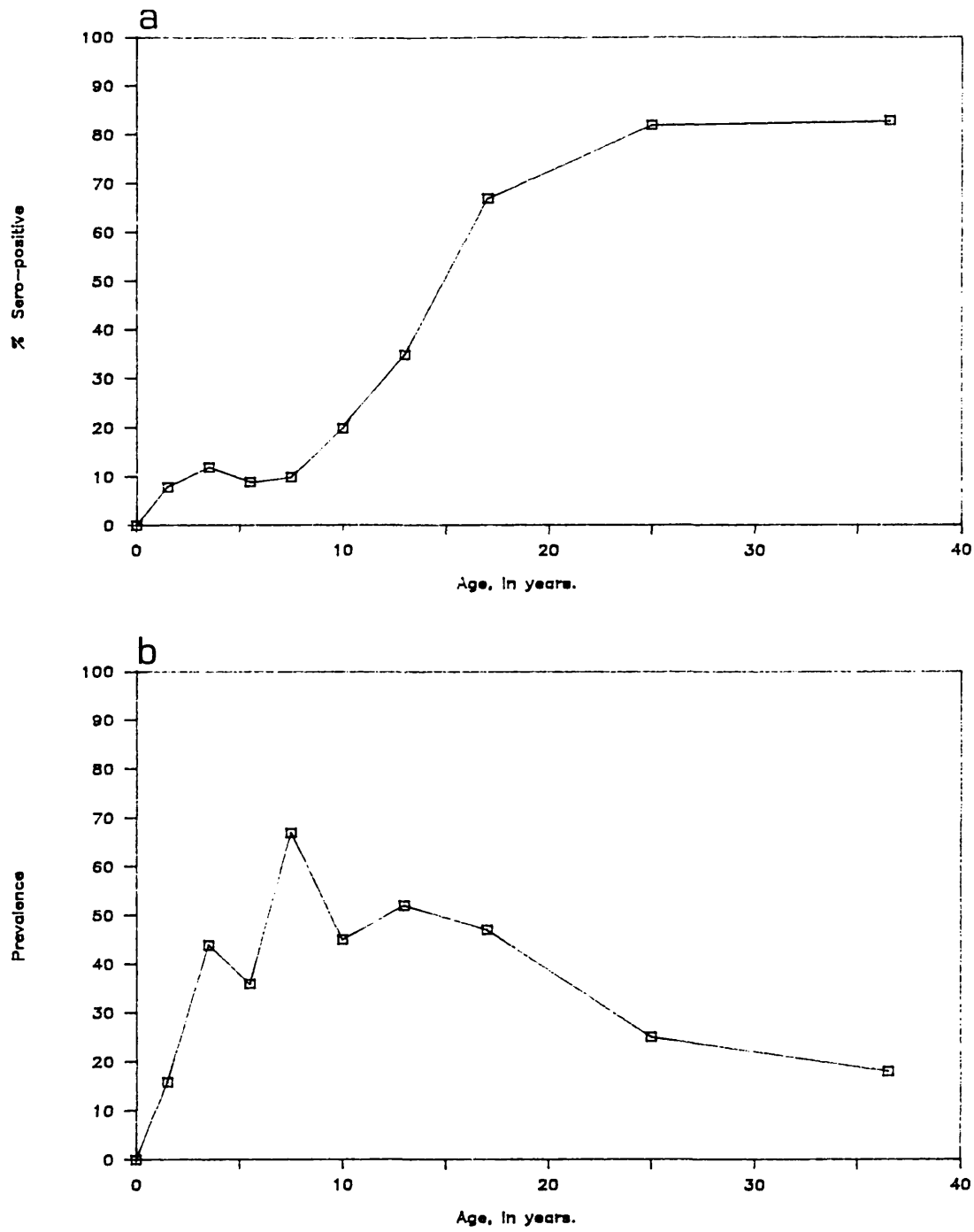


Fig. 6.12.
(a) Rise in seropositivity for anti-sporozoite antibodies with age, (measured by radio-immune assay of anti-$(NANP)_3$ antibodies), (b) Age-prevalence profile for P.falciparum asexual parasites, recorded during August (the beginning of the transmission season) in a rural Gambian population of moderate endemicity, (Marsh et al,1988).

Druilhe et al(1986) found that in a low transmission area of Northern Senegal anti-sporozoite antibodies were not detectable in children under 10 years, after which the levels rose very slowly with age, reaching only very low maximum levels in adults. In the much higher transmission area of Burkina-Faso, high levels were detectable in children as young as 2-5 years. Intermediate patterns, similar to those of Marsh et al(1988) have been found in other areas subject to moderate transmission.

The dependence on transmission rate may help to explain differences between the age-prevalence profile and the rise in functional immunity (indicated by seropositivity to CSP) recorded in Gambia (fig. 6.12) compared to those generated by the model (fig. 6.11). The rise in functional immunity appears to be less rapid and the maximum prevalence occurs at a older age in the Gambian study, which probably indicates that the transmission rate used for the model (L = 0.05/week) is higher than that experienced by the Gambian community. The unexpectedly high level of seropositivity among the very young in the Gambian study is possibly a reflection of seasonal changes in transmission rate to which infants are particularly sensitive. Additionally the anti-sporozoite response was seen to be transient among these children, so that children seropositive in the August survey (fig. 6.12 (a)) were invariably seronegative by the following January.

The rise in both functional immunity and also that of the anti-sporozoite response would appear therefore not to be simply a function of age but of the accumulated past experience of infections. At present with the transfer from the "non-immune" state to the functionally immune state $\delta(a)$, dependent only on the age of the individual, almost all adults are predicted to be functionally immune irrespective of the transmission rate (L), which is clearly too simplistic an assumption. Under fairly constant transmission conditions the accumulated past experience of infections can be crudely represented by the product of age and transmission rate, so that the transfer from the "non-immune" state to the functionally immune state $\delta(a)$, can be more realistically represented by $\delta(a,L)$, where $\delta(a,L)$ is a function of both age and transmission rate.

6.4 (iii) (c) Functional immunity acquired at a rate which is a function of total experience of past infections

The functional form for $\delta(a)$ above, in which $\delta(a)$ is a linear function of age, can be easily modified to incorporate the effects of the transmission rate (L).

$$\delta(a,L) = Ma + C \qquad (6.23)$$

where,

$$M = M_0 \left(1 - \exp -(bL) \right) \qquad (6.24)$$

so that for high transmission rates, $\delta(a,L)$ rises more rapidly with age than for smaller values of L. A wide range of relationships exist between the value of $\delta(a,L)$ and age, dependent on the choice of M_0 and b and which vary widely with L (fig. 6.13).

In order to fully reflect the important affects of the transmission rate on the other rate parameters with regard to age-prevalence patterns as highlighted by the simple transmission dependent loss of immunity model introduced in section 5.4 (ii), the recovery rates r_1 and r_2 can be adapted to reflect the effects of superinfection (according to eq.(4.7)), where r_{01} and r_{02} are the recovery rates from a single infection for "non-immunes" and functionally immune individuals respectively. The loss of immunity (π_2) amongst the functionally immune can also be adapted to reflect acquired immunity boosted by re-exposure (eq.(5.36)).

The effect of the parameter M_0 on the prevalence profile and the rise in functional immunity is illustrated in fig. 6.14. Larger values of M_0 allow functional immunity to be acquired at a faster rate, the estimate for this parameter therefore determines in part (along with $\pi(L)$) the rate of decline in prevalence with age and also the level of infection amongst adults.

Figure 6.15 (a) illustrates the age-prevalence generated by the model for three different levels of transmission, which roughly correspond to low, moderate and high endemicity. Most of the features of observed age-prevalence profiles appear to be captured by the model. The prevalence among children increases with transmission rate, the highest adult prevalences are seen at intermediate levels of transmission, prevalence gradually declines with age and is most pronounced when transmission is very high, and, at the lowest level of transmission the prevalence rises monotonically with age, and there is no decrease in

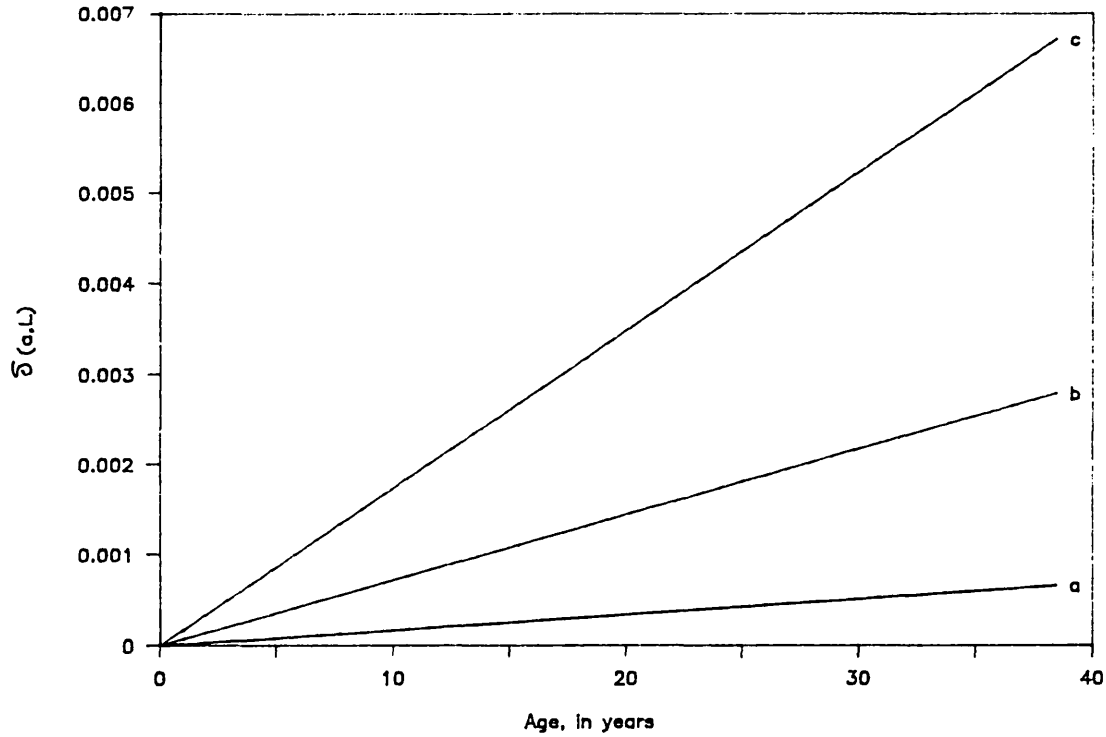


Fig. 6.13. The effect of transmission rate (L) on the rise in the value of $\delta(a,L)$ with age according to eq.(6.24) in the text. Where (a) L = 0.006/week, (b) L = 0.025/week and (c) L = 0.06/week
(Parameters b=0.02 and M_0=0.0028)

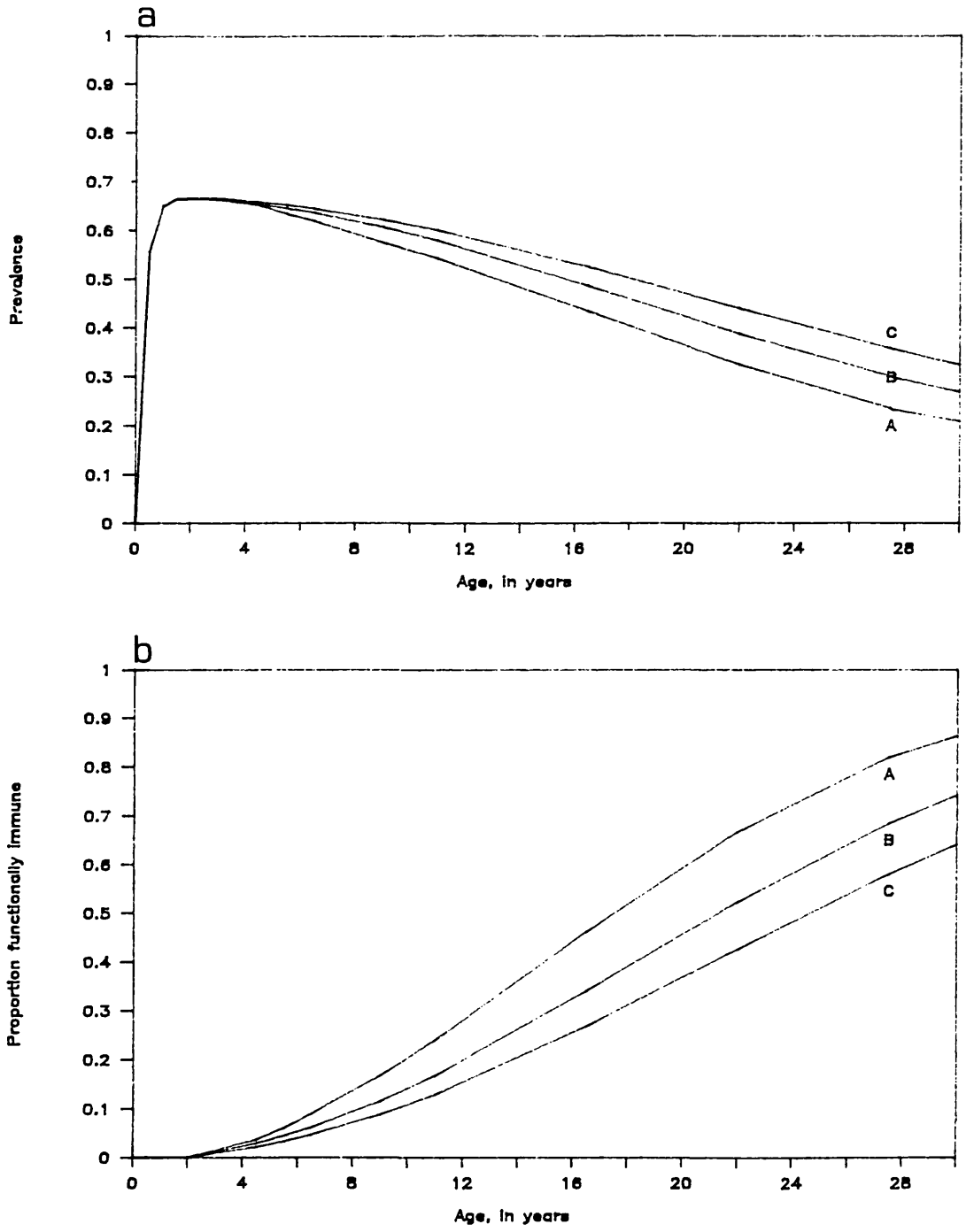


Fig. 6.14. The influence of the estimated value of M_0 on,

(a) the age-prevalence profile, given by eq.(6.22)
(b) the rise in the proportion functionally immune ($PX_2+PY_2+PZ_2$) A. M_0 = 0.0028, B. M_0 = 0.002, C. M_0 = 0.0015. (Parameters L=0.05/week, r_{01}=0.04/week, r_{02}=0.125/week, π_2=0.041/week and b=0.02)

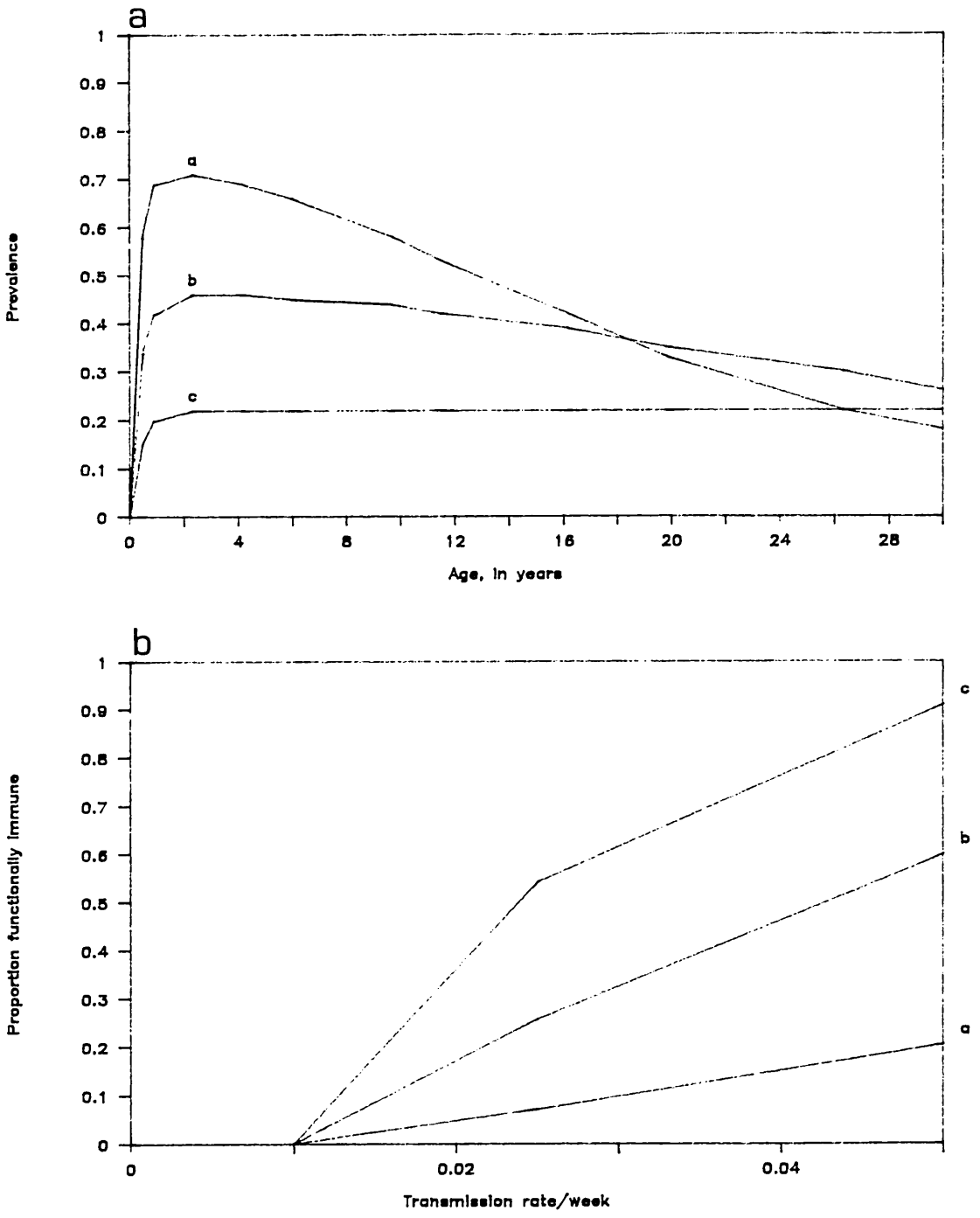


Fig. 6.15.
(a) The age-prevalence predictions for the model defined by the eqs.(6.17)-(6.22) and (6.24), for three levels of transmission: (a) L = 0.05/week, (b) L = 0.025/week and (c) L = 0.01/week, (b) The proportion of the population predicted to be immune at, (a) age 10 years, (b) age 20 years and (c) adult. (Parameter values r_{01}=0.04/week, r_{02}=0.125/week, π_2=0.416/week, b=0.02 and $M_0=2.8 \times 10^{-3}$).

prevalence with age. When the transmission rate is very low (L=0.01/week), the prevalence of infection is low in all the age-groups and, as can be seen in fig. 6.15 (b), essentially nobody becomes functionally immune to infection. For the intermediate transmission rate the prevalence rises more steeply among children and some people become functionally immune (7 % by the age of 10 years, 25.6 % for those aged 20 years rising to 54 % among adults, fig. 6.15 (b)), which results in a decrease in prevalence with age. As the transmission rate gets larger, functional immunity is acquired at a much faster rate, hence the steeper decline in prevalence in age. Also, prevalences among adults fall to very low levels due to the extended periods spent in the immune class (Z_2) as a result of the frequent re-exposure and boosting of acquired immunity.

The profiles in fig. 6.15 (a), although much more realistic still do not reproduce two important features of age prevalence profiles. The maximum prevalence seen in fig. 6.15 appears to occur at the same age, irrespective of the transmission rate, and high prevalences are not maintained over wide enough age-ranges. Observed age-prevalence profiles indicate that the age of maximum prevalence is extended to older age-classes under conditions of low to moderate transmission.

Comparison of the age-prevalence profiles recorded in the Garki study in Northern Nigeria (Molineaux and Gramiccia,1980), with the profile generated by the model for a comparable transmission rate highlights the problem (fig. 6.16 (a)). The shape of the profile may however be considerably improved if in the estimates for r_{01} and M_0 are lowered slightly, which can be done without making the estimate for the recovery amongst "non-immunes" (r_{01}) unrealistic (fig. 6.16 (b)). Further comparison of age-prevalence profiles from central and suburban Accra, Ghana (Colbourne and Wright,1955) and Kisuma, Kenya (Molineaux et al,1978b) with predictions from the model using the new estimates for r_{01} and M_0 also show generally good agreement (fig. 6.17). The model however is still unable to predict the age of maximum prevalence with any accuracy, and the model seems less precise at predicting both the amount of malaria in a locality and also specific changes in prevalence in age in areas where malaria transmission is intensely seasonal e.g. the examples shown in figs. 6.16 (b) and 6.17 (c).

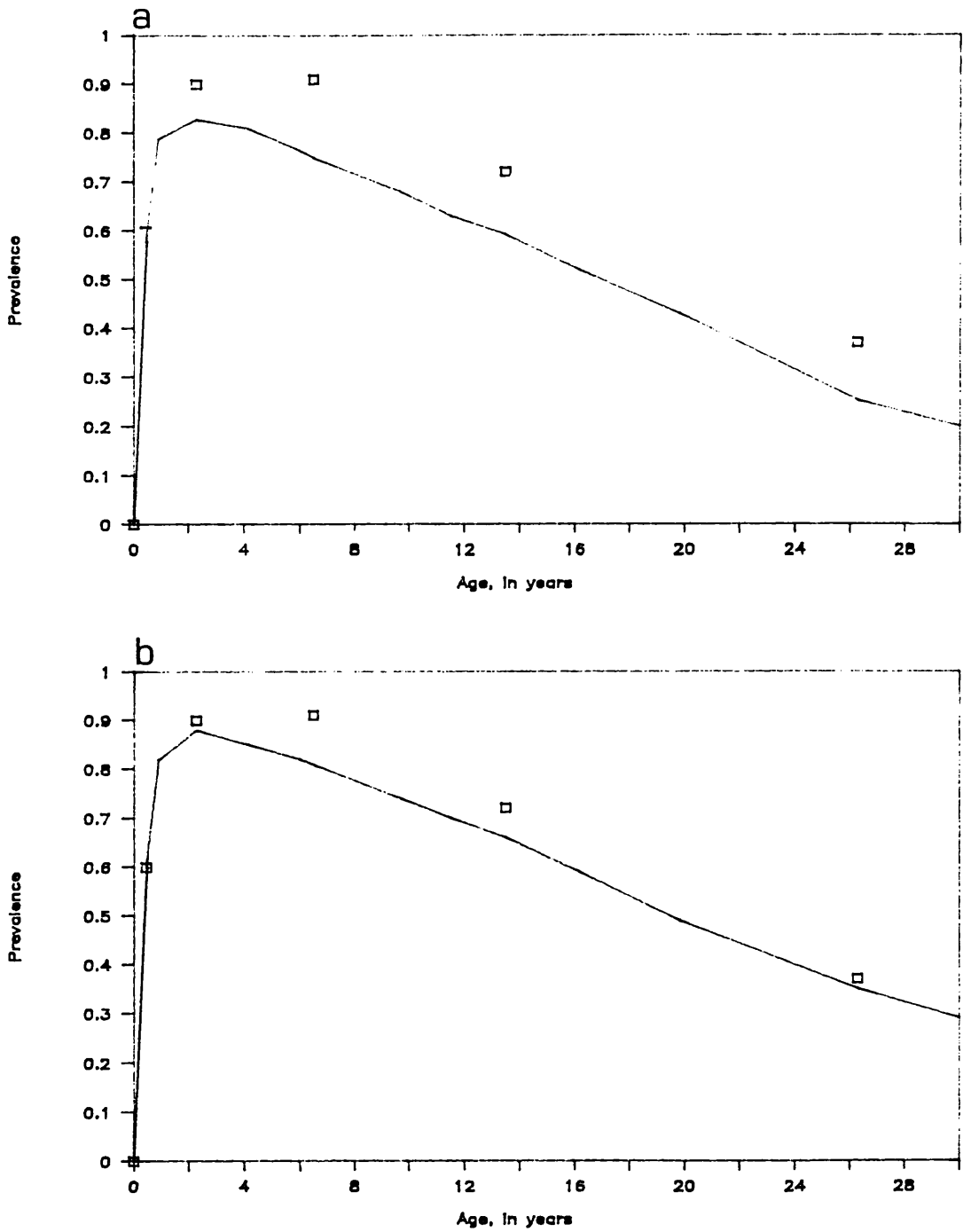


Fig. 6.16. The observed age-prevalence profile of P.falciparum from the Garki region of N. Nigeria (Molineaux and Gramiccia,1980), (symbols) compared with the profiles generated by the model (solid line), for an estimated transmission rate (L) of 0.065/week, (a) r_{01} = 0.04/week and M_0 = 2.8 X 10^{-3}, (b) r_{01} = 0.035/week and M_0 = 1.5 X 10^{-3} (Parameters r_{02} =0.125/week, $\overline{11}_2$= 0.416/week and b= 0.02).

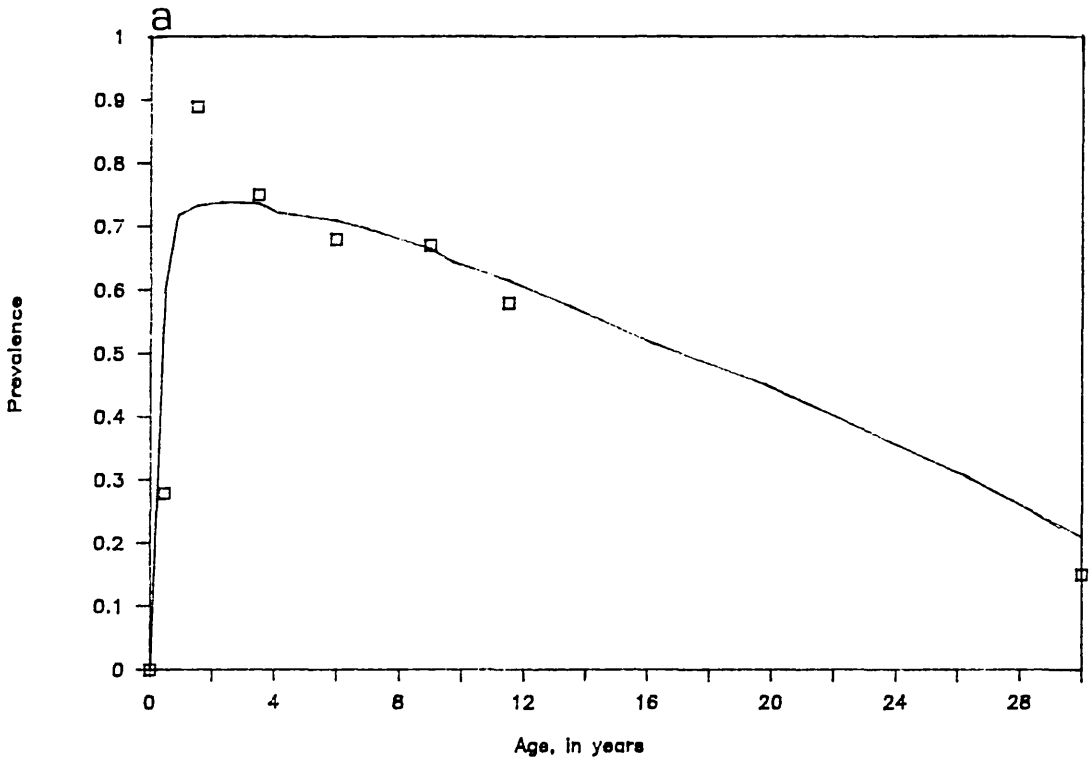
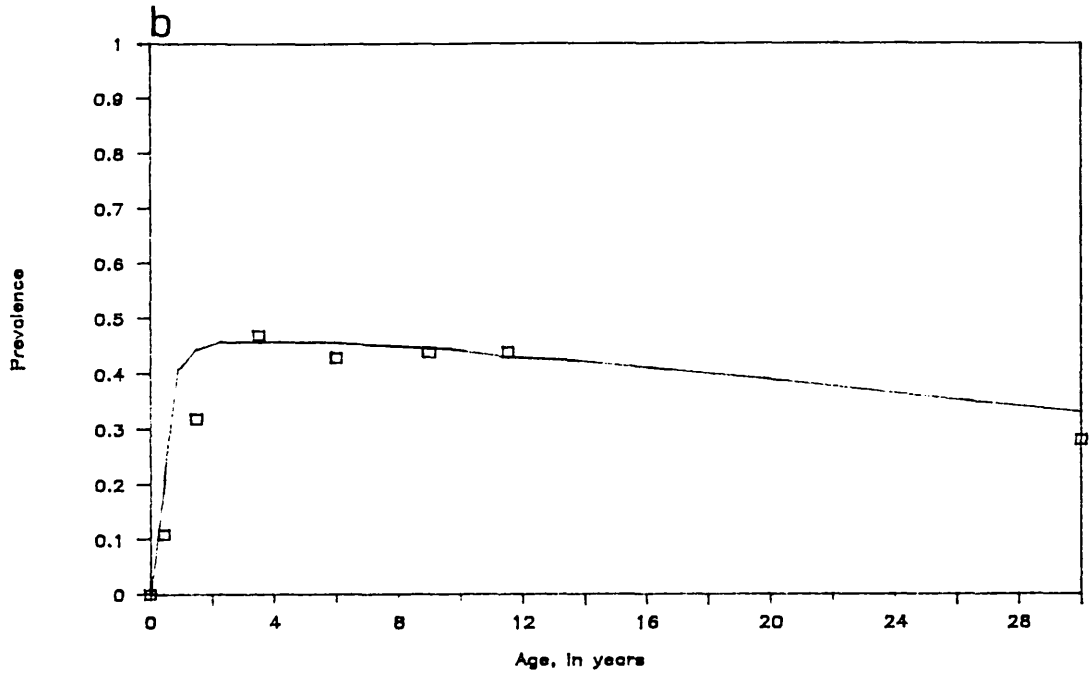
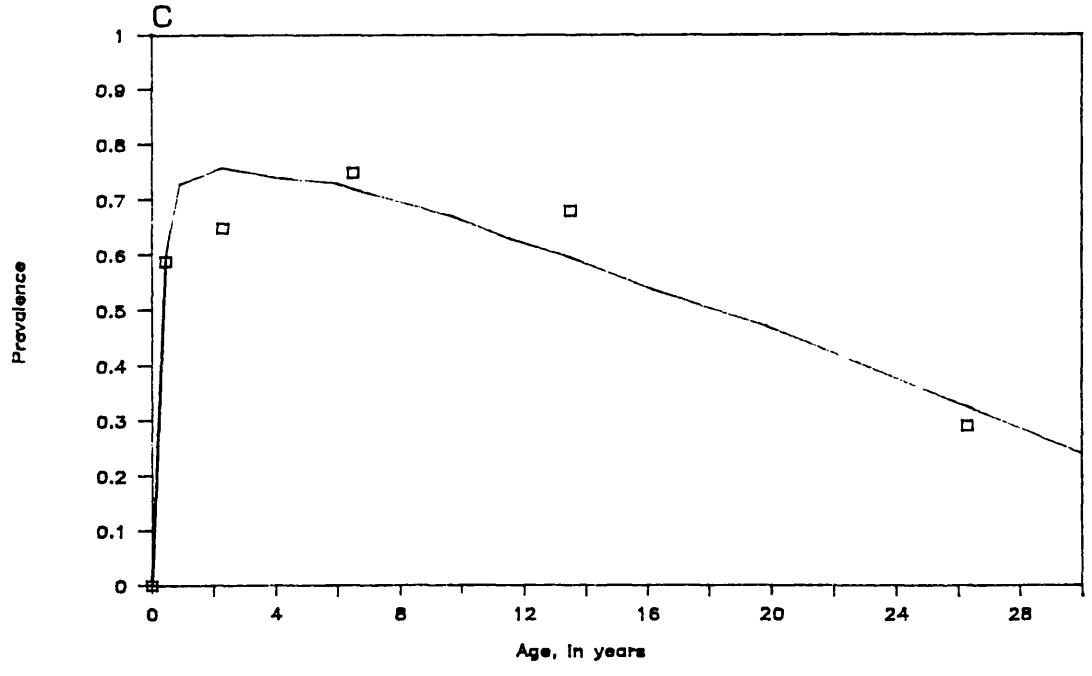


Fig. 6.17. Comparison of the age-prevalence profiles (symbols) recorded in surveys from:

(a) Suburban Accra, Ghana (Aug-Sept) (Colbourne and Wright,1955) - estimated transmission rate (L), 0.0578/week, (b) Central Accra, Ghana (Aug-Sept) (Colbourne and Wright,1955) - estimated transmission rate, 0.0202/week, (c) Kisumu, Nyanza Province, Kenya (Dec-Jan) (Molineaux et al,1978b) - estimated transmission rate, 0.0612/week) with profiles predicted by the model (solid line). (Parameters r_{01}= 0.035/week, r_{02}= 0.125/week, π_2= 0.416/week, M_0 = 1.5 X 10^{-3} and b= 0.02)


b
Prevalence
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
4
8
12
16
20
24
28
Age, in years


c
Prevalence
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
4
8
12
16
20
24
28
Age, in years

6.4 (iv) Summary discussion

The model outlined in this section appears to include many of the important features associated with the acquisition and maintenance of functional immunity in the population. It thus captures the basic differences in dynamics of infection among children and adults. In addition to the important effects that the magnitude of the current infection rate has on the maintenance of the immune status (Z) and on the extension of recovery periods due to superinfection already mentioned, the new model illustrates the critical relationship between the accumulated past exposure to infection and the acquisition of functional immunity. To summarize, infection among the non-immune children reflects the observed tendency for children to suffer extended periods of infection (r low) with relatively short intervals between infections (no apparent immunity to re-infection). A higher "immune status" represented by the classes X_2, Y_2 and Z_2 is acquired at a rate linearly related to the accumulated past experience of infection; thus transfer to this higher "immune status" is negligible among infants and young children, but becomes increasingly rapid among older children and particularly among those adults that have not already transferred to the higher immune state. Furthermore, since this transfer is related to past exposure and is not a simple constant or dependent merely on age, the rate of acquisition of immunity is able to vary with the magnitude of the transmission rate. This pattern of acquisition of immunity reflects not only the observation that long periods of exposure to malaria are required before anti-sporozoite response/functional immunity becomes significant, but also that the rate of development of functional immunity varies with, and reflects, the intensity of malarial transmission in a locality. The higher "immune status" is reflected in a pattern of infection where recovery from patent infection is much more rapid and there are extended periods between successive infections. This results in much lower prevalences among this section of the community. The model is able to predict reasonably well not only changes in prevalence with age but also age-prevalence patterns and levels of infection among areas of quite different infection rates.

Although the model captures the basic features of infection which affect prevalence rates, there are of course many additional aspects of immunity that the model makes no attempt to take into account. Mortality studies clearly show that the structure of the population with regard to the innate ability to control infection is not the same at birth as it is in older age-groups, hence the heterogeneous distribution of such innate mechanisms may have an important consequence on the pattern of infection amongst non-immunes.

Similarly the heterogeneous distribution of immunological competence with regard to the ability to mount an effective functional response may be important in determining the level of infection amongst adults. Infection rates and the effect of superinfections cannot realistically be assumed to be independent of immune status, and the effect of maternally acquired immunity has been shown to clearly increase recovery rates for the first 3-6 months of life; these features will only alter qualitative features of the predicted profiles. What is more important is to identify the underlying reasons why the generated age-prevalence profiles appear unable to predict accurately either the age of maximum prevalence or the level of infection among children of intermediate age, particularly in areas subject to intense seasonal changes to the infection rate.

These problems are undoubtedly associated with the over-simplifications involved in assuming that two distinct sets of compartments, representing the non-immune and functionally immune portions of the population with a single rate of transfer between the two, can adequately represent the gradual acquisition of immunity observed to occur among children as the grow older. This framework allows reasonably realistic age-prevalence profiles to be produced, simply due to functional immunity being gradually acquired by members of a particular age-class, which is not the same as immunity being gradually acquired by the individual. Within the framework of the model, functional immunity for an individual is either present or absent, whereas in reality there is a gradual development of immunity dependent on accumulated past experience with a graded response to infection reflected in a observed gradual increase in both the recovery rate and also the duration of time between infections (Segal et al,1974; Krafsur and Armstrong,1978; Bekessy et al,1976), and probably a gradual decrease in the effects of superinfection too. Furthermore, since within the framework of the model immunity is assumed to be "all or nothing", small losses of immunity seen to occur during the dry season or during periods of control when the level of transmission drops cannot be reflected in the model. The observed prevalence rates among children of intermediate age, where the changes due to the acquisition of immunity are occurring most rapidly, are more likely to be sensitive to small changes in infection and recovery rates. The predictions made by the model are therefore less accurate among these age-groups.

CHAPTER 7 A model of acquired immunity to malarial infection

7.1 Introduction

Previous chapters examined the evidence to support the notion that acquired immunity to many aspects of infection does develop in man. Adults in malaria-endemic areas carry only very low levels of blood parasites, and are generally clinically immune despite repeated sporozoite challenges. These challenges are often at very high rates because vectors "prefer" to select larger hosts (Section 2.1 (i) (b)). Children are seen to gradually develop functional immunity, which is expressed as a reduction in both disease-related mortality and morbidity with increasing age and also an enhanced ability to control and eliminate parasites. Passive transfer studies with antibody from immune African adults have demonstrated that anti-malarial anti-bodies confer immunity to susceptible children (Cohen et al,1961) and maternally derived antibody has also been shown to have significant effects on the control and elimination of blood parasite populations. All detailed descriptions of the development of immunity, whether under controlled experimental conditions in a hospital or under natural conditions, indicate that functional immunity develops gradually, and is not an "all-or-nothing" phenomena. Experimental studies by Ciuca et al(1934; cited by Brumpt,1949) involving P.vivax infections, demonstrated a clear decline in the severity of clinical symptoms from the first inoculation to the fifth and last. The gradual acquisition of immunity has also been demonstrated by Zuckerman(1974) using experimental animal models. Immune sera from laboratory rats became more and more effective at controlling infections in non-immune rats as the number of infections given to the immune donor rats increased. Under natural conditions, true functional immunity to all aspects of infection, both clinical and parasitological, appears to take many years to develop. Immunity is rarely observed to be complete (infections still occur even among the most highly immune members of the population) and requires continuous exposure to infective bites. Maintenance may also require the existence of sub-patent infections (Howard,1986). The importance of gradually acquired immunity to infection in determining both the level of infection in a population and also age-specific prevalences was demonstrated, (albeit in a very simplified way), in the previous chapter.

Acquired immunity effects several well defined aspects of infection, which are important determinants of the

dynamics of transmission. First, it can reduce the probability that a bite by an infected mosquito results in a patent infection, either a new infection, a superinfection or a reinfection. Second, it may increase the rate of elimination of the asexual forms of the parasite, which is equivalent to an increase in the rate of recovery from infection. Third, it may cause reductions in the level of infectivity by affecting the "quality" of the gametocytes, (in particular their ability to infect mosquitoes). These three aspects of immunity correspond with the three basic types of vaccines which are currently under development; vaccines against sporozoites, the blood-stage parasites (merozoites and schizonts) and the sexual-stage parasites (gametocytes and ookinetes) respectively. To investigate fully the dynamics of acquired immunity and also to assess the future epidemiological implications of candidate vaccines, all three aspects need to be considered and included in a suitable mathematical framework. This framework needs to not only describe infection in both the human community and also the vector population, but additionally it must accurately reflect the subtle balance between transmission and the development of immunity.

Although the gross effects of acquired immunity on infection and recovery rates mentioned above are quite obvious in the field, very little is known about the exact mechanisms involved or what features of infection are important to their generation. An important question that remains unanswered is: " Why does functional immunity to the various aspects of infection develop so slowly in man ? " Many ideas have been suggested including; the lower vector-man contact rates among children, the antigenic complexity of malaria parasite, genetic variability in the parasite population and evasion strategies by the parasite, including antigenic variation and modifications to the immune responsiveness in the host, (such as immunodepression and polyclonal lymphocyte activation). Other theories are based on the notion that sporozoites are of low immunogenicity/viability. Studies by Nussenzweig & Chen(1974) and Verhave(1975) clearly show that earlier crops of sporozoites are significantly less viable or immunogenic than older sporozoites. When considered with the limited longevity of the vectors, this feature may be of epidemiological importance. An age-dependent ability to mount an effective immune response, similar to that seen among rats infected with P.berghei (Nussenzweig et al (1966b); Verhave(1975)) has also been suggested. However, before this questioned can be answered with any certainty, a much more detailed understanding of the interactions between parasite and host responses and the balance between host defenses and parasite survival is required. Epidemiological models, may be used to

investigate the gross dynamic effects of continued exposure, suggest ways in which immunity may be developed, maintained or lost and also provide a theoretical framework for the collection of new data concerning the development and maintenance of acquired immunity.

The model presented in this chapter aims to investigate two of the three aspects of immunity mentioned above: (i) acquired immunity and the reduction of the probability that a bite by an infected mosquito results in a patent infection, and, (ii) acquired immunity and the increase in the rate of elimination of the asexual forms of the parasite. Two further factors that modify the immunresponsiveness of the host, superinfection and immunodepression are also considered. These aspects of immunity are important determinants of the incidence of infection and recovery from infection. The primary aim of this chapter, therefore, is to investigate how age-specific incidence and recovery rates may be determined by these immunological processes. Comparison of predicted age- and seasonal-dependent transition rates will be made with data collected by Krafsur & Armstrong(1978) and Bekessy et al(1976).

The effect of different rates of contact between vectors and man and seasonal variations in the potential for transmission will also be considered. Since incidence and recovery rates are also major determinants of the prevalence of infection, age- and seasonal-specific predictions for the prevalence of infection are also examined. Appendix G.1 summarizes the parameter notation used in this chapter.

7.2 Malarial incidence and recovery rates by age and season

7.2 (i) Method of estimation

Bekessy et al(1976) outline a method of estimating malarial incidence rates (I) and recovery rates (R) from longitudinal data, which can reveal age-related and season-related variations. The method is based on the assumption that patent parasitaemia can be represented by a reversible two-state catalytic model (Muench,1959). Generally, the longitudinal study of infants (who are assumed negative at birth) is used to estimate the incidence rate of malaria (Macdonald,1950b; Pull & Grab,1974) and the study of individuals removed from exposure to infection (Earle et al,1939; Macdonald & Göckel,1964) is used to estimate the recovery rate. For both methods any age- or seasonal-related changes in the incidence or recovery rate that might be operational under conditions of natural malarial transmission

is unlikely to be revealed. The method introduced by Macdonald(1950a) can be adapted to calculate age-related changes in the incidence rate. However, the estimates made using both this method and also the methods by Pull & Grab(1974) and Macdonald(1950b) mentioned above, are very sensitive to the value of R used in the calculations. If R is wrongly approximated, estimates for I will be seriously in error. Furthermore, the calculations are based on the assumption that only one type of transition is possible between two consecutive surveys - from negative to positive for the estimation of I and from positive to negative for R. In view of the observed tendency for parasitaemias to fluctuate between patency and sub-patency over quite short time periods (Bruce-Chwatt,1963a; Collins et al,1971), this type of assumption could lead to errors in the estimation of the rate parameters.

Bekessy et al(1976) describe a method in which simultaneous estimates of I and R can be made from the longitudinal study of a population of mixed patents and sub-patents. The estimation procedure can be applied to any age-group, and is equally applicable to surveys covering any season, hence both age-related and seasonal variations can be revealed. Furthermore, the procedure allows for several transitions from patency and non-patency to occur between consecutive surveys.

The study population is surveyed at defined intervals, and classified as patent or non-patent by a standard method of blood examination. In a given time interval, it is assumed that negatives become positive at a rate I and positives become negative at a rate R, between consecutive surveys two transition frequencies are calculated α (proportion negative at first survey and positive at the second) and β (proportion positive at the first and negative at the second). The relationship between transition rates (I and R) and transition frequencies (α and β) is described by a Markcov process such that:

$$I = \frac{\alpha}{t(\alpha + \beta)} \quad In \quad \frac{1}{1 - (\alpha + \beta)} \qquad (7.1)$$

$$R = \frac{\beta}{t(\alpha + \beta)} \quad In \quad \frac{1}{1 - (\alpha + \beta)} \qquad (7.2)$$

where t is measured in days for the estimation of daily rates. For the calculation of daily rates the probability of more than one transition is assumed negligible.

7.2 (ii) Seasonal and age-related trends in R(a) and I(a)

This model has been applied to three sets of longitudinal data collected from areas of high but seasonal transmission in West/East Africa, (i.e. areas of hyperendemic malaria). From West Africa - the Garki District, Kano State, Northern Nigeria (Bekessy et al,1976) and the town of Gambela, Western Ethiopia (Krafsur & Armstrong,1978), and from East Africa - the villages of Tago and Kongodjan in Burkina Faso (Gazin et al,1988). The study by Bekessy et al(1976) is very much more comprehensive than either of the other two studies, involving larger sample sizes, and a more detailed description of the population according to age. The study also involves a more accurate detection level for examination of blood smears. Figures 7.1 (a) and (b) illustrate the seasonal averages for I(a) and R(a), measured as daily rates, for the study by Bekessy et al(1976). Figures 7.2 (a)-(e) and 7.3 (a)-(e) illustrate the daily incidence and recovery rates respectively according to season. Figures (a), (d) and (e) show the transition rates recorded in the dry season, and (b) and (c) are those of the wet season. Figure 7.4 illustrates the age- and seasonal-related data collected by Krafsur & Armstrong(1978); figs. 7.4 (a) and (b) show the seasonal variations recorded in the incidence, among children under 15 years (fig. 7.4 (a)) and among adults (fig. 7.4 (b)). Similarly figs. 7.4 (c) and (d) show the seasonal variations in the recovery rates among children under 15 years and adults respectively. Figure 7.5 shows the seasonal variations in the incidence rate (fig. 7.5 (a)) and the recovery rate (fig. 7.5 (b)) recorded by Gazin et al(1988) in Tago and Kongodjan, Burkina Faso, among children aged 6 months to 15 years.

Three further studies have involved the use of the model, these include two from Equatorial Africa, one in the People's Republic of the Congo (Carnevale,1979 cited in Gazin et al,1988), the second in Burundi (Coosemans,1985). The third study was in China (Deng Da et al,1985 cited in Gazin et al,1988). The situations found in these studies were very different from those observed in East or West Africa. Transmission was found to be either non-seasonal or else very low and/or unstable.

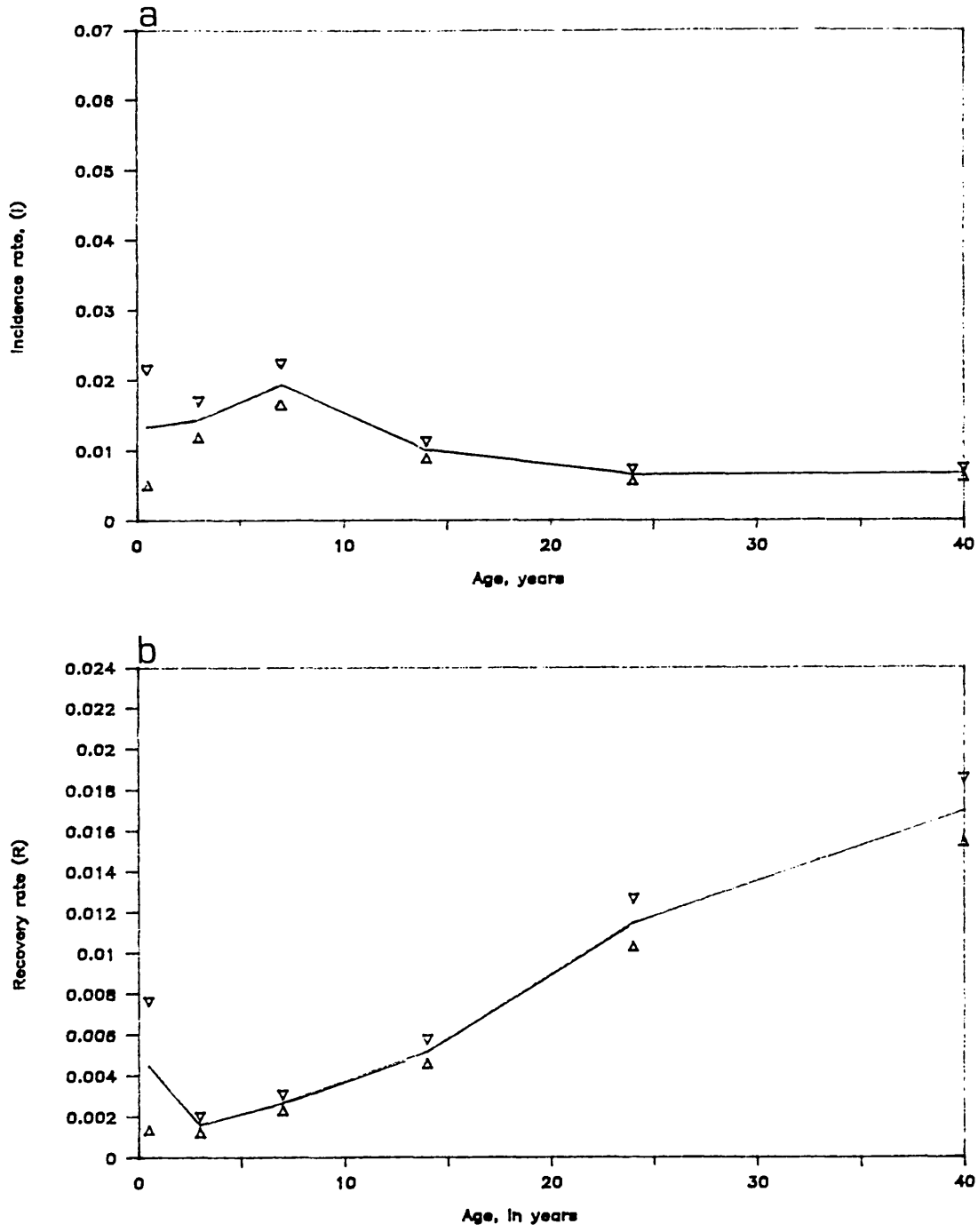


Fig. 7.1. Estimated yearly average daily transition rates (with 95 % C.I.) of P.falciparum parasitaemia by age, recorded in Garki, N.Nigeria, (a) Incidence rate, I, (b) Recovery rate, R, (Bekessy et al(1976)).

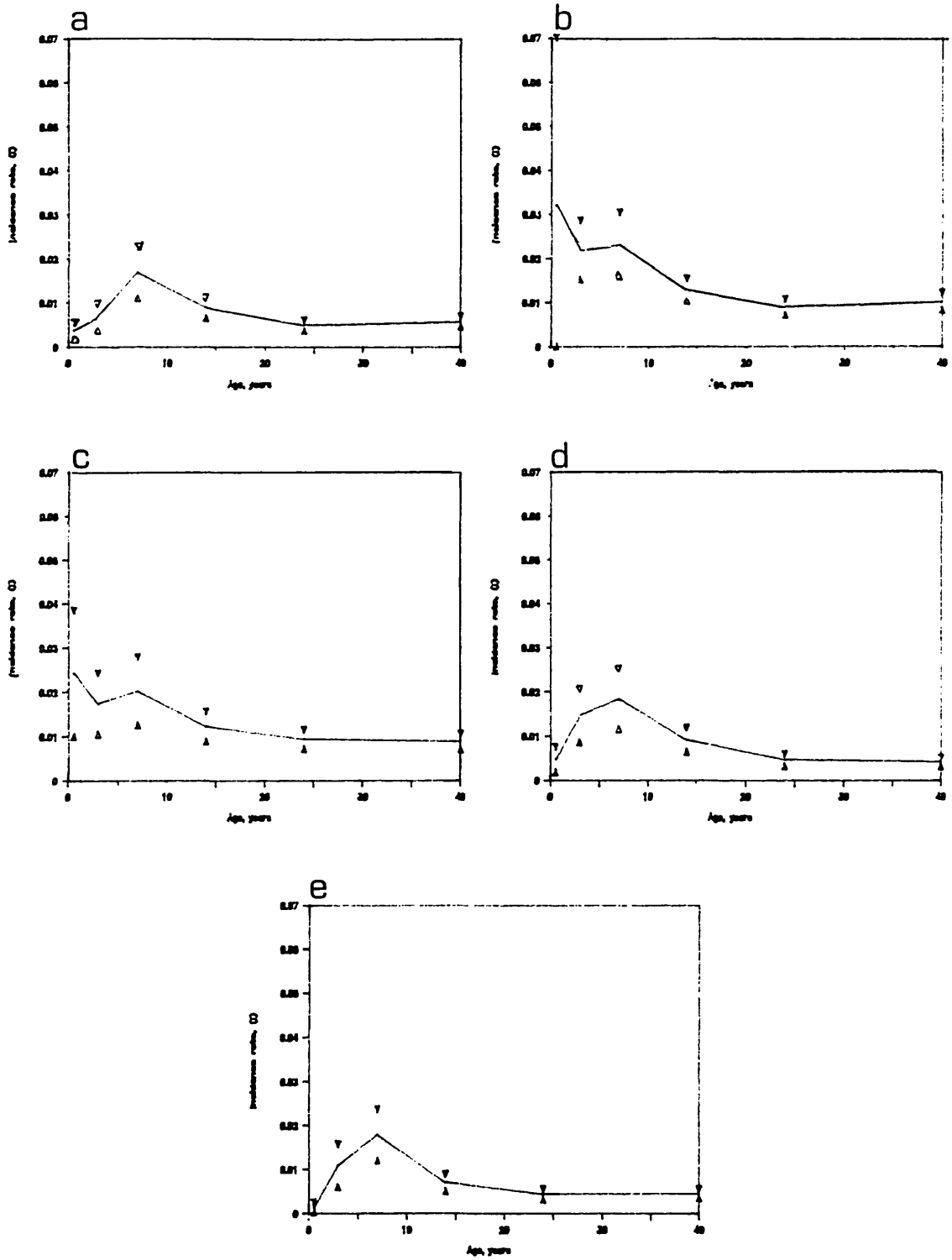


Fig. 7.2. Estimated daily incidence rate (with 95 % C.I.) of P.falciparum parasitaemia by age and season, recorded in Garki, N.Nigeria, 1971-1972, (a) Survey 3-4, dry season, (b) Survey 4-5, wet season, (c) Survey 5-6, wet season, (d) Survey 6-7, dry season, (e) Survey 7-8, dry season. Surveys conducted at 10 week intervals (Bekessy et al(1976)).

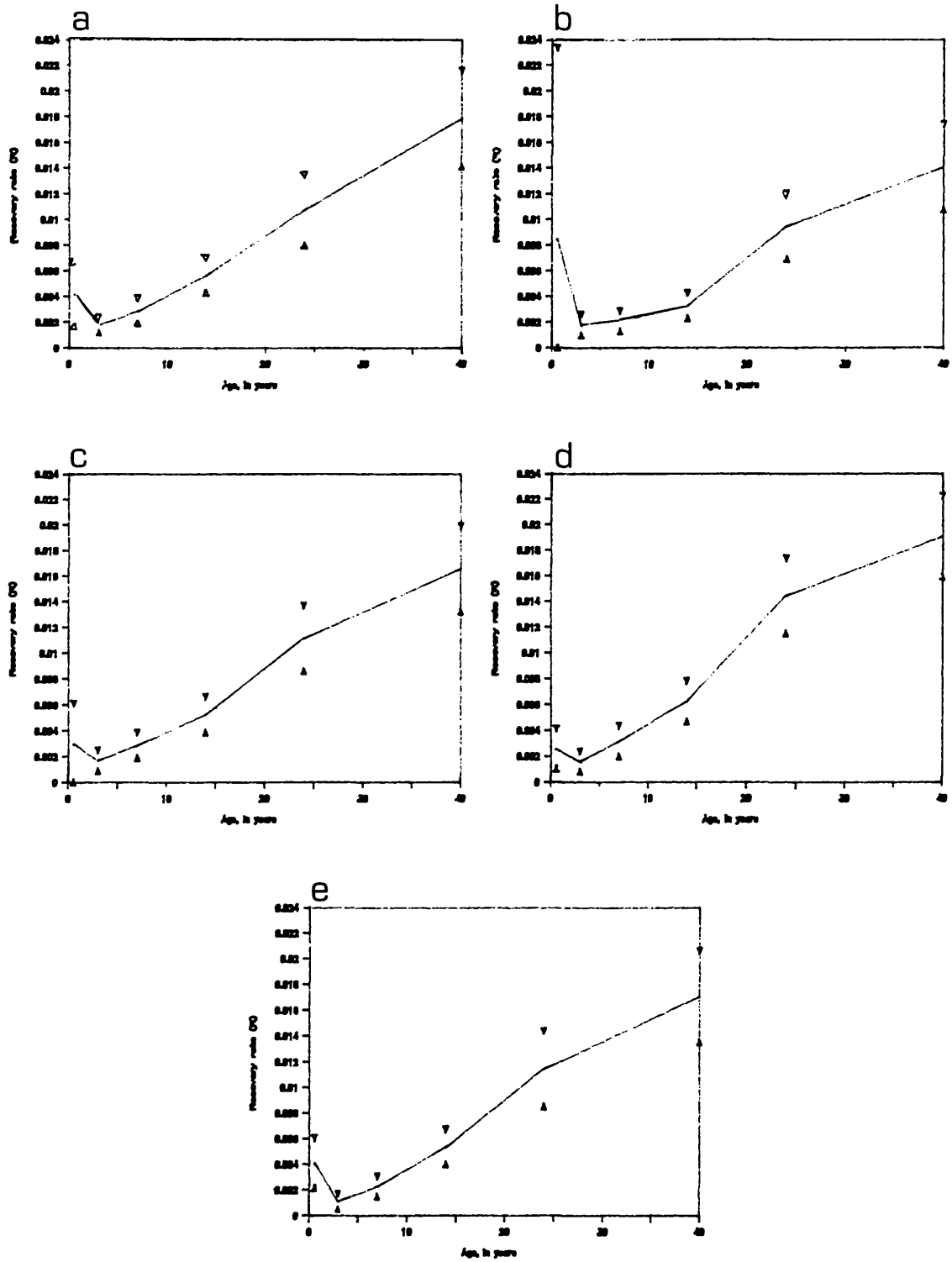


Fig. 7.3. Estimated daily recovery rates (with 95 % C.I.) from P.falciparum parasitaemia by age and season, recorded in Garki, N.Nigeria, 1971-1972, (a) Survey 3-4, dry season, (b) Survey 4-5, wet season, (c) Survey 5-6, wet season, (d) Survey 6-7, dry season, (e) Survey 7-8, dry season. Surveys conducted at 10 week intervals (Bekessy et al(1976)).

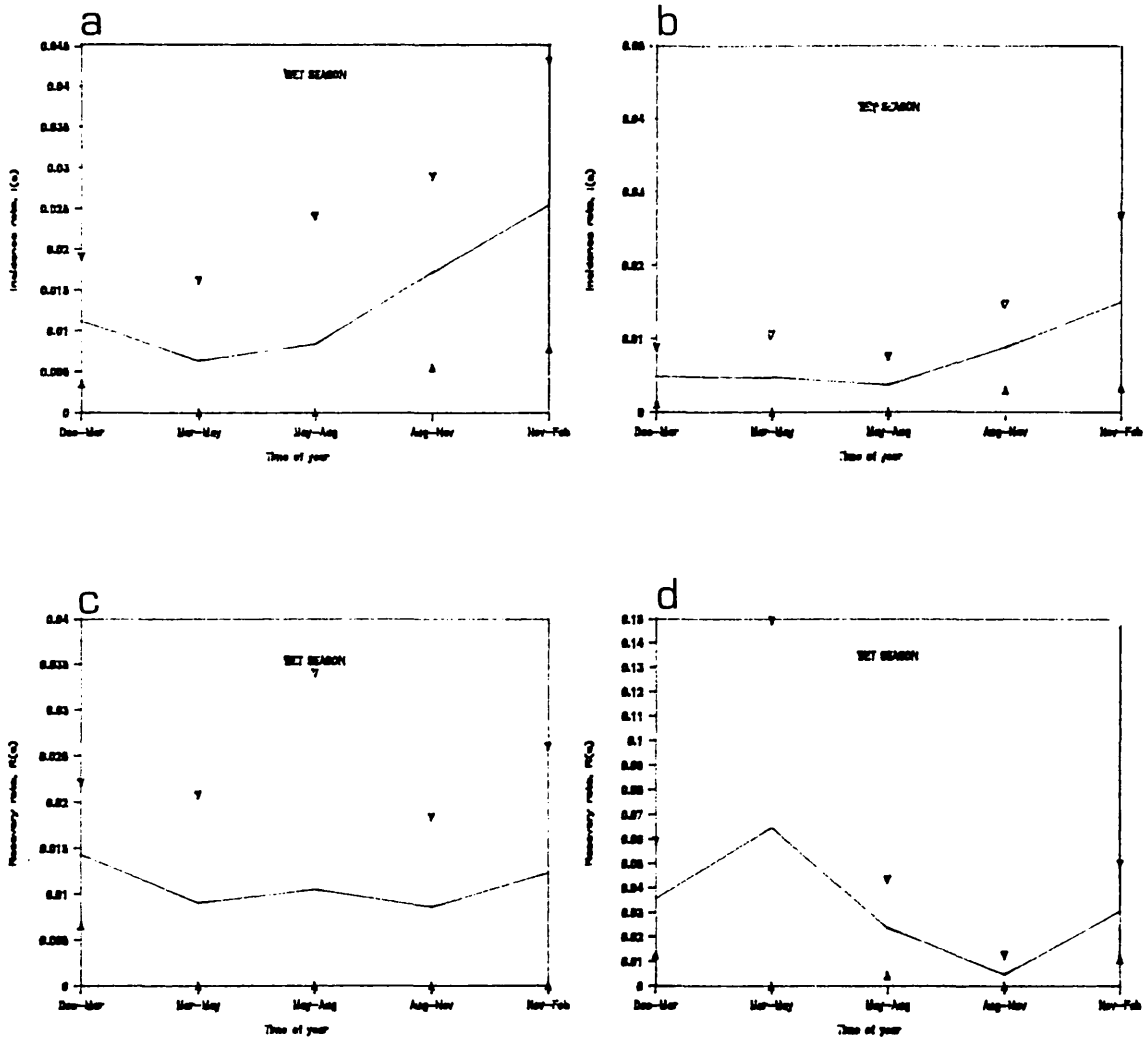


Fig. 7.4. Parasitologically estimated mean daily incidence and recovery rates (with 95 % C.I.) of P.falciparum by season, recorded among the Anuak population in Gambela, W.Ethiopia, Dec 1967-Feb 1969, (a) Incidence rate, children < 15 years, (b) Incidence rate, adults > 15 years, (c) Recovery rate, children < 15 years, (d) Recovery rate, adults > 15 years. Surveys conducted at 28 day intervals (Krafsur & Armstrong,1978).

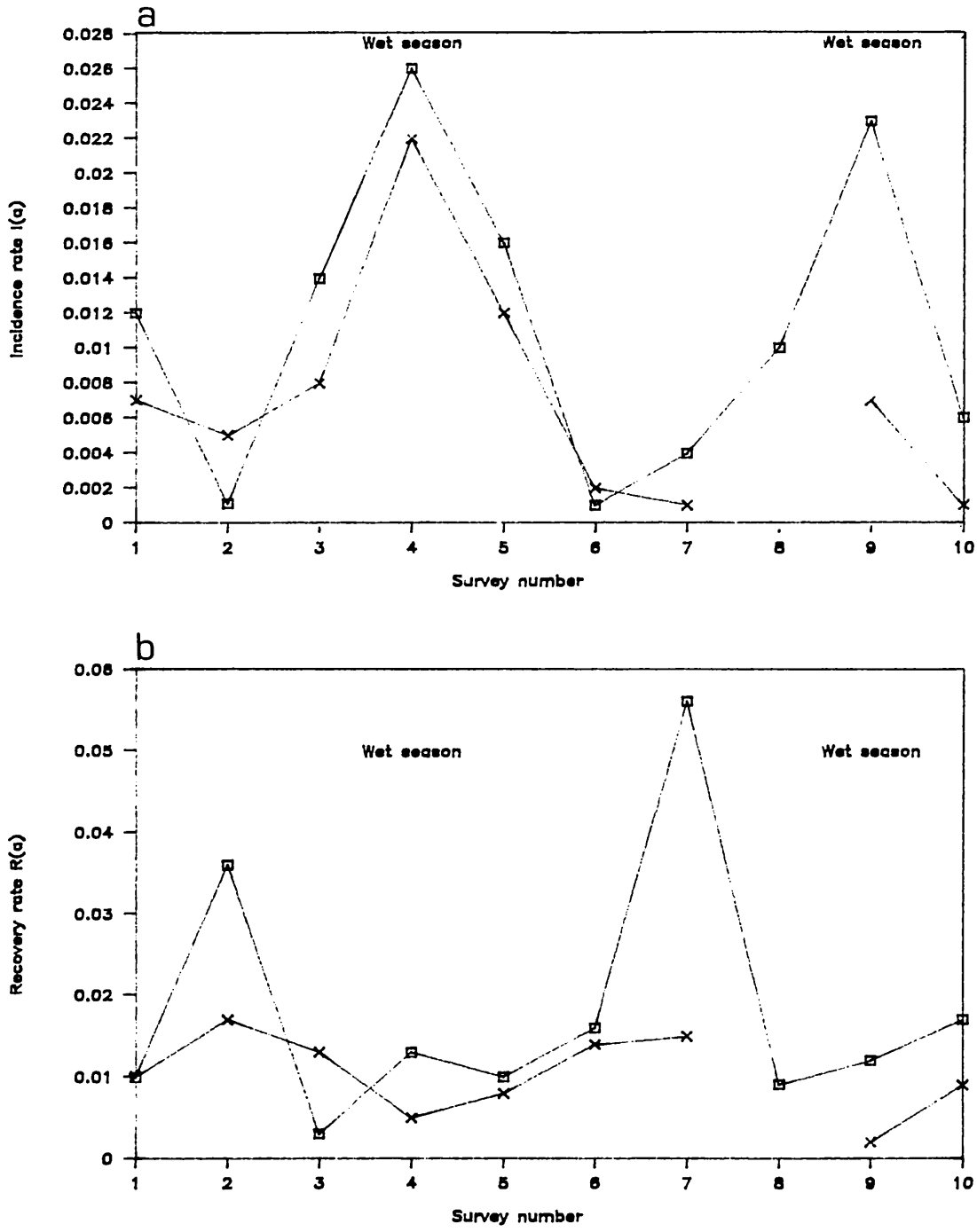


Fig. 7.5. Parasitologically estimated mean daily incidence and recovery rates of P.falciparum by season recorded in the villages of Tago and Kongodjan, Burkina Faso for children aged 6 months to 15 years, (a) Incidence rate, (b) Recovery rate. (□ - Tago, X - Kongodjan). Surveys conducted at intervals of 60-120 days (Gazin et al,1988).

7.3 The deterministic framework for the acquired immunity model

The basic structure of the model introduced in this chapter is very simple, it is a deterministic compartmental model in which individuals can either be non-patent (X), (i.e. no detectable blood parasites in the peripheral blood), or patent (Y). The number of individuals of age a, at time t, who are non-patent and patent are given by the variables X(a,t) and Y(a,t). The numbers in each epidemiological class are assumed to vary with both age and time as individuals move between the classes according to defined rates of flow. The transfer from the non-patent to patent state is determined by the apparent incidence rate (I), and the apparent recovery rate (R) determines transfer back to the non-patent state. The apparent incidence rate is assumed to include both the acquisition of new infections (h) and also the incidence of parasitological recrudescences (f). The incidence rate among infants is assumed to include only new infections, (i.e recrudescences amongst infants and congenital infections are assumed to be negligible). The apparent recovery rate is determined by both "true" recovery from new infections and also recovery from recrudescences. The partial differential equations describing these assumptions are:

$$\frac{\delta X(a,t)}{\delta t} + \frac{\delta X(a,t)}{\delta a} = - I(a,t)\, X(a,t) + R(a,t)\, Y(a,t) \quad (7.3)$$

$$\frac{\delta Y(a,t)}{\delta t} + \frac{\delta Y(a,t)}{\delta a} = + I(a,t)\, X(a,t) - R(a,t)\, Y(a,t) \quad (7.4)$$

Equations (7.3) and (7.4) can be solved by numerical methods to provide estimates for seasonal- and/or age-specific prevalence rates. However, the stochastic model used by Bekessy et al(1976) to estimate the malaria incidence and recovery rates from longitudinal data provides a much simpler method of estimating the proportion patent (PY).

If the time interval over which the estimates for I and R are to be calculated is sufficiently small, the possibility of more than one transfer during the interval can be considered to be negligible compared to the probability of a single transition. Additionally, if, in a given time interval, the transition rates I and R are assumed to be constant, a direct relationship exists between the stochastic model by Bekessy et al(1976) and the model described above. At equilibrium, the proportion patent (PY) and the rate

parameters I and R, in the stochastic model are defined by the following relationship:

$$PY = \frac{I}{(I + R)} \qquad (7.5a)$$

This simple expression can be used directly to provide estimates for the equilibrium prevalence of infection among individuals of a given age, for known values of I(a) and R(a). Furthermore, if the transition parameters are assumed to be subject to seasonal influences, but that these seasonal variations do not vary significantly between years, the prevalence of infection for different age-groups at various points in the year can also be estimated.

$$PY(a,t) = \frac{I(a,t)}{(I(a,t) + R(a,t))} \qquad (7.5b)$$

In view of the complexity involved in the calculation of the incidence and recovery rates, the use of this much more straightforward expression has the advantage of making the calculations involved in making season- and age-specific prevalence predictions much more manageable.

7.4 Calculation of incidence rate of new infections, h(a,t)

In section 5.2 (ii), the incidence of new infections was shown to depend on both the rate of contact between susceptible humans and the infected portion of the vector population and also the probability that such contact resulted in infection (eq.(5.9)). For the purposes of the new model various modifications are required to both this definition of the incidence rate of new infections and also the parameters used in its calculation.

To allow for both seasonal- and age-dependent variations, the incidence of new infection is now assumed to depend on: the age-and seasonal-dependent rate of contact between non-patent humans and the infected portion of the vector population and the age-and seasonal-dependent probability that such contact results in a patent infection. Thus,

$$h(a,t) = MBR(a,t) \times \frac{Y''(t)}{N''(t)} \times b(a,t) \qquad (7.6a)$$

and, for conditions of constant transmission:

$$h(a) = MBR(a) \times \frac{Y''}{N''} \times b(a) \qquad (7.6b)$$

7.4 (i) Estimation of parameters

7.4 (i) (a) The rate of contact of vectors and the human population, MBR(a,t)

The rate of contact between the uninfected and infected portions of the vector population and the human population are assumed to be identical, as is the rate of contact between the vector population and the patent and non-patent portions of the human population. Past experience of infection is also assumed to have no effect on the contact rate. The rate of contact is however assumed to vary with the age of the human host, (see section 2.1 (i) (b)) and also be dependent on seasonal variations in the density of potential vectors.

A simple way of representing the parameter MBR(a,t) is as an exponential function of age (a):

$$MBR(a,t) = MBR(0) \exp(C_1 a) \times SEASN \qquad (7.7a)$$

Where MBR(0) is the average biting-rate (or vector-man contact rate) for infants and C_1 is a measure of the relative increase in the biting-rate with age. The value of the parameter C_1 was estimated to be 0.0567, where age is in weeks (data by Carnevale et al,1978, see fig. 2.1). The parameter SEASN represents the seasonal variation in the vector population. For areas where the density of potential vectors does not vary significantly throughout the year or for average contact rates, the parameter SEASN may be omitted or represented by the value of one.

$$MBR(a) = MBR(0) \exp(C_1 a) \qquad (7.7b)$$

However, if regular seasonal variations in the climatic conditions produce regular seasonal variations in the number of potential vectors, the expression $MBR(0)\exp(C_1 a)$ represents average age-dependent contact rates and the variations in the vector population can be represented by annual sinusoidal cycles in the value of SEASN.

$$SEASN = (Av + (S \times \sin (2\pi t)) \quad (7.8)$$

where t is measured in fractions of one year and (Av + S) represents the magnitude of the seasonal effect when vector densities are at a maximum.

The functional form of MBR(a) is considered further in section 7.4 (ii) (b).

7.4 (i) (b) The probability that contact between infected vector and non-patent host results in a patent infection, b(a,t)

Many uncertainties surround the understanding of exactly what aspects of malarial infection generate acquired immune responses that affect the probability that an infected bite produces a patent infection in man. Nonetheless, several of the dynamic features of continued exposure to human malaria outlined in the literature, seem to indicate that the probability that potentially infective contacts give rise to patent infection is dependent on humoral anti-sporozoite responses. (A potentially infective bite is defined as an infected bite that would give rise to a patent infection in an naive person or an infant with no acquired immune response, see Appendix G.2). Recent studies using animal models have however shown that in the absence of antibody, two cell-mediated responses (gamma interferon and tumour necrosis factor) will also protect hepatocytes and animals against sporozoite challenge (Schofield et al,1987; Ferreira et al,1986).

The circulation time of sporozoites has been used as an *in vivo* expression of anti-sporozoite activity. Following intravenous inoculation of sporozoites into naive humans, blood samples taken at regular intervals and sub-inoculated into further naive hosts, indicate that potentially infective sporozoites circulate in the blood for up to one hour (Fairley,1947). Studies using mice have shown that passive transfer of immune sera, or prior inoculation with irradiated sporozoites reduces circulation times from one hour to only 5-10 minutes (Rivera-Ortiz & Nussenzweig,1972). Naturally occurring anti-sporozoite immune responses however have rarely been shown to completely prevent the invasion of hepatocytes by sporozoites. Typically, a recent study in W.Africa showed that human sera with the highest anti-sporozoite antibody titres still left 12-18% of sporozoites able to enter hepatocytes and develop normally (Mellouk et al,1986). Several surveys have shown that even adults with the highest levels of anti-sporozoite antibodies often have patent infections (Marsh et al,1988; Mellouk et al,1986;

Hoffman et al,1987). It would appear that natural anti-sporozoite immune responses therefore fail to prevent parasite establishment but considerably reduce the circulation time and therefore the number of sporozoites that successfully invade hepatocytes (i.e. they reduce the life expectancy by increasing the death rate of the sporozoites). This factor must be related in some way to the ability of individuals with high antibody titres to prevent the majority of potentially infective bites from producing patent infections despite the fact that a single sporozoite is capable of producing a patent infection.

Studies in sporozoite-induced malaria in man suggest that an inverse relationship exists between the size of the sporozoite inoculum and the duration of pre-patency (Jeffery et al,1959). This relationship has been quantitatively documented in various animal models (Nussenzweig et al,1966; Vanderberg et al,1968) and more recently by Nardin et al(1982) and Schmidt et al(1982). In mice infected with a known number of sporozoites of P.berghei, the pre-patent period was considerably lengthened when the inoculum was reduced from 5×10^3 to 10 sporozoites (Nussenzweig,1967). Furthermore, as mentioned in the experiments outlined in Chapter 3, reducing the size of the sporozoite dose administered to experimental murine hosts increases the probability that the inoculum will fail to produce patent infections, even in naive hosts. It would appear therefore that the critical factor which determines the probability that potentially infective bites produce patent infections is the duration of the time delay before parasites reach sufficient numbers to "breakthrough" and produce patent infections.

One possible explanation is that the longer the time that infections remain at sub-patent levels the longer various immune responses have to develop and then successfully control infections by either eliminating all the parasites or maintaining them at sub-patent levels. The occasional patent infections among adults could therefore simply be the result of an inoculum containing unusually large numbers of sporozoites which the immune response is unable to cope with.

It is unlikely however that immune responses affecting the magnitude of b(a,t) are dependent on the accumulated past experience of bites that merely contain sporozoites i.e. infected bites. Numerous studies from both experimental infections and also naturally occurring malaria indicate that not all sporozoites are viable (Vanderberg,1975; Vanderberg,1977). Nussenzweig & Chen(1974) have found that immature sporozoites are considerably less immunogenic with regard to anti-sporozoite reactions. Additionally, several factors may prevent sporozoites ever reaching the blood; an

interrupted bloodmeal, immediate re-ingestion of sporozoites during the bloodmeal (Yorke & Macfie,1924), some sporozoites inoculated into the skin and subcutaneous tissues may be lost via lymphatic drainage (Boyd & Kitchen,1939) or cleared by lymphoid-macrophage cells (Huff & Coulston,1948). A much more accurate description of the development of immune responses which affect b(a,t), is the accumulated exposure to potentially infective bites (Appendix G summarizes the terminology concerning infected and infective bites). Not only are these bites potentially immunogenic but in partially immune individuals the sub-patent infections that may result from them may be an important source of antigenic stimulus. Antigenic stimulus of this type may be required to maintain functional immunity. This suggestion is consistent with the notion that protective anti-malarial immunity requires continuous antigenic stimulus (Howard,1986; Maegraith,1973).

The general form for b(a,t) is therefore expressed as follows:

$$b(a,t) = F\left[\, b(0,t) \times \overline{MBRY}(a,t) \,\right] \qquad (7.9)$$

where, $\overline{MBRY}(a,t)$ records the accumulated sum of past experience of infected bites in a human aged a, at time t, where,

$$\overline{MBRY}(a,t) = \frac{Y''}{N''} \int_0^a MBR(a'',\ t-(a-a'')\ )\ da'' \qquad (7.10)$$

b(0,t) is the probability that an infected bite is potentially infective and capable of producing patent infection in a host with no acquired immunity. The function F denotes the functional relationship between b(a,t) and the accumulated past experience of potentially infective bites.

The relationship between age and the magnitude of b(a,t) can vary considerably. It is not only dependent on the assumptions concerning the functional form of F, but also on localized features such as the density of potential vectors, the relative seasonal changes to this density due to climatic conditions and the sporozite rate amongst the vectors in an area.

For simplicity, the sporozoite rate is assumed to be constant and the relationship between b and age will initially be considered for non-seasonal conditions. Under these conditions, eq.(7.9) reduces to an equation for changes in b with age:

$$b(a) = F [b(0) \times \overline{MBRY}(a)] \quad (7.11)$$

where, $\overline{MBRY}(a)$ is the accumulated past infection of infected bites for a person aged a, under conditions of constant transmission, according to the equation.

$$\overline{MBRY}(a) = \frac{Y''}{N''} \int_0^a MBR(a'')\, da'' \quad (7.12)$$

7.4 (i) (c) The probability that an infected bite produces patent infection in a naive infant, b(0).

The value of b(0) can be estimated from data on the rate of acquisition of infection (I) for infants. Since all infections among infants can be assumed to be new infections the value of I(0) will be identical to that of h(0). From eq.(7.6b) we know that b(0) is related to h(0) according to the following equation.

$$b(0) = \frac{h(0)\ N''}{MBR(0)\ Y''} \quad (7.13)$$

Estimates for infant infection rates made during the Garki Project (Molineaux & Gramiccia,1980) were used in the estimation of b(0). Although these infection rates are not strictly the infection rates for infants aged 0, since the estimates include infants up to the age of 45 weeks, the estimated infection rates were found to be approximately the same in the first few weeks of life as later. Acquired immunity obviously has little effect on the acquisition of infections during the first year of life, so that the infection rates for infants up to 45 weeks can be confidently used to estimate b(0). Nevertheless, it might be assumed that infection rates measured in the villages with the lowest vectorial capacity will give the most accurate estimates for b(0).

Table 7.1 summarizes the infection rates (ICR) and rates of contact between infected vectors and man (EIR) obtained by the Garki Project for villages with different levels of vectorial capacity. Table 7.1 also shows the estimates for b(0), made by assuming no reduction in MBR, a 50% reduction and a 75% reduction.

Table 7.1

Entomological inoculation rates and Infant conversion rates for P.falciparum, estimated in the District of Garki, Kano State, Nigeria, (Table 17, Molineaux & Gramiccia,1980)

Vectorial capacity	Daily entomological inoculation rate (EIR)	Daily infant conversion rate (ICR)	Estimated value of b(0), (% reduction made to EIR)		
			0 %	50 %	75 %
Lowest	0.229	0.0128 (+/-0.003)	0.056	0.111	0.223
Moderate	0.252	0.0163 (+/-0.0027)	0.065	0.120	0.259
Highest	0.940	0.0124 (+/-0.0017)	0.013	0.026	0.053

These reductions are made to allow for the fact that the estimated EIR in the Garki Project are not based on infant man biting-rates, but are calculated using estimates of the number of bites received by young adults. The number of bites received by infants is known to be considerably less than that of older individuals; unless allowance is made for this fact the value of b(0) could be considerably over-estimated. A 50 % reduction in man-biting rates was considered reasonable, and the probability that an infected bite received by a naive infant (i.e. with no acquired immunity) gives rise to a patent infection, b(0), was estimated to be 0.1. The value of b(0) is assumed not to vary significantly between seasons so that $b(0,t) = b(0)$ for all values of t.

It is interesting to note that whereas the estimates for b(0) from villages with low or moderate vectorial capacities are in good agreement with each other, the estimate from the high vectorial capacity area is considerably lower. It is possible that the higher infection rates in these areas mean that infants under one year have already acquired some functional immunity which is reflected in ICR that are considerably lower than those of the area with the lowest vectorial capacity. Even allowing for the development of some functional immunity amongst these infants the estimate for b still appears to be very low. Since maternally derived antibody has been found to have little effect on the acquisition of new infections it has been suggested that areas with high transmission potential may give rise to higher levels of "population immunity" which results in a reduction in the "quality" or quantity of gametocytes available to infect the vectors: a negative correlation was found in the villages between vector density and density of gametocytes.

Previous estimates for b(0) are, on average a degree of magnitude smaller than those made above. Davidson & Draper(1953) estimated b(0) to be 0.01, Pull & Grab(1974) concluded $0.015 < b(0) < 0.026$ from their data and Nedelman(1985) estimated similar values using a different method of analysis for the same data. Using the data in table 7.1, Nedelman(1984) estimated the values of b(0) for the villages of highest and lowest mosquito densities to be 0.012 and 0.086 respectively. All of these estimates have, however failed to consider in their calculations the very much reduced vector-man contact rates among infants. Port et al(1980) recalculated the estimate for b made by Pull & Grab(1974), by taking into consideration a correction factor to account for the differences in the number of feeds on infants and adults. The new estimate for b was calculated as $0.054 < b < 0.093$.

Some of the factors responsible for reducing the probability that an infected bite produces a patent infection

in naive individuals to 0.1 have already been discussed i.e. sporozoite viability, interrupted bloodmeals and various innate properties of the skin that can prevent parasite establishment. Section 2.1 (iii) outlines further mechanisms of innate resistance involving several cell and tissue types which are able to restrict parasite establishment and the development of patent infections.

7.4 (ii) Acquired immunity functions for new infections

If one assumes that the age-dependent infection rates (I(a)) recorded in the dry season by Bekessy et al(1976) (figs. 7.1 (a), (d) and (e)) are almost exclusively recrudescences of infection acquired in the previous wet seasons and that the infection rates during the wet seasons (figs. 7.1 (b) and (c)) are predominately the result of newly acquired infections, it appears probable that new infection rates (h(a)) steadily decline with age until the age of about 13 years after which the rates remain constant. Recrudescent infection rates (f(a)) however appear to be highest among the 5-8 year olds, and at very low levels among both infants and adults. It is probably true to say that the convex pattern for average infection rates (covering one year), is simply a reflection of this seasonal effect (fig. 7.1 (a)). It is therefore assumed that for the youngest children and also among adults the observed average incidence rates are approximately equivalent to the average rate at which new infections are acquired. The observed infection rates amongst the other age-classes reflect both the rate at which recrudesces appear and also the rate at which new infections are acquired.

7.4 (ii) (a) Simple linear immunity function

The simplest assumption concerning the functional relationship between age and b(a) is that the probability, b(a) is a linear function of the accumulated past exposure of potentially infective bites. This assumption can be represented by:

$$b(a) = b(0)\left[1 - \frac{\underline{Y}''}{N''} g_1 \int_0^a MBR(a'')\, e^{-\sigma_1(a-a'')}\, da''\right] \qquad (7.14)$$

g_1 denotes the strength of acquired immunity which reduces the probability that infected bites produce patent

infections as the accumulated experience of potentially infective bites increases. $1/\sigma_1$ represents the average duration of "immunological memory" of past exposure to potentially infective bites. For example, if $\sigma_1 = 0$, memory is life-long; if $\sigma_1 \longrightarrow \infty$ memory is absent.

The linear dependency however is a very simple approximation, and has the disadvantage that for certain values of g_1:

$$\frac{1}{g_1} < \frac{Y''}{N''} \int_0^a MBR(a'') \, e^{-\sigma_1(a-a'')} \, da'' \qquad (7.15)$$

the net rate of new infections becomes negative in older individuals (fig. 7.6 (a)).

The linear model (eqs.(7.6b), (7.7b) and (7.14)) describing the rate of acquisition of new infections is able to produce a wide variety of patterns, (fig. 7.6), ranging from little or no variation in h(a) with age, through convex curves to steady increases or decreases in h(a) with age. For a given severity of acquired response (g_1) the curves tend to produce convex patterns if memory is of shorter duration ($1/\sigma_1$ small) or the human-vector contact is moderate. The values for h(a) rise to a peak in older children and then decline rapidly to negative levels among adults. However for very small values of g_1 (less acquired immunity), if $1/\sigma_1$ is small (duration of immunological memory negligible) or transmission is very low the infection rates are seen to rise with age. For realistic values of σ_1 and g_1, h(a) is seen to decline steadily with age; this decline tends to be very rapid when human-vector contact is high and less rapid if the contact rate is lower (with a tendency toward convexity) (fig. 7.7). For very low contact rates however the relationship between age and b(a) becomes a positive one. These predictions tend to reflect what one might expect to happen for natural infections in human communities: a more rapid decline in h(a) in areas of high contact rates, and, in areas where vector-human contact is too low to generate functional immunity, the higher contact rates amongst older individuals causes an increase in h(a) with age.

Although the model predicts convex patterns for h(a) similar to the patterns seen for the average yearly infection rates (I(a)) in fig. 7.1 (a), the peak appears to be predicted to occur in much older age groups. The convex profiles predicted by the model are determined by the interaction of the acquired immune response (g), the duration of immunological memory ($1/\sigma_1$) but particularly reflect the relationship of MBR(a) with age. The functional form of MBR(a) given by eq.(7.7) means that the vector-man contact

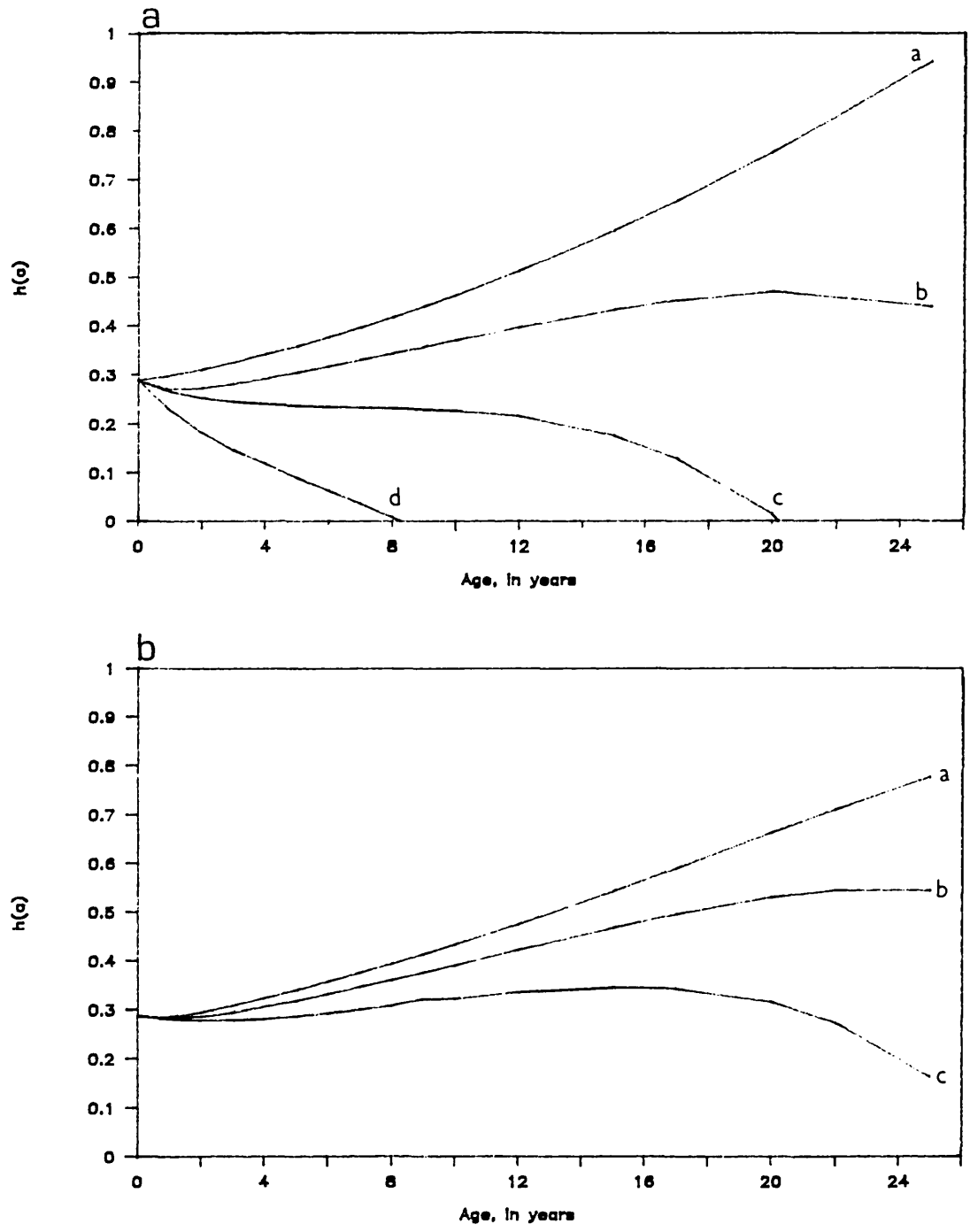


Fig. 7.6. Age-specific equilibrium new infection rates (h(a)) generated by a linear function for acquired immunity, eqs.(7.6b), (7.7b) and (7.14) in the main text. Parameter values (all /week), (a) profile a - g_1=0.000161, σ_1=0.008, profile b - g_1=0.0012, σ_1=0.0213, profile c - g_1=0.001, σ_1=0.008, profile d - g_1=0.002, σ_1=0.008. (b) profile a - g_1=0.00066, σ_1=0.0213, profile b - g_1=0.00066, σ_1=0.01332, profile c - g_1=0.00066, σ_1=0.008.(additional parameters MBR(0)=96.4, C_1=0.0567, b(0)=0.1, Y"/N"=0.03)

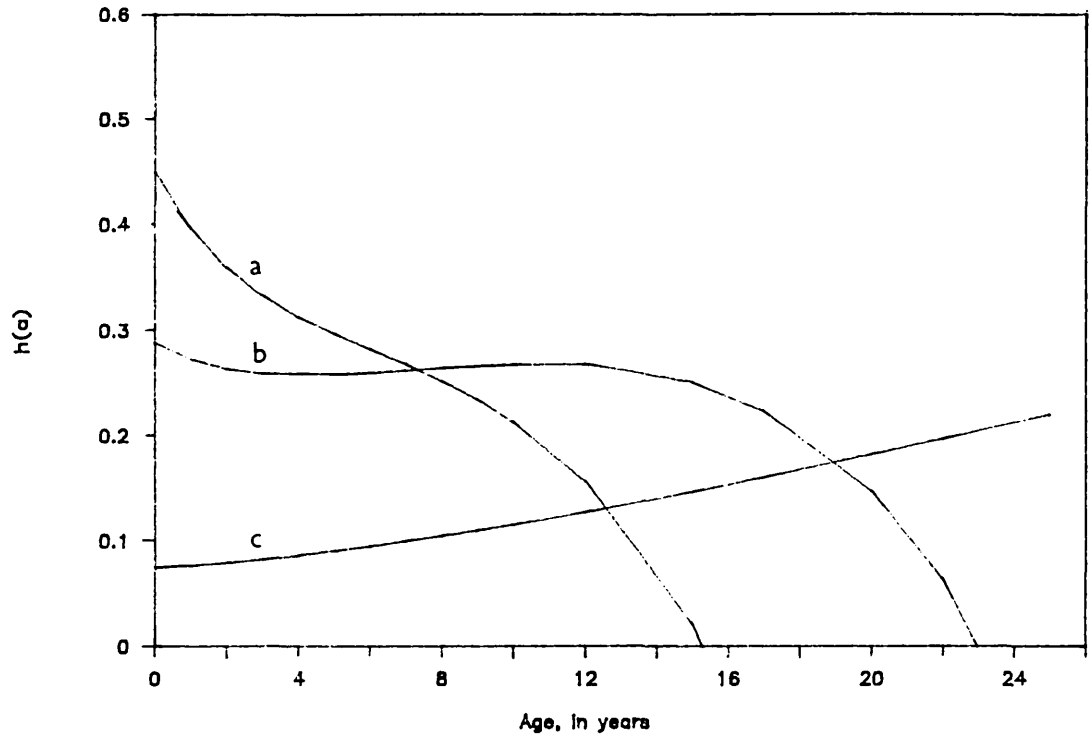


Fig. 7.7. Age-specific equilibrium new infection rates (h(a)) generated by a linear function for acquired immunity, eqs.(7.6b), (7.7b) and (7.14) in the main text. Parameter values (all /week),(a) MBR(0)=150; (b) MBR(0)=96.4; (c) MBR(0)=25. Additional parameters g_1=0.00085, σ_1=0.008, C_1=0.0567, b(0)=0.1, Y"/N"=0.03.

continues to rise with age with no upper limit. The change in the relationship of b(a) with age, from positive to negative simply reflects the age at which, in a given time interval, the losses to the accumulated past experience of potentially infective bites due to the finite duration of immunological memory is more than compensated by newly acquired potentially infective bites.

The assumption of linear dependency of the function F is clearly not a good reflection of the acquisition of functional immunity which reduces the value of b(a). The predicted negative infection rates among adults is a clear indication that the reduction in b(a) with age cannot be produced by an immune response that can continue to improve throughout the whole life-time of an individual. It is probable that a threshold is reached sometime in late childhood/early adulthood after which the immune response to new infections cannot be improved on. Furthermore the relationship between h, MBR and b in eq.(7.6) indicates that this threshold must coincide with a similar threshold effect for MBR(a) so that the acquisition of new infections remain constant, similar to observed rates, and do not start to rise in the oldest age groups.

7.4 (ii) (b) More complex immunity functions

The problem with the contact rate between man and vectors can be overcome by the function MBR(a,t) assuming a slightly more complex form where, for example,

$$MBR(a,t) = [MBR_{max} - \exp^{-C_2 a}(MBR_{max} - MBR(0))] \times SEASN \qquad (7.16a)$$

where MBR_{max} is the maximum rate of contact between man and vectors and C_2 is a measure of the increase in the contact rate with age. The value of C_2 was estimated to be 0.00175 when age a, is measured in weeks (data from Carnevale et al,1978). This function for MBR(a,t) generates man-biting rates that rise steeply with age, as suggested by the available data (see Section 2.1 (i) (b)), but which rise to an upper plateau MBR_{max}, amongst adults. The parameter SEASN is the same as that defined previously by eq.(7.7a). For non-seasonal man-biting rates equation (7.16a) reduces to,

$$MBR(a) = [MBR_{max} - \exp^{-C_2 a}(MBR_{max} - MBR(0))] \qquad (7.16b)$$

The function F also takes a more complex form,

$$b(a) = b_{min} + (b(0) - b_{min}) \exp\left[\frac{g_2}{g_3} (1-\exp (g_3 \bar{\bar{W}}(a))) \right] \quad (7.17)$$

where,

$$\bar{\bar{W}}(a) = b(0) \frac{Y''}{N''} \int_0^a MBR(a'') e^{-\sigma_1(a-a'')} da'' \quad (7.18)$$

b_{min} is the probability that an infected bite produces a patent infection when the acquired immune response is at its maximum level and the parameters g_2 and g_3 determine the strength of the acquired immune response to potentially infective bites. This more complex immunity function overcomes the problem of negative infection rates generated by the simpler linear function above.

This immunity function can also generate a variety of patterns, describing the change in b(a) with past experience of potentially infective bites ($\bar{\bar{W}}(a)$); the exact relationship being critically dependent on the parameters controlling the strength of the acquired immune response (g_2 and g_3)(fig. 7.8). Figure 7.9 (a), clearly shows that if the development of immunity against potentially infective bites is rapid (profile a), the age of at which the new infection rate is maximum, lies amongst the infants and youngest children despite the lower contact rates between children of this age and the vector population. However, if immunity develops more slowly, the model predicts a higher rate of acquisition of new infections amongst some or all of the age groups and also a shift in the maximum new infection rate h(a) to an older age class (profiles b and c).

Since most aspects of malarial infection appear to indicate that immunity against new infections develops only slowly with increasing age, due to the apparently low immunogenicity of the sporozoites and/or the low contact rates between vectors and children, one might expect that the relationship between b(a) and W(a) would to be similar to that of profile b or c in fig. 7.8. The predicted values for h(a) from such a relationship are however much too high; compare the infection rates for profile b and c in fig. 7.9 (a) with the average daily infection rates obtained by Bekessy et al,(1976) for an area of high transmission, fig. 7.1 (a).

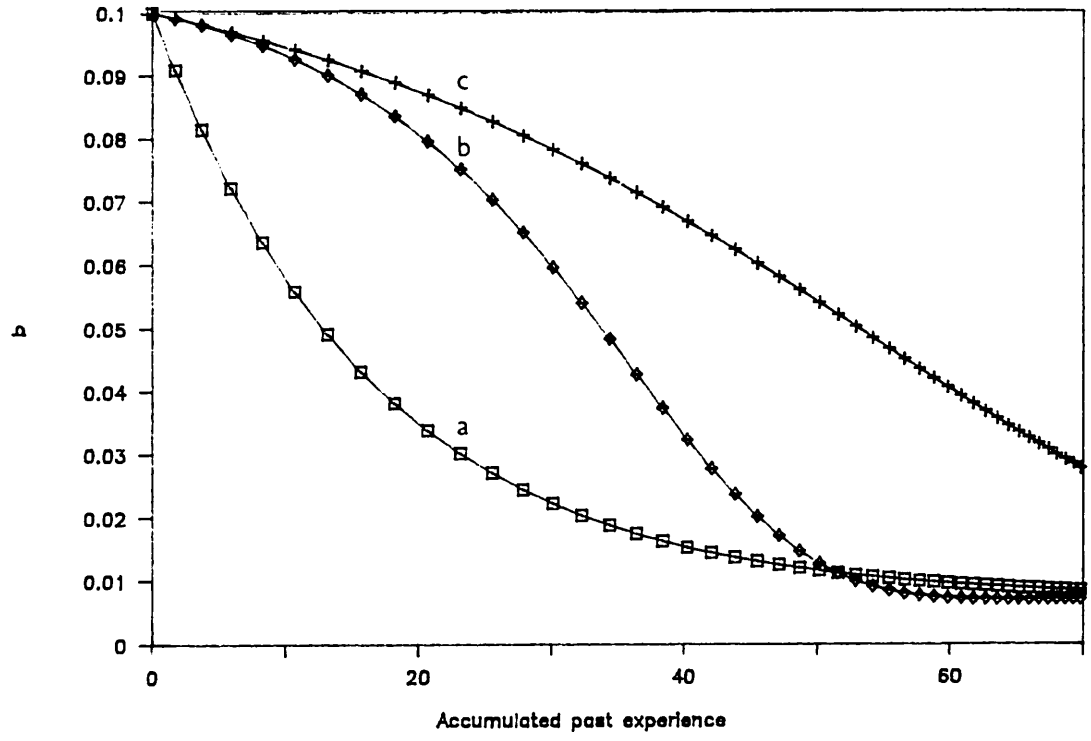


Fig. 7.8. The relationship between accumulated past experience of potentially infective bites and the probability that a infected bite results in patent infection (b), predicted by eq.(7.17) in the main text. Parameter values (all/week), profile a, g_2=0.005, g_3=0.075; profile b, g_2=0.06, g_3=0.0001; profile c, g_2=0.005, g_3=0.035. (Additional parameter values b(0)=0.1, b_{min}=0.007).

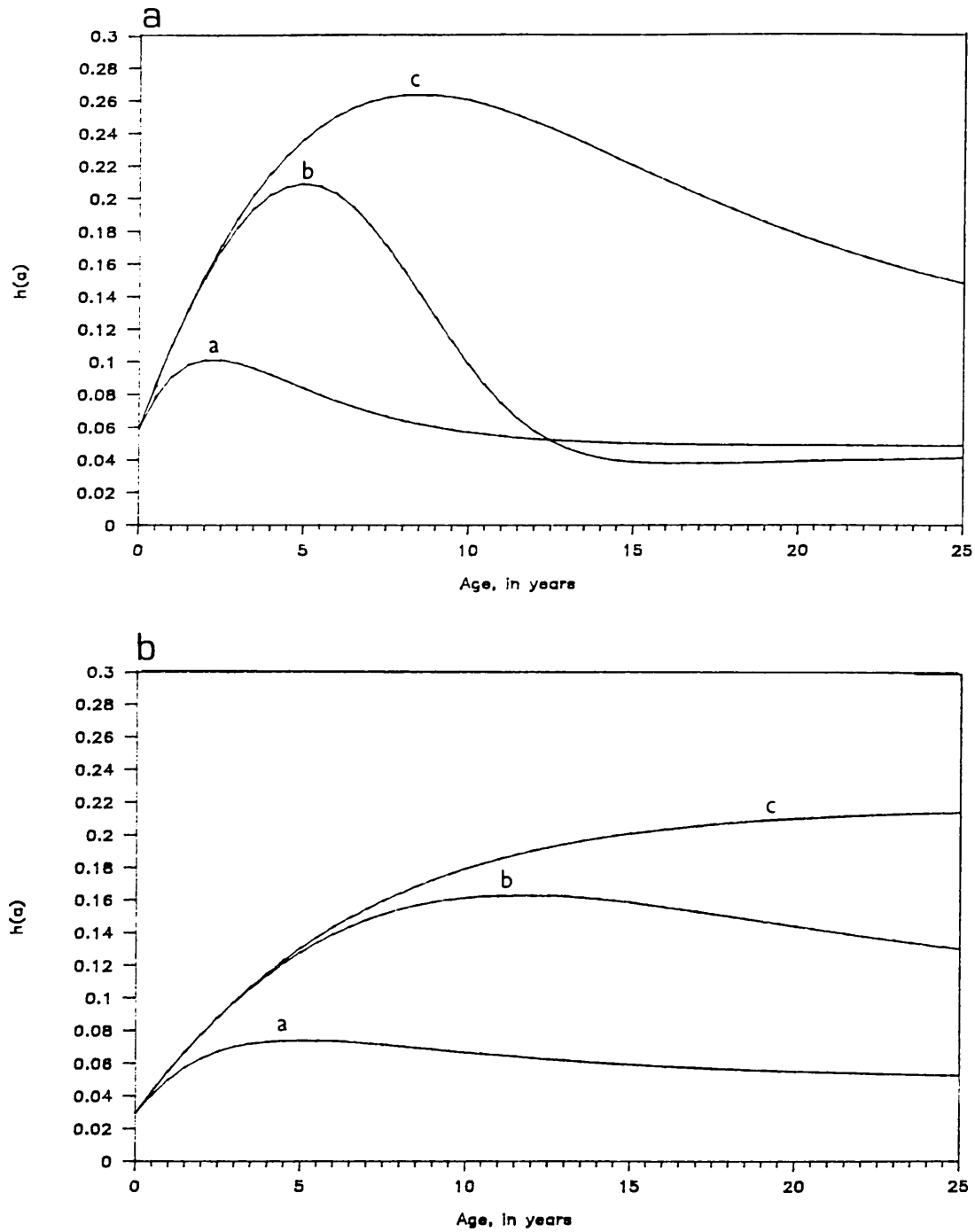


Fig. 7.9. Age-specific equilibrium new infection rates (h(a)) generated by the immunity functions shown in Fig. 7.8 and eqs.(7.6b) and (7.16b) in the main text. Parameter values (all /week),(a) MBR(0)=20, MBR_{max}=220, (b) MBR(0)=10, MBR_{max}=110.(Parameters as in Fig. 7.8 also C_2=0.00175, Y"/N"=0.03 and σ_1=0.008).

Furthermore, these types of relationship between b(a) and W(a) also predict high infection rates even when the contact rate between vectors and man is considerably reduced i.e. transmission potential less (fig. 7.9 (b)). Clearly, immunity toward new infections must develop quite significantly among even the youngest children. The relationship between age and the level of immunity toward potentially infective bites may more closely resemble that of profile a in fig. 7.8.

Figure. 7.10 (a) illustrates the possible age-related change in the value of b(a) which produces th change in the rate of acquisition of new infections with age, observed by Bekessy et al(1976). The profile assumes that the average contact rate between man and vectors during the year is 20 per week for infants (MBR(0)), rising to 220 contacts per week amongst adults (MBR_{max}) and the sporozoite rate among the vectors is assumed to be 0.03. Both these estimates are realistic representations of the transmission conditions in the area studied by Bekessy et al(1976). Figure 7.10 (b) shows the predicted changes in the rate at which new infection are acquired for the conditions mentioned above, compared to the average infection rates recorded by Bekessy et al(1976). The model appears to be able to predict reasonably well the level of infection among young children and adults, where infection rate is predominately due to new infections. The difference between the predicted and observed infection rates for individuals between the ages of 4-20 years reflects the additional effect of recrudescences (f(a)) among these age-groups which will be considered in the next section.

Figure 7.11 (a) shows the changes in b(a) with age, using the same estimates for immunological memory (σ_1), and acquired responses (g_2 and g_3), used in fig. 7.10 (a), for three levels of contact between vectors and man. For the highest level of contact (MBR(0)=20, MBR_{max}=220) the maximum limit of acquired response is achieved by the age of 18 years, whereas for the lowest level of contact (MBR(0)=2, MBR_{max}=22) the maximum limit of acquired response is never achieved, and the adults living under these conditions can be considered to be as susceptible to new infections as children aged 2 years who live in areas where vector-man contact is intense. Figure 7.11 (b) illustrates the changes in h(a) with age predicted for the three levels of vector-man contact. For the two highest contact rates, the rate at which new infection are acquired increases with age, a peak occurs among young children, after which the rate then decreases with age. This decline with age is most pronounced when vector-man contact is intense. For low vector-man contact rates, the rate at which new infections are acquired rises monotonically with age to an upper plateau amongst adults. The age at which the peak rate of new infection occurs therefore appears to increase as the contact rate decreases.

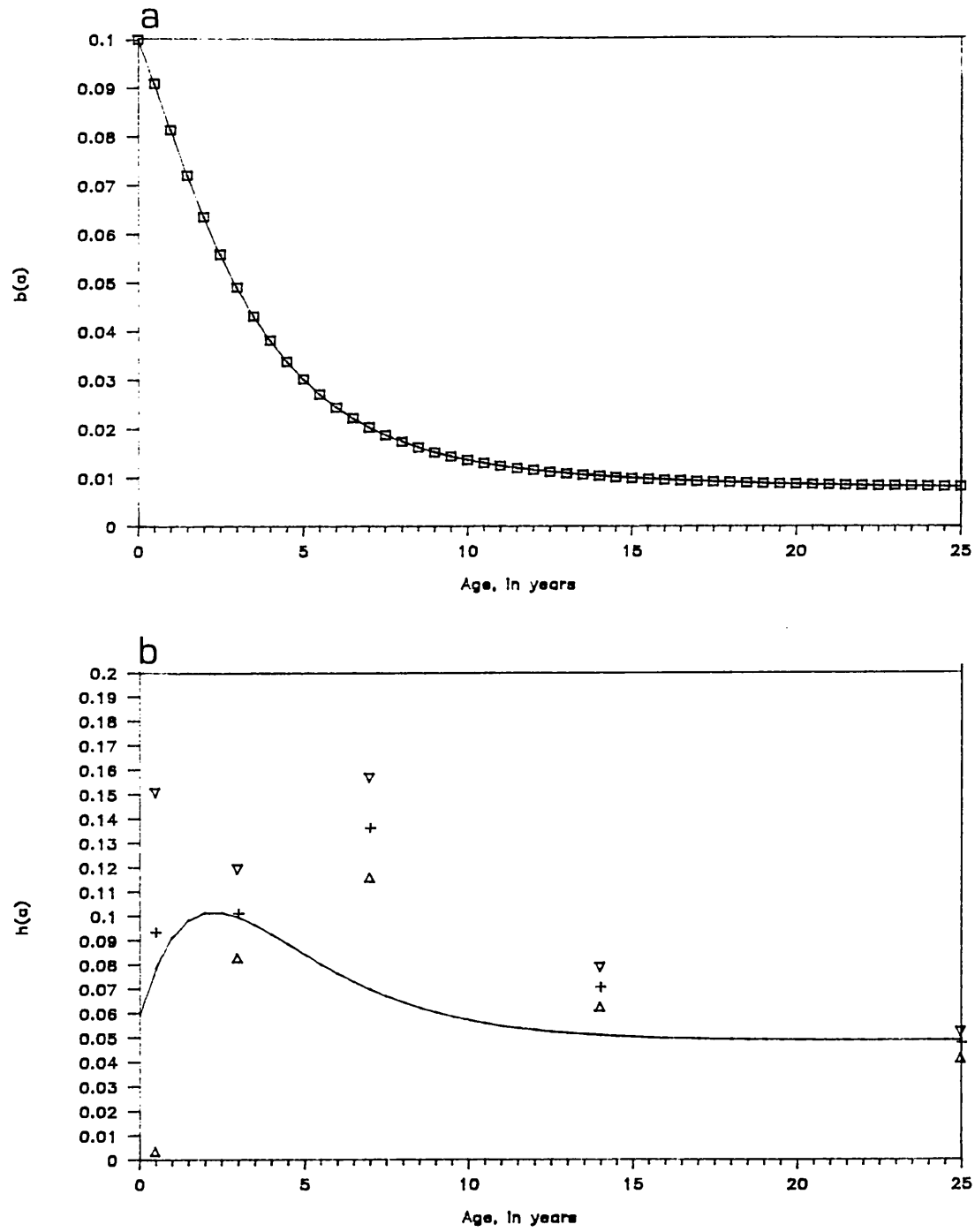


Fig. 7.10.
(a) Age-specific probability that a infected bite results in patent infection (b(a)), eqs.(7.16b)-(7.18) in the main text, (b) Age-specific equilibrium new infection rates (h(a)), eqs.(7.6b) and (7.16b) in the main text, compared with the yearly average daily incidence (I(a)) rates (with 95 % C.I.), recorded by Bekessy et al(1976).(Parameter values (all /week), $b(0)=0.1$, $b_{min}=0.007$, $\sigma_1=0.008$, $C_2=0.00175$, Y"/N"=0.03, $MBR(0)=20$, $MBR_{max}=220$, $g_2=0.0475$ and $g_3=0.005$).

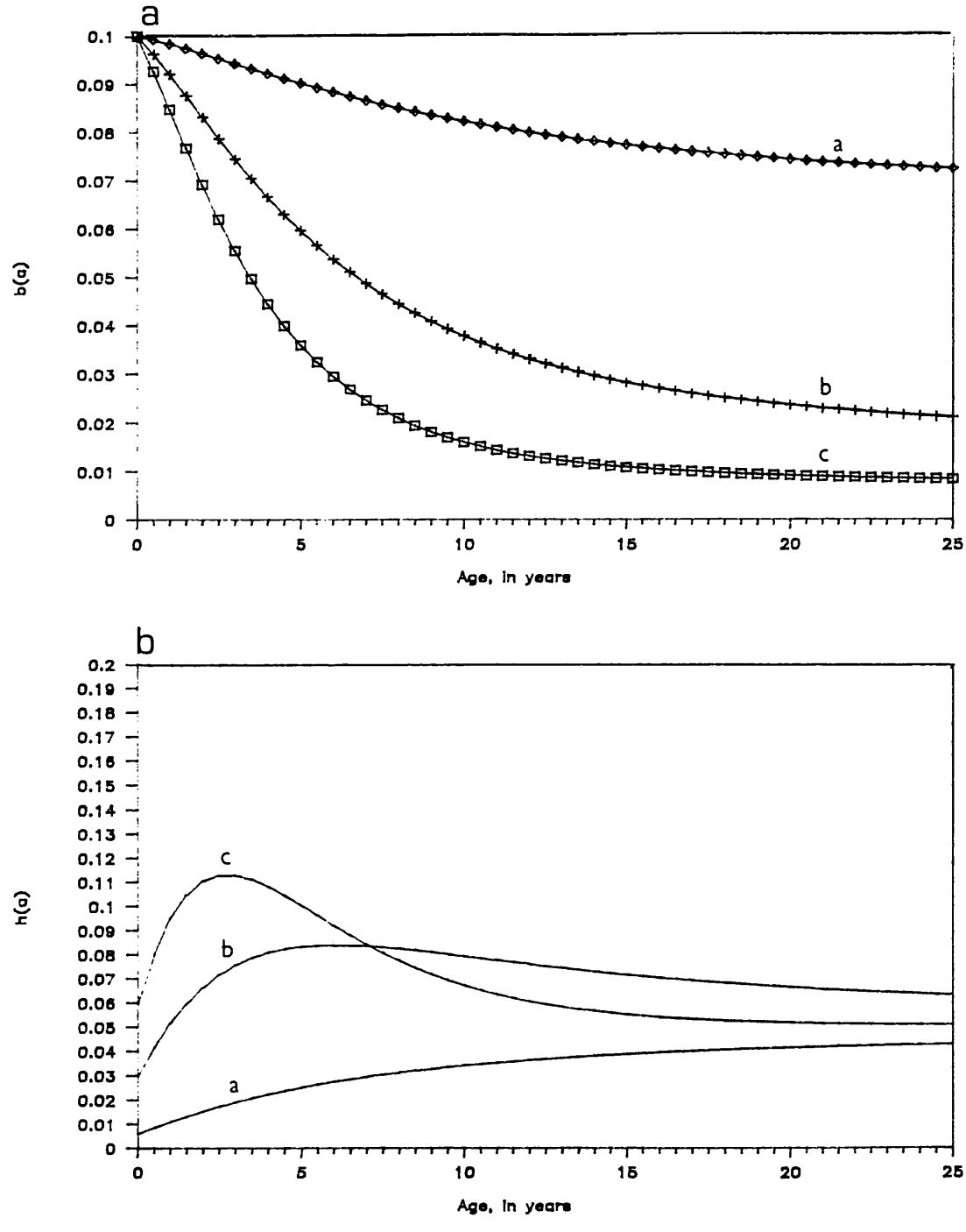


Fig. 7.11. The effect of the degree of contact between vectors and humans, (a) Age-specific probability that a infected bite results in patent infection (b(a)), eqs.(7.16b)-(7.18) in the main text, (b) Age-specific equilibrium new infection rates (h(a)), eqs.(7.6b) and (7.16b) in the main text. Parameter values (all /week), MBR(0), MBR_{max}: Profile a - 2,22 ; profile b -10, 110; profile c -20,220. (Additional parameter values, b(0)=0.1, b_{min}=0.007, σ_1=0.008, C_2=0.00175, g_2=0.0475 and g_3=0.005)

7.5 Calculation of incidence of parasitological recrudescences, f(a,t)

Malarial infections in their natural hosts are often of long duration, and a host which recovers from the acute phase of a sporozoite infection may subsequently carry a chronic and intermittently patent parasitaemia. For sporozoite-induced P.vivax infections in man a renewal of activity in the liver and a secondary exo-erythrocytic phase gives rise to true relapse parasitaemia in the blood in about 50 % of individuals (Krotoski et al,1980; Pampana,1969). Following recovery from the primary attack other types of malarial infections in man and, in other mammals, also give rise to further phases of patent parasitaemia. These secondary attacks, unlike those of P.vivax are produced by renewed activity of subpatent blood infections caused by the survival of some erythrocytic forms of the parasite.

The rate at which these recrudescences occur f(a,t), will be the product of the rate at which new infections were acquired by an individual at time t-ø, age a-ø , h(a-ø, t-ø) (where ø is the average time period between the onset of the primary infection and the onset of the recrudescence), and the probability c(a,t), that primary infections acquired at time (t-ø) give rise to a recrudescent infections at time t. Thus,

$$f(a,t) = h(a-\phi, t-\phi)\, c(a,t) \qquad (7.19a)$$

$(a < \phi)$, $h(a-\phi, t-\phi) = 0$.

For non-seasonal conditions eq.(7.19a) becomes,

$$f(a) = h(a-\phi)\, c(a) \qquad (7.19b)$$

7.5 (i) The probability that patent new infections produce a recrudescence, c(a,t)

The persistence of blood infections and the occurrence of recrudescent infections reflects a complex balance between the effect of host-generating immune responses which are potentially lethal to the parasite population, and other mechanisms employed by the parasite in an attempt to evade the host's immune response. Little information is available however concerning either the aspects of infection that influence the probability that patent infections recrudesce

(c(a,t)), or the relationship between the onset of recrudescences and the levels of acquired immunity in either man or experimental animal models. Serological studies have revealed a decline in the levels of antimalarial antibody during the period between patent parasitaemias in some individuals but not others (Kuvin et al,1962). Similar results were found in rats experimentally infected with P.berghei (Zuckerman et al,1969). Sequestering of P.yoelii in the kidney (an immuno-privileged site) has also been observed (see Chapter 3), although it is not known whether these parasites can give rise to recrudescences. Antigenic variation has been described for a number of plasmodium species (P.knowlesi, Brown & Brown,1965; P.berghei, Cox,1962; P.falciparum, Voller,1971), and it is assumed that parasites can survive in semi-immune hosts by undergoing periodic antigenic variations.

A study by M^{C}Lean et al(1982) examined the relationship between acquired immunity and the onset of recrudescences using P.chabaudi in the mouse. The protective immune response was found to be unable to reach a level which was adequate for the total elimination of the parasites following primary parasitaemia, and furthermore a decline in the level of resistance to the erythrocytic stages of the parasite was found to relate to the onset of recrudescences. This suggests that the emergence of recrudescences may, at least in part, directly result from depression of acquired immunity. Furthermore, work by Orjih & Nussenzweig(1979) have shown that the immunosuppressive nature of blood infections can have a severe affect on the development and maintenance of several antiparasitic responses in P.berghei in mice. Brown et al(1985) have found that although prior immunization of P.chabaudi in CBA/Ca mice showed some ability to control primary parasitaemia, recrudescences were in fact significantly enhanced over control levels. Furthermore, Marsh & Greenwood(1986) found that parasitization with blood-stage malaria parasites is immunosuppressive with regard to a number of antigens in natural human infections. This appears to be indicative of a suppressor effect of prior infections on the long-term efficiency of the protective response.

Study of the natural history of malaria infections in man demonstrates quite clearly that the level of acquired immunity rises slowly with increasing age, and reaches a stable level only after decades of continual exposure to infection. However, recrudescences appear to be concentrated on the 5-8 year old age group, which tentively suggests that the major factors controlling the level of "resistance" to other clinical and parasitological aspects of disease are not the same as those that control the occurrence of recrudescences.

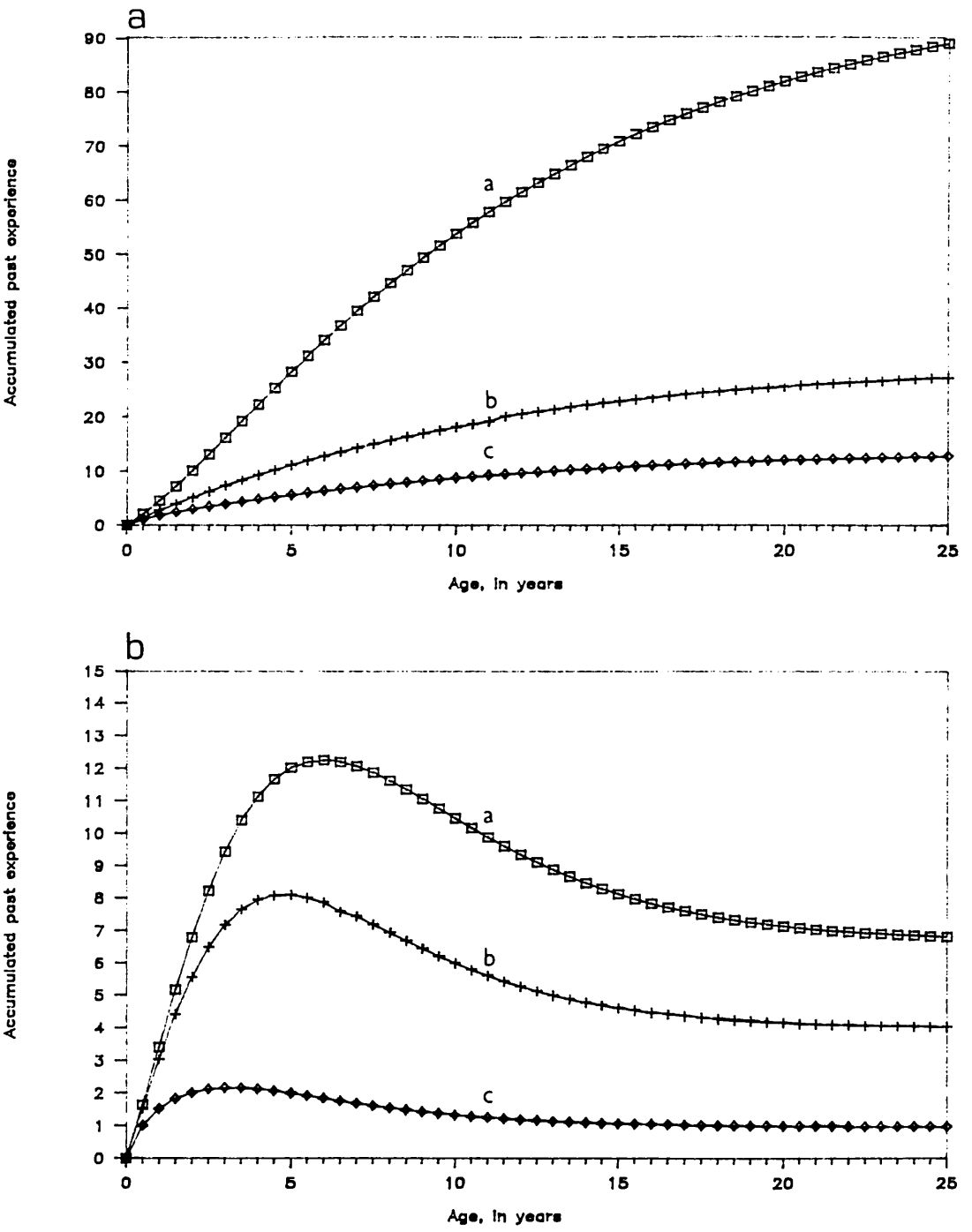


Fig. 7.12. The relationship between age and the accumulated past experience of, (a) potentially infective bites (profile a - σ_1=0.0081; profile b - σ_1=0.0215; profile c - σ_1=0.0451), (b) primary patent infections (profile a - σ_2=0.0075; profile b - σ_2=0.0125; profile c - σ_2=0.05). (Additional parameter values as for Fig. 7.10, parameter values all /week)

Figure 7.12 shows that whereas the accumulated past experience of potentially infected bites increases monotonically with age, the profile for the accumulated past experience of primary patent infections, (assuming that a duration of immunological memory, $1/\sigma_2$ in the region of 0.5-2.5 years is reasonable) tends to be convex, rising to a peak among children and then declining at older ages. The longer the duration of immunological memory, $1/\sigma_2$, the higher the age at which the profile peaks. The differences between the two sets of profiles increases with age and can be explained by the development of immunity. With increasing age more and more potentially infective bites fail to produce patent infection. It is however interesting to see that the peak in the accumulated experience of patent infections appears to occur in approximately the same age groups as the highest observed rates of recrudescences.

Recrudescence, at least in experimental animal models has been correlated with; (i) the inability of protective immune response to reach an adequate level for the total immune elimination of the parasites of the primary parasitaemia, and (ii) a decline in the level of anti-parasitic activity toward the erythrocytic stages of the parasite. It is possible therefore that the occurrence of recrudescences in man may, at least in part, result from immunodepression of acquired immunity. The level of past experience of past patent infections among children aged 4-13 years may affect the development and maintenance of antiparasitic immunity and thereby allow recrudescences to occur more frequently than in other age groups.

It is therefore assumed that there is a negative relationship between the accumulated past experience of past patent infection, both primary infections and recrudescent infections, and the probability c(a,t) that new patent infections recrudesce. The general form for c(a,t) is therefore expressed as follows:

$$c(a,t) = V \left[\overline{H}_f(a,t) + \overline{F}_f(a,t) \right] \qquad (7.20)$$

where, $\overline{H}_f(a,t)$, the accumulated past experience of past primary infections, which affects the rate of recrudescence, for an individual aged a, at time t, is given by,

$$\overline{H}_f(a,t) = \frac{Y''}{N''} \int_0^a MBR(a'',t-(a-a'')) \; b(a'',t-(a-a'')) \; e^{-\sigma_2(a-a'')} \, da'' \qquad (7.21)$$

and $\bar{F}_f(a,t)$, the accumulated past experience of past recrudescent infections which affects the rate of recrudescence is expressed as,

$$\bar{\bar{F}}_f(a,t) = \frac{\underline{Y}''}{N''} \int_0^a MBR(p)\ b(p)\ c(a'',t-(a-a''))\ e^{-\sigma_3(a-a'')}\ da'' \qquad (7.22)$$

where $p = (a''-\phi,\ t-(\phi+a-a''))$, $1/\sigma_2$ and $1/\sigma_3$ are the duration of immunological memory of past infection for primary infections and recrudescent infections respectively and the vector-man contact rate MBR(a,t) is defined by eq.(7.7a).

Under conditions of constant transmission eqs.(7.20)-(7.22) reduce to equations which predict changes with age only.

$$c(a) = V\ [\ \bar{\bar{H}}_f(a) + \bar{\bar{F}}_f(a)\ ] \qquad (7.23)$$

and,

$$\bar{\bar{H}}_f(a) = \frac{\underline{Y}''}{N''} \int_0^a MBR(a'')\ b(a'')\ e^{-\sigma_2(a-a'')}\ da'' \qquad (7.24)$$

$$\bar{\bar{F}}_f(a) = \frac{\underline{Y}''}{N''} \int_0^a MBR(a''-\phi,t-\phi)\ b(a''-\phi,t-\phi)\ c(a'')\ e^{-\sigma_3(a-a''))}\ da'' \qquad (7.25)$$

7.5 (ii) The acquired immunity function for recrudescences

The functional relationship between age and c(a) is assumed to be similar to that of the complex immunity function for b(a) (section 7.4 (ii) (b)), except that whereas the relationship between b(a) and accumulated past experience in eq.(7.17) was a positive one, the relationship between accumulated past experience of patent infection and c(a) is assumed to be negative. The functional form V therefore takes the form:

$$c(a) = c_{max} - (c_{max}-c_{min})\ \exp\left[\ \frac{g_4}{g_5}\ (1-\exp\ g_5\ (\bar{\bar{H}}_f(a)+\bar{\bar{F}}_f(a))\ \right] \qquad (7.26)$$

where $\bar{H}_f(a)$ and $\bar{F}_f(a)$ are defined by eqs.(7.24) and (7.25), c_{max} is the maximum probability that a primary infection will recrudesce, c_{min} is the minimum probability that a primary infection will recrudesce and g_4 and g_5 represent the strength of the immunosuppression. It is important to note that although all infections in infants are assumed to be new infections ($f(0) = 0$), c_{min} does not have to be equal to zero; $f(a)$ will be zero, irrespective of the value of $c(a)$, for all values of $a < \phi$ (eq.(7.19)).

From the seasonal data by Bekessy et al(1976) the average weekly dry season incidence rate of 0.0238 +/- 0.00763 for children < 1 year indicates that a few new infections are acquired and that some active transmission must occur during these months. Bearing in mind the higher vector-man contact rate among older children (3-5 times that of infants) and therefore a greater risk of acquiring new infections during the dry season it is probably reasonable to assume that the maximum probability that a primary infection recrudesces (c_{max}) is about 0.55 and the minimum probability (c_{min}) is about 0.02.

In view of the estimated long duration of each positive episode (1/R) particularly among the age classes where recrudescences are most prevalent, it is probable that the duration of immunological memory $1/\sigma$ (measured from the onset of the primary infection) is likely to be of considerable length. Figure 7.13 clearly illustrates that if the duration of immunological memory is short the rate of maximum recrudescence occurs among younger children (5 years of age) instead of the observed 5-8 year age-group. The estimate for the length of the time period ϕ, from the onset of primary infection to the occurrence of a recrudescence however appears to have less effect on the position of the peak and has only a minor effect on the maximum rate at which recrudescences occur. The shape of these profiles are very reminiscent of the patterns observed by Bekessy et al(1976) in the dry season. Figure 7.14 (a) shows the type of relationship between the value of $c(a)$ and age for plausible values of ϕ ,g_3, g_4, σ_2 and σ_3 that is able to give generate average non-seasonal recrudescence rates (fig. 7.14 (b)) that not only occur in the right age-groups but appear to be of the correct magnitude.

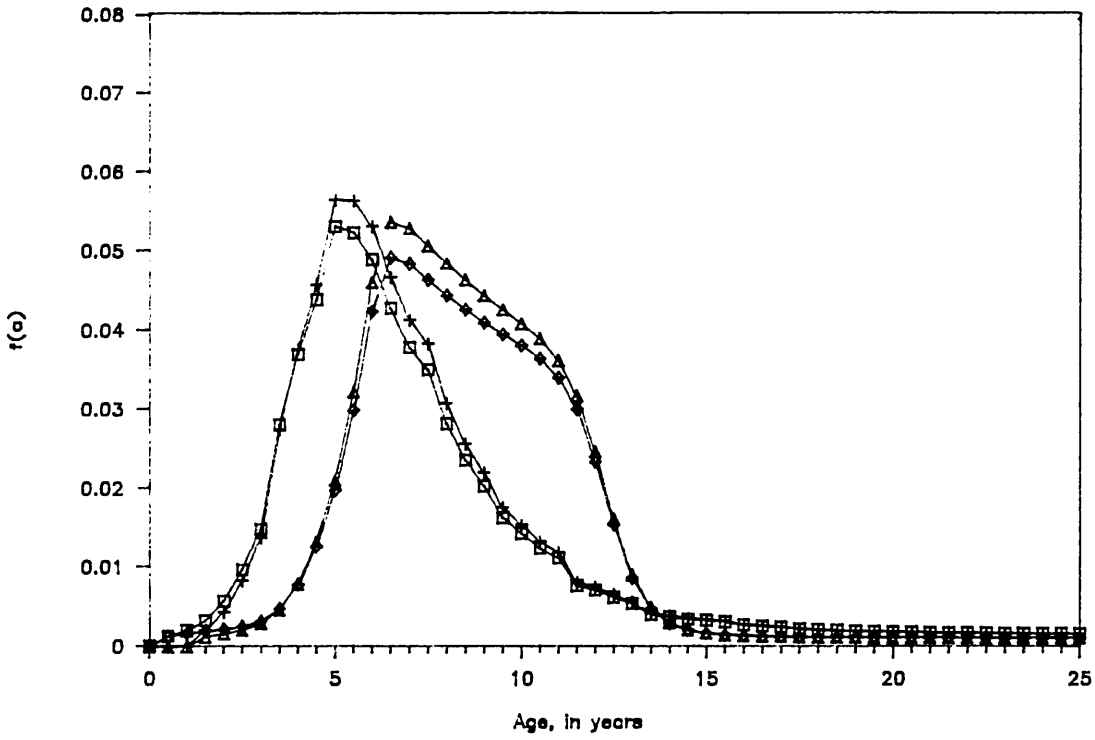


Fig. 7.13. Age-specific equilibrium recrudescence rates (f(a)) generated by eqs.(7.19b), (7.6b) and (7.24)-(7.26), in the main text. Parameter values (all /week), σ_2=0.0125, ϕ=26 weeks (symbol □); σ_2=0.0125, ϕ=78 weeks (symbol +); σ_2=0.0075, ϕ=26 weeks (symbol ◇); σ_2=0.0075, ϕ=78 weeks (symbol △). In all cases $\sigma_2 = \sigma_3$.(Additional parameters as Fig. 7.10 plus c_{max}=0.55, c_{min}=0.02, g_4=7.5 X 10^{-7}, g_5=1.075)

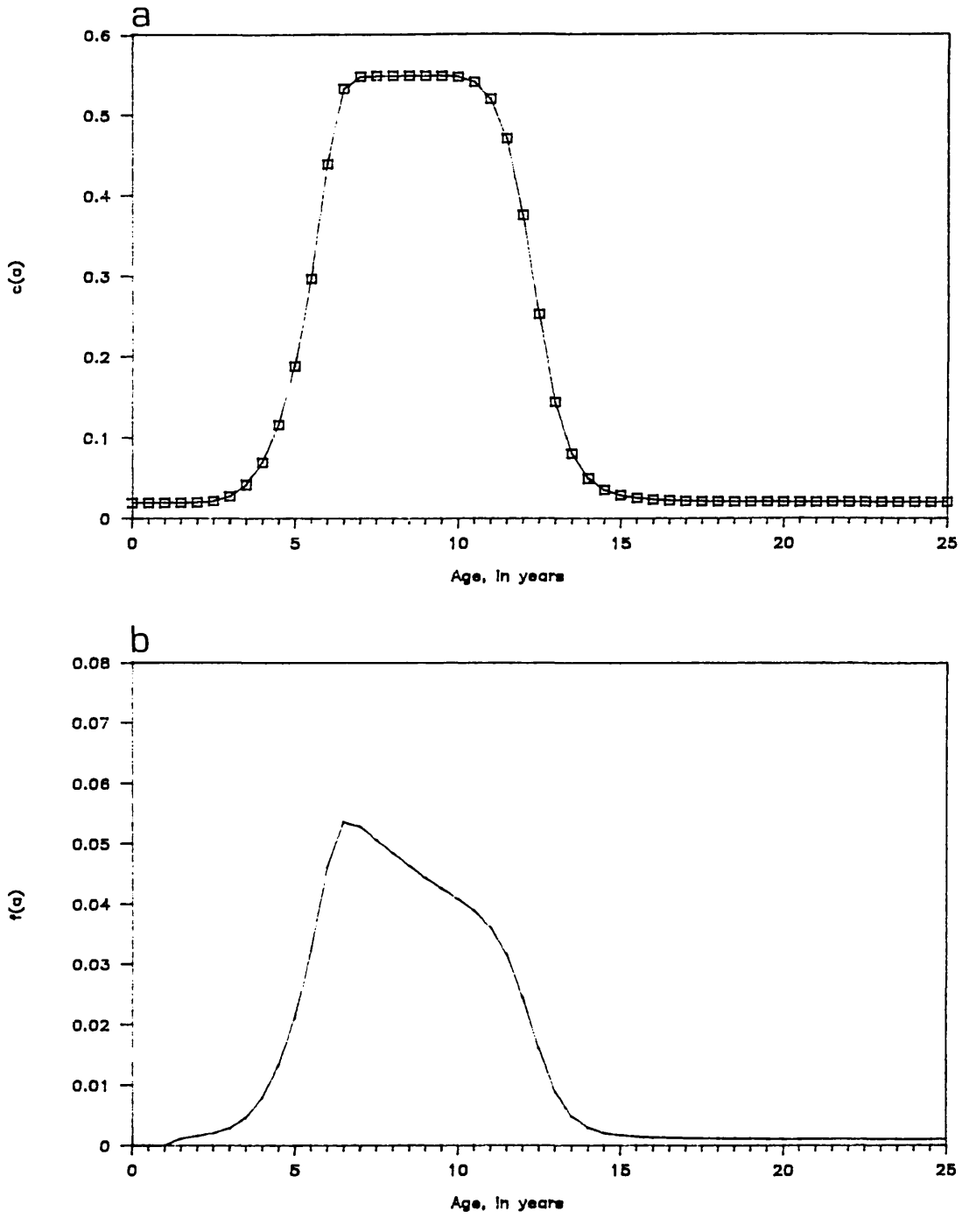


Fig. 7.14.
(a) Age-specific probability that a primary infection recrudesces (c(a)), eqs.(7.24)-(7.26) in the main text, (b) Age-specific equilibrium recrudescence rates (f(a)), eqs.(7.19b), (7.6b) in the main text.(Parameter values (all /week), b(0)=0.1, b_{min}=0.007, σ_1=0.008, C_2=0.00175, Y"/N"=0.03, MBR(0)=20, MBR_{max}=220, g_2=0.0475, g_3=0.005, c_{max}=0.55, c_{min}=0.02, g_4=7.5 X 10^{-7}, g_5=1.075, ø=78 weeks, σ_2 =σ_3 = 0.008)

For areas where man-vector contact is less intense, the model predicts that recrudescences become increasingly less significant. This is presumably because of the combined effect that fewer patent infections have on the factors that determine the value of f(a). When the rate at which new infections are acquired is low (fig. 7.11 (b)) the potential source for recrudescent infections is also low. Additionally, a low new infection rate will make the immuno-depressive effect less intense and the probability that primary infections recrudesce (c(a)) will therefore be also be less. The net result is that the rate of recrudescence rapidly becomes a negligible in comparison to the rate at which new infections are acquired.

7.6 The total rate of transition from negative to positive, I(a)

The combined incidence rate I(a) (h(a)+f(a)), generated by the model appears to simulate fairly accurately the observed rate recorded by Bekessy et al (1976) (fig. 7.15). The complex variations in the incidence rate observed among children are particularly well represented. The rise in the incidence rate with age to the peak rate among children age 5-8 years, and the slow decline with age thereafter are all accurately reflected by the model.

As discussed above when vector-man contact is less intense the contribution of f(a) to the total incidence rate (I(a)) becomes less significant. The incidence rates for vector-men contact rates of 10-110 and 2-22 ($MBR(0)-MBR_{max}$) were not found to be significantly different from that of the new infection rate h(a) shown in fig. 7.11 (b).

7.7 The recovery rate from patent infection, R(a,t)

7.7 (i) Observed patterns

There are several quite remarkable features of the dynamic affects of extended exposure to malaria on the rate of recovery from patent infection as observed by Bekessy et al(1976) and Krafsur & Armstrong(1978). First there appears to be an approximate ten-fold increase in the recovery rate between the age-group 1-4 years and the adult age groups (fig. 7.1 (b)). This implies that the expected duration of a positive episode decreases from about 600 days to about 50 days probably as a direct result of the development of functional immunity.

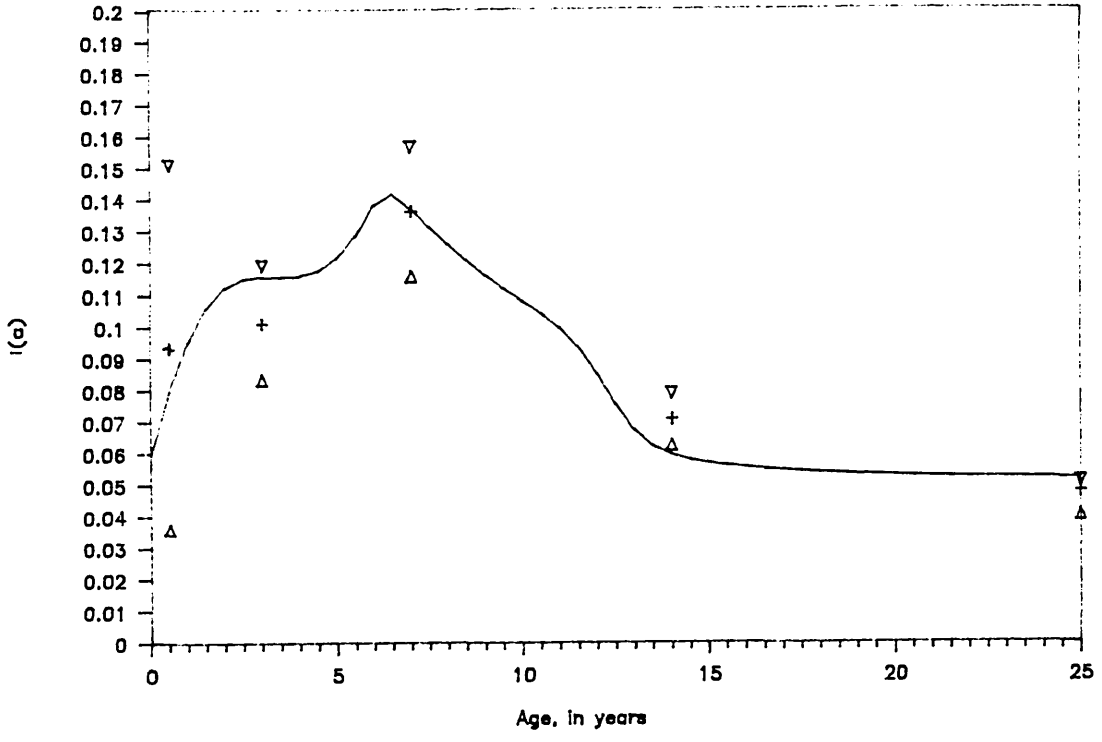


Fig. 7.15. The equilibrium age-specific total incidence rate, I(a) = (h(a) + f(a)) generated by eqs.(7.24)-(7.26), (7.19b), (7.16)-(7.17) and (7.6b) in the main text compared with the estimated yearly average daily incidence rates (I(a)) of P.falciparum parasitaemia by age (with 95 % C.I.), recorded in Garki, N.Nigeria (Bekessy et al(1976).(Parameter values (all /week as for Fig. 7.14).

Second, the recovery rate fails to reach a stable upper plateau although it does appear that the recovery rate among the oldest age groups is approaching some sort of upper limit. This could imply that the duration of immunological memory relevant to the immunologically mediated reduction in parasite survival and/or ability to multiply within the human host may be particularly long. If it is assumed that the increase in recovery rate is related to past experience of patent infection, the fact that recovery rates do not reach an upper plateau even in the adult classes implies that patent infections are being acquired at a rate greater than the losses to the accumulated past experience of patent infections resulting from the finite duration of immunological memory. The third notable feature concerning the age-and seasonal-related changes in the recovery rate is that there are only very minor variations to the recovery rate between wet and dry seasons (fig. 7.3). This feature also points to the existence of immunological memory of long duration.

7.7 (ii) Immunity

The very significant increase in recovery rate with age is without doubt related in some way to immunity based on continued exposure to infection and the accumulated past experience of patent infections. Each patent infection presents the immune system with an array of antigenic stimulus relating to all stages of the life-cycle. The accumulated past experience of patent infection will therefore be a much more accurate measure of the total antigenic stimulus received as a result of malarial infection than for example potentially infective bites, many of which never reach patency. Since the recovery rate is a measure of the ability of the immune system to control the multiplication of the parasite population within the host and then finally eliminate or maintain parasites at sub-patent levels, it is important that the past experience on which the recovery rate is based reflects the possibility that the immunologically-mediated reductions in parasite multiplication can occur both at the sporozoite and asexual blood-stage. Thus the general form for the age- and season-related changes in recovery rate can be expressed as:

$$\overline{R}(a,t) = K \left[\overline{F}_r(a,t) + \overline{H}_r(a,t) \right] \qquad (7.27)$$

where, $\overline{F}_r(a,t)$ and $\overline{H}_r(a,t)$ are the accumulated past experience of new infections and recrudescences respectively which affect recovery.

For average non-seasonal recovery rates eq.(7.27) becomes:

$$R(a) = K \left[\overline{F}_r(a) + \overline{H}_r(a) \right] \qquad (7.28)$$

where,

$$\overline{H}_r(a) = \frac{\underline{Y}''}{N''} \int_0^a MBR(a'')\, b(a'')\, e^{-\sigma_4(a-a'')}\, da'' \qquad (7.29)$$

$$\overline{F}_r(a) = \frac{\underline{Y}''}{N''} \int_0^a MBR(a''-\phi,t-\phi)\, b(a''-\phi,t-\phi)\, c(a'')\, e^{-\sigma_5(a-a''))}\, da'' \qquad (7.30)$$

Figure 7.16 ($\sigma_4 = \sigma_5 = 0.001$) shows that the principle features of the change in recovery rate with past experience cannot be captured by a model in which the recovery rate from patent infection is a linear function of the accumulated experience of patent infection. The relationship between the recovery rate and past experience of patent infection is clearly non-linear. When past experience is fairly low (i.e. among children), quite large changes to the level of past experience create only small variations in the recovery rate. For higher levels of past experience there appears to be a threshold after which the recovery rate increases rapidly with quite small changes to the past experience. This information seems to relate quite well to the observation that whereas there appear to be quite large seasonal variations in the incidence rate (and therefore that rate at which past experience is gained) amongst children, and minor variations among adults, the recovery rates of neither children nor adults show marked seasonal variations. These observations can be represented by the relationship:

$$R(a) = R(max) - R^*(a) \qquad (7.31)$$

where,

$$R^*(a) = (R(max)-R(0))\, \exp\left[\frac{g_6}{g_7} \left(1 - \exp\left(g_7 \left(\overline{F}_r(a) + \overline{H}_r(a)\right)\right)\right)\right] \qquad (7.32)$$

($\overline{F}_r(a)$ and $\overline{H}_r(a)$ are given by eqs.(7.29) and (7.30)).

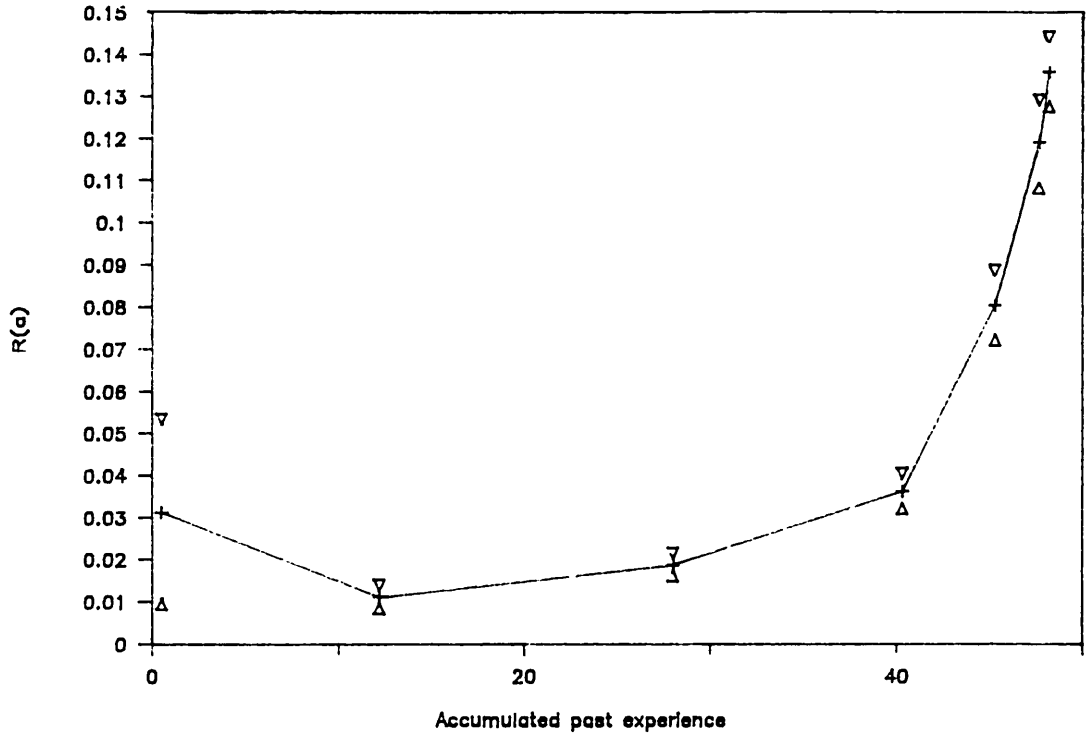


Fig. 7.16. The relationship between the estimated yearly average daily recovery rate (R(a)) by age (with 95 % C.I.) recorded in Garki, N.Nigeria by Bekessy et al(1976) and the accumulated past experience of patent infection predicted by eqs.(7.29)-(7.30) in the main text.(Parameter values (all /week) as for Fig. 7.14 plus $\sigma_4 = \sigma_5 = 0.001$)

The value of $R^*(a)$ varies between 0 and (R(max) - R(0)) so that when past experience relating to recovery is very high $R^*(a)$ will be zero and R(a) will be equivalent to R(max). When past experience is negligible, $R^*(a)$ will be equal to (R(max) - R(0)) and R(a) will be equivalent to R(0). The recovery rate for intermediate levels of past experience is determined by the severity of the rise of the immune response (g_6 and g_7) affecting parasite multiplication as the accumulated past experience of patent infection increases. A variety of functional relationships between the recovery rate and past experience of patent infection are possible. The relationship shown in fig. 7.17 (a) generates average age-dependent recovery rates very similar to those observed by Bekessy et al(1976), (fig. 7.17 (b)). It is interesting to see that when vector-man contact is intermediate, past experience of patent infections amongst adults and older children is higher than when vector-man contact is more intense (fig. 7.18 (a)). This observation can be explained by the fact that when vector-man contact is less intense, the accumulation of past experience of potentially infective bites is not so great and the value of b(a) (the probability that potentially infective bites produce patent infections) does not decline as rapidly. Hence more patent episodes are experienced in this situation. When vector-man contact levels are very low, despite the higher probability that potentially infective bites will produce a patent infection, less patent episodes are experienced. These differences are also reflected in the recovery rates predicted for areas of different vector-man contact rates (fig. 7.18 (b)). When vector-man contact is very low, past experience is slowly accumulated with continued exposure and the gradual increases in the level of immunity causes the recovery rate steadily rises age. When vector-man contact is moderate the model predicts that the recovery rate rises sigmoidally with age, to a upper plateau almost double that predicted when man-vector contact rate are twice as intense.

Although no detailed age-related recovery rate data for areas of widely differing transmission conditions is available for comparison, it is unlikely that recovery rates in areas of moderate transmission do actually rise to the high levels predicted by the model. The recovery rates obtained by Krafsur & Armstrong(1978), from an area where transmission could be described as moderate, suggest that adult recovery do possibly achieve high levels under such conditions. The average weekly adult recovery rate was recorded as 0.2247 +/- 0.0658. The sample sizes in the study were however very small which, together with the low mean densities of parasitaemia among adults probably exaggerate the values of the transition frequencies α and ß and therefore the values of I and R.

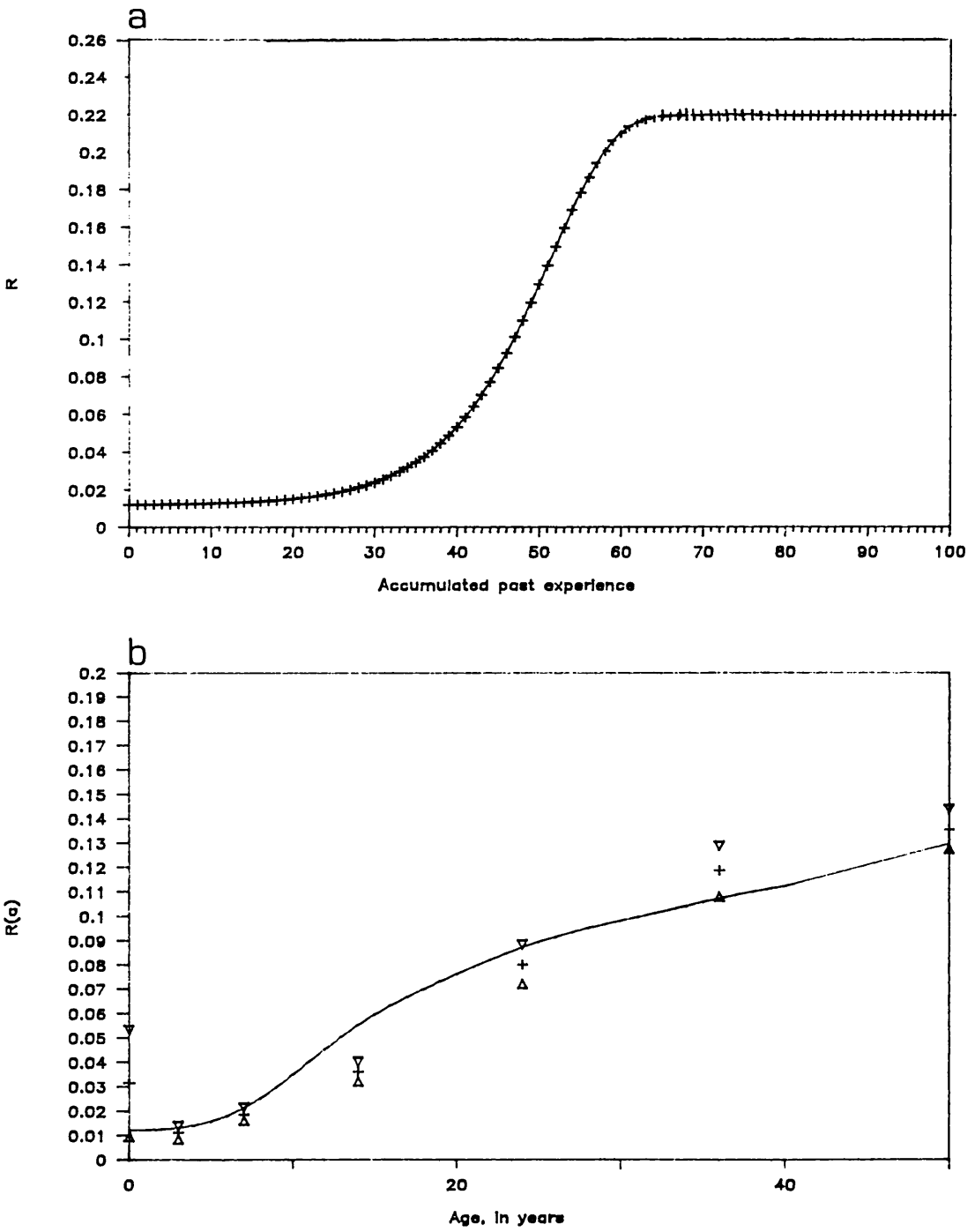


Fig. 7.17.
(a) The relationship between accumulated past experience of total patent infection and the recovery rate (R), eqs.(7.29)-(7.32) in the main text, (b) Age-specific equilibrium recovery rate (R(a)) generated by the immunity function above, compared with yearly average daily recovery rate by age (with 95 % C.I.), recorded by Bekessy et al(1976). (Parameter values (all /week), as for Fig. 7.14 plus $\sigma_4 = \sigma_5 = 0.001$, R(0)=0.0125, R(max)=0.22, $g_6 = 0.00015$ and $g_7 = 0.132$).

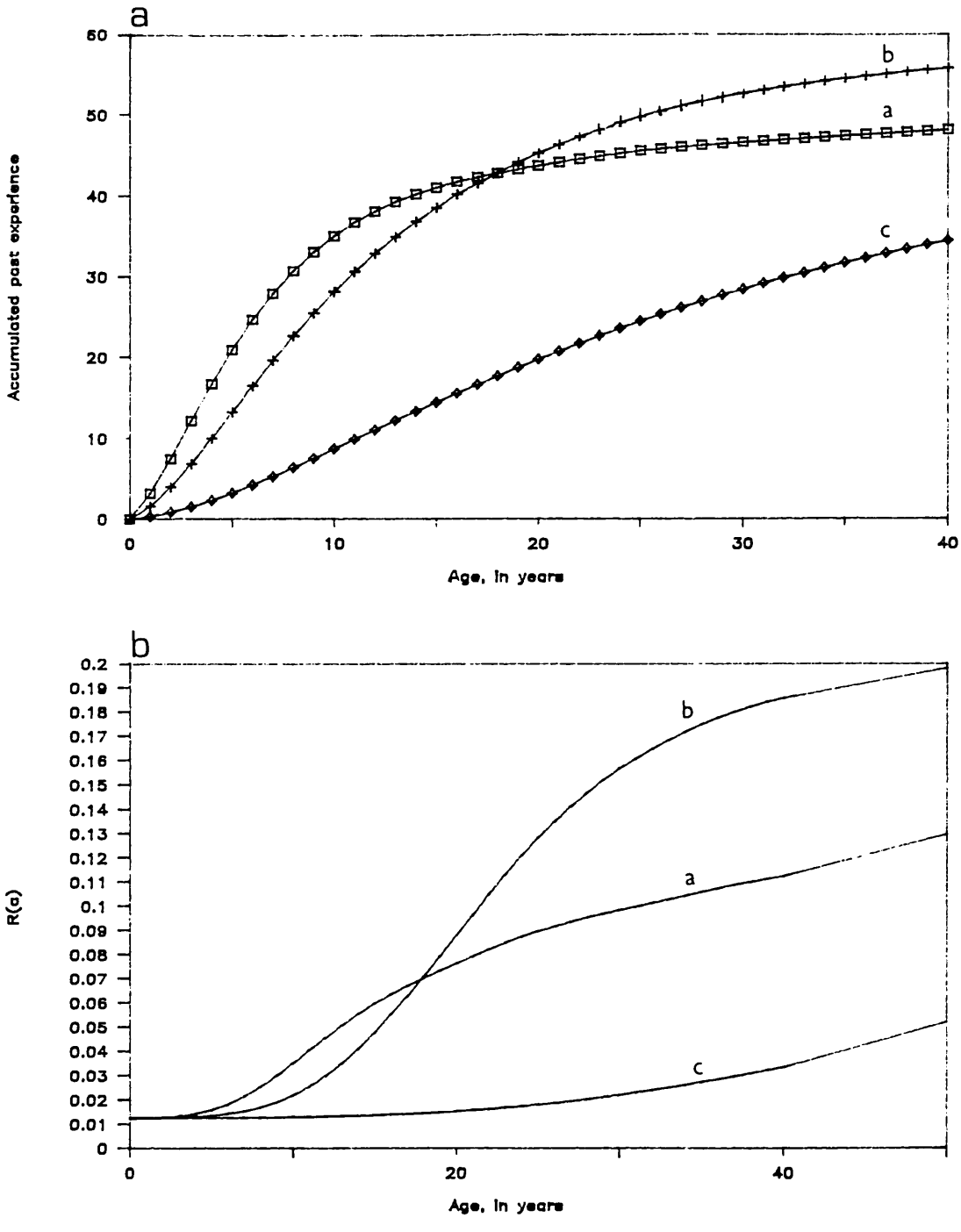


Fig. 7.18. The effect of the degree of contact between vectors and humans.
(a) The relationship between age and the accumulated past experience of patent infection, eqs.(7.29)-(7.30) in the main text, (b) Age-specific equilibrium recovery rates, eqs.(7.29)-(7.32) in the main text).(Parameter values (all /week), MBR(0), MBR_{max}: Profile a - 20,220; b - 10,110; c - 2,22. Additional parameters as for Fig. 7.14, also $\sigma_4 = \sigma_5 =$ 0.001, R(0)=0.0125, R(max)=0.22, g_6= 0.00015 and g_7=0.132)

7.8 Prevalence predictions

The predicted equilibrium proportion positive (PY(a)) for vector-man contact rates MBR(0)=20 and MBR_{max}=220 (fig. 7.19 (a)), appears to be very close to the one actually observed by Bekessy et al(1976), excepting the infants. For different levels of transmission the prevalence patterns (fig. 7.19 (b)) are very similar to those described by Boyd(1949) (fig. 5.14). There are however two important points of note arising from these predictions. First, it is surprising to find how similar the prevalence profiles for the two highest transmission conditions are, remembering that the vector-man contact rates of the highest profile (a) is twice that of the second highest profile (b). It appears that when transmission conditions are less intense, but still quite high, the lower incidence rates (which one might expect to give lower prevalences) are offset by a slower acquisition of immunity with age and thus lower recovery rates. Hence prevalences are very similar to those when transmission is much more intense but where the immune status of individuals of all ages is generally higher. Similar patterns were observed during the Garki Project (an area subject to high transmission), in two of the villages surveyed. Despite an almost one hundred fold difference in the man-biting rates recorded in the two villages, age-specific prevalence profiles for P.falciparum were very similar (Molineaux & Gramiccia,1980; figs. 26 and 77).

The second observation concerning fig. 7.19 (b) is the shape of profiles c and d. These profiles predict quite high prevalences (especially in the adult age-class 20 years) despite the vector-man contact rate being very low. Under these conditions the level of immunity acquired is very low, and infections are long-lived. These two profiles however do predict much higher prevalences than might be expected, a problem which is discussed further in section 7.10.

The profiles illustrated in fig. 7.19 (b) quite clearly show the importance of the balance between transmission and immunity in generating prevalences patterns.

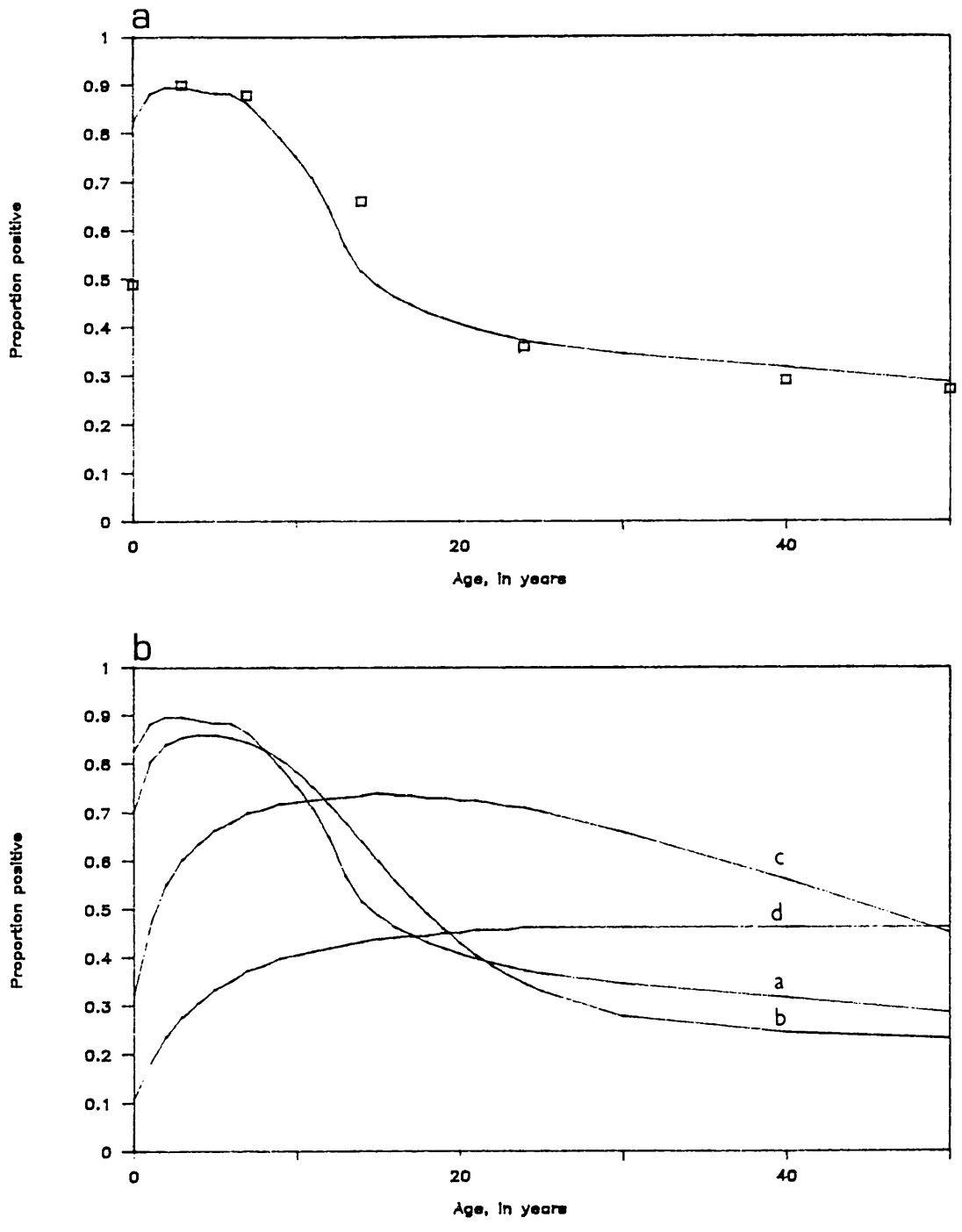


Fig. 7.19.
(a) The equilibrium age-prevalence profile, (solid line) (eq.(7.5a) in the main text), compared with the estimated average prevalence by age, recorded in Garki, N.Nigeria (Bekessy et al(1976) (symbols).(Parameter values (all /week), as for Fig. 7.17), (b) Predicted equilibrium age-prevalence profiles for various vector-man contact rates. Parameter values (all/week), MBR(0),MBR_{max}; Profile a:20,220; b:10,110; c:2,22; d:0.5,5.5.(Additional parameters as for Fig. 7.17)

7.9 The effects of seasonal variations in the contact rate between vectors and man

In the model previously described, all population parameters are assumed to be constant throughout the year and independent of time. However, vector density, or the rate of contact between vectors and the human population, in many endemic areas is clearly not constant throughout the year. In addition, seasonal changes affect many other determinants of transmission, such as the interval between blood meals, the duration of the extrinsic cycle or the rate of vector mortality (see section 2.1 (iv)). Seasonal variations in the potential for transmission are therefore frequently observed in endemic areas. This variation is reflected in a pattern of disease prevalence and transition rates which changes with season. The differences are particularly striking in areas of high transmission which are subject to large seasonal variations in climatic conditions.

The effects of basic seasonal variations in the contact rate between the vectors and the human population can be investigated quite easily by using the seasonal forms of the equations for the man biting-rate, MBR(a,t) (eq.(7.7a)); the incidence rate, h(a,t) (eq.(7.6a)); the incidence of parasitological recrudescences, f(a,t) (eq.(7.19a)); the recovery rate, R(a,t) (eq.(7.27)); and prevalence, PY(a,t) (eq.(7.5b)). Although this method is simple it captures the essential features of seasonal variation in vector density.

The seasonal variation in the vector-man contact rate, SEASN (eq.(7.8)) was assumed to vary between 0 and 2, with an average of 1. T = 13 and T = 39 represent the periods of maximum and minimum vector density respectively. A time/age step of two weeks was used in all simulations, this step length was found to give accurate predictions and also allow a much wider age range to be investigated. All other model parameters were assumed to be the same as described for the previous model, except the probability of a parasitological recrudescence is now assumed to vary between 0.02 and 1.

7.9 (i) Seasonal predictions and observed patterns - Transition rates

The vector-man contact rate was seasonally varied in three simulations, for three different average vector-man contact rates: MBR(0)=20, MBR_{max}=220; MBR(0)=10, MBR_{max}=110; MBR(0)=2, MBR_{max}=22. The 3-dimensional variations in the transition rates by age and season are shown in figs. 7.20, 7.21 and 7.22.

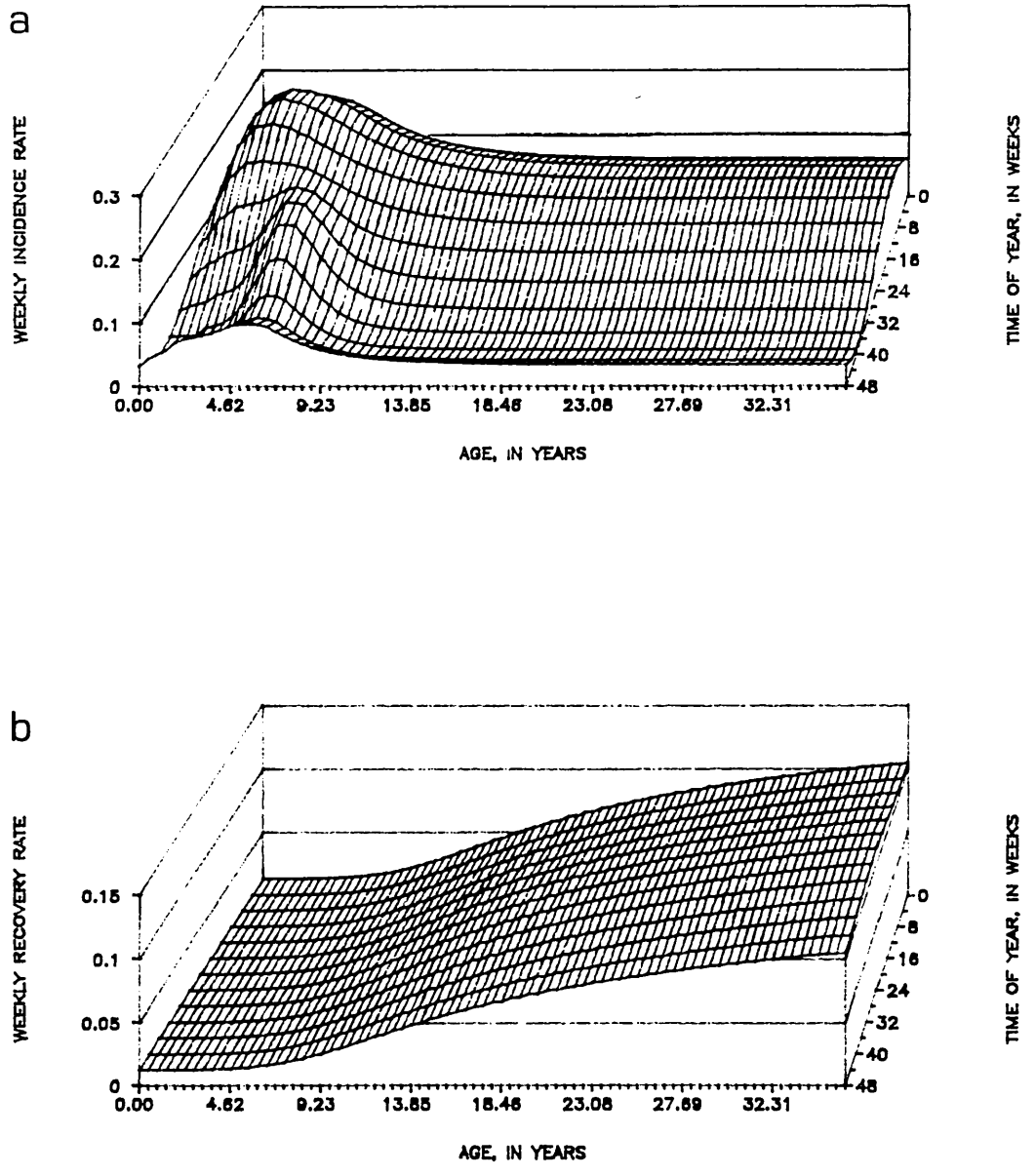


Fig. 7.20. Three-dimensional representations by age and time of year of: (a) the equilibrium total incidence rate, $I(a,t) = h(a,t) + f(a,t)$, (b) the equilibrium recovery rate, $R(a,t)$ generated by eqs.(7.16a), (7.6a), (7.19a) and (7.27) in the main text, for conditions of high vector-man contact rates and large seasonal fluctuations in vector density. (Parameter values (all /week) as for Fig. 7.19, except $MBR(0)=20$, $MBR_{max}=220$, $Av=1$, $S=1$, $c_{max}=1.0$).

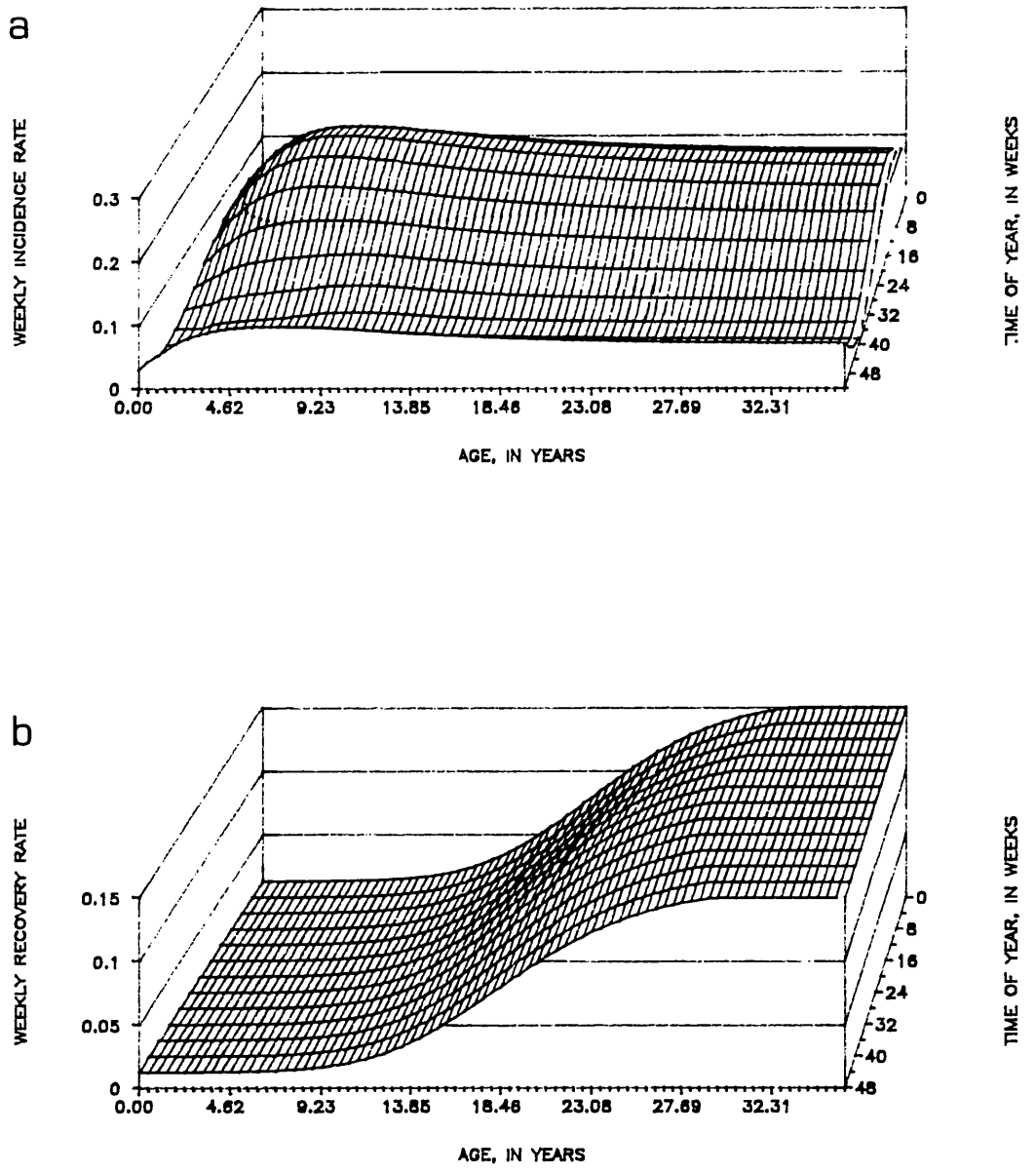


Fig. 7.21. Three-dimensional representations by age and time of year of: (a) the equilibrium total incidence rate, I(a,t)= h(a,t) + f(a,t), (b) the equilibrium recovery rate, R(a,t) generated by eqs.(7.16a), (7.6a), (7.19a) and (7.27) in the main text, for conditions of moderate vector-man contact rates and large seasonal fluctuations in vector density. (Parameter values (all /week) as for Fig. 7.19, except MBR(0)=10, MBR_{max}=110, Av=1, S=1, c_{max}=1.0).

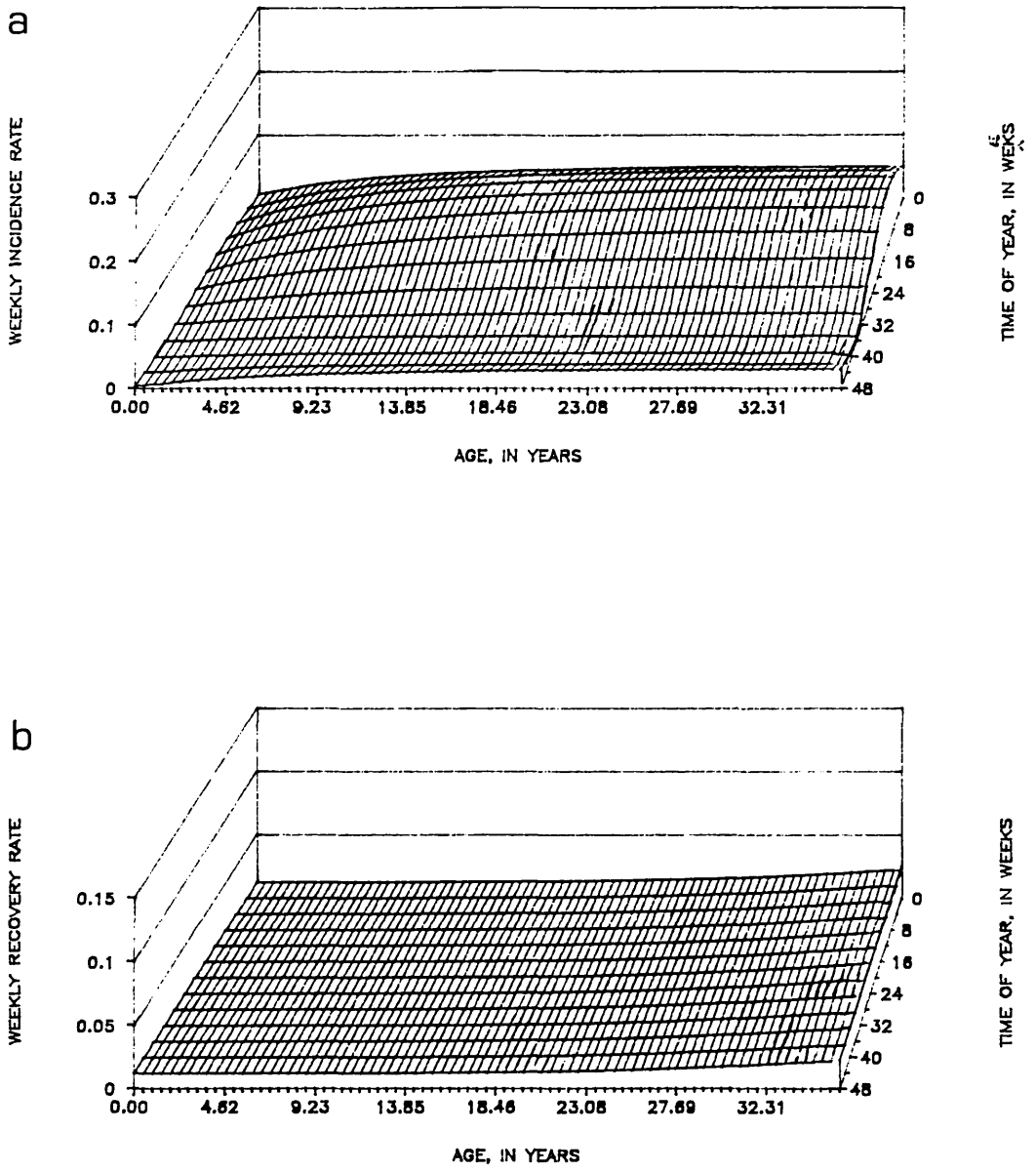


Fig. 7.22. Three-dimensional representations by age and time of year of: (a) the equilibrium total incidence rate, I(a,t)= h(a,t) + f(a,t), (b) the equilibrium recovery rate, R(a,t) generated by eqs.(7.16a), (7.6a), (7.19a) and (7.27) in the main text, for conditions of low vector-man contact rates and large seasonal fluctuations in vector density.
(Parameter values (all /week) as for Fig. 7.19, except MBR(0)=2, MBR_{max}=22, Av=1, S=1, c_{max}=1.0)

7.9 (i) (a) Recovery rate

The results for the recovery rate (R(a,t)) at all transmission levels (figs. 7.20 (b), 7.21 (b) and 7.22 (b)) show very little seasonal variation. The features of age-related recovery and the effects of increasing immunity with exposure, appear to be very similar to those already described for non-seasonal transmission in section 7.7.

7.9 (i) (b) Incidence rate

The results for the incidence rate (I(a,t)) for all levels of transmission show a distinct annual cycle, as expected, due to fluctuations in the potential for new infections. These variations are most obvious when transmission is high (fig. 7.20 (a)).

When transmission is moderate (fig. 7.21 (a)) or low (fig. 7.22 (a)) it appears that although the incidence of infection in a particular age group varies seasonally, the rate relative to other age groups does not appear to differ markedly. The model therefore appears to suggest that for areas with lower man-vector contact rates, parasitological recrudescences are considerably less significant than the acquisition of new infections. In contrast, for the highest level of transmission, the relative differences between seasons of high and low transmission does not appear to be constant with age. Looking at this feature in more detail, fig. 7.23 (a) shows an alternative way of illustrating the age-related variation in incidence by season. The relative difference between seasons is seen to be greatest in the children below 4 years, at a minimum among those ages 5-8 years, and increases again in the older age groups. The explanation for this pattern is that from birth to the age of 5-8 years, the probability that new patent infections recrudesce gradually increases, these recrudescences are less dependent on season, hence the magnitude of the relative seasonal variation decreases. In older age groups, recrudescences are less frequent, so the seasonal variation again increases. The magnitude of this variation is however considerably less than in the infant age classes since acquired immunity among adults reduces the rate of acquisition of new infections during the wet season. Figure 7.23 (b) shows the incidence rate of patent P.falciparum parasitaemia by age and season estimated by Bekessy et al(1976) from the Garki Project in N.Nigeria. The level and seasonal variation in vector-man contact rate for this area is comparable to the conditions assumed in the simulation above where the average vector-man contact rate was $MBR(0)=20$, $MBR_{max}=220$.

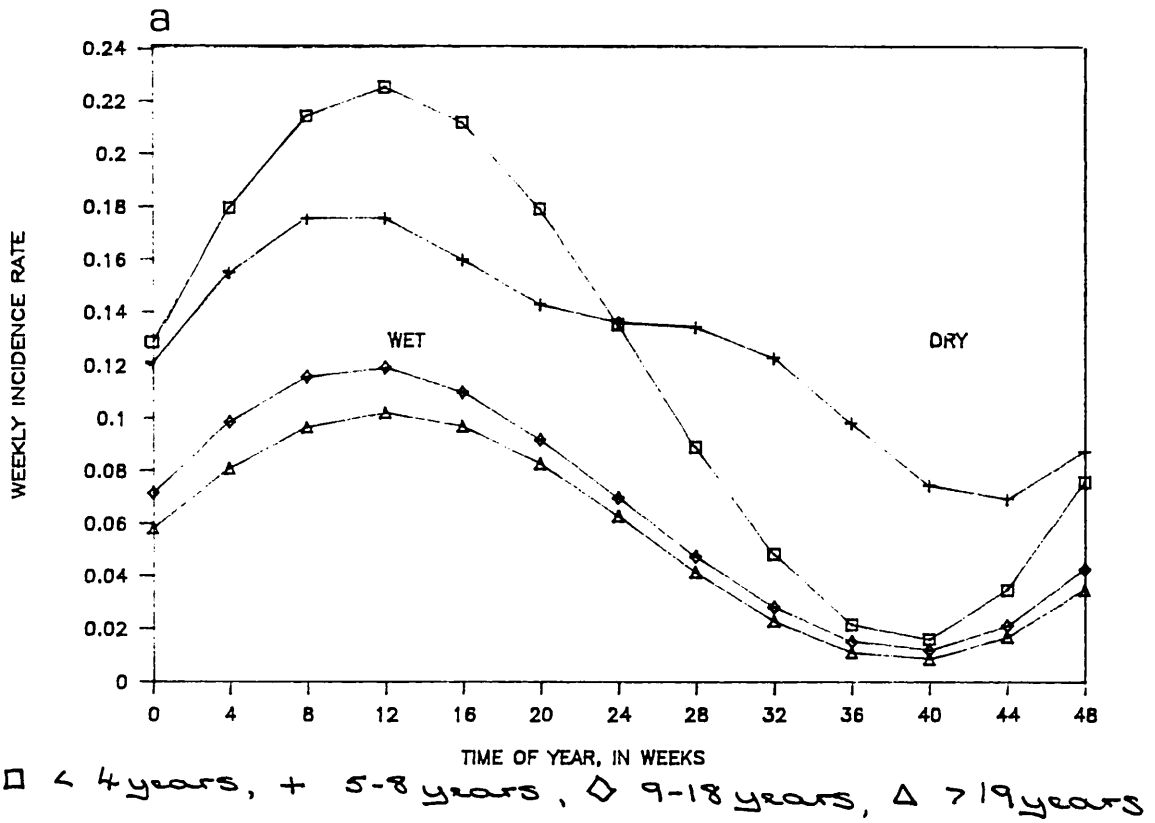
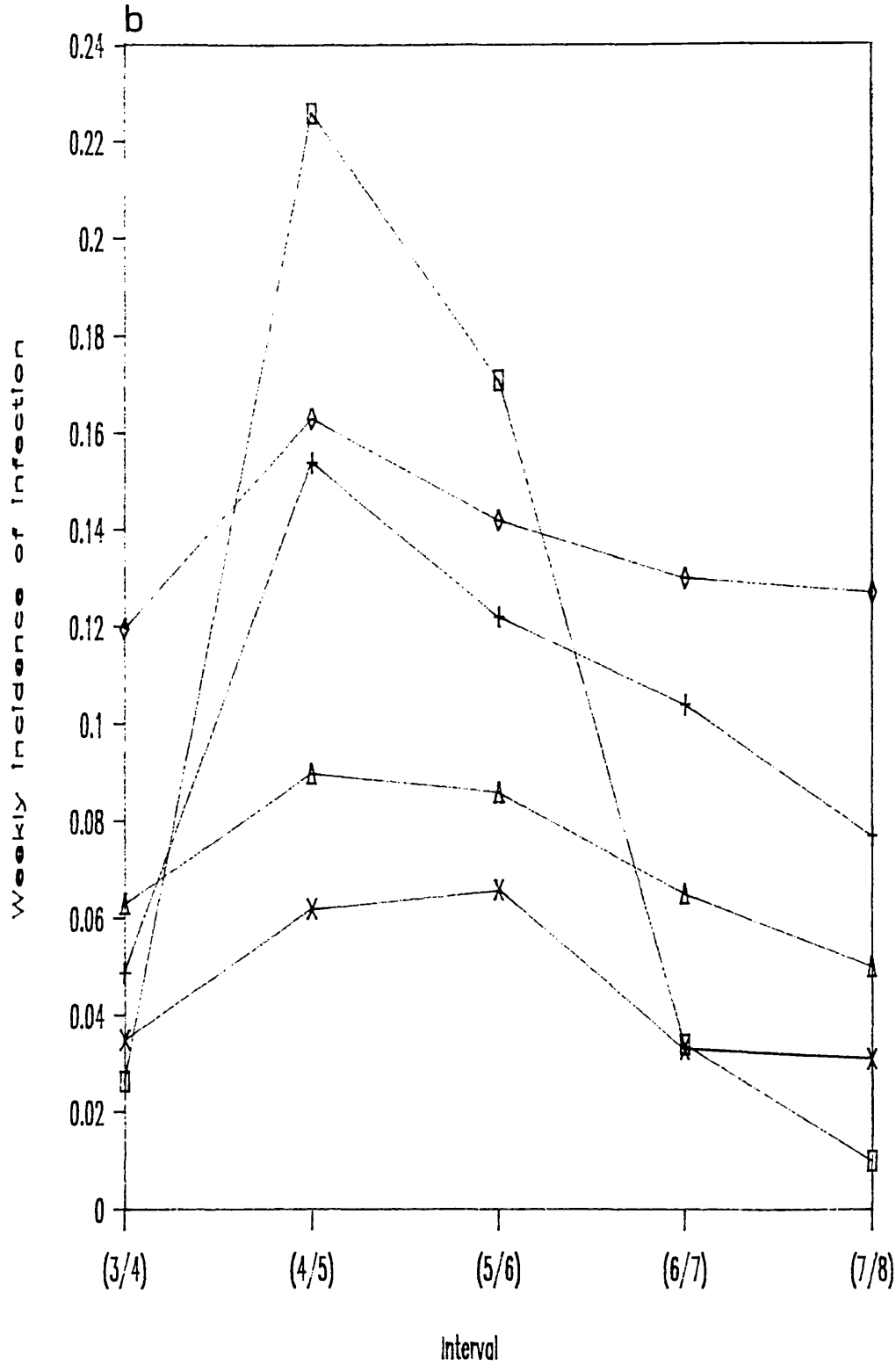


Fig. 7.23.
(a) The age-related variation to the equilibrium weekly incidence rate of infection, according to the time of year, for conditions of high vector-man contact rates and large seasonal fluctuations in vector density, generated by eqs.(7.6a) and (7.19a) in the main text. T = 13 represents the period of maximum vector density. (Parameter estimates (all /week) as for Fig. 7.20 (a))
(b) Weekly incidence of P.falciparum parasitaemia by age and season, estimated by Bekessy et al(1976), from 5 surveys at 10 week intervals, in the Garki District of N.Nigeria. Survey intervals (4/5) and (5/6) correspond with the period of maximum rainfall and vector densities.
(□ - < 1 year, + - 1-4 years, ◇ - 5-8 years, △ - 9-18 years, × - > 19 years)


b
0.24
0.22
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Weekly Incidence of Infection
(3/4)
(4/5)
(5/6)
(6/7)
(7/8)
Interval

The observed patterns and trends seem to be represented fairly well by the simulation, although the decline in incidence during the dry season does appear to less dramatic than that predicted by the model. One possible explanation for this could be that the estimates made by Bekessy et al(1976) were based on transition frequencies in 16 villages. Shidrawi(1972) indicates that in the Garki district, and generally in Northern Nigeria, localized ecological factors are thought to create variations to the time period of the annual seasonal variations in vector density. This effect is quite clearly illustrated by Molineaux & Gramiccia (1980; figs. 7, 10 and 11). This type of variation between villages, would tend to obscure some of the seasonal-dependent changes to the incidence rate, particularly the decline in new infections during the period of minimum vector-man contact. Additionally, the observations on which Bekessy et al(1976) based their estimates were conducted at 10 week intervals, the dynamic effects of the rapid changes in vector densities and vector-man contact rates therefore might easily be missed.

7.9 (ii) Prevalence patterns

The age- and seasonal-dependent variations in prevalence calculated using eq.(7.5b), also show some interesting patterns (figs. 7.24 (a) and (b) and 7.25 (a)). For the lowest vector-man contact rate, MBR(0)=2, MBR_{max}=22, prevalences rise monotonically with age, irrespective of the time of year (fig. 7.24 (a)). Prevalences follow the same seasonal cycle as the rate of contact between vectors and man, with maximum and minimum levels occurring in all age groups at T = 13 and T = 39 respectively. Moderate levels of transmission, MBR(0)=10, MBR_{max}=110, (fig. 7.24 (b)) show the classical prevalence pattern expected for moderate transmission, a rapid rise in prevalence with age up to aged 5 years followed by a steady decline. There appears to be very little deviation from this pattern throughout the yearly cycle, although there does appear to be a slight shift in the age of maximum prevalence, so that the maximum prevalences during the periods of higher transmission are found among younger children compared to the dry season. Figure 7.25 (a) illustrates the prevalence pattern for the highest level of vector-man contact, (MBR(0)=20, MBR_{max}=220), which reflect the variations in the incidence of infection with age between wet and dry seasons discussed above. Prevalences amongst adults and infants follow the annual variations in the vector-man contact rate. Prevalences amongst children aged 4-12 years however remain quite high even during the periods of negligible contact between vectors and man, due to parasitological recrudescences.

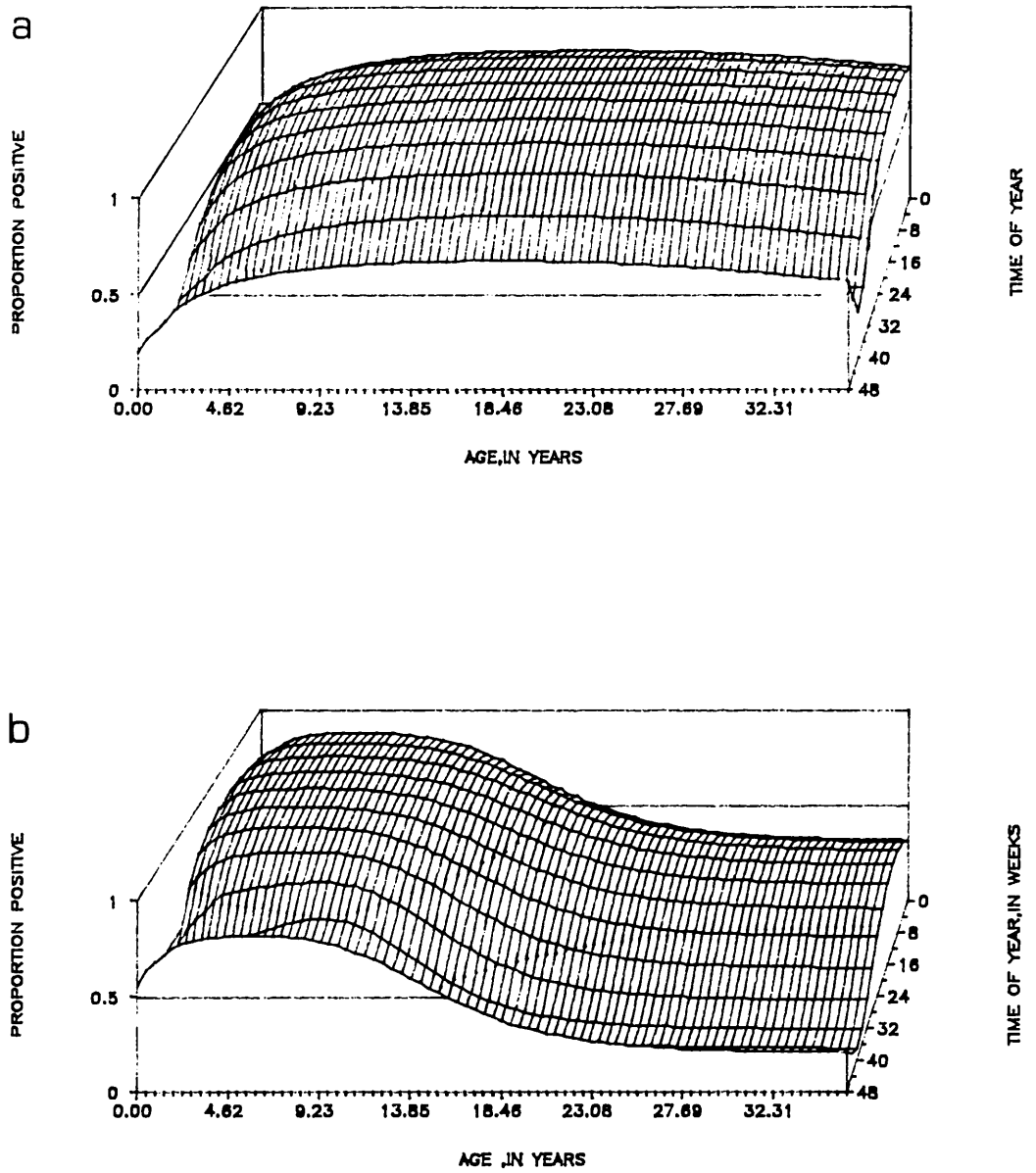


Fig. 7.24. Three-dimensional representations the of equilibrium prevalence rate by age and time of year for conditions of large seasonal fluctuations in vector density, and,(a) low vector-man contact rates (MBR(0)=2, MBR_{max}=22) (b) moderate vector-man contact rates (MBR(0)=10, MBR_{max}=110) generated by eq.(7.5b) in the main text.(Parameter values (all /week) as for Fig. 7.19, except Av=1, S=1, c_{max}=1.0)

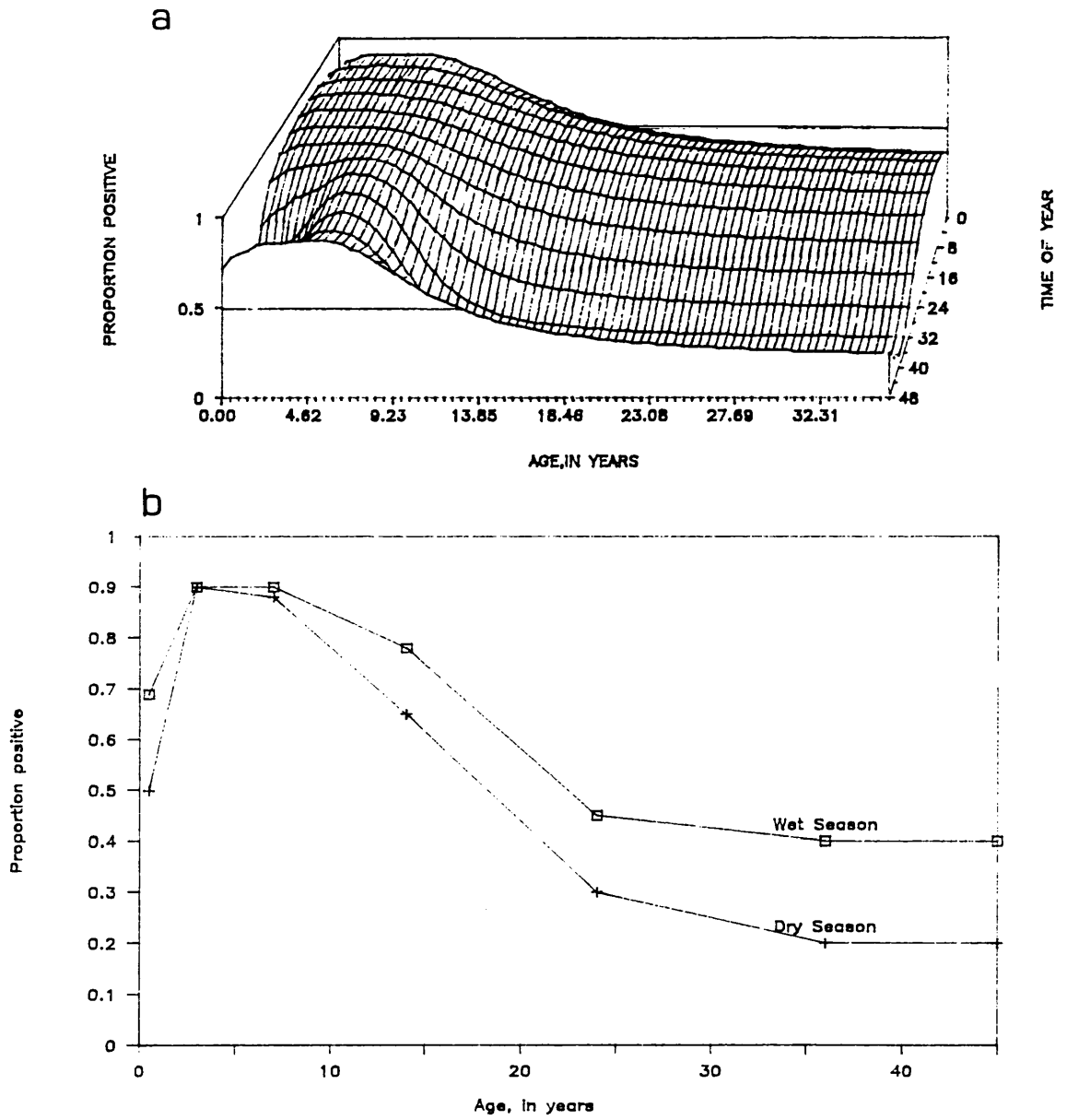


Fig. 7.25.
(a) Three-dimensional representation of the equilibrium prevalence rate by age and time of year (high vector-man contact rates and large seasonal variations) eq.(7.5b) in the main text.(Parameter values (all /week) as for Fig. 7.20).
(b) Observed prevalence by age and season in 16 villages of the Garki District, N.Nigeria (Molineaux & Gramiccia, 1980).

Figure 7.25 (b) shows the highest and lowest prevalence rates of P.falciparum recorded during the Garki Project for the 16 villages used in the estimation of transition rates by Bekessy et al (1976). Allowing for the regional variations to the time period of the annual seasonal fluctuations in vector density, discussed above, which probably obscures the low level of infection during the period of minimum vector-man contact, the simulations seem to reproduce observed prevalences fairly accurately.

7.10 Superinfection and the rate of recovery

One factor which effects the rate at which individuals recover that has already been mentioned in previous chapters, but which so far has not been considered in the new acquired immunity model is superinfection.

For diseases such as malaria which confer little immunity, or more precisely, invoke an immune response which is slow to develop, new infections may be acquired before clearance of an earlier infection. The period of patency resulting from multiple infections is therefore likely to be considerably longer than that of a single infection. Estimation and measurement of the recovery rate are therefore complicated by superinfection. The superinfection effect may not only produce seasonal variations in recovery rates, (recovery rates should decrease as infection rates increases), but also create age-related variations according to which age-groups receive more or fewer superinfections.

The longitudinal studies by Bekessy et al(1976) and Krafsur & Armstrong(1978) indicate that only adults show the expected seasonal variation, with recovery being slightly faster when transmission is reduced. Aron and May(1983) suggest that the apparent discrepancy arises because individual infections are longer-lived in children, and infections acquired during the transmission season persist through the low transmission season. In addition however, is the fact that incidence rates among many children does not vary markedly between the seasons. The rate at which recrudescent infections occur during the dry season being almost equivalent to the infection rate during the transmission season. Wide seasonal variations in the incidence rate is observed among infants but the seasonal effects of superinfection are still not apparent. A possible explanation for this is that the rapid infant recovery rate together with the low vector-man contact rates among infants, significantly reduces the number of superinfections experienced during the transmission season.

The simple non-seasonal version of the acquired immunity model can be used to investigate the possible effects of

superinfection. The severity of the superinfection effect is assumed to depend on the accumulated experience of patent infections, where the average duration of memory for these infections is quite short (ϵ = 0.016). The revised model for the recovery rate therefore combines the effects of both immunity and superinfection.

$$R(a) = [R(max) - R^*(a)] \chi [z_{max} - z(a)] \qquad (7.33)$$

where $R^*(a)$ is given by eq.(7.32), and,

$$z(a) = (z_{max} - 1) \exp [\frac{k_1}{k_2} (1 - \exp (k_2 (\bar{F}_z(a) + \bar{H}_z(a))))] \qquad (7.34)$$

$\bar{\bar{F}}_z(a)$ and $\bar{\bar{H}}_z(a)$ are the accumulated past experience of patent infections from recrudescences and primary infections respectively relating to superinfection.

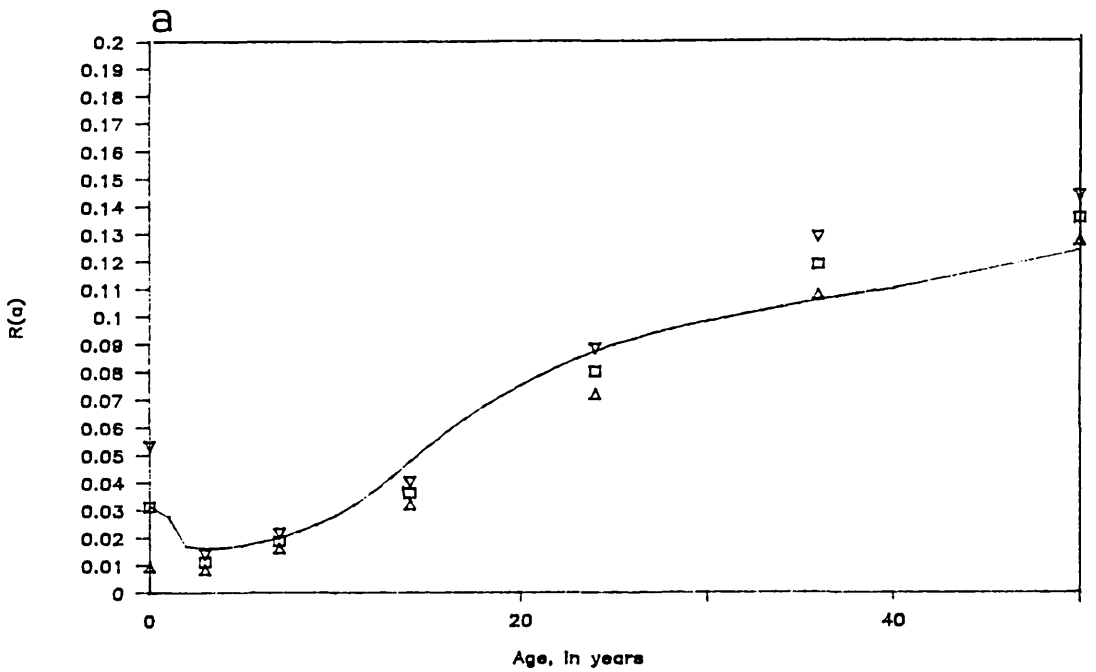
$$\bar{\bar{H}}_z(a) = \frac{Y''}{N''} \int_0^a MBR(a'') \, b(a'') \, e^{-\epsilon(a-a'')} \, da'' \qquad (7.35)$$

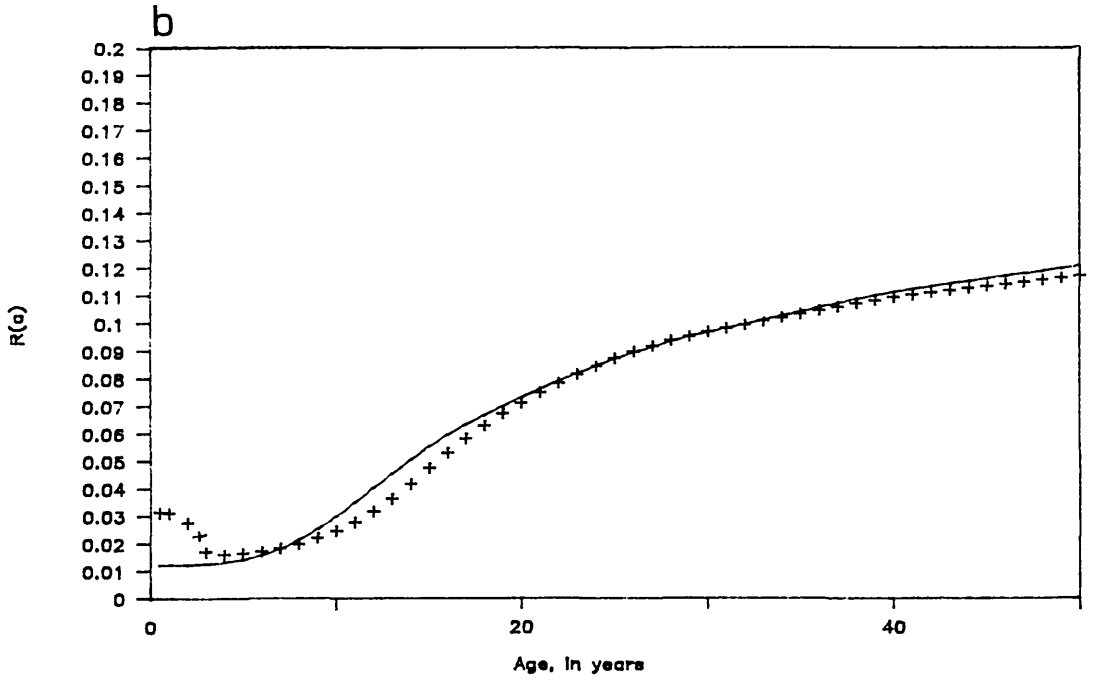
$$\bar{\bar{F}}_z(a) = \frac{Y''}{N''} \int_0^a MBR(a''-\phi, t-\phi) \, b(a''-\phi, t-\phi) \, c(a'') \, e^{-\epsilon(a-a''))} \, da'' \qquad (7.36)$$

The value of z(a) varies between 0 and (z_{max} - 1) so that when the number of patent infections very high z(a) will be zero and the reduction to the recovery rate due to superinfection will be at a maximum i.e. z_{max}. When the number of patent infections is negligible, z(a) will be equal to (z_{max} - 1) and there is no reduction to R(a). The superinfection effect for intermediate levels of past experience is determined by the severity of the rise of the superinfection effect (k_1 and k_2) as the accumulated past experience of patent infection increases.

Figure 7.26 (a) shows the changes in recovery rate with age predicted by eq.(7.33) compared with observed rates under similar conditions observed by Bekessy et al(1976). Figure 7.26 (b) shows that the revised model predicts age-related recovery rates that are broadly similar to those described for the simpler model in the previous section (eqs.(7.31)-(7.32)).

Fig. 7.26.
(a) Age-specific equilibrium recovery rate (R(a)) (including both immunity and superinfection factors), eqs.(7.33), (7.34)-(7.36) and (7.29)-(7.30) and (7.32) in the main text), compared with yearly average daily recovery rates by age (with 95 % C.I.), recorded in Garki, N.Nigeria (Bekessy et al(1976).(Parameter values (all /week), as for Fig. 7.14. Also $\sigma_4 = \sigma_5 = 0.001$, R(0)=0.0315, R(max)=0.22, g_6= 0.000106, g_7=0.15, z_{max}=0.5, k_1=0.03125, k_2=1.25,ϵ =0.016),
(b) Comparison of the age-specific equilibrium recovery rates generated by the immunity factor alone (solid line), and by the modified recovery function (including both immunity and superinfection factors)(symbols).(Parameter values as above, except R(0)=0.0125, g_6= 0.00015 and g_7=0.132 for the immunity factor only (solid line)).


a
R(a)
0.2
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
20
40
Age, in years


b
R(a)
0.2
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
20
40
Age, in years

However, this more complex model is able to simulate quite accurately the lower recovery rate among children aged 1-8 years observed by Bekessy et al(1976) which the simpler model was not able to do.

For different levels of contact between vector and man the effect of superinfection depends on the age-dependent pattern for the past experience of patent infections when ϵ = 0.016. Figure 7.27 (a) reveals that for moderate and intense transmission the greatest reduction to the recovery rate will occur among the youngest children. The higher the transmission rate the younger the age at which superinfection has its maximum effect. The superinfection effect appears to then decrease with increasing age, with high transmission rates appearing to exaggerate the decline. When transmission is very low the effects of superinfection rises monotonically with age. The greatest superinfection effect among adults is seen when transmission is moderate. The effect of these patterns on the apparent recovery rate are illustrated in fig. 7.27 (b). For low levels of transmission, recovery rates still rise with age. The increase in the recovery rate due to immunity appears to more than compensate for the fact that the effects of superinfection increase with age. When transmission is moderate the minimum recovery rate, as expected, occurs in an older age group than when transmission is more intense. The rates are lower in the intermediate age-groups, and higher among adults. The differences between the recovery rates for adults for the three levels of transmission are considerably less than those described for the simpler model (eqs.(7.31)-(7.32)) in fig. 7.18 (b).

Figure 7.28 (a) shows the equilibrium proportion positive (PY(a)) generated using vector-man contact rates MBR(0)=20 and MBR_{max}=220 and the revised recovery function. The differences between this profile and that generated using the simpler recovery function with no infection appear to be quite minor. However, in comparison with the profile obtained by Bekessy et al(1976), in an area with similar vector-man contact rates, the revised recovery function appears to predict prevalence rates among the infant portion of the population much more accurately.

For different levels of transmission the prevalence patterns (fig. 7.19 (b)) are again broadly similar to those described by Boyd(1949) (fig. 5.14). Inclusion of the superinfection effect appears to produce lower, probably more realistic prevalence-rates among the youngest age groups for all levels of transmission and also generate lower prevalence-rates for all age groups for the two lowest levels of transmission (much more representative of what might be expected for the levels of transmission). These two features are associated with the new higher estimate used for R(0), which reflects the observation that recovery in young

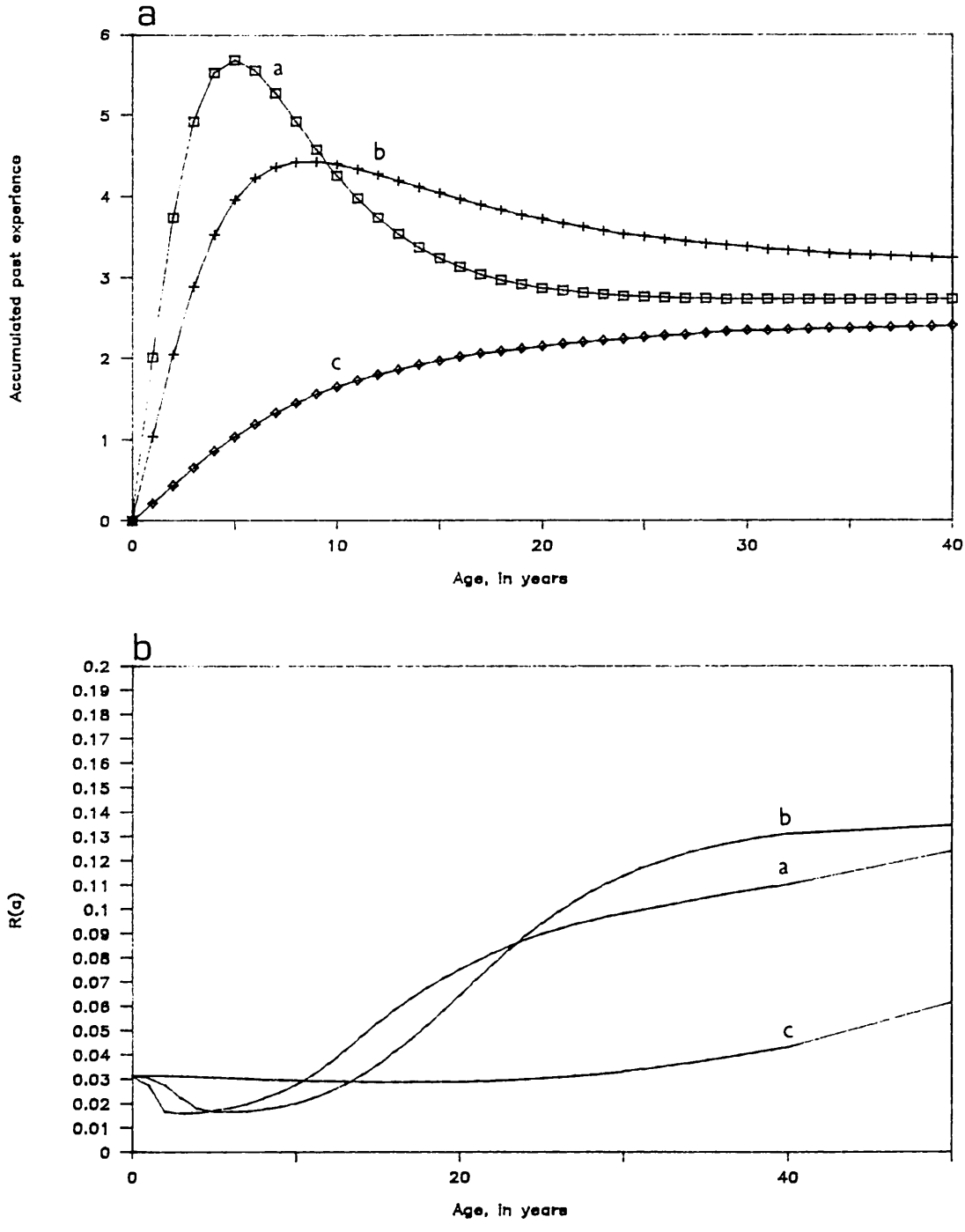


Fig. 7.27. The effect of the degree of contact between vectors and humans.
(a) The relationship between age and the accumulated past experience of patent infection, eqs.(7.35)-(7.36) in the main text, (b) Age-specific equilibrium recovery rates, predicted by The modified recovery function, eqs.(7.33), (7.34)-(7.36) and (7.29)-(7.30) and (7.32) in the main text. Parameter values (all/week), MBR(0), MBR_{max}; a:22,220; b:10,110;c:2,22. (Additional parameters as for Fig. 7.26a).

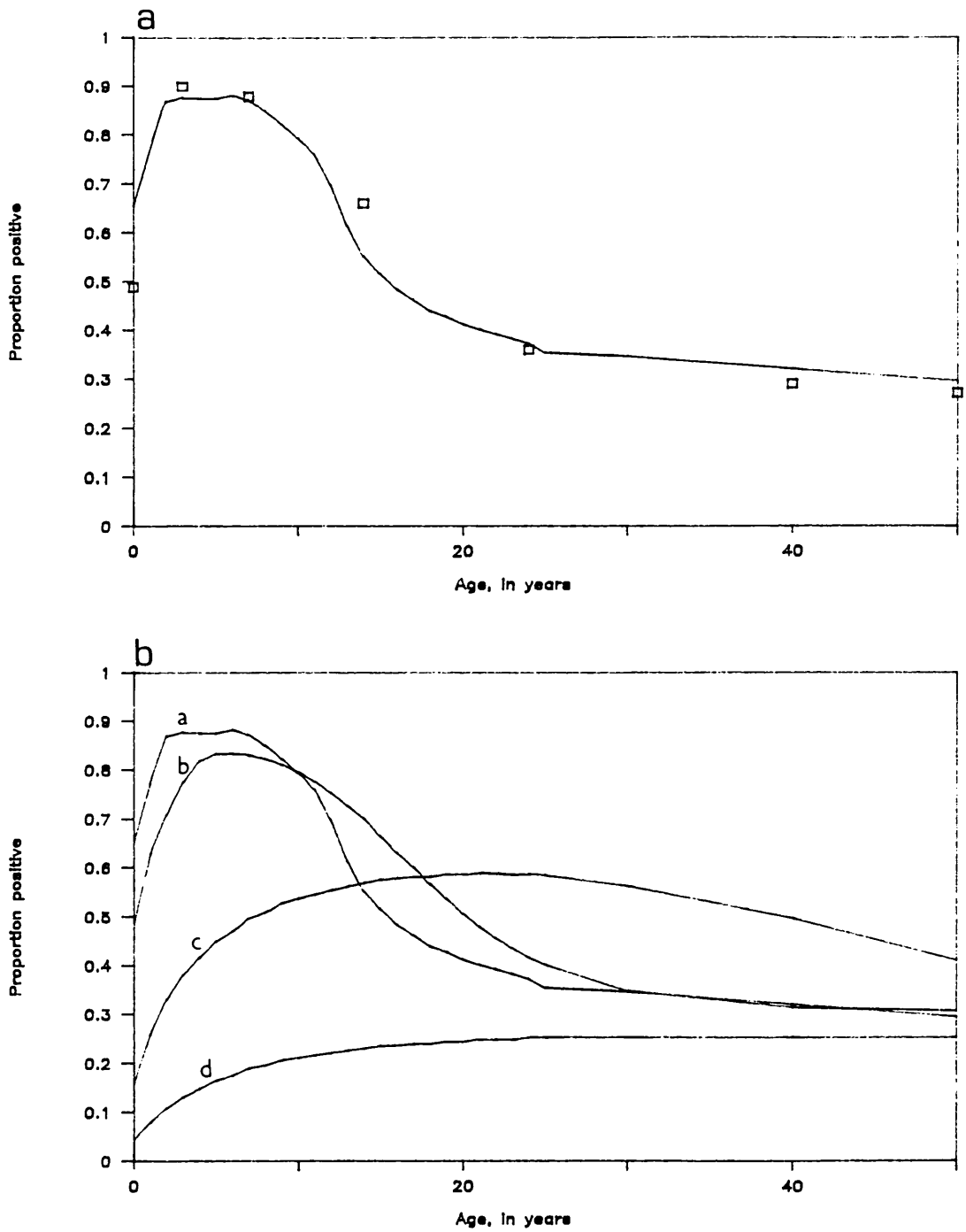


Fig. 7.28.
(a) Equilibrium age-prevalence profile, eq.(7.5a) in the main text (solid line), using the modified recovery function, compared with average prevalences by age, recorded by Bekessy et al(1976) (symbols), (b) Equilibrium age-prevalence profiles for a range of vector-man contact rates, using the modified recovery function. Parameter values (all/week), MBR(0),MBR_{max} a:22,220; b:10,110; c:2,22; d:0.5,5.5. (Parameter values (all /week), as for Fig. 7.26 (a))

children (and presumably individuals with low functional immunity subject to negligible superinfection) is quite rapid. For profile b, and to a lesser extent profile a however, superinfection appears to have a differential effect, affecting some age groups more than others. In particular the decline in prevalence as a function of age appears to be much slower.

Comparison of figs. 7.19 (b) and 7.28 (b) also serve to illustrate how sensitive age-prevalence predictions are to the estimate for the recovery rate.

7.11 Conclusions

Diverse issues have been considered by the acquired immunity model developed in this chapter. They include age-dependent vector-man contact rates, gradual development of functional immunity according to past experience of infection and/or parasite antigen, immunity acquired at different rates to different aspects of infection, loss of immunity and maintenance of immunity by continual exposure, immunodepression, superinfection and the effects of fluctuating mosquito populations. The model has aimed to improve on past acquired immunity models by attempting to explore the role of gradually acquired immunity in determining transition rates and shaping age-prevalence profiles. The model also attempts to mirror the relationship between the infected portion of the vector population and the development of immunity in the human community. The model is based on a more detailed representation of the human response to malarial parasites and allows for the influence of heterogeneous contact rates. The critical importance of the relationship between transmission and "immune status" has also been demonstrated.

Although the model is able to account for the both the development, maintenance and loss of immunity, and their affects on transition rates, the third and final aspect of acquired immunity, concerning how it affects gametocyte "quality" and reduces infectiousness to mosquitoes still needs to be considered. Inclusion of all three aspects of immunity in a dynamic model of acquired immunity to malarial infection would then allow an examination of the impact of control measures (chemotherapy and vaccination) on transmission and population immunity.

The model developed in this chapter is able to describe epidemiological patterns that are similar to those actually observed. Predictions generated for a variety of different transmission potentials under conditions of both constant and seasonal transmission all appear to be broadly similar to observed patterns. However, the immunity functions used in

the model are speculative and require clarification and development before a more comprehensive model can be developed.

It is interesting, however, to compare some of the findings made by Aron(1981) on detectability, with the results illustrated in this chapter. The observed decrease in I(a) and the increase in R(a) with age is accounted for in the acquired immunity model by the existence of acquired immunity affecting both the probability of acquiring patent infection and also the apparent recovery rate from patent infection. Aron(1981) states that this trend can also be explained by everyone being infected and that the average detectibility of infection declining as immunity develops (indicated by the decline in the density of parasites with age). At present no method is available with which to disentangle the effect of reduced rate of infection and increased recovery rate from that of lower detectability, although it is probable that immunity affects all three aspects of infection. However, it is important to note that by dividing the human population into patent and non patent, and describing the changes in transition rates with age as apparent infection and recovery rates (as is the case with the model described in this chapter), the effects of acquired immunity on the detectability of infection and also actual transition rates are both accounted for.

CHAPTER 8 Conclusions

Recent problems surrounding the control and eradication of malaria (i.e. drug and insecticide resistance), have focussed increased attention on the need for a better understanding of the complicated dynamics of naturally acquired immunity to malaria. Such an understanding is of particular importance in research on vaccines development. The significance of chronic, asymptomatic infections as a source of infection for mosquitoes, and the immune processes that enable malarial infections, (which are typically acute in young children), to become gradually less severe with increasing age, are however poorly understood at present. Current experimental infections of animals fail to reproduce many of the features of human malaria - malarial parasites either kill the host or the immune system eliminates the parasites. Furthermore, conditions of naturally transmitted malaria including continual re-infection and superinfection, infection by mixed parasite populations and seasonal variations in potential for transmission, are not easily reproduced in the laboratory. Analysis of naturally occurring human malaria is therefore necessary for the characterization of endemic malaria. However, interpretation of epidemiological data is difficult. Many of the features of infection are inter-related and current data often lacks the detail to determine the exact effect of functional immunity on the various aspects of infection. Age-stratified data including infection, recovery and prevalence rates, gives a much more accurate picture of how immunity develops and how it is maintained. The collection of such data is very time-consuming and unfortunately little data of high quality is available at present.

One fact that has become clear, however, is that prevalence rates are not a good determinant of infection. They are not sufficiently sensitive to accurately assess the impact of control or the potential impact of a future vaccine. The prevalence of infection, and also surprisingly, the intensity of infection, are not good indicators of the extent of "disease" within a defined population. Many individuals possess parasites in the blood which, in children may be of high density, but suffer no other symptoms of disease. Furthermore, prevalence rates do not give a true indication of the actual proportion of people infected. Detectability is known to vary considerably between people according to their "immune status", and, additionally parasite numbers are seen to fluctuate quite widely from day to day. Prevalences often appear, in all age-groups, to be considerably higher if more than one blood-smear is taken or if the survey is repeated daily for 3-4 days. Modern

serological methods, such as RIA and ELISA (Bidwell & Voller,1981; Mackey et al,1982) and DNA hybridization (Franzén et al,1984) may well indicate that all individuals have parasites for most of their life, albeit at very low levels for the vast majority of the time. A more accurate picture of the extent of "disease" and the affect of vaccines may be obtained from such determinants as age-dependent death or morbidity rates. These will indicate exactly how many people in which age-groups will be directly affected (be it beneficial or not) by the use of vaccines. Independent measurement of immunity via indicator variables in the immune system may also be of epidemiological value. Modern techniques and advances in the fields of immunology, molecular biology and biochemistry area are steadily improving the reliability, range and interpretation of such data. They raise the possibility of the use and incorporation of such data into epidemiological models. The models introduced in Chapters 6 and 7 represent a small step toward this goal.

The various models described in this thesis, are clearly very simple interpretations of the complexity of immunological responses to malaria parasites and the dynamic interactions between transmission and immunity. Some of the models are able to generate epidemiological trends and patterns broadly similar to those actually observed. The acquired immunity model in Chapter 7 appears to reproduce many of the features associated with the dynamics of continued exposure to malaria. However, the predictions made by the model must be treated with caution. Although this model represents an advance in several aspects of malaria modelling, it is based on an incomplete knowledge of either the mechanisms of the immune response (currently based mainly on experimental infections of laboratory animals) or the immuno-epidemiological effects of continued exposure to malaria. Characterization of many of the components of the immune processes are admittedly rather empirical or overly simplified. A much greater understanding of the dynamics of acquired immunity is required before models of this type may be improved and extended. Many of the assumptions made in the construction of the model may well be applicable only to areas of highly seasonal transmission in African countries. Conditions of constant transmission and/or genetic and environmental differences between countries may involve quite different immune mechanisms (Jensen et al,1983).

There are several areas in which future research should be directed. As discussed above, future modelling efforts should be concentrated on developing models which incorporate more accurately the major elements of acquired immunity, particularly those relating to the parts of the malaria life-

cycle which would be directly affected by the use of vaccines. It is important that the effects of immunity on the complete cycle of malaria transmission are included so that the models may be used for dynamic predictions. Future dynamic/genetic models may then be used not only to assess the potential impact of control measures but also to investigate further the possible role of parasite antigenic variation and genetic heterogeneity on the long-term persistance of malaria within the host community. The importance of competition among malarial parasites, as opposed to host immune responses, in regulating parasite populations is another potential area for future research. However, more detailed research is also needed to extend the data base on which predictions from such models could be tested and to refine further the structure of the models. Specifically, more comprehensive information is required as to the best determinant with which to measure the success of vaccines. More accurate methods of estimating how immunity is acquired and maintained in human population are required, which ideally should be applicable to widely spaced geographical locations and different transmission conditions. The significance of continued antigenic stimulus in the form of potentially infective bites and sub-patent infections to the acquisition, maintenance and loss of immunity must be addressed. The pattern of infectiousness and loss of immunity needs to be characterized, so that the duration of immunity and infectiousness in the absence of re-exposure and also under natural conditions can be estimated. The effects of maternally derived antibody on infection and immune processes among infants needs greater attention in future studies. Methods need to be developed which are sensitive enough to detect changes to infection rates, infectiousness and recovery rates among infants. The identification of stabilizing influences on the association between parasites, vectors and humans, such as heterogeneity in immunological competence or exposure to infection also requires investigation. One further need is a detailed examination of the effect of infection on the vector, in terms of the estimation of both mortality and also the parameters that describe the feeding behaviour and/or frequency of feeding.

Finally, issues such as genetic and spatial heterogeneity and evolutionary aspects of the host-parasite interaction also need to be considered. These are of particular importance in areas where transmission potential is intense and stable such that control measures may take a considerable time to become effective. Genetic and spatial variability are both factors which will tend to stabilise and enhance transmission and therefore complicate the process of effective control. It must be remembered that evolution not only has short-term effects in the form of drug- and

insecticide-resistance but will continue to affect parasite-host adaption on a much longer-term time scale. Such co-evolutionary issues, in human populations subject to infection by a highly genetically heterogeneous parasite population, will be of great importance in the design, implementation and monitoring of any malaria vaccine trial.

APPENDICES

APPENDIX A

A.1 The life-cycle of human malaria

The general course of the Plasmodium life-cycle, for species that infect man is shown diagrammatically in fig. A.1, and may be described as follows.

A.1 (i) The exo-erythrocytic stages in the human host

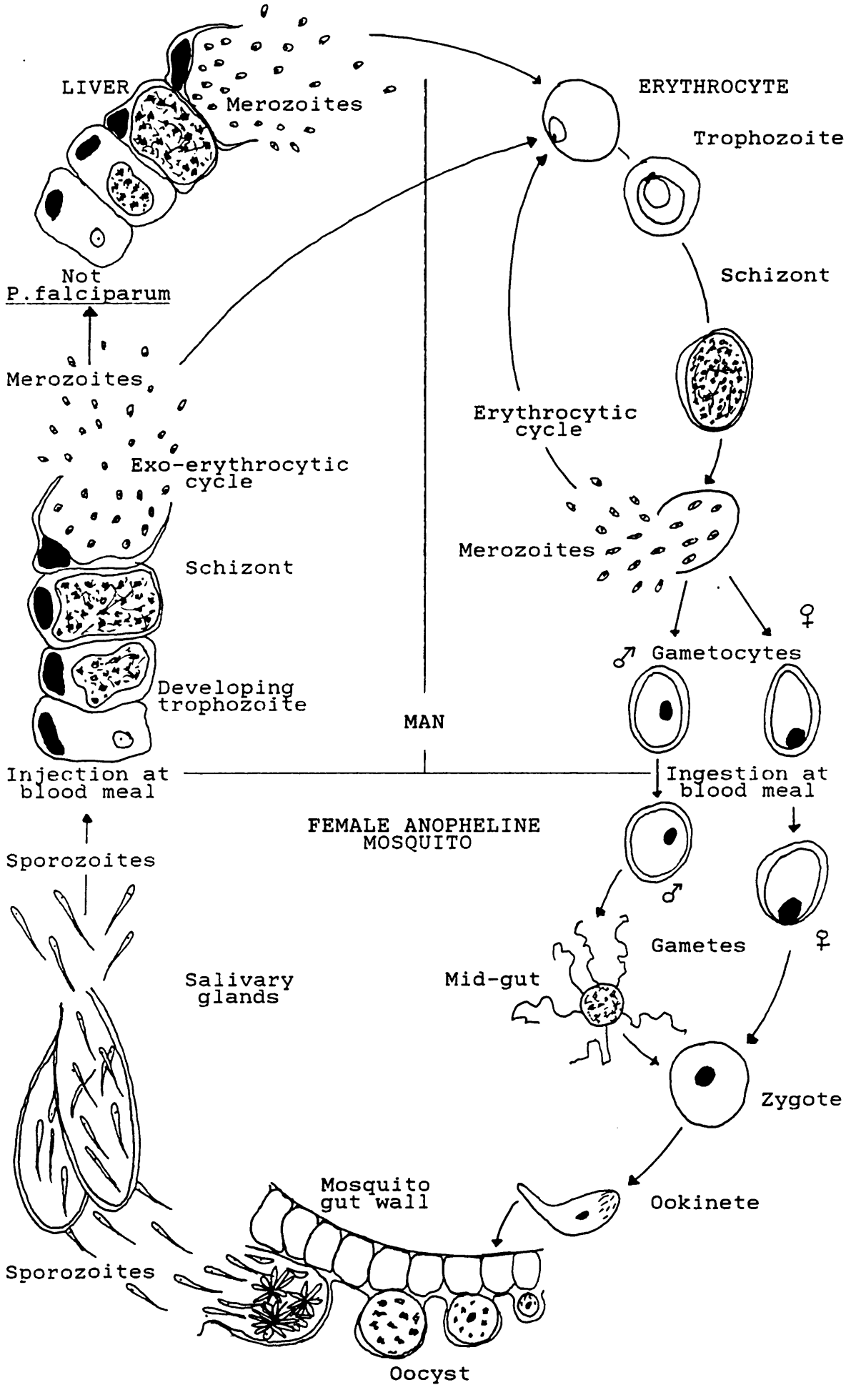
Following the inoculation of sporozoites into the bloodstream the first developmental stages in the human host are those of the exo-erythrocytic cycle, which occurs in the parenchyma cells of the liver. Sporozoites are completely cleared from circulation within 30 minutes to one hour after inoculation. After entering the parenchyma cells the parasite undergoes division and develops into a tissue schizont, which contains several thousand merozoites. When the schizont is mature these exo-erythrocytic merozoites are released directly into the bloodstream where they rapidly invade the red blood cells and initiate the erythrocytic phase of the life-cycle. Between 10 000 and 30 000 merozoites are produced per schizont, the exact number being species-dependent.

The erythrocytic parasites may appear as early as $5^1/_2$ days after the sporozoites were introduced, as is the case for P.falciparum. Blood parasites however appear later in the other species; after 8 days for P.vivax, 9 days for P.ovale and 15 days for P.malariae. Excluding P.falciparum the human malaria species all have secondary exo-erythrocytic cycles and can develop dormant forms in the liver which are able to give rise to relapses at a later date. Tissue schizonts have never been observed in P.falciparum infections after a patent infection has developed.

A.1 (ii) The erythrocytic cycle in the human host

Each malarial species has a marked intrinsic preference for red cell types of a particular age. P.malariae selects mature cells whereas P.vivax prefers to invade the younger reticulocytes; P.falciparum appears to have less strong preferences and has been observed in all red cell types at all stages of maturity.

Fig. A.1
The lifecycle of the malaria parasite in the mosquito and in the human host.


LIVER
Merozoites
ERYTHROCYTE
Trophozoite
Schizont
Not
P.falciparum
Merozoites
Erythrocytic
cycle
Exo-erythrocytic
cycle
Schizont
Merozoites
♀
♂ Gametocytes
Developing
trophozoite
MAN
Injection at
blood meal
Ingestion at
blood meal
FEMALE ANOPHELINE
MOSQUITO
Sporozoites
♂
Gametes
♀
Salivary
glands
Mid-gut
Zygote
Mosquito
gut wall
Ookinete
Sporozoites
Oocyst

Mitotic division among the growing erythrocytic parasites results in the development of a blood schizont, which, when mature ruptures releasing erythrocytic merozoites (8-32). Once in the bloodstream the merozoites rapidly invade other red blood cells and initiate additional asexual cycles in the erythrocytes. The entire cycle from merozoite through schizont to merozoite takes about 48 hours for P.falciparum, P.vivax and P.ovale (tertian malaria) and 72 hours for P.malariae (quartan malaria).

Certain of the erythrocytic merozoites are not destined to become blood schizonts. Having entered an erythrocyte they develop into gametocytes, the sexual stages of the parasite, instead. Mature microgametocytes (male) and macrogametocytes (female) do not develop any further in the human host, and are the only forms of the parasite that are infective to susceptible Anopheline mosquitoes.

A.1 (iii) Sexual developmental stages in the Anopheline mosquito

Only species of Anopheline mosquitoes are capable of supporting the development of the malaria parasite, and, of the 400 known species of Anopheles only 60 are proven vectors of human malaria. Female mosquitoes require bloodmeals to aid the maturation of their eggs and after feeding on an infected human host, whose blood contains mature gametocytes, further development of the parasite is possible.

Within a few minutes, after digestion of the bloodmeal has started in the midgut of the mosquito the red cell membranes surrounding each gametocyte begins to degenerate and the gametocytes become extracellular. The matured macrogametocytes released from their red cells can then transform into macrogametes and are then ready for fertilization. The transformed microgametes however require one further developmental process, that of exflagellation, before fertilization can occur. Within ten minutes of ingestion the microgametes can be seen energetically thrusting their way out of the microgametocyte (six to each cell) along threadlike structures formed by the parent microgametocyte. The microgametes quickly break away from the parent body and move vigorously through the partially digested bloodmeal. Fertilization of a high proportion of the macrogametes occurs within the first few minutes after exflagellation.

The resultant zygote rapidly elongates and matures into a motile ookinete, which then forces its way through one of the epithelial cells lining the mosquito mid-gut to take up its final position on the external wall of the epithelial cell, about 24 hours after the bloodmeal was ingested. Further development and meiotic division results in the formation of an oocyst with a thick cyst wall.

A.1 (iv) The extrinsic developmental period in the mosquito host

Several mitotic divisions gives rise to a rapid growth in the size of the oocyst and its protrusion into the haemocoel of the mosquito. When mature the cyst ruptures releasing thousands of spindle-shaped motile sporozoites into the haemocoel. The sporozoites migrate through the haemocoelonic fluid to the salivary glands, which they then penetrate. They finally come to rest in the lumen of the salivary glands. Sporozoites can remain in a viable condition almost indefinitely, until they are either inoculated into the bloodstream, together with anti-coagulant salivary fluid, during a bloodmeal or until the mosquito dies.

The extrinsic cycle in the mosquito is dependent on environmental conditions and cannot normally be completed if the temperature is below 16^{o} C (60^{o} F) or above 33^{o} C (91^{o} F). Between these two limits development proceeds faster at higher temperatures, thus the entire cycle in the mosquito takes about 2 weeks in tropical lowlands but 3 weeks or longer in cooler upland areas. Since the female mosquito is continually feeding every 2-4 days throughout its adult life it is not unusual to find examples of all the developmental stages of the extrinsic cycle in a single mosquito.

A.2 Classification of endemicity and stability

A.2 (i) Endemic malaria

The term endemicity refers to the amount or severity of malaria in a locality. Malaria is described as endemic when there is a constant incidence of cases over a period of many successive years. Epidemic malaria is the term used to describe periodic or occasional sharp increases in the amount of malaria in a given indigenous population. During epidemics all age groups are more or less equally affected.

Degrees of endemicity of malaria have been adopted by the WHO on the basis of either the spleen rate (the proportion with enlarged spleens) or the parasite rate (the proportion with parasites in the peripheral blood), calculated from a statistically significant sample of the population (see also Boyd(1949a, p595)). These degrees of endemicity are classified as follows:

(i) Hypoendemic - rates in children (2-9 years) not exceeding 10 %. Denotes areas where there is little transmission and the effects of malaria on the general population are unimportant.

(ii) Mesoendemic - rates in children (2-9 years) between 11 % and 50 %. Areas typically include small rural communities in the sub-tropics, with varying intensity depending on local circumstances.

(iii) Hyperendemic - rates in children (2-9 years) constantly over 50 % , rates in adults over 25 %. Typical of areas with intense but seasonal transmission, where immunity is insufficient to prevent the effects of malaria in the older age groups.

(iv) Holoendemic - rates in children (2-9 years) constantly over 75 % , rates in adults low. Perennial transmission of a high degree, resulting in a considerable degree of immune response, especially in the older age groups. (Bruce-Chwatt,1980).

A.2 (ii) Stability

Malaria terminology includes a more general classification of endemic and epidemic disease patterns, that of stable and unstable malaria (Macdonald,1957). For stable malaria the amount of transmission is usually high without any marked fluctuations over the years, although seasonal fluctuations may exist. For unstable malaria the amount of malaria varies considerably from year to year. For stable malaria, the collective immunity of the population is high and epidemics are unlikely; unstable malaria results in collective immunity that is much lower and epidemics are the typical pattern of disease.

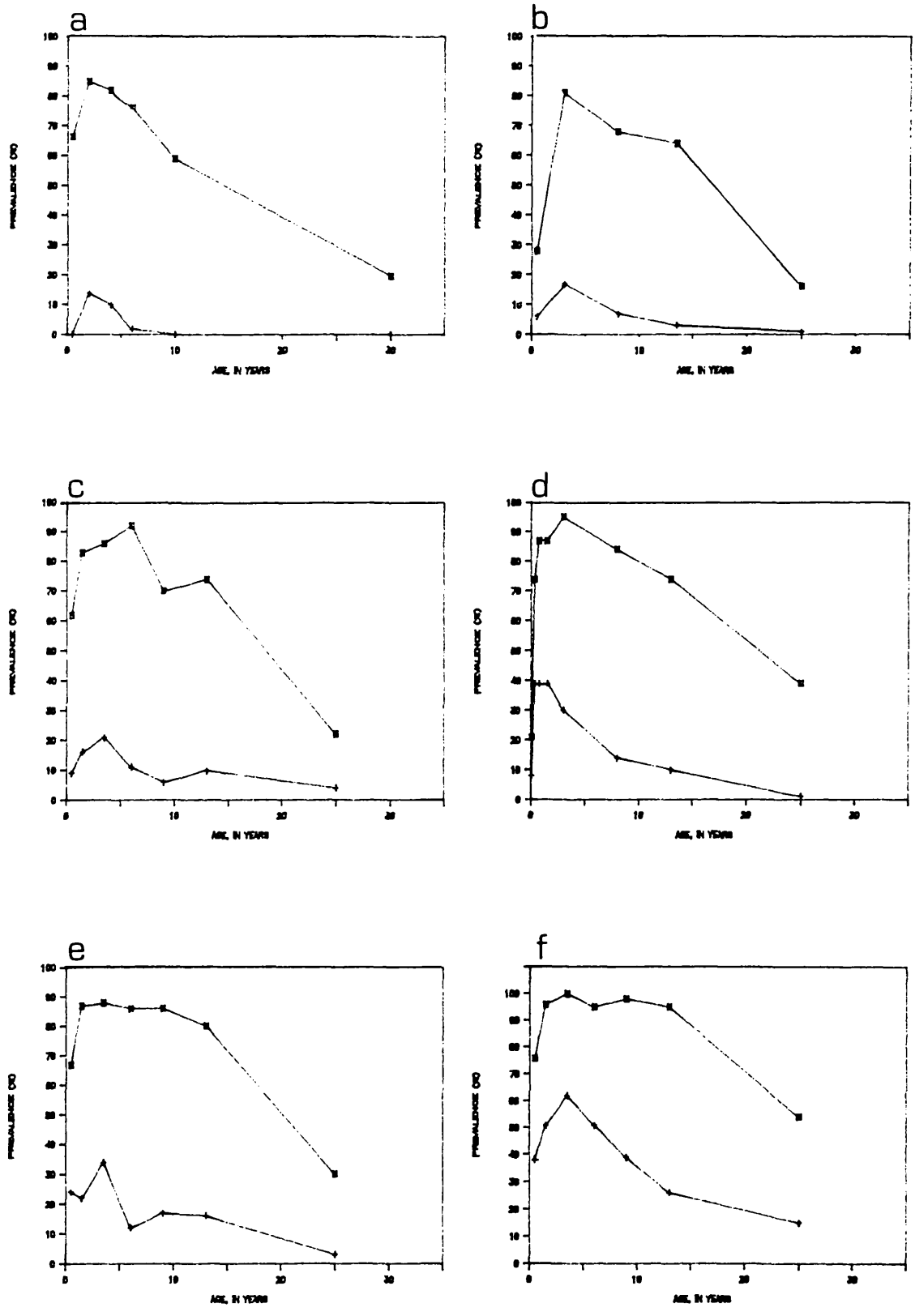
These two terms represent extremes of a continuum of situations, the degree of stability being determined by climatic and vectorial factors. Stable malaria is characterised by highly efficient vector populations (high anthrophily and longevity) and climatic conditions which favour more or less continuous transmission. As malaria becomes more unstable, climatic conditions have an

increasingly pronounced affect on the incidence of disease. Fluctuations in incidence are typically marked and uneven, with epidemics occurring when climatic conditions are suitable. The vector populations generally have a low potential for spreading disease (low anthrophily and moderate to low longevity). Unstable malaria is normally amenable to control or eradication through imagocides and larvicides combined with chemotherapy. Daily vector mortality of 20-25 % may be adequate for control of transmission (Macdonald,1957). Stable malaria is very difficult to control, especially in rural areas. Eradication/control is unlikely unless socio-economic conditions are favorable. Daily vector mortality of at least 50 % needed for a degree of control (Macdonald,1957)

APPENDIX B

B.1 Age-prevalence and age-intensity patterns

B.2 Age-related morbidity and mortality rates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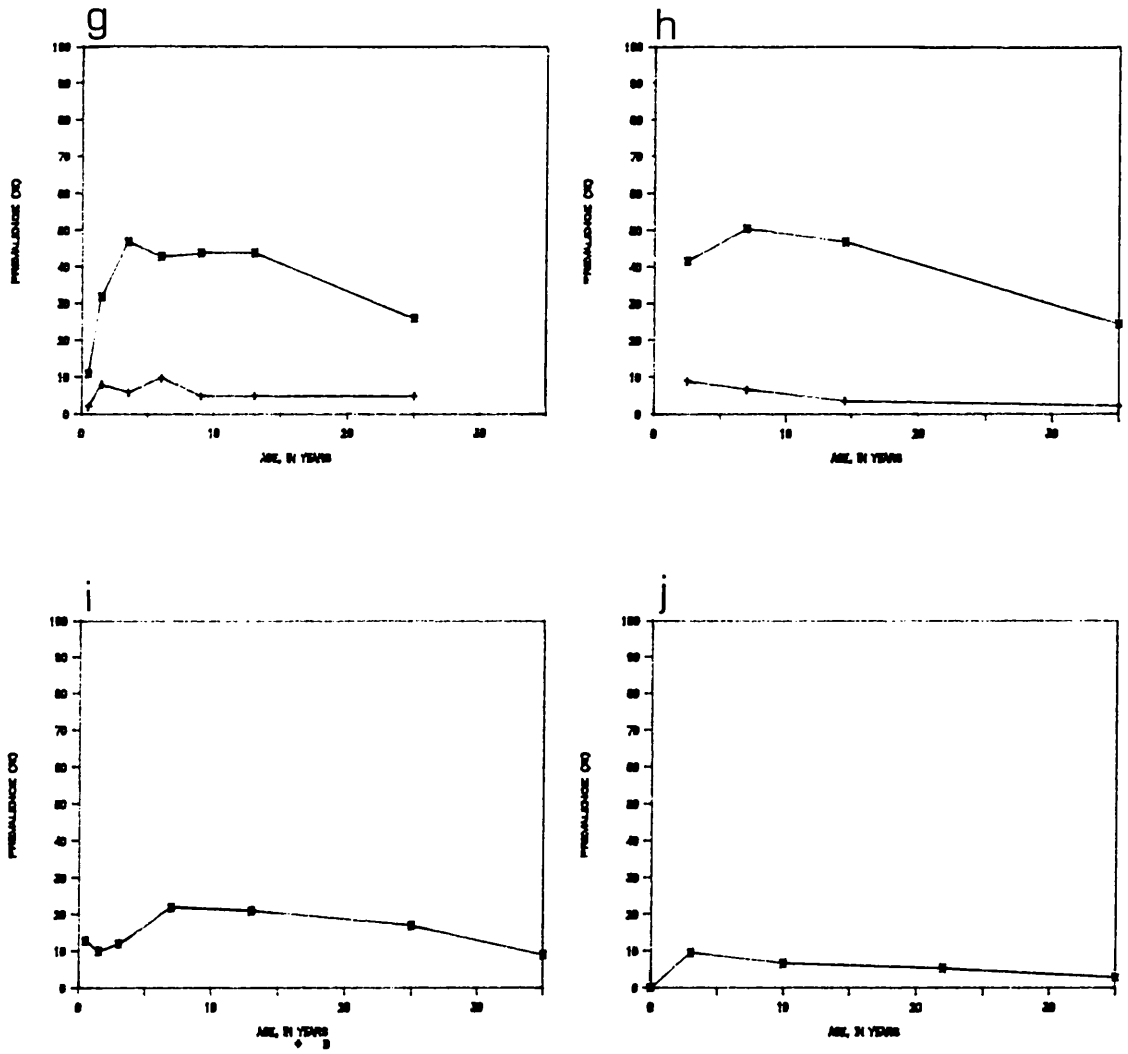
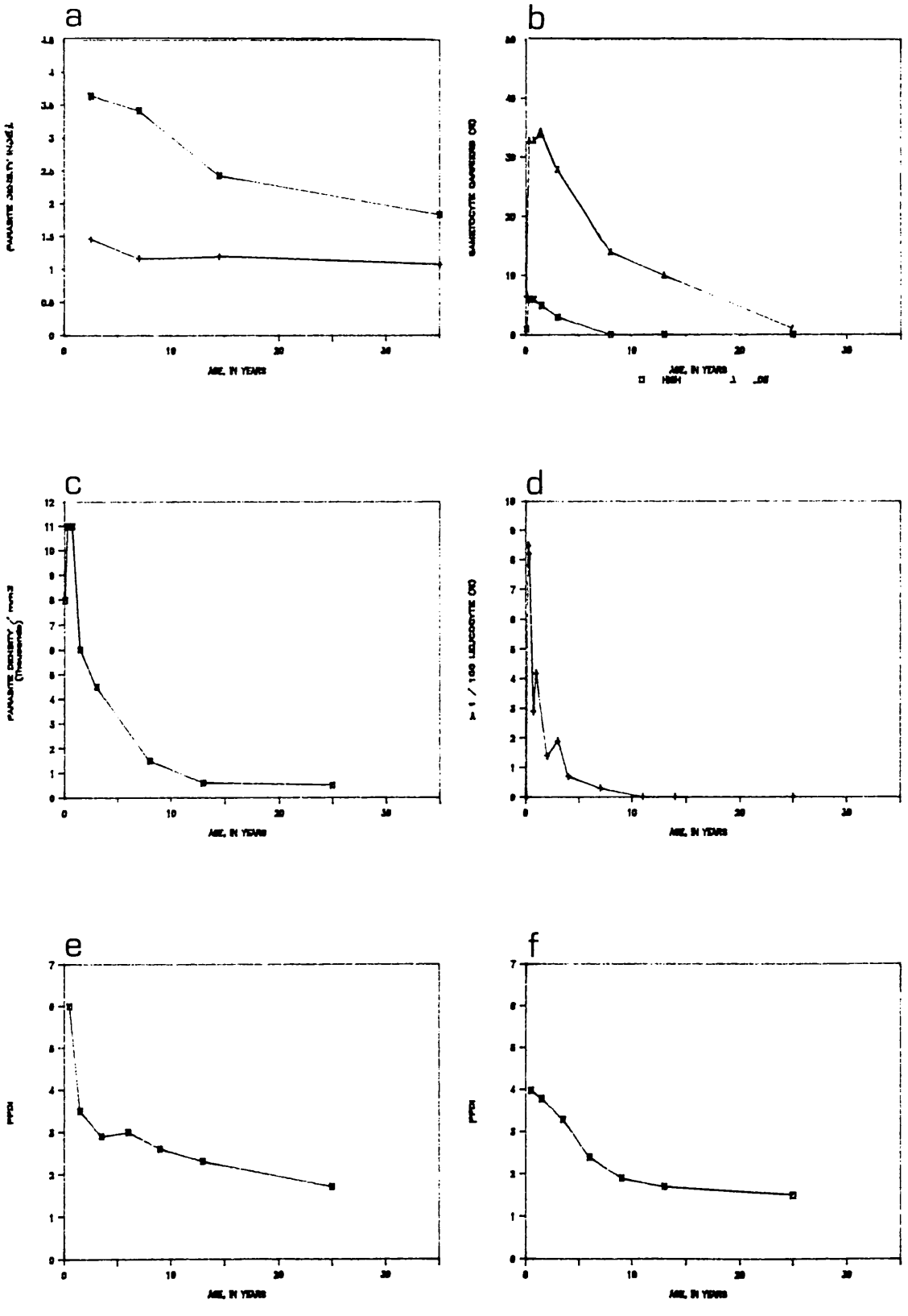


Fig. B.1. Age-prevalence patterns. (a) Mamfe, South Cameroons, (Bruce-Chwatt,1947, published in Gibson,1958). (b) Yoruba country, South-West Nigeria, (Draper,1953). (c) Bomfa, Gold Coast (Feb.), (Colbourne & Wright,1955). (d) Tanganyikan coast, (Davidson & Draper,1953). (e) Bomfa, Gold Coast (Nov.), (Colbourne & Wright,1955). (f) Yorugu-Bolgatanga, Gold Coast (April), (Colbourne & Wright,1955). (g) Maraga, Papua New Guinea, (Graves et al,1988). (h) Butelgut, Papua New Guinea, (Graves et al,1988). (i) East Africa, (Draper, Voller & Carpenter,1972). (j) Kalapara, Bangledesh, (Lobel et al,1973). Total prevalence (sexual and asexual stages) - □ , Gametocyte prevalence - + .

Fig. B.2. Age-intensity of infection patterns. (a) Madang, Papua New Guinea, (Graves et al,1988). (b) High (>100 / mm^3) and low (<100 / mm^3) density gametocyte carriers, Tanganyika, (Davidson & Draper,1953). (c) Mean (arithmetic) positive P.falciparum density, Tanganyika, (Davidson & Draper,1953). (d) Southern Nigeria, (Barber & Olinger,1932). Positive parasite density index, (e)-(f) The Gold Coast; Central Accra (e), Yorugu-bolgatanga (f), (Colbourne & Wright,1955). Total parasite intensity (sexual and asexual stages) - □ , Gametocyte density - + .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AGE, IN YEARS

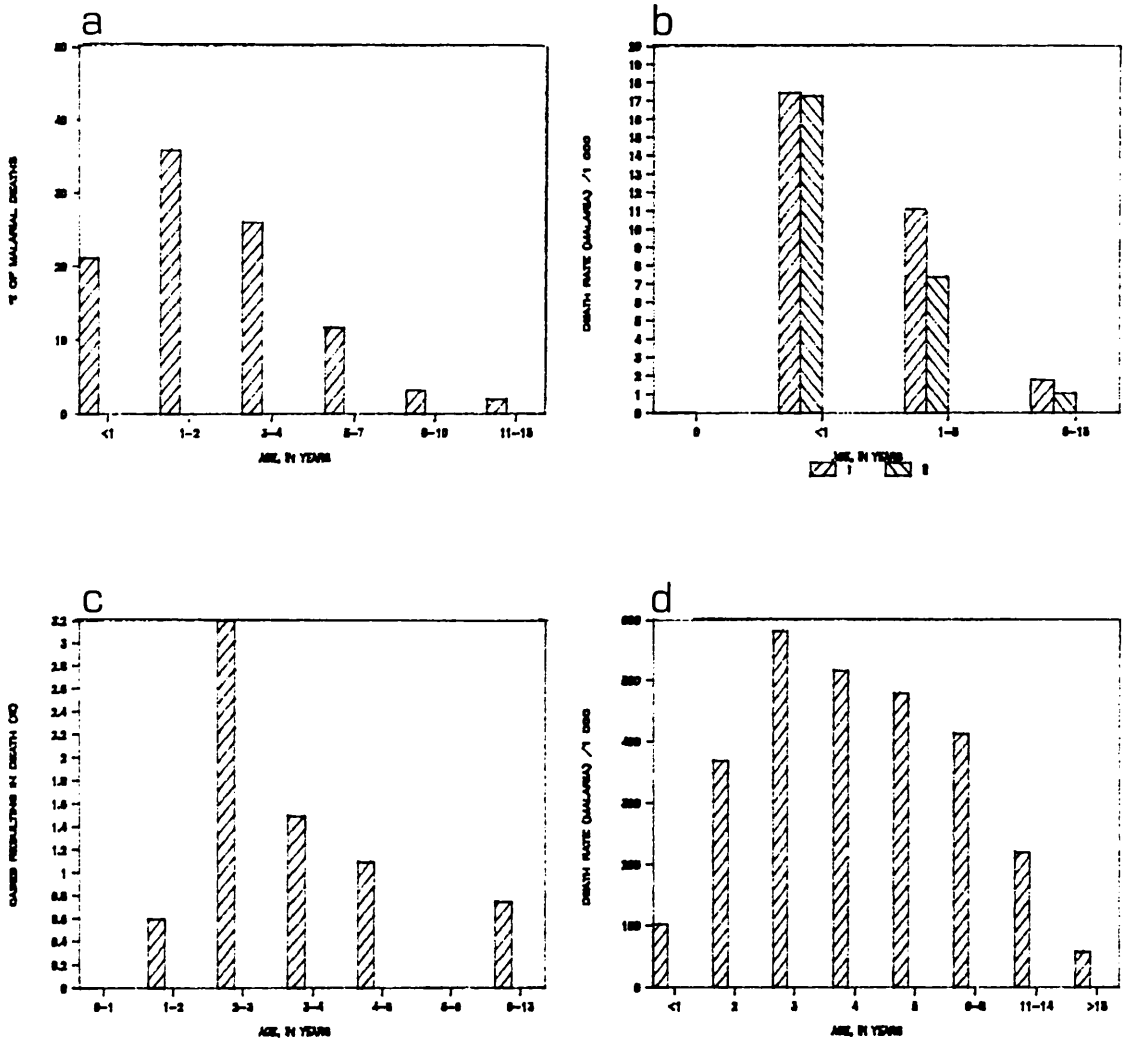

Fig. B.3. Age-related mortality. (a) Lagos, Nigeria, (Bruce-Chwatt,1952b). (b) Accra, (central (1), suburban (2)) Gold Coast, (Colbourne & Eddington,1954). (c) Fajara, Gambia (Marsh, unpublished). (d) Guyana (Giglioli,1972).

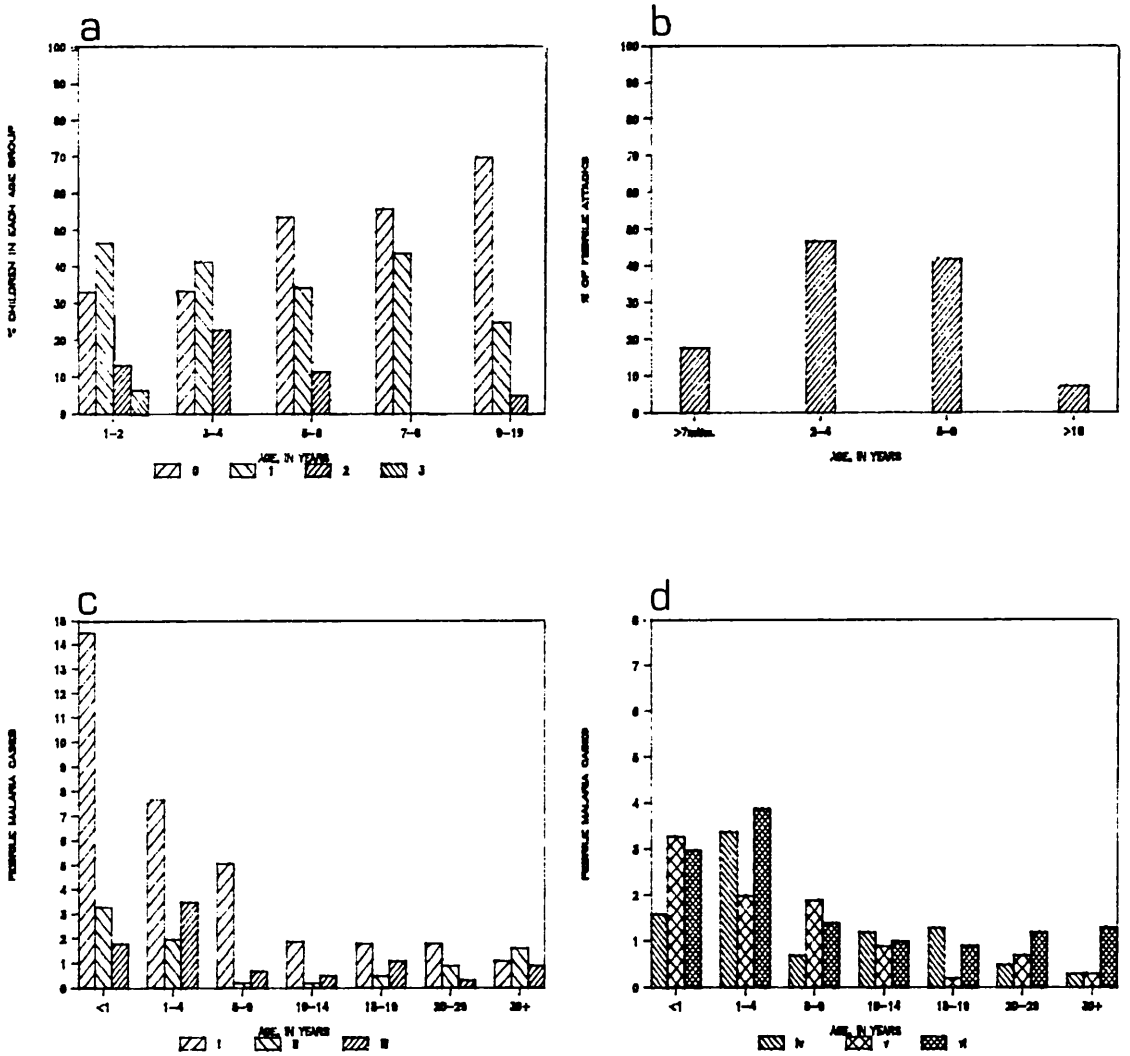

Fig. B.4. Age-related morbidity. (a) Number of clinical episodes of malaria per year, Gambia (Marsh et al,1988). (b) Distribution of febrile cases by age, Burkina Faso, (Baudon et al,1985). (c)-(d) Madang Province, febrile malaria cases per year, by village and age-group; Butelgut(1), Mebat(2), Budlip(3), Maraga(4), Dogia(5) and Sah(6), (Cattani et al,1986).

APPENDIX C

The results of infection and superinfection with small inoculums of P.y.yoelii sporozoites in BALB/c mice

Mean parasitaemias, for groups of 10 mice infected with 250 sporozoites on day 0 and superinfected on days 2 (Group A_1) and 15 (Group A_2), as compared to control mice with no superinfection.

(* denotes at least some of the mice positive by thick blood smear)

Days post-infection	% Parasitaemia		
	control	A_1	A_2
0	0	0	0
1	0	0	0
2	0	0	0
3	*	*	*
4	0.313	0.238	0.351
5	0.60	0.87	0.572
6	1.28	1.53	1.22
8	3.81	4.59	4.12
10	11.40	12.87	11.37
11	19.01	21.75	26.80
12	28.44	31.04	26.08
13	24.21	29.51	23.40
14	21.31	20.67	22.47
16	8.97	8.51	9.76
17	3.67	0.043	4.31
18	*	0	2.21
19	*	0	0.095
20	0	0	0.034
21	0	0	0
22	0	0	0
23	0	0	0
24	0	0	0
25	0	0	0

APPENDIX D

Parameter notation used in Chapters 5 and 6

$X(a,t)$	Number of susceptibles, aged a, at time t.
$H(a,t)$	Number of latents, aged a, at time t.
$Y(a,t)$	Number of infectious individuals, aged a, at time t.
$Z(a,t)$	Number of immunes, aged a, at time t.
$N(t)$	Total population size at time t.
L	The per capita infection rate of humans (= transmission rate).
σ	The per capita rate of transfer from latent to infectious class.
r	The per capita rate of becoming non-infectious.
π	The per capita rate at which acquired immunity is lost.
μ	The per capita natural mortality rate.
α	The per capita disease-induced mortality rate.
b	The probability that an infected bite produces an infection in the human host.
a^{max}	The maximum age for any individual.
$N''(t)$	The size of the female mosquito population, at time t.
$X''(t)$	The number of susceptible female mosquitoes, at time t.
$H''(t)$	The number of latent female mosquitoes, at time t.
μ''	The mosquito death rate.
β	The transmission coefficient, between man and mosquitoes.
MBR	The total number of bites, per unit time.
IMBR	The number of bites, per unit time taken by one mosquito.
U	The feeding frequency.
P	The probability that a human host is chosen for the blood meal.
L''	The mosquito infection rate.
β''	The transmission coefficient, between mosquito and man.
b''	The probability that a bite on an infected human host results in an infection in the vector.
$\overline{Y}(t)$	The equilibrium number of infectious humans.
$\overline{Y}''(t)$	The equilibrium number of infectious vectors.

$\bar{L}(Y)$	The equilibrium transmission rate.
τ''	The duration of the extrinsic cycle in the vector.
N(0)	The Birth rate of the human population.
CP(a)	The cumulative prevalence of individuals aged a.
MA(a)	The number of infants, aged a, partially protected by maternal antibodies.
L1	The per capita infection rate among infants protected by maternal antibodies.
C	The per capita rate at which maternally derived antibodies are lost.
τ	The average duration of acquired immunity.
r_0	The per capita rate of recovery from a single infection.
$\pi(L)$	The per capita rate of loss of acquired immunity, boosted by re-exposure.
δ	The rate of development of functional immunity by the population.

APPENDIX E

E.1 Summary of the relationship between parameters involved in the calculation of the transmission coefficients ß and ß"

E.1 (i) Transmission coefficient from mosquito to man, ß

$$ß = MBR \times b \tag{E.1}$$

$$MBR = m \times IMBR \tag{E.2}$$

$$IMBR = U \times P \tag{E.3}$$

where, m is the number of female mosquitoes per human host.

E.1 (ii) Transmission coefficient from man to mosquito, ß"

$$ß" = IMBR \times b" \tag{E.4}$$

E.2 Equilibrium results for the mosquito model

E.2 (i) Analytical solutions to the model for infection in the mosquito population

The analytical solutions to the basic mosquito model defined by eqs.(5.16) - (5.18) in the main text are:

$$X"(t) = \mu"N"(t)\, e^{-(L"+\mu")t} \tag{E.5}$$

$$H"(t<\tau") = \mu"N"(t)\, e^{-\mu"t}\,(1 - e^{-L"t}) \tag{E.6a}$$

$$H"(t>\tau") = \mu"N"(t)\, e^{-(L"+\mu")t}\,(e^{L"t} - 1) \tag{E.6b}$$

$$Y"(t<\tau") = 0 \tag{E.7a}$$

$$Y''(t>\tau'') = \frac{L''\mu''N''(t)e^{L''\tau''}}{L''} - (L''+\mu'')t\left(e^{L''(t-\tau'')} - 1\right) \quad \text{(E.7b)}$$

subject to the initial conditions given in the main text.

E.2 (ii) Equilibrium level of infected mosquitoes

The equilibrium number of infected mosquitoes $\bar{Y}''(t)$ is given by:

$$\bar{Y}''(t) = \int_{\tau''}^{\infty} Y''(t)\, dt \quad \text{(E.8)}$$

using eq.(E.7b) above,

$$\bar{\bar{Y}}''(t) = \frac{L''}{(L''+\mu'')} N''(t)\, e^{-\mu''\tau''} \quad \text{(E.9)}$$

re-arranging and using eq.(5.13) in the main text, an expression for the equilibrium proportion of infected mosquitoes is obtained:

$$\frac{\bar{\bar{Y}}''(t)}{\bar{N}''(t)} = \frac{\beta''\,\bar{Y}(t)\, e^{-\mu''\tau''}}{\beta''\,\bar{\bar{Y}}(t) + \mu''\bar{N}(t)} \quad \text{(E.10)}$$

E.3 Equilibrium age-distributions for the human model

This appendix presents the analytical solutions to the age-prevalence model defined by eqs.(5.21)-(5.25) in the main text.

The age-distributions, subject to the initial conditions given in the main text, are:

E.3 (i) For constant age-independent death rate, μ

$$X(a) = N(0)\, e^{-(L+\mu)a} \qquad (E.11)$$

$$H(a) = \frac{N(0)\, L}{(\sigma - L)} \left(e^{-(L+\mu)a} - e^{-(\mu+\sigma)a} \right) \qquad (E.12)$$

$$Y(a) = \frac{N(0)\, L\sigma}{(\sigma - L)} \left(\frac{e}{h}^{-(L+\mu)a} - \frac{e}{f}^{-(\sigma+\mu)a} \right)$$

$$+ \frac{N(0)\, L\sigma}{hf} e^{-ga} \qquad (E.13)$$

$$Z(a) = \frac{N(0)\, Lr}{(\sigma - L) f} \left(e^{-(\sigma+\mu)a} - e^{-\mu a} \right)$$

$$\cdot\ N(0)L\sigma\left(\left(\frac{e}{(\sigma - L)h}^{-(L+u)a} - e^{-\mu a} \right) + r \left(\frac{e}{fh(\alpha + r)}^{-ia} - e^{-\mu a} \right) \right) \qquad (E.14)$$

$$N(a) = N(0)\alpha L\sigma \left(\frac{e}{L(\sigma - L)h}^{-(L+\mu)a} - \frac{e}{\sigma(\sigma - L)f}^{-(\mu+\sigma)a} + \frac{e}{(\alpha + r)fh}^{-ha} \right)$$

$$+ N(0)\alpha L\sigma\, e^{-\mu a} \left(\frac{1}{\alpha L\sigma} - \frac{1}{L(\sigma - L)h} + \frac{1}{\sigma(\sigma - L)f} - \frac{1}{(\alpha + r)fh} \right) \qquad (E.15)$$

where, $f = (\alpha + r - \sigma)$, $g = (\mu + r + \alpha)$
$h = (\alpha + r - L)$ and $i = (\mu + r + \sigma)$

E.3 (ii) For age-dependent death rate, $\mu(a)$

When the parameter μ in eqs.(5.21)-(5.25) in the main text is replaced by $\mu(a)$, the new set of equations for the "starred" variables (see eq.(5.26)) with the age dependence factored out are solved in an identical way to the equations with μ (Bailey,1975). The results are similar to those given above, e.g.

$$X(a) = N(0)\, e^{-La}\, D(a) \qquad (E.16)$$

where,

$$D(a) = \exp\left(- \int_0^{a^{max}} \mu(g)\, dg \right) \qquad (E.17)$$

and, a^{max} is the max age limit of 40 years.

For example, with two death rates of μ^1 and μ^2 defined by the upper age limits a^1 and a^{max}, expressions for D(a) are as follows:

$$a \in [a, a^1) \qquad D(a) = \exp -\mu^1 a \qquad (E.18)$$

$$a \in [a^1, a^{max}) \qquad D(a) = \exp -(\mu^1 a^1 + \mu^2 (a - a^1)) \qquad (E.19)$$

E.4 Calculation of the birth rate

E.4 (i) No parasite induced mortality, $\alpha = 0$

When all individuals irrespective of age or disease status are subject to the same death rate, μ, eq.(E.15) simplifies to the expression:

$$N(a) = N(0)\, e^{-\mu a} \qquad (E.20)$$

integration of this expression between age 0 and the max age limit, a^{max} gives an expression for the total population size N, in terms of the birth rate N(0), the death rate μ, and the max age a^{max}.

Rearrangement gives the expression for the birth rate for a population of known size and death rate:

$$N(0) = \frac{N\mu}{(1 - \exp -\mu a^{max})} \qquad (E.21)$$

E.4 (ii) Parasite induced mortality, $\alpha \neq 0$

Integration of eq.(E.15) between age 0 and age a^{max} gives an expression for the total population size N.

$$N = - N(0) \alpha L \sigma \frac{(e^{-(L+\mu)amax} - 1)}{L(\sigma-L)(L+\mu)h}$$

$$+ N(0) \alpha L \sigma \left(\frac{(e^{-(\mu+\sigma)amax} - 1)}{\sigma(\sigma-L)(\mu+\sigma)f} - \frac{(e^{-(r+\alpha+\mu)amax} - 1)}{(\alpha+r)(r+\alpha+\mu)fh} \right)$$

$$- \frac{N(0)\alpha L\sigma(e^{-\mu amax} - 1)}{\mu} \left(\frac{1}{L\alpha\sigma} - \frac{1}{L(\sigma-L)h} + \frac{1}{(\sigma-L)f\sigma} - \frac{1}{(\alpha+r)fh} \right) \qquad (E.22)$$

For a population of known size the above expression contains only one unknown N(0); therefore rearrangement of the above equation will give an explicit expression for the birth rate, N(0).

E.4 (iii) Age-dependent death rate, $\mu(a)$

For simplicity the derivation of the birth rate for a population with age-dependent mortality will not include parasite induced mortality. Following the explanation in section E.3 (ii) above, the expression for the age-distribution of the total population with $\alpha = 0$ and $\mu(a)$ (see eq.(E.17)) is:

$$N(a) = N(0) D(a) \qquad (E.23)$$

For a population with only two death rates μ^1 and μ^2 determined by upper age limits of a^1 and a^{max}, using eqs.(E.18) and (E.19), and integrating between the limits 0 and a^1 and a^1 and a^{max} expressions for the population aged less than a^1 and greater than a^1 can be obtained:

$(a<a^1)$

$$N = \frac{N(0)}{\mu^1} \left(1 - \exp\left(-\mu^1 a^1\right) \right) \qquad \text{(E.24)}$$

$(a>a^1)$

$$N = \frac{N(0)}{\mu^2} \left(\exp -\mu^1 a^1 \left(1 - \exp \mu^2 \left(a^1 - a^{max}\right) \right) \right) \qquad \text{(E.25)}$$

Summing the two portions of the population and rearranging gives an expression for the birth rate.

$$N(0) = \frac{N \mu^1 \mu^2}{\mu^2(1-\exp(-\mu^1 a^1)) + \mu^1 \exp(-\mu^1 a^1)(1-\exp(-\mu^2(a^1-a^{max})))} \qquad \text{(E.26)}$$

E.5 Formulation of immunity dependent on re-exposure

E.5 (i) Assumptions

(a) Immunity lasts for a fixed interval of time Υ, in the absence of re-exposure.
(b) An immune person remains immune until the occurrence of a time interval Υ, without exposure.
(c) Infective exposures arrive randomly at a constant mean rate L.
(d) The risk of mortality is also random, occurring at a rate μ, which is independent of both age and epidemiological status.

E.5 (ii) The relationship between the average duration of immunity and the rate of exposure L

It follows that:

$\exp -(L + \mu)$ = probability of surviving the interval Υ without at least one infective contact

$(1 - \exp -(L + \mu)\Upsilon)$ = probability of surviving the interval with an infective contact before time

According to the assumptions above, an individual remains immune as long as the event of exposure within the interval Υ repeats itself without interruption. If each person receives an average of N exposures, without interruption, the average number of exposures until susceptible has a geometric distribution so that,

$$N = \frac{(1 - \exp -(L + \mu)\Upsilon)}{\exp -(L + \mu)\Upsilon} \qquad (E.27)$$

Until immunity is lost, i.e. when the interval between exposures is greater than Υ, the average time interval between exposures may be given by W, where W is the mean of the negative exponential distribution restricted to the range zero to Υ, so that,

$$W = \frac{1}{(L + \mu)} - \frac{\Upsilon \exp -(L + \mu)\Upsilon}{(1 - \exp -(L + \mu)\Upsilon)} \qquad (E.28)$$

Therefore the average duration of immunity T, is the sum of the time during which exposures are repeated without interruption (WN) and the final interval Υ, in which re-exposure fails to occur.

$$T(L,\Upsilon) = WN + \Upsilon \qquad (E.29a)$$

$$= \frac{(1 - \exp -(L + \mu)\Upsilon)}{(L + \mu) \exp -(L + \mu)\Upsilon} \qquad (E.29b)$$

The average duration of immunity T, where immunity is boosted by re-exposure, is related to the rate of loss of immunity Π, such that $\Pi = 1/T$.

APPENDIX F

The rates of change in the proportions infected with age where there are two separate parasite sub-populations each of which induces homologous immunity

For single infections:

$$\frac{d}{da} PY_i(a) = L_i\, PX(a) - r_i\, PY_i(a) - {}_iL_j\, PY_i(a) \qquad (F.1)$$

$$\frac{d}{da} PY_j(a) = L_j\, PX(a) - r_j\, PY_j(a) - {}_jL_i\, PY_j(a) \qquad (F.2)$$

Infection by both parasite populations:

$$\frac{d}{da} PY_{ij}(a) = {}_iL_j\, PY_i(a) + {}_jL_i\, PY_j(a) - r_{ij}\, PY_{ij}(a) \qquad (F.3)$$

Infection by parasite sub-population i amongst individuals immune to infection by parasite sub-population j.

$$\frac{d}{da} PW_i(a) = L_i\, PZ_j(a) - r_i\, PW_i(a) \qquad (F.4)$$

Infection by parasite sub-population j amongst individuals immune to infection by parasite sub-population i.

$$\frac{d}{da} PW_j(a) = L_j\, PZ_i(a) - r_j\, PW_j(a) \qquad (F.5)$$

APPENDIX G

G.1 Parameter notation used in Chapter 7

X(a,t)	The number of humans aged a, non-patent at time t.
Y(a,t)	The number of humans aged a, patent at time t.
PY(a,t)	The proportion of humans aged a, patent at time t.
R(a,t)	The apparent recovery rate for individuals aged a, at time t.
I(a,t)	The apparent incidence rate for individuals aged a, at time t.
h(a,t)	The rate at which new patent infections are acquired among individuals aged a, at time t.
f(a,t)	The rate at which old infections recrudesce amongst individuals aged a, at time t.
MBR(a,t)	The rate of contact between the vector population and individuals aged a, at time t.
MBR_{max}	The maximum rate of contact between the human and vector population in a given locality i.e. the rate of biting on adults.
SEASN	The parameter controlling the seasonal variation in the number of vectors.
C_1, C_2	The rate of increase with age of the contact rate between vectors and humans.
g_1, g_2, g_3	The parameters controlling the strength of the acquired immune response which reduces h.
g_4, g_5	The parameters controlling the strength of the acquired immune response which reduces f
g_6, g_7	The strength of the acquired immune response reducing R.
σ_1	The duration of immunological memory, affecting the immune responses to infected bites.
σ_2, σ_3	The duration of immunological memory, affecting the immune responses associated with recrudescences.
σ_4, σ_5	The duration of immunological memory, affecting the immune responses associated with recovery.
b(a,t)	The probability that an infected bite on a susceptible human aged a, at time t, results in a patent infection.
b(0)	The probability that an infected bite on a naive infant i.e. aged 0, results in a patent infection.

b_{min}	The lower limit for the probability that an infected bite produces a patent infection.
c(a,t)	The probability a old infection will recrudesce in an individual aged a, at time t.
c_{min}	The minimum probability that an old infection will recrudesce.
c_{max}	The maximum probability that an old infection will recrudesce.
ø	The average time period between the onset of the primary infection and the onset of a recrudescence.
R(max)	The maximum rate of recovery from patent infection.
z(a)	The magnitude of the superinfection effect for an individual aged a.
z_{max}	The maximum superinfection effect possible.
k_1, k_2	The determinants of the strength of the superinfection effect.
$1/\epsilon$	The average duration of time over which past patent infections contribute to the superinfection effect.
EIR	The inoculation rate, determined from entomological data (= entomological inoculation rate).
ICR	The inoculation rate, determined from parasitological examination of infants (= infant conversion rate).
α	The transition frequency, negative to positive, between consecutive surveys (the proportion of persons negative in the first survey but positive in the second).
ß	The transition frequency, positive to negative, between consecutive surveys (the proportion of persons positive in the first survey but negative in the second).
$\overline{MBRY}(a,t)$	The accumulated past experience of infected bites, for an individual aged a, at time t, given by eq.(7.10) in the main text (see below).
$\overline{W}(a)$	The accumulated past experience of potentially infected bites for a person aged a, given by eq.(7.18) in the main text (see below).
$\overline{H_f}(a)$	The accumulated past experience of primary patent infections for a person aged a, relating to the rate at which recrudescences occur, as given by eq.(7.24).
$\overline{F_f}(a)$	The accumulated past experience of patent recrudescences for a person aged a, relating to the rate at which recrudescences occur, as given by eq.(7.25).

$\overline{\overline{H_r}}(a)$ The accumulated past experience of primary patent infections for a person aged a, relating to the affect of acquired immunity on the rate of recovery, as given by eq.(7.29).

$\overline{\overline{F_r}}(a)$ The accumulated past experience of patent recrudescences for a person aged a, relating to the affect of acquired immunity on the rate of recovery, as given by eq.(7.30)

$\overline{\overline{F_z}}(a)$ The accumulated past experience of patent recrudescences for a person aged a, relating to the affect of superinfection on the rate of recovery, as given by eq.(7.36).

$\overline{\overline{H_z}}(a)$ The accumulated past experience of primary patent infections for a person aged a, relating to the affect of superinfection on the rate of recovery, as given by eq.(7.35).

G.2 Definitions used to describe bites from sporozoite positive vectors

G.2 (i) Infected bites

Any bite received from a sporozoite positive mosquito. For an individual aged a, at time t, the number of infected bites received per unit time is expressed as:

$$MBR(a,t) \times \frac{Y''(t)}{N''(t)} \qquad (G.1)$$

G.2 (ii) Potentially infective bites

Any infected bite that is capable of producing a patent infection in a naive individual with no acquired immunity. For an individual aged a, at time t, the number of potentially infective bites received per unit time is expressed as:

$$MBR(a,t) \times \frac{Y''(t)}{N''(t)} \times b(0,t) \qquad (G.2)$$

G.2 (iii) Infective bites

Any infected bite that produces a patent infection.
For an individual aged a, time t, the number of infective bites per unit time is expressed as:

$$MBR(a,t) \times \frac{Y''(t)}{N''(t)} \times b(a,t) \qquad (G.3)$$

The number of infective bites received per unit time is equivalent to the incidence of new infections (eq.(7.6a) in the main text.
For a naive human with no acquired immunity, the number of infective bites received is identical to the number of potentially infective bites received.

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