MALARIA PARASITAEMIA AND HUMORAL IMMUNE RESPONSES TO SOME DEFINED *Plasmodium falciparum* ANTIGENS IN NEWBORNS, INFANTS AND ADULT NIGERIANS.

BY

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A THESIS IN THE DEPARTMENT OF CHEMICAL PATHOLOGY SUBMITTED TO THE FACULTY OF BASIC MEDICAL SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY, UNIVERSITY OF IBADAN, NIGERIA.

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DEDICATION

TO the Almighty for His love and blessings.

TO my parents for their love and patience.

TO Aduni and Erica for their love and endurance.

ABSTRACT

A cohort of mothers and their newborns at Igbo-Ora, Oyo State was studied longitudinally for 12 months to determine the incidence of malaria parasitaemia, episodes of clinical malaria and their humoral immune response to malaria infection. Cross-sectional studies were also performed on adults at the Government Technical College, Igbo-Ora and blood donors at the University College Hospital, Ibadan during the rainy and dry seasons.

Peripheral and cord blood samples were collected from 116 women at delivery and maternal-newborn malariometric indices were recorded. Infants were monitored fortnightly to detect episodes of clinical malaria and serial blood samples were collected at bi-monthly clinics.

Blood samples were collected from 100 volunteers at the G.T.C. Igbo-Ora in July, 1991 and 33 of these volunteers in February, 1992; 224 blood donors at the U.C.H., Ibadan between October and November, 1991 and in 192 donors in March, 1992.

Immunological assays included single radial immunodiffusion assay for IgG, IgM and IgA; immunofluorescence assay for antibodies to total blood stage antigens; erythrocyte membrane

immunofluorescence (EMIF) assay to detect antibodies to the Pf155/RESA; and an enzyme-linked immunosorbent assay (ELISA) for antibodies to four synthetic peptides.

Malaria parasites were detected in 2.5% of cord blood samples and in 22.4% of the parturient women. The malaria parasite rates and densities of the study infants increased significantly with increasing age. Parasite rates at the July and February surveys at the G.T.C. were similar ($P>0.50$) while parasite density was higher ($P<0.01$) at the July survey. The parasite rate of blood donors at the October-November survey was higher ($P<0.001$) than at the March survey while parasite density in March was higher ($P<0.001$) than at the October-November survey.

Cord blood IgG was significantly lower than maternal IgG levels and a correlation was observed between cord and maternal IgG but not IgM levels. During the first year of life, IgM but not IgG and IgA was significantly higher in malaria positive infants compared with negative infants.

Antibodies to total blood stage antigens were detected in all sera tested. Malaria-specific IgM was detected in 5.8% of cord blood samples. There was a correlation between maternal and cord blood antibody titres to the Pf155/RESA ($P<0.001$) antigen. In addition a correlation was obtained between maternal and cord blood ELISA

(OD_{405}) values to the $(EENV)_6$, LJ5 and MAP2 peptides but not $(NANP)_6$ peptide.

There was no correlation between cord blood IgG, IgM, anti-Pf155 antibody titres, ELISA (OD_{405}) values to the $(EENV)_6$, $(NANP)_6$, LJ5 and MAP2 peptides and duration of onset of malaria in the infant. Cord blood seropositivity for antibodies to the Pf155/RESA and $(NANP)_6$ antigens or $(EENV)_6$ and $(NANP)_6$ peptides did not influence age of onset of clinical malaria. However, infants with haemoglobin AS whose cord blood was seropositive for antibodies to the Pf155/RESA and $(NANP)_6$ antigens or $(EENV)_6$ and $(NANP)_6$ peptides showed delayed onset of clinical malaria compared with AA infants.

In adults, anti-Pf155 antibody titres and ELISA seroreactivities to the $(EENV)_6$, LJ5 and MAP2 peptides showed a wide variation and individual levels were similar on consecutive surveys. Seroreactivity to the $(NANP)_6$, was higher at the end of the rainy season than at the end of the dry season. The presence and level of antibodies to the Pf155/RESA, $(EENV)_6$, $(NANP)_6$, LJ5 and MAP2 antigens did not influence the presence and density of malaria parasites.

Parasitological data in infants suggest some relative protection within the first 2 - 3 months of life. However, maternally acquired antibodies alone may not be responsible for this observation. The

presence of malaria-specific IgM in cord blood suggest intrauterine sensitization of the foetus by malarial antigens. Although no relationship was observed between malarial antibody levels and parasite rates/densities in the adult subjects, these antibodies may still play a role in immune protection against malaria.

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useful information and for assistance rendered during my stay in Stockholm. I am equally grateful to all the Postgraduate students and Staff of the malaria unit, Immunology Department, Stockholms Universitet for their warm hospitality and brain storming seminars during my sojourn in Stockholm. I am most grateful to Prof. G. Pasvol of Saint Mary Hospital, London for his keen interest in this study and for providing me with monoclonal antibodies to the MNSsU blood group antigens. My thanks are also due to Dr. O. J. M. Oduola of the Postgraduate Institute for Medical Research & Training, Ibadan for useful suggestions and facilities extended during this study. The skillful technical assistance of Mr. I. Ekanem of Immunology Unit, U.C.H. Ibadan, Margareta Hagstedt and Ingegard Andersson of the Department of Immunogy, Stockholms Universitet is gratefully acknowledged.

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CERTIFICATION

We certify that this work was done by Mr. ACHIDI ERIC AKUM in the Immunology Unit of the Department of Chemical pathology, University of Ibadan.

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ABBREVIATIONS

ADCC	Antibody-Dependent Cellular Cytotoxicity
Ag332	Antigen 332
BSA	Bovine Serum Albumin
°C	Degree Centigrade
CRP	C-Reactive Protein
CSP	Circumsporozoite Protein
CTL	Cytotoxic T-Lymphocytes
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diaminetraacetic Acid
(EENV)6	A synthetic Peptide from the C-terminal repeat region of the PF155 molecule.
ELISA	Enzyme-Linked Immunosorbent Assay
EMIF	Erythrocyte Membrane Immunofluorescence
FSV-1	Falciparum Sporozoite Vaccine -1
gp	Glycoprotein
G6PD	Glucose-6-Phosphate Dehydrogenase
GTTC	Government Technical College IgboOra
HLA	Human Leucocyte Antigen
IFN	Interferon
Ig	Immunoglobulin

IgA	Immunoglobulin A
IgD	Immunoglobulin D
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
Kd	Kilodalton
Kg	Kilogram
LJS	Synthetic Peptide of a non-repeated sequence (MQTLWDEIMINKRK) from the N-terminal of the Pf155 molecule.
MAP2	Synthetic Peptide made up of 8 branches of the 11 amino acid repeat (SVTEEIAEEDK) from Ag332 coupled to an oligo-lysine backbone.
MHC	Major Histocompatibility Complex
Mr	Relative Molecular Mass
MSP1	Merozoite Surface Protein 1
$(NANP)_6$	Synthetic Peptide (Asparagine-Alanine-Asparagine-Proline)$_6$ from the CSP repeat region
Nk	Natural Killer Cell
NO	Nitric Oxide

OD	Optical Density
Pf	*Plasmodium falciparum*
Pf155	Antigen located in the membrane of red cells infected with ring forms of *Plasmodium falciparum* of molecular weight 155Kd
Pf195	*Plasmodium falciparum* antigen of molecular weight 195Kd (Merozoite surface antigen)
Pf EMP1	*Plasmodium falciparum* Erythrocyte Membrane Protein 1
Pf HRP1	*Plasmodium falciparum* Histidine Rich Protein 1
Pf HRP2	*Plasmodium falciparum* Histidine Rich Protein 2
PARIF	Parasite Immunofluorescence
PCV	Packed Cell Volume
PMN	Polymorphonuclear leucocytes
RESA	Ring-infected Erythrocyte Surface Antigen also known as Pf155
ROI	Reactive Oxygen Intermediates
SSP2	Sporozoite Surface Protein 2
TBH	Tris-Buffered Hank's solution
TNF	Tumour Necrosis Factor
U.C.H	University College Hospital, Ibadan
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Malaria a disease rooted in antiquity, remains the most important of the tropical diseases. Malaria parasites are transmitted from infected people to susceptible people by the bite of female mosquitoes of the genus Anopheles and in some rare cases, congenitally through the placenta (Airede, 1991), and also by blood transfusion from an infected blood donor (Guerrero et al., 1983). It was reported by WHO (1990a) that 267 million people are infected with malaria, with 107 million clinical cases annually affecting 103 countries while 2100 million people are considered at risk of being infected. Malaria is estimated to be responsible for the deaths of over 1 million children in Africa annually, the majority of them being children less than five years of age (WHO, 1990a). Greenwood et al. (1987) identified malaria as the probable cause of 4% of infant deaths and of 25% of deaths in children aged 1 to 4 years in The Gambia.

In malaria endemic areas, where falciparum malaria is holo- or hyper-endemic, children below 5 years of age and pregnant women are more vulnerable to the disease than are other age groups (WHO, 1974). The morbidity and mortality caused by this parasitic infection in young children living in malaria endemic areas, is in sharp contrast to the almost lack of patent parasitaemias in African infants during the first few weeks

of life (Nardin et al., 1981). The protection of the newborn against the malaria parasite has been attributed to various non-immunological malariostatic mechanisms such as the milk diet deficient in p-aminobenzoic acid (Hawking, 1965), haematological factors such as an ageing red cell population and the presence of erythrocytic foetal haemoglobin (Wilson et al., 1977; Pasvol and Wilson, 1982), and selective biting by mosquitoes among different age groups (Muirhead-Thompson, 1951).

The single most important factor thought to be responsible for the specific resistance to malaria parasites by infants is the presence in neonatal blood of anti-malarial antibodies transferred across the placenta (Bruce-Chwatt, 1952; Gilles and McGregor, 1959; Biggar et al., 1980; Nardin et al., 1981). Such antibodies have been documented in both maternal and cord blood and are essentially immunoglobulin G (Ibeziako et al., 1980). Moreover, the gammaglobulin fraction of cord serum when administered to acutely infected children has been shown to reduce parasitaemia (Edozien et al., 1962) and to inhibit malaria parasite growth *in vitro* (Chizzolini et al., 1991). This serological inheritance provides partial protection during the first few weeks of life and forms a biological shield under the protection of which the child can start raising its individually acquired immunity.

In malaria endemic areas, infants under 6 months of age rarely contract the disease. However, after six months of age unprotected infants suffer repeated and severe attacks that become milder with time as they

grow, due to acquired immunity. By the age of 5 years immunoprotection is reflected by the decrease in the clinical manifestations of the disease despite the dense evident parasitaemia, and later by the decrease in the mean parasite density with age (McGregor, 1986).

Although it was exceptionally rare for an infant in a malaria endemic area to contract the disease before the age of 6 months, this is now being observed (Tijani and Adeleye, Personal communication). Whether this observation is due to the emergence of chloroquine resistant malaria, genetical differences that renders some individuals more susceptible to infection, increased transmission intensity or some innate factors remains to be elucidated. One important factor that warrants investigation is the use of chemoprophylaxis in pregnancy to alleviate the morbidity experienced by pregnant women, following recommendations from previous field studies (WHO, 1986b; Greenwood et al., 1989). Studies conducted in The Gambia (Voller and Wilson, 1964), Uganda (Harland et al., 1975) and Nigeria (Bradley-Moore et al., 1985b) however, showed that chemoprophylaxis exerts an immunosuppressive effect on the humoral immune response to crude malaria antigens. Chemoprophylaxis in pregnancy is being recently embraced by our primary health care system. Chemoprophylaxis lowers the humoral immune response to crude malaria antigens and African newborns partially depend on transplacentally acquired malaria antibodies for protection during the first weeks of life. Hypothetically it therefore follows that chemoprophylaxis in pregnancy will lower the level of

transplacental malaria antibodies and consequently hasten the duration of onset of clinical malaria in the infant.

Protection of the African newborn has also been linked with innate factors and malarial control measures. It has been reported that foetal hemoglobin provides a less suitable environment for the development of human plasmodia (Allison, 1954; Gilles, 1957, Friedman and Trager, 1981). Sickle cell heterozygotes in malarious zones have been known to be relatively protected against malaria (Wilson et al., 1977; Friedman and Trager, 1981). In addition, Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency in heterozygous state also confers a powerful protection against *P. falciparum* malaria, which accounts for their high frequency in nearly all parts of the world where malaria is or has been common (Bienzle et al., 1972; Usanga and Luzatto, 1985; WHO, 1989).

The protective role of these innate factors have been investigated individually in children and adults. There is need to investigate the extent and possible synergistic protective role of these innate factors especially with regards to malaria in infants.

Another innate factor worthy of consideration in the evaluation of protection against malaria are the erythrocyte sialoglycoproteins. It has been established that merozoite invasion of red blood cells occurs through its binding to the erythrocyte membrane sialoglycoprotein receptors, and genetic variants of these glycoproteins differentially resist merozoite invasion (Pasvol et al., 1982a,b; Facer, 1983; Pasvol et al., 1984; Mitchell et al., 1986). It is also known that during merozoite

invasion of red cells, a parasite protein of Mr 155-kd is deposited in the erythrocyte membrane (Perlmann et al., 1984). Hypothetically, it would be expected that individuals deficient in any of the sialoglycoproteins would be relatively protected against malaria and their levels of antibodies to the Mr 155-kd parasite protein would be lower compared to individuals with normal sialoglycoproteins. An investigation of the frequency of genetic polymorphism of the erythrocyte sialoglycoproteins and its correlation with protection from malaria may shed some light on the possibility of an evolutionary selection pressure on the genetic variants that protects inhabitants in malarious areas just as is the case with the sickle cell trait.

For several decades treatment and control of malaria especially that caused by *P. falciparum* have been unsatisfactory in many areas, partly because of the growing problems of drug resistance of both the parasites and the mosquito vectors. Following this development, attention has been focussed on immunoprophylaxis as a possible solution to control the malaria scourge.

A major achievement in the search of a malaria vaccine has been the identification of some plasmodial antigens accessible to the immune system of the host and capable of inducing protective immunity. The structure of the immunodominant epitopes of these antigens has been defined, thus opening the way to the development of malaria vaccines using chemically synthesized or genetically engineered molecules (Miller et al., 1986). These antigens envisaged as potential malarial vaccines

represent a powerful tool for the dissection of the specific immune response of the host to the parasite. Prominent amongst these antigens is the circumsporozoite protein (CSP), a single polypeptide with repeat and non-repeat regions which covers the surface of the sporozoite. Antibodies to the CSP immunodominant repeat region has been demonstrated in the sera of immune individuals using both recombinant-R32tet32 (Hoffman et al., 1986) and synthetic - $(NANP)_n$ peptides (Chizzolini et al., 1988). Seroepidemiologic studies conducted in Indonesia (Hoffman et al., 1986), Tanzania (Del Giudice et al., 1987), Kenya (Campbell et al., 1987) and The Gambia (Snow et al., 1989) demonstrated that anti-CSP specific antibodies increase with age and may contribute to immune protection against malaria in humans. On the contrary Marsh et al. (1988) in The Gambia and Burkot et al. (1989) in Papua New Guinea reported that the humoral immune response to the CSP repeat region (NANP) does not play a major role in the development of immunity to clinical malaria in the population studied.

The ring-infected erythrocyte surface antigen (RESA) also known as Pf155, located in the membrane of erythrocytes infected with ring-forms of *P. falciparum* (Perlmann et al., 1984), is one of the malaria candidate vaccines under investigation. Anti-Pf155/RESA antibodies have been reported to inhibit parasite growth *in vitro* (Wahlin et al., 1984). In epidemiologic studies, anti-Pf155/RESA antibodies increase with age and transmission (Wahlgren et al., 1986; Marsh et al., 1989; Chizzolini et al., 1989; Deloron and Coz, 1990) except in early childhood

(Bjorkman et al., 1991, Hogh et al., 1991), and may be related to the acquisition of protective immunity. However, there are conflicting reports as regards the protective role of anti-Pf 155/RESA antibodies. In Liberia, Petersen et al. (1990) and Bjorkman et al. (1991) reported some correlations between anti-Pf 155/RESA antibodies and lower parasitaemia. On the contrary, Marsh et al. (1989) in The Gambia and Bjorkman et al. (1990) in Liberia reported that there was no correlation between anti-Pf 155 antibodies and protection against malaria

It is evident from the various seroepidemiologic investigations aimed at ascertaining the protective potentials of the CSP and Pf155/RESA antigens that, the factors governing the acquisition of antibodies to these two malaria candidate vaccines are still inadequately understood given the inconsistencies in their findings. Consequently there is need to carry out more field surveys so as to clearly define the functional relation between antibodies to these defined antigens and protection against malaria.

So far only two cross-sectional studies have been conducted in Nigeria pertaining to the protective role of the above two malaria candidate vaccines. Williams et al. (1987) reported that there was no correlation between anti-$(NANP)_{40}$ and anti-Pf 155/RESA antibodies in a large population of Nigerians. Achidi (1989) in a cross-sectional study observed an age progressive increase in anti-Pf155/RESA seropositivity with a corresponding reduction in parasite prevalence rate. However, there was no sufficient evidence to suggest that anti-Pf155 antibodies

protected against clinical malaria. There is need therefore to conduct more field studies preferably executed longitudinally involving cohorts so as to ascertain the protective role of these antigens and to examine whether seasonal variation affects seroprevalence to the CSP and Pf155/RESA antigens.

Seroepidemiological studies provide useful information on the endemicity and transmission rates and the success of malaria control projects (WHO, 1974) which is vital for planning of appropriate public health measures. It is also considered necessary in identifying non-immune individuals from endemic areas and in the selection of test populations for early vaccine field trials.

There are previous seroepidemiological data mainly from a few specific study areas in Nigeria. This includes the Malumfashi area in the Northern Savannah where epidemiological studies were undertaken during the early 1980's (Williamson and Gilles, 1978; Gilles et al., 1983). These data indicated holoendemic transmission of malaria with significant seasonal variation. Main malaria vectors were identified as *Anopheles gambiae* and *A. funestus* . In Igbo-Ora, Oyo State, there are some data indicating meso to hyperendemicity with perennial transmission although marked seasonal variation was observed. The species found were *P. falciparum* (about 90%), *P. malariae* (5-8%) and *P. ovale* (2%); *Anopheles gambiae* and *A. funestus* were main vectors (Lawrence, 1965). Voller and Bruce-Chwatt (1968) in a seroepidemiological survey in Northern Nigeria reported a seropositivity

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rate of 92% in all sera tested. In a study of the epidemiology and control of malaria, conducted in the Garki District of Kano State, Voller et al. (1980) measured malaria antibody levels by the ELISA technique in two different populations, one exposed to intense malaria transmission and the other protected. They observed an increase in ELISA values with age in the unprotected population reflecting the development of immunity to malaria. Malaria control activities reduced ELISA values in the protected population.

All the above seroepidemiological studies were carried out when the various malaria candidate vaccines had not been fully characterized and consequently there were no adaptable field techniques to measure their seroprevalence. Antibodies directed to the total blood stage antigens are known as non-reliable indicators of protective humoral immunity (Voller and Bruce-Chwatt, 1968; Achidi, 1989; Marsh et al., 1989). However, with the recent elucidation of the fine structure of many plasmodial antigens envisaged as potential malarial vaccines, it is relevant to use these defined antigens to dissect the specific immune response of individuals inhabiting malarious areas. Information from such a study will shed more light on the protective role of malaria candidate vaccines and also provide baseline epidemiological data for future vaccine field trials.

To advance the progress towards the development of a malaria vaccine and to help measure its potential impact, field studies are needed to clearly define the mechanisms involved in the development and

maintenance of naturally acquired immunity to envisaged malaria candidate vaccines. Cross-sectional studies in which comparisons are made between different age groups within a community has a role to play in identifying factors worth further investigation as possible indicators of protective immunity. However, it does not permit analysis of individual responses to infection. Longitudinal field studies allow a better assessment of protection and of its relationship in the immune response to putative protective antigens. In general, the follow-up of a community submitted to natural conditions of exposure to malaria represents a better means to investigate the variations of the immune response of the individuals in relation to a wide variety of environmental changes.

Since infants and young children account for the highest morbidity and mortality rates from malaria in endemic areas, studies on the passive transfer of maternal immunity and the development of immunity to malaria are of great importance in vaccine developments. It is not clear whether malaria vaccination would be suitable and effective when applied early in life. This consideration is relevant as it is known that maternally acquired antibodies inhibit the immunological process induced by vaccination against measles, rubella or mumps, when these vaccines are administered before the age of one year (Ajjan, 1988).

1.1 RESEARCH OBJECTIVES

Sequel to previous inconsistent findings (as regards the protective role of antibodies to malaria candidate vaccines) and the recent observations of malaria in infants under six months of age (Spencer et al., 1987), contrary to previous findings (Gilles, 1957; Nardin et al., 1981) this study intends to:

1. Determine the level of transplacental malarial antibodies and its duration of protection against clinical malaria in the infant.
2. Investigate the development of malaria parasitaemia/clinical malaria and the humoral immune response to malaria in infants during the first year of life.
3. Determine the malaria parasite rates/densities and the effect of seasonal variation on antibody levels to some defined *Plasmodium falciparum* antigens in an adult study population.
4. Investigate the existence of a possible relationship between antibodies to some defined *P. falciparum* antigens and protection from malaria.

The objectives of this study would be achieved as follows:

1 Passive Transfer of Malaria Immunity

The level of transplacental transfer of malaria immunity would be determined using maternal/cord paired samples. The level of total immunoglobulin isotypes (IgG, IgM and IgA) would be estimated.

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Antibodies to the total blood stage antigens would be measured. Specific antibodies to the Pf 155/RESA, MAP2 and the immunodominant repeat regions of the CSP and Pf155/RESA antigens would be assayed.

Results from these investigations would provide baseline information on the level of transfer of malarial antibodies from mother to infant. This information would be required to arrive at a conclusion whether chemoprophylaxis in pregnancy has any effect on the level of transplacental malarial antibodies as previously suggested and whether the level of such antibodies at birth has any effect on the duration of onset of clinical malaria in the infant.

2. The Development of Malarial Antibodies to *P. falciparum* Antigens.

The ontogeny of malarial antibodies in the study infants would be investigated by closely monitoring these infants from birth till one year of age, during which time serial blood samples would be collected at bi-monthly intervals. Infants would be regularly screened for malaria parasites and episodes of clinical malaria recorded. Analysis of total immunoglobin isotypes and malarial antibody levels to the above test antigens (total blood stage antigens, CSP, MAP2 and Pf155/RESA antigens) would be carried out.

Results from these investigations would determine the duration of onset of clinical malaria in the study infants and help authenticate the recent observation of malaria in infants under 6 months of age by some

clinicians. It would also provide useful information on the seroconversion period of the study infants which will represent when maternally derived antibodies are on the wane and the child is therefore exposed to malaria parasite attacks.

3. Humoral Immune Response to *P. falciparum* Antigens and Protection.

To investigate the humoral immune response to the above test antigens and the existence of a possible relation between antigen-specific antibodies and protection against malaria, two cross- sectional surveys would be carried out (during the rainy and dry seasons respectively) involving the adult study subjects. The mothers of the study infants would be sampled along with their infants. Analysis of total Ig isotypes and malarial antibodies to the above test antigens would be carried out as previously discussed.

Results from these investigations would establish adult parasite rates/densities and seropositivity rates to test malarial antigens. Using malaria parasite rates/densities and antigen-specific antibody levels, an attempt would be made to find out if there is any correlation between antibody levels and protection from malaria. It would be possible to establish whether seasonal variation has any effect on seropositivity rates to the test antigens.

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Results from these investigations would establish adult parasite rates/densities and seropositivity rates to test malarial antigens. Using malaria parasite rates/densities and antigen-specific antibody levels, an attempt would be made to find out if there is any correlation between antibody levels and protection from malaria. It would be possible to establish whether seasonal variation has any effect on seropositivity rates to the test antigens.

4. Innate Protective Factors and Artificial Malaria Control Measures.

In the assessment of the protective role of malaria specific antibodies, it is desirable that study subjects be characterized for any likely confounding factors. These include innate factors known to protect against malaria morbidity and/or affect the development of malaria antibodies. For the purpose of this study the following confounding factors would be determined:

a. Innate factors: (i) haemoglobin genotype
(ii) MNSsU(Ge) blood group

b. Artificial control measures- use of chemoprophylaxis.

CHAPTER TWO

2.0 LITERATURE REVIEW

Malaria is caused by single-celled protozoan parasites of the genus Plasmodium. Human malaria is identified with four Plasmodium species - widespread throughout the tropics and also in some temperate zones as shown in Fig. 2.1. They include: *Plasmodium falciparum* (malignant tertian malaria), *P. vivax* (benign tertian malaria), *P. malariae* (quartan malaria) and *P. ovale* (ovale tertian malaria).

2.1 Life Cycle

The life cycle of the malaria parasite is essentially similar in all species of plasmodia and involves two hosts: invertebrate (sexual phase) and vertebrate (asexual phase).

2.1.1 The Life Cycle in the Mosquito (Sporogony)

Blood ingested by a mosquito from an infected individual may contain asexual stages in addition to sexually differentiated gametocytes. The former are digested in the midgut of the mosquito. The gametocytes undergo gametogenesis in the lumen of the stomach resulting in their transformation into large macrogametes and thread-like microgametes as shown in Fig. 2.2. These gametes fuse in fertilization to produce a motionless zygote. After 18-24 hours the zygote elongates and becomes mobile forming an ookinete which migrates through the stomach wall and rounds up to form an oocyst lying between the columnar

Fig. 2.1. Approximate Distribution of Malarious Regions (▨). (WHO, 1990a).

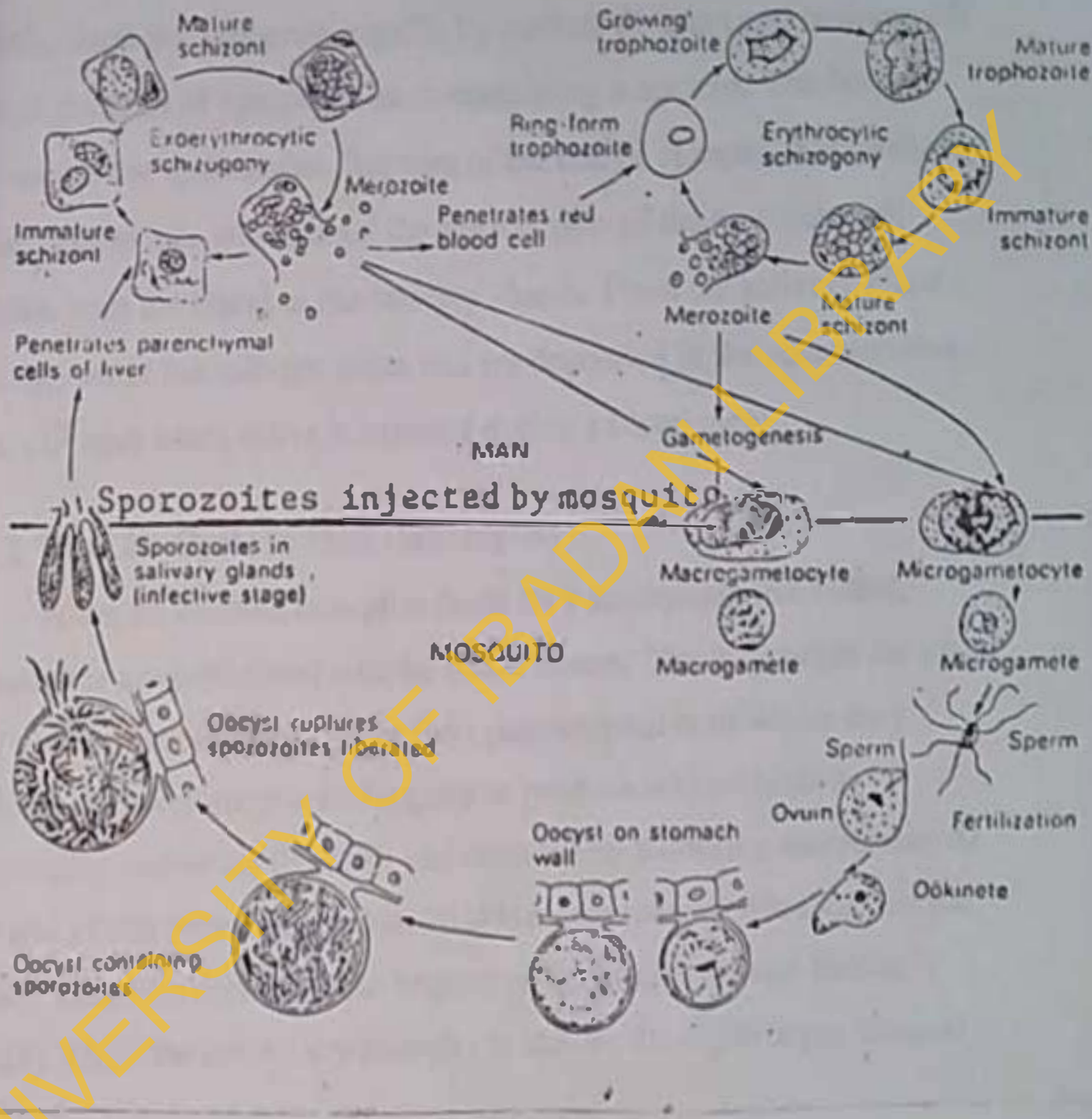

Fig. 2.2. Life cycle of the malaria parasite in man (Deans and Cohen, 1983).

epithelium and the elastic membrane covering the outside surface of the stomach, these oocysts grow rapidly by nuclear division and budding off of small portions of cytoplasm each containing a nucleus. The budded portions are the sporozoites. Rupture of the oocyst releases thousands of mobile sporozoites which enter the haemocoele of the mosquito and circulate with the blood to the salivary glands. From the salivary gland cells they enter the salivary ducts and are deposited in the subcutaneous tissues of man when saliva is injected during a blood meal.

2.1. 2 The Life Cycle in Man (Schizogony)

When an infected mosquito feeds on a susceptible individual, sporozoites are introduced into the blood sueam. These circulate for about half an hour before invading the liver parenchymal cells where they undergo exo-erythrocytic schizogony to produce schizonts each developing within the liver cell and bounded by a limiting membrane. At the end of this period, more than 20,000 merozoites are released into the blood stream by rupture of the hepatocytes (Hockmeyer and Ballou, 1988). These invade the erythrocytes to initiate the erythrocytic asexual phase.

The parasite begins as tiny rings and develops into trophozoites which consist of a cytoplasm and a nucleus. As the parasite grows it becomes actively amoeboid and granules of a brown pigment called haemozoin, formed from haematin and denatured protein, appear in the cytoplasm. The trophozoites mature into schizonts. When schizont-infected erythrocytes ruptured many new merozoites are released each

capable of invading another erythrocyte. It is this cycle of invasion, multiplication and reinvasion of erythrocytes that results in the disease that is clinically recognized as malaria. However, some of the ring-forms develop into gametocytes (male and female).

2.2 Invasion of Erythrocytes by Malaria Parasites

Research findings suggests that *P. falciparum* merozoites recognize and attach to clusters of carbohydrates on the surface of most erythrocytes by means of lectin-like bonds (Pasvol et al., 1982; Facer, 1983). These discoveries suggest the possibility that erythrocyte receptors for *P. falciparum* might involve a specific family of molecules, the glycophorins, shown in Fig. 2.3. Blockage of receptor-ligand interaction, might prevent the survival of the blood-stage malaria parasite, which is an obligate intracelular parasite. Hadley et al. (1986a) suggested that malaria parasite receptors can be used as immunogens to induce antibodies that block merozoite invasion of red cells. However, it is not known whether anti-receptor antibodies are important in the acquisition of naturally acquired immunity.

2.2.1 Morphologic Studies on Invasion

Initial attachment of a merozoite to an erythrocyte occurs between any two points on the surface of the merozoite and erythrocyte (Jungery, 1985). Non-specific electrostatic forces might provide adhesiveness during this initial contact (Pasvol et al., 1984). After initial attachment, the merozoite reorients itself so that its apical end is in apposition to the

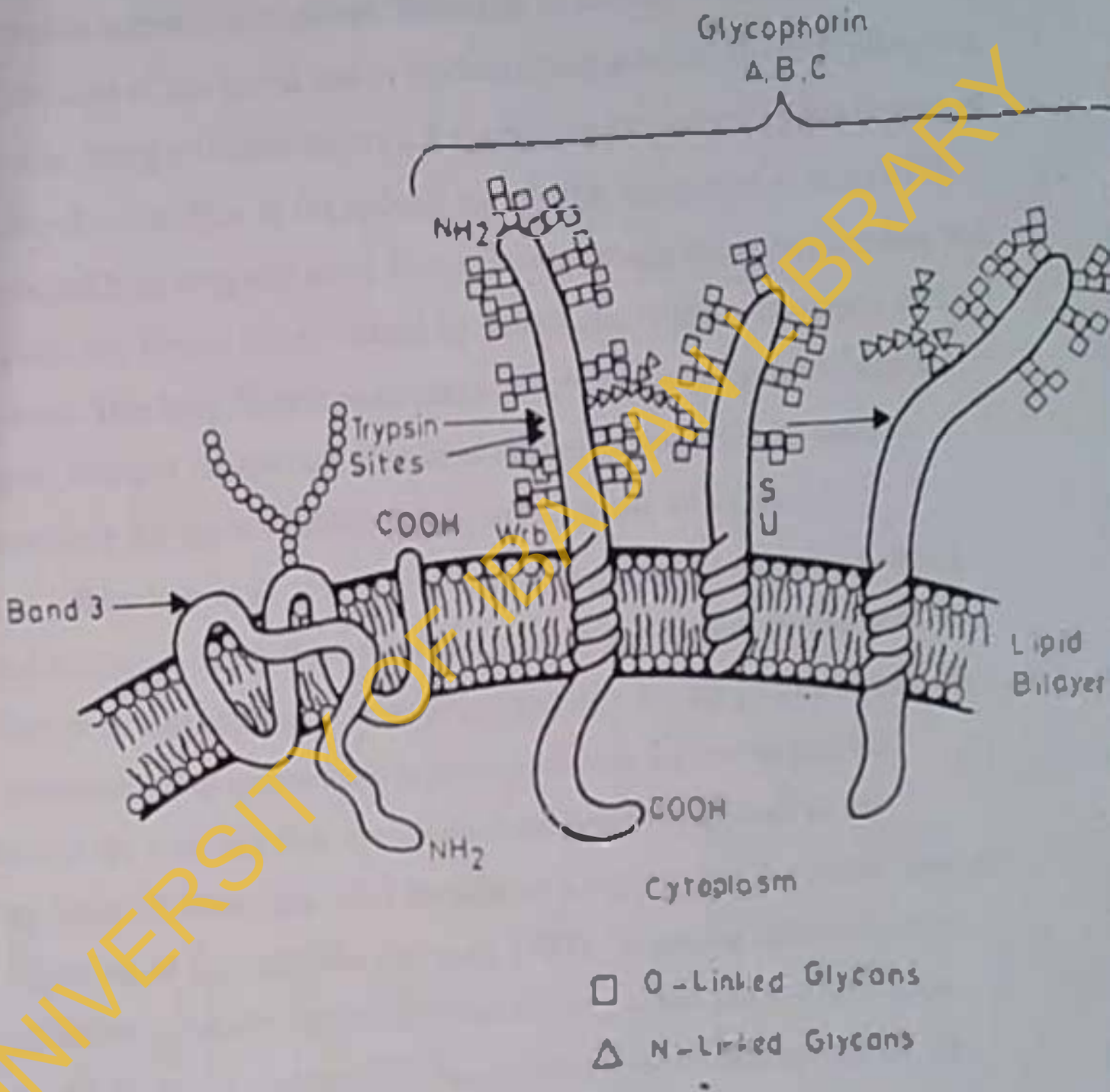

Fig. 2.3. Schematic diagram of the red cell glycophorins (Jungery, 1985).

erythrocyte. The apical end of the merozoite is characterized by an apical prominence, a pair of apical organelles termed rhoptries and adjacent organelles termed micronemes. Bannister et al. (1977) observed that the surface coat of the apical end of the merozoite attaches to the erythrocyte and that strong adhesive forces are exerted, as evidenced by the degree of spasmodic distortion in the red cell membrane. Bannister et al. (1977) described both long and short distance connections between parasites and red cell, the former characterized by extensions of the merozoite's surface bristles. This long-distant attachment occurs between the merozoite's apical end and the red cell. Fibrils of the merozoite's bristly surface, especially the thicker fibrils, appear to be the site of attachment.

Bannister et al. (1977) also noticed a pattern of sporadic bending and relaxation along the erythrocyte surface at points of attachment, as though the merozoites were adhering and then letting go in a zipper-like movement. This observation is consistent with the notion that the merozoite connects first to carbohydrate groups (Fig. 2.4.) at the glycophorin N-terminal, and then forms a stronger bond with the internal segments of the molecule (Jungery, 1985). Following deformation on attachment, the erythrocyte membrane invaginates and the merozoite enters the cavity so formed and the erythrocyte membrane reseals. The merozoite then releases the contents of the apical organelles resulting in the formation of the parasitophorous vacuole (Aikawa et al, 1978). Entry of the merozoite into the parasitophorous vacuole is characterized by the movement of the junction (formed at the area of aposition between the

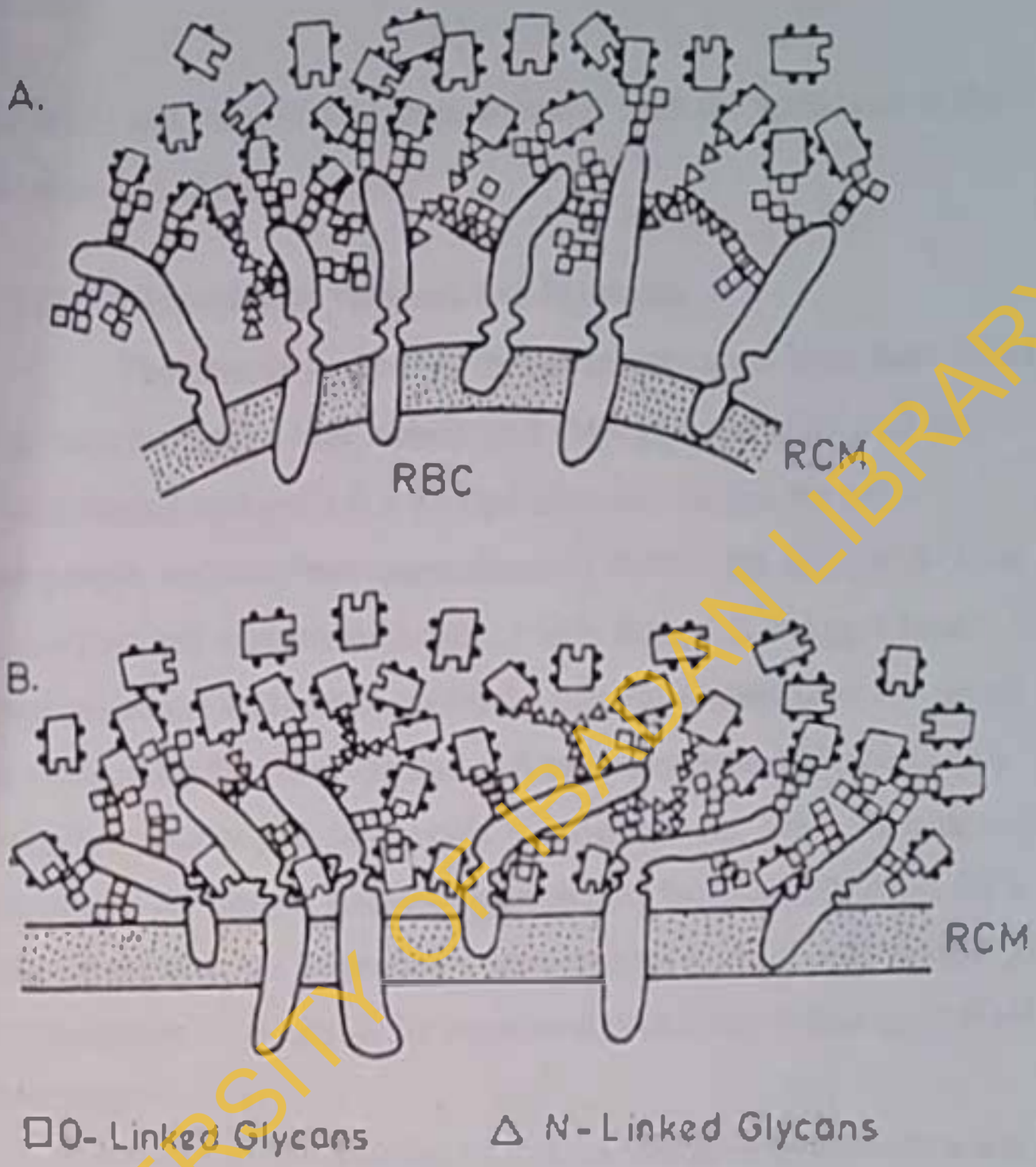

Fig. 2.4 Proposed model of two stage recognition process. Part A: The parasite lectin-like protein binds to carbohydrates on the glycoprotein molecules; Part B: Carbohydrate binding is followed by parasite attachment to epitopes of glycophorin A and/or B and C close to the cell membrane (RCM) (Jungery 1985).

merozoite and the erythtrocyte membrane) from the apical end to the posterior end of the merozoite.

2. 2. 2 Glycophorin Variants and Invasion.

The normal erythrocyte membrane carries at least three distinct glycophorins (gps) which include gpA (MN glycoprotein), gpB (Ss glycoprotein) and gpC ($\beta + \gamma$ - glycoprotein). GpA is the major component and contributes approximately 60% of the sialic acid of the normal red cell membrane (Anstee, 1980). Both gpB and gpA have identical amino acid sequences for the first 26 residues from the amino terminus, with the exception that gpA expresses M and N blood group antigens, whereas gpB carries only N antigen (Issitt, 1981). Genetic variants of erythrocyte sialoglycoprotein have been described and these include erythrocytes lacking gpA [homozygous En (a-)] cells, normal gpB (homozygous S-s-) cells or the absence of both A and B (the rare M^kM^k homozygous cells).

Previous studies with En(a-) erythrocytes gave invasion rates that were 50% (Miller et al., 1977), 37% (Facer, 1983) and 10% (Pasvol et al., 1982 a, b) of those obtained with normal erythrocytes. Perkins (1981) reported a 90% or greater reduction rate. En(a-) cells have increased glycosylation of band 3 and an overall decrease in sialic acid (important for optimal invasion) and lack the Wright (Wr^b) antigen (Anstee, 1981).

Pasvol et al. (1982 b) showed that invasion of S-s-U- (gp B deficient) cell was 28% less than that in control cells. Facer (1983) reported a significant reduction in invasion of S-s- homozygous variant

cells. The U antigen resides close to the erythrocyte membrane (see Fig. 2.3) on gpB normal erythrocytes, and this portion of gpB is not involved in invasion (Facer, 1983). However, the above findings indicate that gpB as present in normal erythrocytes, may also carry determinants necessary for recognition.

Pasvol et al. (1984) reported that the extent of invasion of *P. falciparum* into gpC-deficient cells is on the average 57% of that of normal human red cells. Trypsin treatment of normal red cells reduced invasion to about 34% of the control while treatment with gpC-deficient cells reduced invasion from 57% to 22%. GpC carries similar oligosaccharides to those found on A and B. This suggests that the oligosaccharide components of gpC may play a role in the initial binding between red cell and merozoite.

Since the antigens on the gps exhibit a high degree of polymorphism with some variants resisting merozoite invasion of erythrocytes, it is worthwhile investigating in this environment the frequency of occurence of these variants in a cross-sectional study and the possibility of relative protection from malaria through a longitudinal study. Individuals deficient in some of these gp antigens would be expected to have lower antibodies to the Pf 155/RESA antigen since this antigen is deposited in the erythrocyte membrane during merozoite invasion.

2.3 Specific Cellular Acquired Immunity

2.3.1 T - Cell Numbers

In man, numerous studies have reported alterations in the proportion of peripheral T and B cells during malaria infection. Wyler (1976) reported that both the percentage and concentration of T cells were decreased in malaria while the percentage but not concentration of B cells was increased. Both the percentage and concentration of 'null' cells were increased in malaria. They suggested that these alterations may be due to sequestration of T cells in the spleen or other organs.

Ade-Serrano and Osunkoya (1977) in a study of Nigerian children with acute malaria observed a marked fall in the differential and absolute counts of T cells but little or no change in B cell numbers. They suggested that the apparent decrease in circulating T cells was due to T cell mobilisation (to extravascular sites) necessary for effective host resistance against infection. Wells et al. (1979) in a study of Thai adults with malaria reported a decrease in both percentage and concentration of T cells, increased percentage but not concentration of B cells and an increase in the 'null' cell percentage but a decrease in the absolute number of null cells.

Salimonu and Akinyemi (1986) in a study of Nigerian children aged 2-10 years with acute malaria confirmed previous findings of depressed T - lymphocyte numbers. They observed normal levels of B cells. They suggested that the decrease in T-lymphocyte numbers may be due to sequestration of this lymphocyte subpopulation in specific areas of some

lymphoid organs. It has been reported recently that both the percentage and total number of γ/δ T-cells increase significantly in acute malaria infection (Ho et al., 1990). It has been suggested that γ/δ T-cells may function *in vivo* by inhibiting the development of the parasite's liver stages (Tsuji et al., 1993).

In all these studies, a significant decrease in both percentages and total numbers of circulating T cells was reported. Phenotyping of T cell subsets has revealed a decrease in the helper/inducer subsets and, in some studies, also of the cytotoxic/suppressor subsets (Ho et al., 1986; Merino et al., 1986). The clinical significance of these changes is unknown, but the general decrease in T cell pool has been postulated to be due to the effect of lymphocytotoxic antibodies which preferentially reacts with T cells (Merino et al., 1986).

2.3.2 Cell - Mediated Immunity

Accumulating evidence suggests that immunity to malaria is mediated by additional mechanisms which can act in concert with or independently of protective antibodies. Observations supporting this concept include: (a) the inability of sera from immune host to transfer protection (Jayawardena et al., 1978); (b) the diminished effectiveness of immune sera transferred to splenectomized or T cell deprived recipients to protect against infection (Brown and Phillips, 1974); (c) the ability of B cell-deficient hosts to spontaneously resolve malarial infections or to resist

infection (Weidanz and Grun, 1983) and (c) the adoptive transfer of immunity to malaria with T cells but not B cells (Cavacini et al., 1986).

Cytotoxic T Lymphocytes (CTL) of the $CD4^+$ and $CD8^+$ phenotype have been implicated in immunity to the hepatic stages of the malaria parasite. This is because CTL recognize T epitopes in association with class I major histocompatibility gene products present on the surface of most nucleated cells. CTL plays no role in immunity against the erythrocytic stages since human erythrocytes do not express class I MHC antigens.

Greenwood et al. (1977) also Brown and Smalley (1980) described increased nonspecific and specific antibody-dependent cellular cytotoxicity (ADCC) in malarious Gambians. Erythrocytes infected with mature malarial parasites carry surface plasmodial antigens and may therefore be destroyed by ADCC. The nature of the effector cells in ADCC include killer cells, monocytes and polymorphonuclear neutrophil leucocytes (Greenwood et al., 1977; McDonald and Phillip, 1978).

Gilbreath et al. (1983) observed a significantly impaired lectin-induced cellular cytotoxicity and spontaneous cell-mediated cytotoxicity in malarious subjects. No change in ADCC was observed. They concluded that malaria patients have defective T cell and natural killer cell cytotoxicity capabilities but do not exhibit defective killer cell function. Riley et al. (1989) reported that cellular immune response to malarial antigens are suppressed during acute falciparum malaria, suggesting that parasite derived factors may be directly immunosuppressive.

Results from previous studies suggest that the major pathway of cell-mediated immunity in plasmodial infection involves the release from antigen-activated T cells of lymphokines such as IFN-γ and interleukin I, which then stimulate cells of other cell systems (e.g., the mononuclear phagocytic cell system) to exert antiparasitic effects.

2.3.3 Serum Inhibitory Substances to Cellular Immunity in Malaria

In a study of Nigerian children from Zaria, Greenwood et al. (1972) observed a selective form of immunosuppression during acute *P. falciparum* infections. Children with acute malaria showed a diminished antibody response to the H-antigen of *Salmonella typhi* and their cellular immune response was normal. In Ibadan Nigeria, Osunkoya et al. (1972) reported a significantly higher "spontaneous" transformation to blast cells *in vitro*, of lymphocytes from malarious children compared with controls. They suggested that the blast transformation observed may be due to lymphocyte activation by immune complexes.

Moore et al. (1977) observed that in several cases of malaria and protein energy malnutrition (PEM), the ability of lymphocytes to transform was depressed in autologous plasma. Tests of two malaria plasma indicated that depresssion was due to inhibition rather than lack of essential nutrients. They suggested that plasma inhibitors induced during an acute attack of malaria interfere with the development, or expression of an effective protective immunity.

Plasma inhibitors of lymphocyte transformation occur in a number of diseases (Whittaker et al., 1971; Kumar and Taylor, 1973; Heyworth et al., 1975). Whittle et al. (1978) in a study of Nigerian children with acute measles from Zaria observed that depletion of T cells, an inhibitor of lymphocyte proliferation and possible defect in antigen processing, interacts to depress cell-mediated immunity in measles. Salimonu et al. (1982) demonstrated that sera from malnourished children inhibit sheep erythrocyte rosette formation by lymphocytes. The sera of malnourished children having inhibitory substance did not inhibit autologous lymphocytes whereas the E rosette formation of homologous lymphocytes were inhibited. They suggested that the E rosette inhibitory substance(s) present in the blood of some malnourished children either sterically hinders or cross reacts with the T cell receptor *in vivo* and *in vitro*, thus reducing the proportion of T cells that can form E rosettes in sheep erythrocytes *in vitro*. They postulated that the inhibitory substance is likely to be either soluble immune complexes, endotoxin or α_2-macroglobulin.

Salimonu et al. (1986) demonstrated that the presence of E rosette inhibitory substance in patients with acute malaria, measles and kwashiorkor. They also observed that most of the patients who had soluble immune complex levels >52mg/dL had serum E rosette inhibitory substances in their sera and they invariably had low percentages of E-rosettes. Children infected with malaria or measles had low levels of

circulating E rosetting lymphocytes; detectable serum E rosette inhibitory substance, and elevated levels of circulating soluble immune complexes.

2.3.4 The Role of T - Cells In Humoral Immune Response to Malaria

Existing evidence suggests that T cells function as helper cells in the production of protective antibodies. Good et al. (1987b) observed that T cell stimulation with selected constructs containing T cell epitopes of the circumsporozoite protein of *P. falciparum* allowed such mice to produce IgG antibodies following challenge. Troye-Blomberg and Perlmann (1988) exposed T cells to *P. falciparum* antigen preparations. They observed that at very low doses, these antigens induced IgG secretion in autologous B cells, whereas the control antigen did not. Exposure of B cells in the absence of T cells gave no increment in IgG secretion, indicating that it was T helper-dependent. In control lymphocytes the antigens induced no immunoglobulin secretion. Very little IgM was found in the *P. falciparum* -exposed cultures. They postulated that IFN-γ increases the expression of MHC class II antigens on antigen-presenting cells which is important for the triggering of T helper cells.

Specific T-dependent B cell activation can be induced in patients with acute malaria, in whom antigen-induced T cell proliferation and interleukin-2 production may be aborted or suppressed (Troye-Blomberg et al., 1985). T cells which mediate protection against the erythrocytic

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stages of rodent malaria parasites are of the helper/inducer (L3T4) phenotype (Cavacini et al., 1986) and, thus, may provide help for antibody production. Antibody may then be necessary for the host to survive acute infection and to clear the blood of parasites during chronic infection.

2.4 Specific Humoral Immunity

2.4.1 Immunoglobulins

West African adults living in hyperendemic malarial areas show elevated levels of IgG and IgM. In these clinically immune subjects the rate of albumin synthesis is similar to that in normal Europeans, but IgG production is almost seven times greater (Cohen and McGregor, 1963). However, much of this antibody response is non-specific since only about 5% of the total IgG in immune serum reacts with plasmodial antigens, and still less is protective (Cohen and Butcher, 1969).

Specific malarial antibody activity has been demonstrated in the IgG, IgM and IgA fractions of immune human sera (Taylor, 1989) but not as yet in the IgD class. Recently antimalarial IgE has been detected in human malaria immune sera (Desowitz, 1989; Desowitz et al., 1993; Perlmann et al., 1994).

In malaria immunotherapy the classes of immunoglobulin responsible for the antiparasitic action observed belongs to the IgG_1 and IgG_2 isotypes (Cohen and Lambert, 1982). Some protection was also found in the IgM fraction. Salimonu et al. (1982) reported that malaria-infected Nigerian patients had elevated levels of IgG and IgG_1 subclass

compared to uninfected controls. They observed a slight diminution in the mean IgG_3 concentration in malarious patients. Wahlgren et al. (1983) observed elevated levels of IgG_1 subclass in both Swedish and Liberian malaria immune sera. Liberian immune sera demonstrated higher IgG_3 levels than the Swedish sera while the latter had strikingly high levels of IgG_2 compared to Liberian sera. Perlmann and Cerottini (1979) reported that IgG_1 and IgG_3 mediate phagocytosis or target cell lysis by monocytes whereas IgG_2 and IgG_4 are inactive or less efficient in this respect.

2.4. 2 Malarial Antibodies

Plasmodial infections rapidly induce a large variety of humoral immune responses. While some of these may be protective, others may help the parasite to evade a protective host response (Anders, 1986) or may give rise to immunopathological reactions harmful to the host (Grau et al., 1987). In general the correlation between total antimalarial antibodies and protective immunity is poor, indicating that most of the antibodies formed have no protective effect.

The role of serum antibodies in acquired immunity to malaria was established by passive transfer studies of hyperimmune serum in human and experimental infection (Edozien et al., 1962, Subcharoen et al., 1991). In acute malaria immunotherapy, transferred antibodies failed to completely suppress parasitaemia. This might be explained by insufficient variant-specific antibody to suppress parasitaemia or the absence of an additional mechanism of resistance necessary for expression of immunity (Brown et al., 1986).

Malarial antibodies may exert protection along different pathways including direct prevention of parasite binding to host cell receptors, e.g in sporozoite/liver cell interaction or invasion of erythrocytes by merozoites. However, antibody-mediated cellular cytotoxicity or phagocytosis appear to play a major role in most instances (Khusmith et al., 1982).

It has been suggested that an important function of the immune system in malaria may be the prevention of disease rather than of infection (Playfair et al., 1990). This "anti-disease" immunity may be due to antibodies against soluble malarial "exoantigens" which are by themselves toxic.

2.5 Non-Specific Cellular Immunity

2.5.1 Phagocytosis

Phagocytosis is a prominent feature of malaria. Early workers observed free parasites, parasitized erythrocytes, uninfected erythrocytes, malaria pigment and erythrocyte debris in the macrophages of the spleen, liver and bone marrow of malarious hosts (Brown, 1969).

Sheagaren et al. (1970) observed enhanced phagocytosis during acute malaria. Following treatment and complete recovery, phagocytosis returned to normal. Celada et al. (1983) confirmedprevious findings that immune sera enhances phagocytosis of *P. falciparum* infected red cells by monocytes and polymorphonuclear leucocytes (PMN) *in vitro*.

Phagocytosis and destruction of antibody coated parasites and parasitized cells is regarded as an important immune effector mechanism

in plasmodial infection. Antibody-mediated phagocytosis of infected red cells by monocytes and PMN may be one of the mechanisms involved in the control of malaria infection.

2.5.2 Macrophage Activity

The malarious host responds to circulating parasitized erythrocytes by a dramatic increase in blood monocytes and the accumulation of macrophages in the liver and spleen. The recruitment of these cells and their activation is mediated by lymphokines (such as macrophage chemotactic factor and IFN-γ) secreted by T cells activated by plasmodial mitogens as well as specific malarial antigens (Allison and Eugui, 1983; Weidanz and Long, 1988).

Macrophage activation can also result from phagocytosis of immune complexes, opsonized parasites and debris. Activated macrophages have been shown to release TNFα and IL-1 which induces the production of reactive free radicals responsible for parasite death (Allison and Eugui, 1983, Liew, 1991). For a long time, it was thought that reactive oxygen intermediates (ROI) such as superoxide and hydrogen peroxide were the major parasite killing mechanism. However, recent studies suggest that nitric oxide (NO) derived from L-arginine and molecular oxygen is the principal effector mechanism (Liew, 1991; Li et al., 1992).

2.5.3 Natural Killer Cells

The possible role of natural killer (NK) cells in providing some protection during malarial infection has been proposed (Eugui and Allison, 1980) and later challenged (Wood and Clark, 1982; Skamene et al., 1983). Eugui and Allison (1980) found that mice with low NK activity were more susceptible to *P. chabaudi* infection. Ojo-Amaize et al. (1981) reported raised NK cell levels in malaria infected children compared to controls. Solomon et al. (1985) reported that beige mutant mice, deficient in NK cells, exhibited a significantly higher parasitaemia than the parental C57Bl/6 strain.

It has been proposed that NK cells may participate in immunity to malaria through the lysis of parasitized erythrocytes and perhaps form the first line of defence against the parasite prior to the development of an immune response(Eugui and Allison, 1980; Solomon et al., 1985). Later during the course of infection, IL-2 and IFNγ produced by activated T cells could contribute to the enhancement of the cytolytic activity of NK cells (Braakman et al., 1986) and the appearance of antibodies could promote the ADCC activity of NK cells.

2.6 Nonspecific Humural Immunity

2.6.1 Complement

Neva et al. (1974) reported a fall in complement levels around the time of schizogony in infected humans. Depletion of complement was associated directly with degree of parasitaemia and presence of

complement-fixing antibody. Depletion involved total haemolytic complement and C4 indicating that complement was being utilized via the classical pathway. Greenwood and Bruce-Chwatt (1974) also noted low levels of C3, C4 and C1q in Nigerian children with acute malaria, again suggesting activation via the classical pathway.

Petchclai et al. (1977) observed a considerable reduction in C3, C4 and C6 levels in Thai malarious subjects while C1q, C3PA, C8 and C9 levels were raised. They concluded that activation of complement through the classical pathway occured in most of the malarious subjects while in a few subjects activation of both classical and alternate pathways did occur. Atkinson et al. (1975) described a cyclical pattern of consumption of early components of the classical complement pathway (C1, C4 and C2) associated temporarily with schizont rupture and suggested that the late-acting components are not required for protective host immunity in malaria. Adam et al. (1981) found hypocomplementaemia and raised C3d in cerebral malaria patients. They suggested that complement activation may be an important factor in the pathogenesis of cerebral malaria.

2.6.2 Interferon

The presence of interferon (IFN) in the sera of humans with malaria has been reported on several occassions (Eugui and Allison, 1982, Allison and Eugui, 1983). Administration of sheep anti-mouse alpha and beta IFN antibodies to mice has been reported to accelerate *P. berghei* infection in mice, although infection was usually fatal even in the

presence of IFN. In children, the presence of IFN, correlated with the degree of parasitaemia (Ojo-Amaize et al., 1981).

IFN-γ produced by antigen-or mitogen-activated T cells is also an important regulatory lymphokine. IFN-γ has been shown to be produced by T cells *in vitro* in respose to *P. berghei* sporozoites (Ojo-Amaize et al., 1984), to *P. falciparum* asexual erythrocytic stages (Troye-Blomberg et al., 1985) and to *P. falciparum* sexual stages (Good et al., 1987 a). Troye-Blomberg and Perlmann (1988) found the highest amounts of IFN-γ to be produced by antigen-stimulated T cells from donors who were clinically immune to *P. falciparum*. IFN-γ has by itself no effect on the erythrocytic stages of the parasite. However, in a recent study, Orago and Facer (1993) reported that IFN-γ retards the growth of parasites *in vitro*. It has been suggested that the target of IFN-γ could be the infected hepatocytes (Manheshwari et al., 1988).

IFN-γ is also important for macrophage activation and for the expression of MHC class II antigens on antigen-presenting cells important for triggering of T helper cells.

2.6.3 Transferrin

Transferrin is a β - globulin and decreases in inflammatory processes. Migasena et al. (1978) reported that transferrin levels remained low in malaria patients four weeks after admission to hospital The behaviour of transferrin during inflammation and haemolysis in malaria supports the observation of Klainer et al. (1969) of an intermittently decreased beta glycoprotein peak.

Mcsawe et al. (1974) reported that patients with negative iron status usually have high levels of serum transferrin and are thus protected against infection. They suggested that the protective factor could be transferrin itself. The Plasmodium parasite requires iron. Ravantos-Suarcy et al. (1982) reported that desferrioxamine (DES) inhibits the growth of *P falciparum* at concentrations readily achievable *in vivo*. This observation was confirmed by Pollack and Fleming (1984) and they suggested that the intraerythrocytic parasite obtains iron from transferrin.

It has been observed that in iron-deficiency anaemia, malarial attacks usually develop only after iron therapy (Byles and D'SA 1970; Mesawe et al., 1974). Iron has a critical modulating influence on the structure and function of the lymphoid apparatus and is necessary for cell-mediated immunity and for efficient neutrophil function (Nurse, 1979). Iron overload inhibits the killing and digestion of phagocytosed parasites (Mesawe et al., 1974).

It has been shown that the malaria parasite can synthesize its own transferrin receptors to supply its iron needs as mature red cells have no transferrin receptor. Rodriguez and Jungery (1986) demonstrated that a protein synthesized by the intracellular parasite and inserted into the erythrocyte membrane of mature infected cells, is a receptor for ferrotransferrin. The parasite receptors has a single high-affinity binding site for human ferrotransferrin.

2.6.4 Globulins

Studies on the alteration of globulin levels in malaria patients demonstrated an increase in the α_1- globulin fraction with a concomitant decrease in the level of the α_2-fraction (Klainer et al., 1969; Murphy et al., 1972; Mousa et al., 1973; Migasena et al., 1978). α_1-antitrypsin was identified as a major, but not sole contributor to the α_1-globulin elevation (Murphy et al., 1972). β-globulin levels was intermittently decreased (Klainer et al., 1969) while γ-globulin remained unchanged (Klainer et al., 1969; Mousa et al., 1973). The decrease in β-globulin levels may have been due to a decrease in the level of transferrin (Migasena et al., 1978). The decrease in α_2-globulin was the result of a decrease in serum haptoglobulin secondary to intravascular haemolysis, although an initial rise preceded the fall in some patients (Murphy et al., 1972). It was suggested that serum globulin changes during malaria appeared to result from an initial inflammatory response with an increase of α_1-antitrypsin in all patients and haptoglobulin in some patients, followed by a precipituous fall in serum haptoglobulin once haemolysis occured.

Chiewslip et al. (1988) reported a significant elevation of β_2-microglobulin levels in malarious compared with control subjects. They suggested that the elevation of β_2-microglobulin levels may have been due to polyclonal activation of both T and B cells.

2.6.5 C-Reactive Protein (CRP)

CRP was first said to be present in the blood of malarious patients in 1954 (Muschel and Weatherwax, 1954). Ree (1971) observed that CRP and *P. falciparum* frequently, but not invariably, coexist in the blood of Gambians. Subjects with high parasite densities or with acute malaria had higher levels of CRP compared with low non-parasitaemic individuals. Follow up studies in untreated children showed that CRP concentrations tend to fluctuate widely and perhaps to reflect changes in parasite density. Following treatment, concentrations fall swiftly to low levels and then persist at these for several weeks. Recurrence of parasitaemia is marked by rapid increase in CRP concentrations.

Naik and Voller (1984) in a study of Zambian children with malaria observed higher serum CRP levels in patients with high *P. falciparum* parasitaemia. However, even African children with lower parasitaemia had higher CRP levels than others without parasitaemia. All the African groups studied had CRP levels above United Kingdom control group.

C-reactive proteins have been shown to reduce the number of subsequent liver schizonts that develop in primary human hepatocytes *in vitro* (Pied et al., 1989). Reduction of schizont load was suggested to be due to macrophages activated by parasite antigen that produced interleukin (IL)-6 and IL-1 which triggered hepatocytes in the vicinity to synthesize C-reactive protein.

2.6.6 Caeruloplasmin

Previous studies have reported elevated levels of caeruloplasmin in malaria patients compared with control subjects (Migasena et al., 1978; Chiewslip et al., 1988). Migasena et al. (1978) observed that 3 weeks after adult malarious patients were discharged from hospital, their mean caeruloplasmin levels was within the same range as the controls and significantly lower than on the day of admission.

2.7 Innate Resistance

There are some genetic traits that influence innate resistance to malaria. For example individuals lacking the Duffy blood group (Fy^{a-} and Fy^{b-}) are protected against *P. vivax* infection, as determinants on this blood group are required for merozoite invasion of red cells (Miller et al., 1976). The absence of some glycophorins in the red cell membrane, as in En(a-), $M^k M^k$ and S-s-U- mutants can confer resistance to *P. falciparum* -merozoite invasion as previously discussed. Intracellular growth of the malaria parasites is also affected by a number of host genetic factors which include the several haemoglobinopathies and glucose-6-phosphate dehydrogenase deficiency.

2.7.1 Hemoglobin S

There is conclusive evidence that the gene responsible for the production of haemoglobin (Hb) S is maintained at high frequency in the tropics because of the biological advantage it confers on heterozygotes (HbAS) through partial protection from *P. falciparum* (Livingstone,

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1971). Bengtsson and Thompson (1981) reported that HbS is associated with a 92% reduction in the relative risk of severe malaria. The clearest indication of the protective effect of the sickle-cell gene is that very few carriers of the gene die from cerebral complications of *P. falciparum* malaria (Friedman and Trager, 1981). Luzzatto et al. (1970) reported that HbS infected cells sickle much faster than uninfected ones.

This observation suggested the following mechanism of protection against malaria in HbAS individuals as described by Friedman and Trager (1981). The parasite in an infected AS cell develops normally until the cell is sequestered in the tissues. Then, given the low oxygen environment and the low intracellular pH, the host cell sickles. The potassium level drops and the parasite dies. However, alternatively infected cells might, for some reason sickle while circulating rather than while being sequestered, and are eliminated by the filtering action of the spleen, by phagocytosis of the cells of the reticulo-endothelial system.

Comille-Brogger et al. (1979) and Molineaux et al. (1979) showed that the distribution of various *P. falciparum* specific antibodies is shifted towards lower values in AS and SS compared to AA individuals, suggesting that they are subjected to less antigenic stimulation by the parasite, presumably as a result of their earlier removal from the circulation. This was not found to be true with AC subjects (Storey et al., 1979). Bayoumi (1987) suggested that the selective advantage of Hb AS individuals is due to earlier acquisition of immunity against *P. falciparum*. Abu-Zeid et al. (1992) reported that HbAS individuals with

clinical malaria had lower plasma IL-2 receptors and parasite densities compared to HbAA subjects.

2.7.2 Haemoglobin F

During the first few months of life, infants are relatively unsusceptible to malaria. Wilson et al. (1977) showed that foetal hemoglobin (HbF) may contribute to this protection. Gilles (1957) in a study of Gambian infants observed an apparent relationship between the disappearance of HbF and the onset of malaria infection (Fig. 2.5). After birth, erythropoiesis is known to cease completely and remains inactive until haemoglobin levels physiological for the newborn are attained. High frequencies (50-90%) of HbF red cells are found in the peripheral circulation at birth. After a few weeks HbF levels decrease linearly to about 5% at 100 days from birth (Wilson et al., 1977).

There is conflicting report as regards the mechanism of protection of HbF. Gilles (1957) suggested that HbF is malariostatic as it may provide less suitable environment for the development of the human plasmodia. On the contrary, it has been suggested that infant protection against malaria is due to an ageing red cell population rather than the presence of HbF (Wilson et al., 1979; Pasvol et al., 1977; Luzzatto, 1979). Wilson et al (1977) observed that *P. falciparum* invades "young" erythrocytes in preference for "older" ones. In the blood of infected infants under six months of age, there was a paucity of parasites in HbF erythrocytes; these cells are "older" on the average than the HbA-containing cells. Heavy and preferential parasitization of HbF-containing

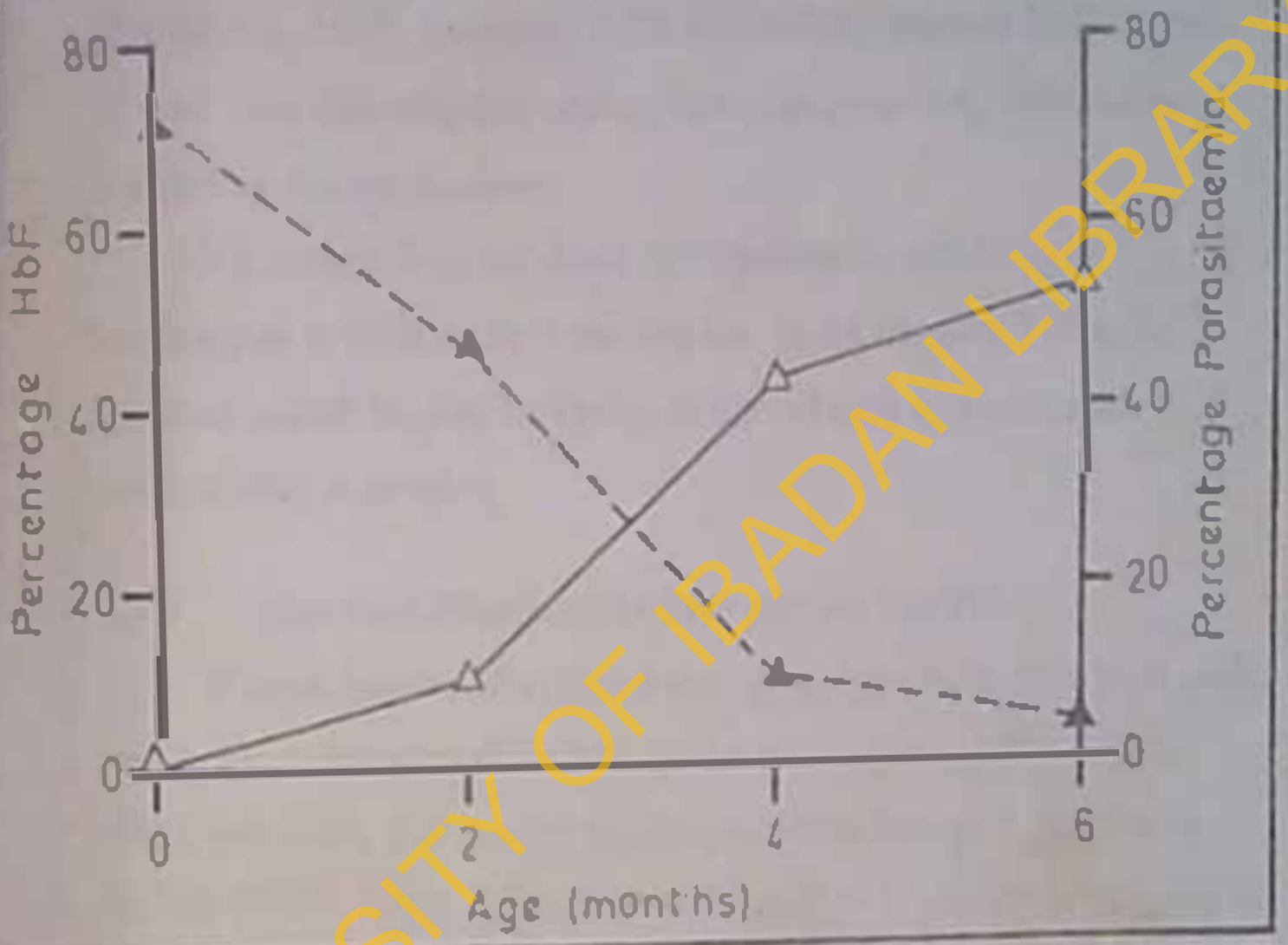

Fig. 2.5. Relationship between the disappearance of foetal haemoglobin and the onset of malaria in breast-fed Gambian infants aged six months and below (Gilles, 1957)

▲ Mean foetal haemoglobin in the various age groups.

△ Parasite rate in the various age groups.

erythrocytes was observed on the other hand when their average age was "younger" as in the umblical cord blood. In adults with hereditary persistence of HbF, red cells are invaded at the same rate as in controls (Pasvol et al.,1977). Luzzatto (1979) successfully infected HbF red cells *in vitro* demonstrating that new erythrocytes containing HbF can be invaded by malaria parasites.

It is evident from the above findings that susceptibility of erythrocytes to invasion by *P. falciparum* is not correlated with the presence of HbF but that the ageing of the red cells decreases their susceptibility to invasion.

2.7.3 Glucose-6-Phosphate Dehydrogenuse (G6PD)

There is impressive evidence through geographical data, field work and *in vitro* studies to show that, apart from the hemoglobinopathies, G6PD deficiency also confers relative protection against *P. falciparum* malaria (WHO, 1989). Friedman and Trager (1981) found that parasites in G6PD deficient cells were highly sensitive to oxidative stress. Bienzle et al. (1972) reported that hemizygous G6PD deficient males do not have any greater resistance to malaria than normals. Homozygous deficient females also showed no evidence of protection. However, heterozygous deficient females had significantly lower parasite counts. G6PD is encoded by an X-chromosome-linked gene. Consequently protection against malaria is manifested in heterozygous females who are genetic mosaics (Usanga and Luzzato, 1985). They observed that in hemizygous G6PD deficient males, *P. falciparum* merozoites emerging from normal

erythrocytes have on the average, an even chance of infecting a normal or G6PD deficient cell. In heterozygous females, the chance to complete successfully the next schizogonic cycle is reduced by approximately 50% thus justifying the reduced parasitaemia actually observed. The existence of two red cell populations ensures the exposure of the parasites alternately to G6PD - rich and G6PD - depleted environment, so that adaptation of the parasite does not occur on a long term basis (WHO, 1989).

2.7.4 Human Leucocyte Antigens (HLA) and Protection from Malaria.

The discovery of MHC restriction of the immune response led to the proposal that MHC polymorphism is maintained by different alleles providing varying degrees of protection against infectious pathogens (Doherty and Zinkernagel, 1975). Heterozygotes for MHC alleles would have an increased capacity to present antigens from a range of pathogens compared with homozygotes.

2.7.4.1 HLA Class I Antigens

In Sardinia, Italy the HLA haplotype A2-BW17 was more frequent in two lowland villages exposed to malaria than in two highland villages never exposed to malaria (Piazza et al., 1972). In north-east Tanzania, the same A2-BW17 (together with A2-AW30) haplotype was found to be more frequent in individuals with high titers of antibodies against *P. falciparum* blood forms. The significance of this possible

linkage between resistance to malaria and HLA Class I antigen is not known.

Hill et al (1991) found that both HLA-A24 and HLA-B14 were more common among Gambian children with severe malaria. They also observed that the frequency of HLA-BW53 was significantly decreased among severe malarious children. The association between HLA-BW53 and protection from severe disease suggest that ,class I-restricted cytotoxic T lymphocytes play an important role in providing protective immunity against liver stage parasite(Hill et al., 1992).

2.7.4.2 HLA Class II Haplotypes

Hill et al (1991) reported that the DRB1*1302 subtype (DRW13 allele) was less frequent among cases of severe malaria anaemia, indicating that it is a protective haplotype. A comparison of the protective efficacies of the two haplotypes DRW13-DQW5 and DRW13-DQW6 against severe malaria anaemia showed that the associated relative risks were similar, suggesting that both are protective and that the DRB1*1302 and the DRB3*0301 alleles that they have in common may be more important than the DQB gene for protection from severe malaria anaemia.

An association between HLA - DR4 and low antibody response to the vaccine immunogen SPf 66 has been reported for Colombian donors (Patarroyo et al., 1991). Troye-Blomberg et al. (1991) did not find any association between T cell responses and HLA -DR or -DQ alleles or DRB -DQA -DQB haplotypes in 145 adult Gambians. Riley et al. (1992)

reported an association between possession of DQA -V/DQB -V1 (DQW2) and high frequency of antibodies to the $(EENV)_6$ peptide of Pf 155/RESA.

2.8 INTERACTIONS BETWEEN CHEMOTHERAPY AND IMMUNITY TO MALARIA

2.8.1 Chemoprophylaxis and Immunity to Malaria

Chemoprophylaxis for risk groups such as children under the age of 5 years, and pregnant women has been suggested for the control of malaria morbidity in malaria endemic areas (WHO, 1974; 1988). In a recent study, Schultz et al. (1993) showed that in a malaria endemic area of Malawi where the prevalence of chloroquine resistance is high, limited use of sulfadoxine/ pyrimethamine resulted in markedly reduced placental infection rates. Critics of this concepts of chemoprophylaxis argue that there is little point in protecting a child from malaria if the child is likely to get life-threatening malaria as soon as prophylaxis is interrupted.

2.8.2 Clinical Protective Immunity

In a study of Nigerian children, no increase in malaria morbidity was observed when chemoprophylaxis was interrupted after 1 - 2 years of drug administration (Archibald and Bruce-chwatt, 1956; Bradley-Moore et al., 1985a). In Liberia, parasitaemia was partly suppressed in children 2-9 years old by monthly doses of chloroquine or chloroproguanil for two years (Bjorkman et al., 1986b). There was no significant change in spleen

rates and sizes, parasite densities and fever episodes when chemoprophylaxis was stopped during the third year of the study. It was concluded that recurrent parasitaemia a few times per year may be sufficient to maintain a certain degree of protective immunity.

In the Pare region of Tanzania, malaria was effectively controlled for a period of 3 years after which there was a tendency of increase of the incidence of clinical malaria in most age groups (Pringle, 1967). In the Garki project of northern Nigeria, effective malaria control was achieved over a period of two rainy seasons by combined vector control and chemoprophylaxis (Molineaux and Grammiccia, 1980). Following the interruption of control measures, the prevalence of malaria parasitaemia gradually rose to the pre-intervention level.

In The Gambia, primigravid women who received maloprim had a lower parasite rate and a significantly higher mean packed cell volume than controls, and their babies were significantly heavier (Greenwood et al.,1989). In multigravidae chemoprophylaxis resulted in lower parasite rate but it had no beneficial effect on haemoglobin level and much less effect on birth weight than was observed in primigravidae.

2.8.3 Antibody Response to Malaria

In a Gambian study, a small number of infants and mothers were protected from malaria by weekly pyrimethamine for 7 months (Voller and Wilson, 1964). Malaria antibodies were found in none of 7 protected infants. In contrast, all 7 control infants had antibodies. In the

protected mothers the mean titer was about one-third of the mean titer found in control women. Harland et al., (1975) in Uganda found that the malarial antibody titers in children who were given monthly pyrimethamine up to the age of 3 years was lower than those of unprotected control children. In a Nigerian study, chloroquine was given weekly to children throughout the first two years of life (Bradley-Moore et al., 1985a). The mean malaria antibody levels were lower in the protected than in the control children, although by the age of 2 years a high proportion of protected children had also developed antibodies. Ibeziako and Williams (1980) reported a decrease in malaria antibody titers in pregnant Nigerian women on pyrimethamine prophylaxis throughout pregnancy. In the Garki Project very low immunofluorescence or ELISA antibody levels were found in a group of protected infants followed from birth until the age of one year as compared to controls (Molineaux et al., 1978).

Taken together, the above studies show that malaria control may produce a reduction of malaria antibody levels in the protected population. This observation has serious implications as newborns depend on transplacental malaria antibodies for protection against malaria during the first few weeks of life.

2.9 MALARIA IN NEONATES, INFANTS AND CHILDREN

2.9.1 Congenital Malaria

Congenital malaria is that which the foetus acquires from its mother, in utero and in which plasmodia are demonstrable in the infant's

blood at birth or soon after birth before the expiry of the malaria incubation period. In endemic areas, despite a high incidence of maternal and placental parasitaemia, congenitally acquired clinical malaria is a rare event. However, there are a few reported cases of congenital malaria in endemic areas (Covel, 1950; Bruce-Chwatt, 1952; Hindi and Azimi, 1980). The incidence in endemic countries has been shown to be low (0.3%) but higher (10%) in women who move from non-endemic areas to endemic ones (Covell, 1950). Airede (1991) reported a case of congenital malaria with chloroquine resistance in a preterm infant born to a 29 year old malaria infected multigravid mother at the Jos University Teaching Hospital.

Low density infections of cord blood are frequently recorded in African newborns; prevalences of 3.8% (Kortman, 1972) and 7.6% (Vleugels, 1984) have been reported in Tanzanian newborns and 21% in babies in the Ivory Coast (Reinhardt et al., 1978).

The placenta is normally an effective barrier against the malaria parasite. However, some researchers believe that the foetus acquires parasites when the placenta is damaged, either during normal delivery or owing to placental paravia or abruptio placenta, when infected red cells are transferred into the foetal circulation (Logie and McGregor, 1970; Ransome-Kuti, 1972).

2.9.2 Malaria in Infants and Children

Very young infants in highly endemic malarious areas appear not to be susceptible to malaria. This protection has been attributed to transplacentally acquired malarial antibodies (Bruce-Chwatt, 1952; Collins et al., 1977; Biggar et al., 1980; Nardin et al., 1981; Chizzolini et al., 1991); and other non-immunological factors such as HbF (Allison, 1954) aversion to young infants by the Anopheline mosquito (Muirhead-Thompson, 1951) and the milk diet deficient in p-aminobenzoic acid (PABA) (Hawking, 1963). PABA is an essential growth factor for malaria parasites because it is required for the synthesis of folic acid.

Protection is however, transient, presumably with the decay of immunological and non-immunological factors. McGregor and Smith (1952) observed that the incidence and density of parasitaemia were maximal in the very young children and declined progressively in the older age-groups, suggesting a gradual acquisition of immunity.

Bruce-Chwatt (1952) in a longitudinal study of 138 African infants in Southern Nigeria observed that during the first quarter year of life the malaria parasite rate was less than 3%. Parasite rate increased to 20% during the second quarter to about 60% during the third quarter, and to over 70% during the fourth quarter. Nearly all the children were infected thereafter. McGregor et al. (1956) reported that malaria exerted its maximal effects in the first 18 months of life. Gilles (1957) reported that the mean parasite rate of Gambian infants increased from 10% in the first

two months of life to 42% in the third and fourth months and to 53% in the fifth and sixth months.

The above observations suggest that the clinical impact of malaria increases as age progresses. Episodes of dense parasitaemia and severe clinical disease reach peak severity in the second year of life when, in addition to pyrexia, faltering growth and marked hepatosplenomegaly are universal features. In the second half of the third year of life, children begin to show marked clinical improvement, despite the persistence of moderately dense parasitaemia. Throughout later childhood, immunity slowly develops, parasite densities diminish, as does the size of the liver and spleen.

2.9.3 The Development of Malarial Antibodies

The African infant emerges from his 9 months sojourn in the normally sterile intrauterine environment into a world swarming with potentially pathogenic organisms. A significant part of the neonate's ability to survive its environment is temporarily provided by the mother via the placenta. For example, Edozien et al. (1962) showed conclusively that highly purified cord γ-globulin had an antiparasitic effect against *P. falciparum* when administered to children with acute falciparum malaria. In El Salvador, Collins et al. (1977) found that 44% of infants born to mothers with a positive IFA response to *P. vivax* had positive IFA response to this antigen. Nardin et al. (1981) detected antibodies to sporozoites of *P. falciparum* in the sera of most babies born to mothers living in endemic areas of The Gambia, Chizzolini et al. (1991) in Gabon

confirmed previous results that antibodies specific for *P. falciparum* antigens are transferred from mothers to newborns.

Gilles and McGregor (1959) observed that the γ - globulin levels of Gambian infants fall steadily from birth for the first 3 - 6 months of life and then begin to rise. Mathew et al. (1976) noticed a slight decline of malarial antibodies in 6 to 8 months-old infants who had no malaria parasites. Children older than 10 months had similar antibody levels irrespective of malaria parasitaemia. Nardin et al. (1981) observed that anti-sporozoite antibodies were gradually lost from the circulation of Gambian infants until 6 months of age when positive reactions against *P. falciparum* sporozoites were no longer detected.

The levels of antimalarial antibodies remain low or undetectable throughout the remainder of the first year of life and, thereafter, rise progressively throughout childhood and into adult life (McGregor, 1986). The rate of increase in early childhood is rapid; it then slows in older childhood and adolescence and plateaus in adult life. This general pattern parallels clinical evidence of gradually increasing resistance. The antibody titres at birth and in the ensuing weeks of life fit well with the observed infrequence of parasitaemia and clinical illness at this time. The low titres that persist into the third year of life corresponds to the period of maximum susceptibility to the disease, while the progressively rising titres thereafter reflect consolidating immunity.

2.10 MALARIA IN ADULTS

In the naturally immunized adult, effector mechanisms function by restricting the replication of blood parasites rather than by preventing the occurence of parasitaemia. Immune adults commonly show low grade asymptomatic parasitaemia (premunition). Bruce-Chwatt (1963) in a 1 - 2 years longitudinal study of Nigerian adults exposed to natural infections, found that, although he could demonstrate parasitaemia in only 25% at any one time, over 90% had blood infections at some time when followed for 1 - 2 years.

Adults in malaria endemic areas usually experience fewer attacks of malaria annually as compared to children below 10 years of age due to acquisition of protective immunity. In cases of clinical malaria attacks, confirmation of malaria parasitaemia by thick smears is usually difficult due to the low grade parasitaemia.

2.11 MALARIA IN PREGNANCY

In highly endemic areas, exacerbation of *P. falciparum* parasitaemia in association with pregnancy has been widely observed (Bruce-Chwatt, 1952; McGregor and Smith, 1952). Parasitization, often very severe, of placental blood is a frequent occurence at parturition. Consequently pregnancy is thought to abrogate previously acquired immunity to malaria and reinstate susceptibility to severe clinical illness. It is suggested that pregnancy associated hormonal changes may depress immune-responsiveness of the otherwise healthy female. Beer and Billingham (1978) found that cortisone produced during pregnancy is

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immunosuppressive. There is also evidence that sera from pregnant women can inhibit the chemotactic responsiveness and phagocytic activity of phagocytes (Bjorksten, 1980).

2.11.1 Malaria and Parity

In malaria endemic areas, parasitaemia is significantly commoner and heavier in primigravids than in multigravids. It appears to peak around the end of the first trimester and fall during the second half of pregnancy (Brabin, 1983). Parasite rates tend to decrease with rising parity and mean parasite rates in pregnant women of parity 3 and above are lower than the overall mean for non-pregnant women of reproductive age (McGregor, 1984). Gilles et al. (1969) found malaria to be an important cause of anaemia in Nigerian primigravids while McGregor (1984) found that depression of haemoglobin levels in pregnant women with malaria parasitaemia diminished progressively as parity increased. McGregor et al. (1983) reported that placental infections are highest in primigravids and declines significantly and progressively with advancing parity. In association with placental infection, mean infant birthweights were reduced overall by 170g.

2.11.2 Immunosuppression of Pregnancy

Serological studies have failed to produce convincing evidence that humoral immune responses to malarial infection are suppressed in pregnancy. McGregor et al., (1970) observed that mean levels of IgG and IgA, but not IgM, fall during pregnancy. Mean values of IgG are

significantly higher in parasitaemic than non-parasitaemic pregnant women (Logie et al., 1973). Similarly, while assays of specific malarial antibody levels in pregnant and non-pregnant women have produced conflicting results, mean levels in pregnant, parasitaemic women are signifantly higher than those of pregnant uninfected women (McGregor, 1984).

Since cell-mediated immune mechanisms are important in the maintenance of immunity to malaria, impairment of this immunity to malaria may in part explain the susceptibility of pregnant women to malaria. Riley et al. (1988) found that women aged 18-45 years were significantly less responsive to malarial antigens than a similar group of men. In The Gambia, Riley et al. (1989) showed that lymphoproliferative responses to *P. falciparum* antigens were depressed in pregnant women compared to parity matched non-pregnant women and that this effect was particularly marked in primigravidae. There was no significant difference in anti-malarial antibody titres between the two groups. Rasheed et al. (1993) found similar malarial antibody levels in maternal peripheral and placental blood. Maternal mononuclear placental cells proliferated less than those from the peripheral blood; these differences were comparable across parity groups. However, primiparae had lower proliferation to malarial antigens.

2.12 BLOOD TRANSFUSION AND MALARIA

The transmission of malaria related to the practice of blood transfusion is of particular interest because of its clinical and public

health aspects. Bruce-Chwan (1980) estimated about 2500 cases of transfusion malaria between 1920 and1980. Guerrero et al. (1983) reported 26 cases of transfusion-induced malaria in the United States from 1972 through 1981. Four of the patients eventually died.

P. vivax infections are most commonly incriminated in accidental infections following blood transfusion: however, *P. falciparum* infections occur not infrequently and more recently *P. malariae* were reported with increasing frequency, because of the asymptomatic, long-term carrier state of donors infected with this Plasmodium. While the longevity of *P. falciparum* in man seldom exceeds one year and *P. vivax* or *P. ovale* usually die out within 3 years, *P. malariae* may remain in the infected host for up to 40 years (Bruce-Chwatt, 1980). The viability of malaria parasites stored in dextrose at 4°C has been put at between one week and 10 days.

Transfusion malaria is of common occurence in the University College Hospital, Ibadan (Ambe and Njinyam,Personal Communication). This could be attributed to the current wave of economic hardship that has transformed voluntary blood donation into a commercial transaction involving mostly the less affluent social class who now see blood donation as a profitable venture.

Following this observation, it is expected that paediatric patients are the most vulnerable victims of blood transfusion malaria as this age group of patients are usually transfused with freshly collected blood. It will therefore be worthwhile to determine the prevalence of malaria

parasites in blood donors so as to evaluate its potential impact on transfusion malaria. Results from such a study may assist in the formulation of guidelines governing the acceptance of donors of whole blood for transfusion or prompt treatment of recipients of malaria infected blood.

2.13 ADVANCES IN MALARIA VACCINE RESEARCH

During the last 40 years, there have been repeated attempts to obtain an effective vaccine against malaria. Clyde et al. (1975) performed the first human trials using sporozoites from irradiated mosquitoes as immunogens. Volunteers were immunized by feeding irradiated sporozoite-infected mosquitoes on the vaccinees. There was a correlation between CSP antibodies and protection against malaria. Vaccinees immunized with *P. falciparum* sporozoites were protected against other strains of *P. falciparum* but not against *P. vivax*. This experiment produced the first clear indication that induced immunity in susceptible hosts was a potential method of controlling the disease. Nevertheless, for protection to be induced, exposure to hundreds of infected mosquitoes was required and consequently the use of this type of immunogen as a vaccine is highly impractical.

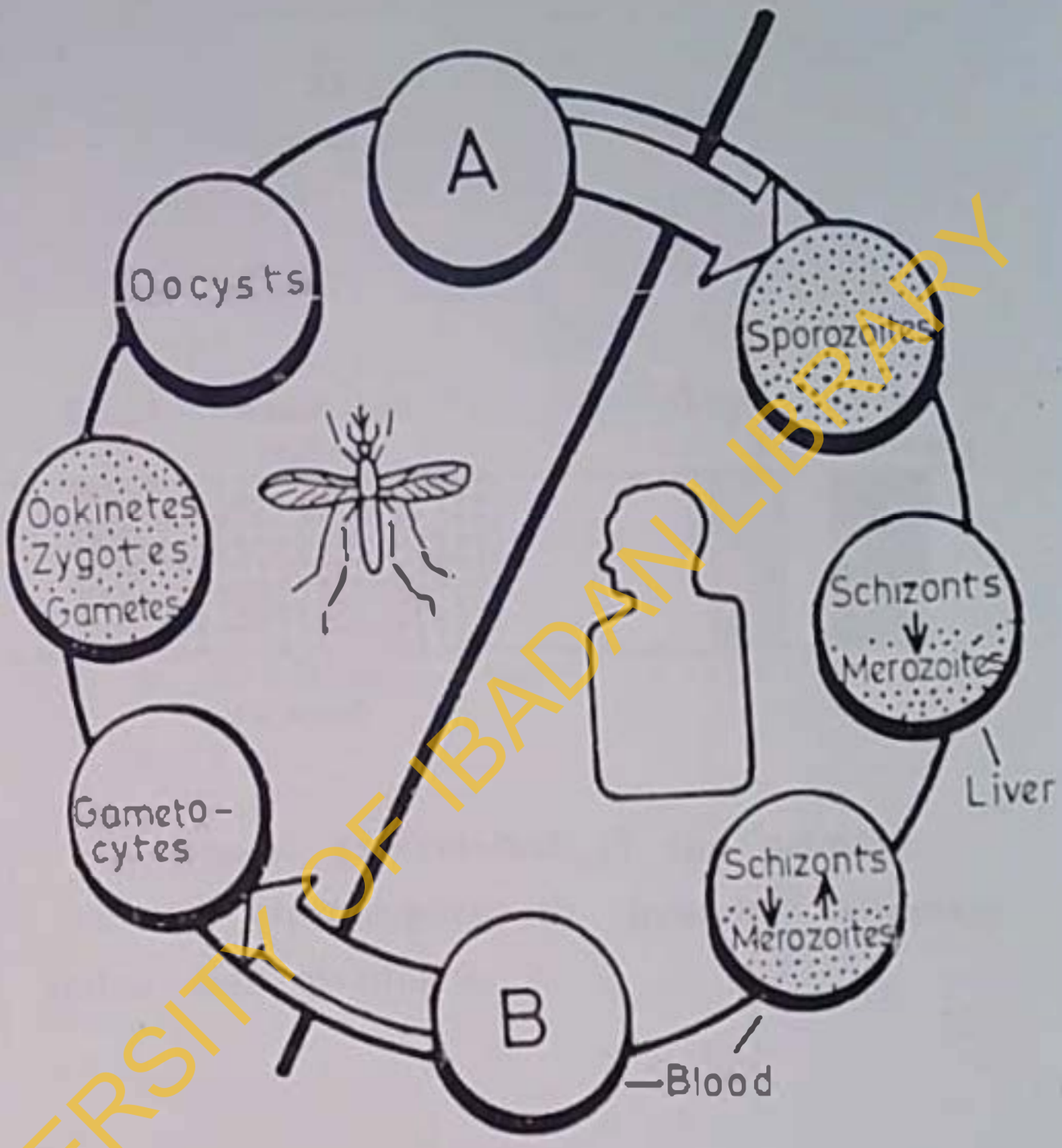
The continuous culture method of *P. falciparum* developed by Trager and Jensen (1976), permitted the preparation of large quantities of antigen without depending exclusively on isolates. This together with recent advances in immunologic and biotechnologic tools has permitted the antigenic characterization of many isolates. Some of these antigens

are now obtainable by both recombinant DNA technology and chemical synthesis and provide a powerful tool for the dissection of the specific immune response of the host to the parasite.

In the search for malaria candidate vaccines, stages in which the malaria parasite is accessible to the immune system of the host have been considered (Fig. 2.6). They include the sporozoite, merozoite, mature infected erythrocyte, exoerythrocyte and gametocyte stages.

2.13.1 Sporozoite Vaccine

The membranes of infective sporozoites are covered by a protein called the circumspomzoite protein (CSP). The CSP genes have been cloned and sequenced from several species of Plasmodium (Enea et al., 1984; Arnot et al., 1985) and all display the same general structure: an immunodominant repeat-coding central domain (Fig. 2.7) flanked by two small conserved sequences on both sides of the immunodominant repeat region referred to as regions I and II. Recently, it has been reported that sporozoites bind to and enter hepatocytes in the space of Disse, and that binding is accomplished by a region II sequence on the CSP gene (Cerami et al., 1992). Hollingdale et al. (1993) demonstrated that a peptide sequence spanning region I of the CSP binds to Hep G2 cells. Antibodies against this sequence inhibit sporozoite invasion of Hep G2 cells suggesting that region I is likely to be involved in sporozoite invasion.



Vaccine targets.

A. Sporozoites infective to humans.

B. Gametocytes infective to Mosquitoes.

Fig 2.6 Malaria parasite life cycle and vaccine targets

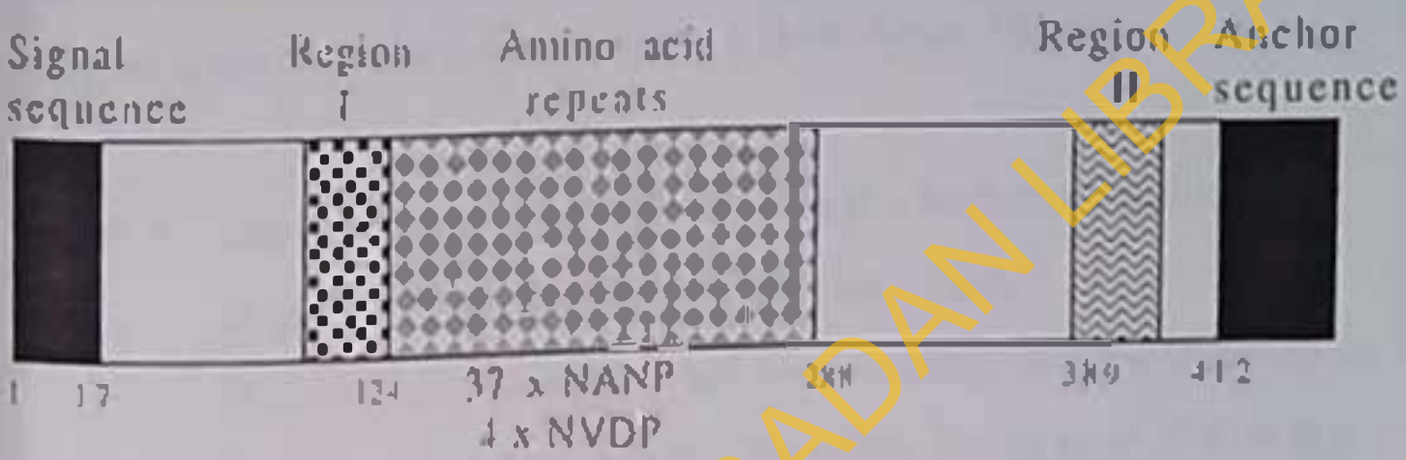


Fig. 2.7. Schematic presentation of the primary structure of the circumsporozoite protein. Numbers indicate amino acid positions.

The gene for the CSP of *P. falciparum* encodes a protein of 412 amino acids, 40% of which are included in 41 tandem repeated tetrapeptides: 37 of these are ASn-Ala-ASn-Pro (NANP) and 4 are ASn-Val-Asp-Pro (NVDP) (WHO, 1986). DNA hybridization studies of 18 geographically distinct *P. falciparum* isolates indicate that the repeat region is highly conserved (Weber and Hockmeyer, 1985).

2.13.2 Host Immune Responsiveness to the Immunodominant Repetitive Epitope of *P. falciparum* CSP.

With the identification of the immunodominant repeat region as the primary vaccine target, it has been shown by Zavala et al. (1985) that after natural infections with *P. falciparum*, the great majority of antibodies produced against sporozoites are directed against the CSP repetitive epitope. Both synthetic-(NANP)n (Del Giudice et al., 1987) and recombinant - R32tet 32 (Hoffman et al., 1986) peptides have been successfully employed in detecting anti-CSP antibodies in sera from immune individuals in malaria endemic areas. Both monoclonal and polyclonal antibodies directed against sporozoites have been shown to inhibit sporozoite invasion of cultured human fibroblast (Hollingdale et al., 1982) and hepatoma (Hollingdale et al., 1984) cell lines in a species specific manner.

There have been conflicting reports as regards the protective role of anti-CSP antibodies in a number of epidemiological studies. Hoffman et al. (1986) in Indonesia reported that anti-CSP antibodies mediated

circumsporozoite - precipitation reactions and blocked sporozoite invasion of hepatoma cells *in vitro* and such reactions have been shown to correlate with protective immunity. The age-specific prevalence of these antibodies correlated with decreased prevalence of malaria suggesting that a vaccine derived from the CSP repeat domain will produce protective immunity. Campbell et al. (1987c) in a study of children 1 month to 10 years from 3 villages in western Kenya observed that the percentage of antibody-positive children increased with age and differed in the three villages. The village with the lowest percentage of antibody-positive children had the lowest percentage of malaria infections. In a longitudinal study of 132 rural Tanzanian children, Del Giudice et al. (1987) reported that anti-$(NANP)_{40}$ antibodies increased with age. A negative correlation was observed between the levels of anti-sporozoite antibodies and both spleen enlargements and malaria parasitaemia.

Deloron et al. (1989a) and Deloron and Cot (1990) in a study in Western Kenya and Burkina Faso respectively found an increase of anti-$(NANP)_5$ antibodies with age and antibody-positive subjects were less likely to be infected with *P. falciparum*. The inverse relation between reactivity to $(NANP)_5$ and prevalence of malaria led them to suggest that these antibodies may play a role in immune protection. However, their observation that individuals with high anti-$(NANP)_5$ antibody titres were infected with *P. falciparum* indicated that these antibodies alone were not sufficient to confer protection against malaria infection. Snow et al.

(1989) in the Gambia reported an increased seropositivity to the $(NANP)_{40}$ peptide with age but not with sex nor ethnic group.

Esposito et al. (1988), using multiple cross-sectional surveys found some evidence for protection in adults at the begining and at the end of the rainy season, when the pressure of infection was low, but not during a period of high malaria transmission.

However, field studies by Hoffman et al. (1987) in Kenya, Pang et al. (1988) in Thailand and Marsh et al. (1988) in The Gambia, as well as sporozoite inhibition studies with West African sera (Mellouk et al., 1986) showed that anti-CSP antibodies are not protective. Webster et al. (1988) reported that CSP antibodies may not be sufficient to confer protection against erythrocytic infection by reducing the number of sporozoites which successfully invade liver cells. They suggested that cell-mediated immunity may however, contribute to protection against sporozoite infection. Burkot et al. (1989) observed a significant trend of increasing anti-CSP antibodies with age but there was no evidence of protection against malaria. Wijesundera et al. (1990) showed that anti-sporozoite antibodies are of short duration, unrelated to recrudescence and independent of the anti-blood-stage antibodies. Rosenberg and Wirtz (1990) reported that intrinsic differences exist in an individuals' ability to respond to the CSP. A plausible explanation for these intrinsic differences is that there is some genetic restriction of the human T cell response to $(NANP)_n$ peptide.

The CSP repeat is known to be included in the immunodominant B cell epitope of this antigen. When injected into H-2 congenic mice, R32tet 32 induced both anti-NANP antibodies and specific T cell proliferation in vitro in 4 of 7 strains (Good et al., 1986). The response to the plasmodial antigen was restricted by MHC class II molecules encoded by the I-Ab genes. Good et al. (1987b) reported that intact CSP contain at least one T helper cell-activating site outside the repeat region, thus emphasizing the necessity of mapping vaccine candidates for T cell antigenic sites. Available evidence indicate that the CSP has a limited number of T helper antigenic sites capable of amplifying the formation of antibodies.

It is evident from the above observations that the factors governing the acquisition of anti-CSP antibodies are still inadequately understood. Consequently there is need to conduct further field studies to ascertain the protective role of these antibodies. Such studies are of tremendous importance in sporozoite vaccine development.

2.13.3 Sporozoite Vaccine Immunization Trials

Several immunization trials with radiation-attenuated sporozoites, synthetic and recombinant CSP have been shown to protect animals and humans against malaria. Campell et al. (1987a) showed that mice immunized with R 32tet32 produced a secondary antibody response after intravenous injection of *P. falciparum* sporozoites. This suggested that boosting of antibody might occur after natural exposure to sporozoites. Del Giudice et al. (1988) showed that C57BL/6 (H-2b) mice

responded strongly to carrier-free $(NANP)_{40}$ but not $(NANP)_3$ nor $(NANP)_4$ peptides. The ability to produce antibodies against $(NANP)_{40}$ was shown to be linked to the presence of the b allele in the I - A subregion of the H - 2 complex.

Khusmith et al. (1991) found that BALB/C mice immunized with irradiated *P. yoelii* sporozoites produced antibodies and cytotoxic T cells against a 140-Kd protein, sporozoite surface protein 2 (SSP2). Mice immunized with either SSP2 or CSP genes were partially protected, while those inumunized with a mixture of SSP2 and CSP transfectants were completely protected against malaria. *P. falciparum* SSP2 (Pf SSP2, Mr 90Kd) has recently been identified (Rogers et al., 1992). Anti-Pf SSP2 antibodies inhibit sporozoite invasion of hepatocytes *in vitro*. Human volunteers immunized with irradiated sporozoites develop antibody and proliferative T cell responses to Pf SSP2 suggesting that it is a target of protective immunity.

Fifteen volunteers with no prior exposure to malaria were immunized with recombinant R32tet 32 adsorbed to alum-falciparum sporozoite vaccine-1 (FSV-1) by Ballou et al. (1987). Antigen-specific antibody was detected as early as two weeks after primary immunization. Antibody response was sustained for 3 - 4 weeks and titers returned to baseline with a calculated half life of 28 days. To determine whether a subsequent booster might increase antibody titers and thus protection, four doses of FSV-1 was administered to 6 of the original volunteers. No evidence of boosting was observed. Five mosquitoes infected with a

chloroquine sensitive NF54 strain of *P. falciparum* were allowed to feed on these six subjects and two controls. Seven of the 8 volunteers developed clinical malaria 9 - 13 days later. Parasitaemia never developed in the individual who had the highest antibody response and the incubation and prepatent periods were prolonged in the two subjects with the highest antibody titers among the subjects who became parasitaemic.

In another vaccine trial, Herrington et al. (1987) immunized 35 healthy males with a *P. falciparum* anti-sporozoite alum-adjuvated vaccine consisting of (NANP)3 -TT. The frequency and magnitude of the antibody response correlated with the vaccine dose. No evidence of boosting was observed nor did antibody titers increase following administration of the 2nd and 3rd doses. Five mosquitoes infected with the chloroquine sensitive NF54 strain of *P. falciparum* were allowed to feed on three vaccinees with the highest antibody titers and four controls. All four controls developed malaria within 7-10 days. Two of the three vaccinated volunteers developed malaria 11 days after exposure. The third volunteer was immune and free of parasitaemia 29 days after challenge (Herrington et al., 1987).

A CSP vaccine called CSP-2 (42/54kd) which is present in *P. falciparum* and *P. berghei* (Brown, 1991) has been identified and the genes cloned. Antibodies to the CSP - 2 antigen protected mice that were infected with *P. berghei* from the disease (Anders et al., 1991). Another CSP vaccine called CSP-3 has also been identified and it is anticipated

that CSP - 3 could be combined with other sporozoite proteins to boost the immune response (Anders et al., 1991).

2.13. 4 Asexual Blood-Stage Vaccines

Numerous *P. falciparum* antigens have been identified that might serve as malaria candidate vaccines. They include the major merozoite surface protein, the ring-infected erythrocyte surface antigen, the S-antigen, Histidine rich proteins, glycophorin-binding proteins, antigens of the parasitophorous vacuole, falciparum erythrocyte membrane proteins, transferrin receptors, Ag332 and many other small molecular weight proteins. For the purpose of this review only the merozoite surface protein, the ring-infected erythrocyte surface antigen and Ag332 will be considered because they are the most studied.

2.13.5 The Major Merozoite Surface Antigen (Pf195)

The major merozoite surface antigen is a glycoprotein synthesized throughout schizogony and transported to the surface of the intracellular parasite. This molecule with a relative molecular mass Mr195-kd (Pf 195) is the precursor of several smaller proteins (83, 42 and 19-kd), some of which are expressed on the merozoite's surface (WHO, 1986).

Anti-Pf 195 antibodies have been demonstrated in the sera of individuals inhabiting malarious areas (Holder and Freeman, 1982; Hall et al., 1984). Antibodies directed against merozoite surface components may inhibit growth *in vitro* in at least two ways. Antibodies may

sterically block merozoite receptors on red cell membrane or alternatively may agglutinate merozoites prior to their dispersal from the mature schizont (Chulay et al., 1981) thereby effectively reducing the numbers of invasive free merozoites.

2.13.6 The Ring - infected Erythrocyte Surface Antigen (RESA)

The RESA was identified as a component of the erythrocyte membrane by Perlmann et al. (1984). The RESA also known as Pf 155 is present in the dense granules in the apical complex of merozoites (Aikawa et al., 1990) and becomes associated with the erythrocyte membrane shortly after invasion. It is not exposed at the external surface of the erythrocyte membrane (Berzins, 1991), but seems to be associated with the intracellular cytoskeleton (Fig. 2.8) and especially with spectrin (Foley et al., 1991). The Pf 155/RESA has also been detected in spent culture medium (Carlsson et al., 1991).

The complete structure of the gene encoding RESA has been determined (Favoloro et al.,1986). An intron in the gene separates a small exon 1 (65 amino acids) from a very much larger (1008 amino acids) exon 2. Within exon 2, there are two regions of repetitive sequences: the 3' repeat region (C-terminus) encodes several tandem repeats (Fig. 2.9) of an 8 amino acid sequence (EENVEHDA) repeated 4-5 times followed by a much more extensive set of tandemly repeated 4-amino acid sequences (Predominantly EENV) repeated 30-40 times. The 5' repeat region (N-terminus) is more degenerate, with an 11-amino acid sequence

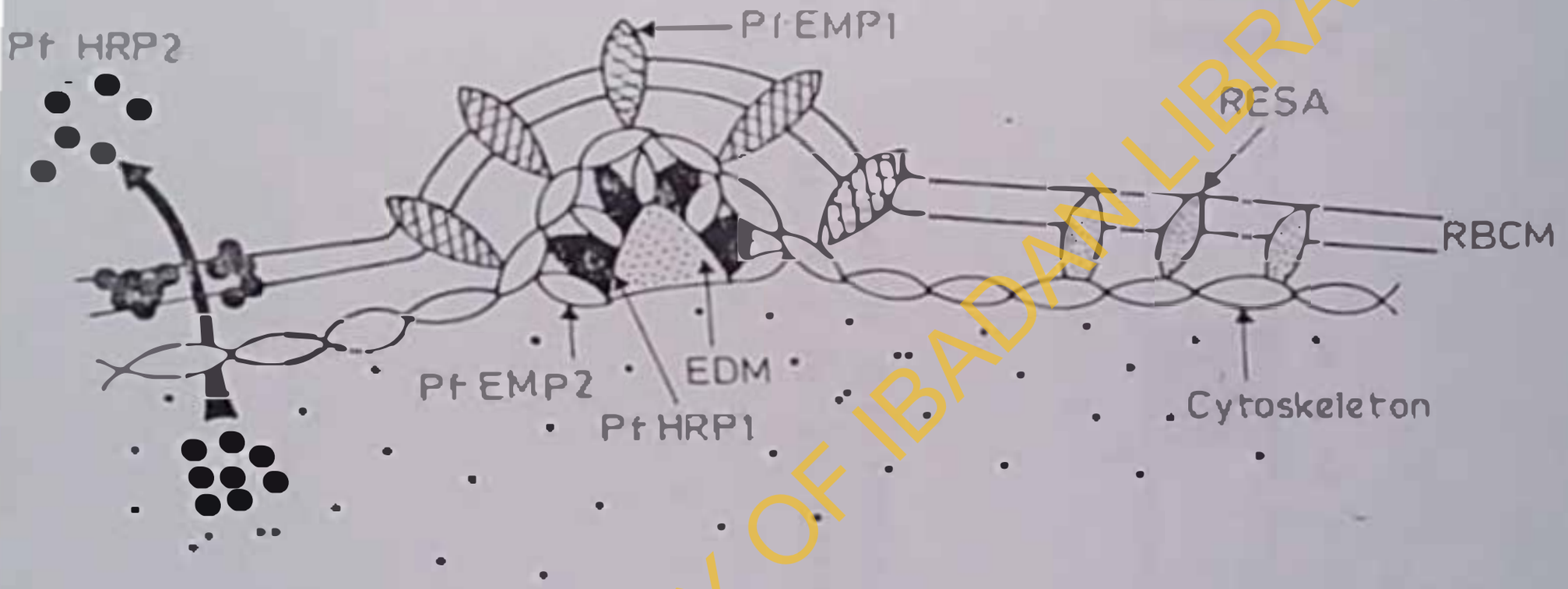


Fig. 2.8 Schematic diagram of the topological distribution of *P. falciparum* proteins (Pf HRP2, Pf EMP2, Pf EMP1, RESA) in the surface membrane of infected erythrocytes. The lipid bilayer of the red blood cell membrane (RBCM) is indicated together with the cytoskeleton and electron-dense material (EDM) under knobs (Howard, 1988)

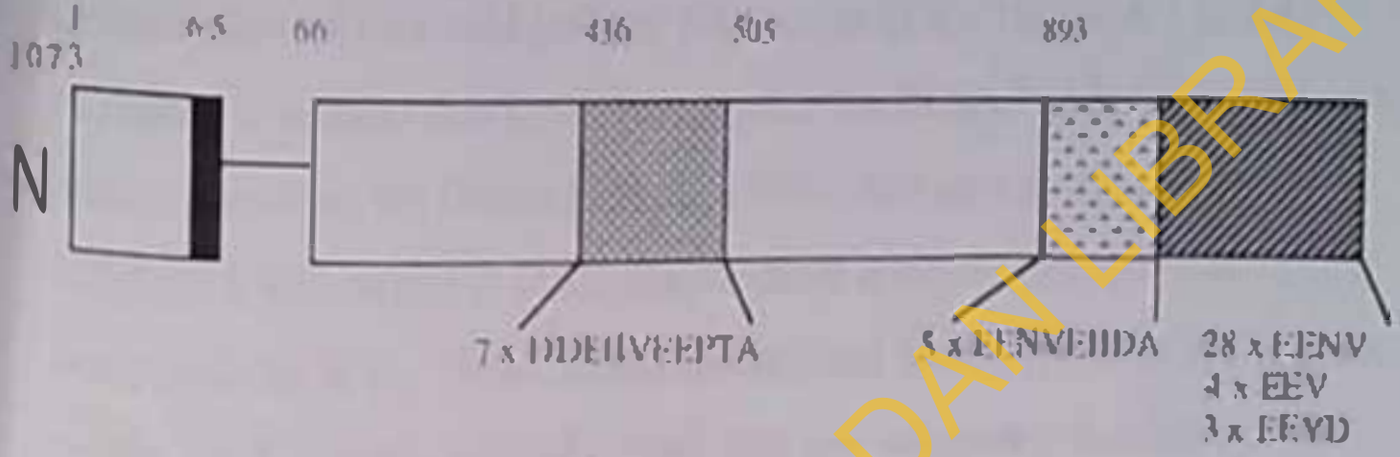


Fig. 2.9. Schematic presentation of the primary structure of the the Pf 155/RESA. Numbers indicate amino acid positions.

(DDEHVEEPTVA) occuring twice and five shorter sequences derived from the 11-mer by deletions and, in some cases, by conservative substitutions.

No antigenic diversity or size variation has been detected between different strains and wild isolates (Perlmann et al., 1987). A 155-kd *P. falciparum* protein that cross-reacts with the RESA has been identified in mature gametocytes (Masuda et al., 1986). Antigens similar to the Pf 155/RESA also occur in other Plasmodium species (Gabriel et al., 1986; Nguyen-Dinh et al., 1988). Immunodominant B-cell epitopes are located within the 3' repeats, central repeats and few sequences located outside the repeat regions (Troye-Blomberg et al., 1989; Chougnet et al., 1992).

The Pf 155/RESA is considered as a prime candidate for a vaccine against the asexual blood stage of *P. falciparum* since both polyclonal and monoclonal anti-Pf 155/RESA antibodies efficiently inhibit erythrocyte invasion by merozoites *in vitro* (Wuhlin et al., 1981, 1992; Berzins et al., 1986), and the development of anti-RESA antibodies in humans appears to correlate with the acquisition of clinical immunity (Wahlgren et al., 1986). However, the role of anti-RESA antibodies in protective immunity against malaria is still questionable since field studies have yielded conflicting results.

In seroepidemiologic studies, anti-RESA antibodies increase with age and transmission (Wahlgren et al., 1986; Deloron et al., 1989; Chizzolini et al., 1989; Baird et al., 1991) except in early childhood (Björkman et al., 1991) and may be related to the acquisition of

protective immunity. Nguyen-Dinh et al. (1987) found low or undetectable levels of anti-Pf 155/RESA antibodies in symptomatic patients and high levels in asymptomatic ones and suggested that these antibodies might play a role in protection against malaria. Deloron et al (1989b) in a study of gravid and nulligravid women in Kenya observed high anti-Pf 155/RESA antibodies in multigravid women, moderate levels in nulligravids and lowest level in primigravid women indicating a pattern consistent with clinically assessed protection against malaria. Petersen et al. (1990) in Liberia observed a positive correlation between anti-Pf 155/RESA antibodies and lower parasitaemia and suggested that high titers of anti-Pf 155/RESA antibodies might play a role in protective immunity in adults.

On the contrary Deloron et al. (1989a) in Kenya found that individuals with high antibody titers to the Pf 155/RESA were infected with *P. falciparum* indicating that these antibodies alone were not sufficient to confer protection against malaria infection. Deloron and Col (1990) reported a similar observation in Burkina Faso. Bjorkman et al. (1990) in a longitudinal study of 32 adult liberians observed no significant correlation between anti-Pf 155/RESA antibodies and parasite densities. A similar observation was reported by Chumpitazi et al. (1991) in Burkina Faso. Marsh et al (1989) in The Gambia found no correlation between anti-Pf 155/RESA antibodies and numbers of clinical episodes.

Antibodies against individual peptides from the Pf 155/RESA also increase to some extent with age (Deloron et al., 1989a). Petersen et al.

(1990) reported a negative correlation between parasitaemia and anti-(EENV)6 antibody levels in Liberian adults. Hogh et al. (1991) did not find any correlation in Liberian children below 5 years of age.

2.13.7 Antigen 332 (Ag332)

A clone, named Ag332, was isolated from a *P. falciparum* expression library based on its reactiviy with a pool of African human immune sera (Mattei et al., 1989a). Ag332 is located in small patches in the erythrocyte cytoplasm and is associated with the membrane of asexually infected erythrocytes. Ag332 is encoded by a single, large gene that is polymorphic in different *P. falciparum* isolates (Mattei and Scherf, 1992). This antigen contains highly degenerated glutamic acid-rich repeats of 11 amino acids.

The recombinant 332 fusion protein reacts with the human mAb 33G2, which is able to inhibit the cytoadherence of parasitized red blood cells on the melanoma cell line C32 (Udomsangpetch et al., 1989a). Furthermore, mAb 332 efficiently inhibits the invasion of erythrocytes by merozoites *in vitro* (Udomsangpetch et al., 1989b; Wahlin et al., 1992), suggesting that the antigen encoded by Ag332 is of potential interest with regards to protective immunity. A series of overlapping synthetic peptides representing one Ag332 repeat (ESVTEEIA) has been synthesized and used to determine the epitope recognized by mAb 332 (Ahlborg et al., 1991). The epitope has been defined as a linear sequence of 5 amino acids, VTEEI.

2.13.8 Immunization Trials with Asexual Blood-Stage Vaccines

Holder and Freeman (1981) immunized BALB/C mice on 3 occassions with the merozoite surface protein and challenged them with 104 *P. yoelii* parasites; all the control group mice died with a fulminating parasitaemia on day 8 after challenge. In the immunized mice, a relatively low grade parasitaemia was cleared by day 10 and all the mice survived. Although immunization induced high antibody titres, serum from immunized mice was not protective on passive transfer thus suggesting that the resistance to infection was not due simply to the antibody response. Other animal immunizations (Hall et al.,1984; Perrin et al.,1984) demonstrate that sterile immunity cannot be achieved by immunization with the Pf 195 antigen. At best a transient, low grade Parasitaemia is observed followed by parasite clearance. So far, no immunization studies have been carried out in humans employing the Pf 195 antigen. However, peptide derivatives of the Pf 195 antigen have been used in human vaccine trials (Patarroyo et al., 1988).

Using β - galactosidase fusion proteins containing the repeat regions of Pf 155/RESA in a vaccination trial in Aotus monkeys, partial protection against *P. falciparum* challenge was observed with some of the immunogens (Collins et al., 1986). Passive immunization of Aotus monkeys with affinity-purified human antibodies reactive with Pf 155/RESA repeats resulted in depressed *P. falciparum* parasitaemia after challenge (Berzins et al., 1991).

Patarroyo et al. (1987a) immunized Aotus monkeys with purified preparations of the Pf 155/RESA protein and other merozoite derived proteins of molecular weights ranging from 115 to 23-Kd. After challenge, animals immunized with the Pf 155/RESA antigen and a merozoite derived protein of Mr 55-kd showed delayed onset of parasitaemia by 5 to 7 days, suggesting a partial protective immunity induced by vaccination. Monkeys immunized with the 83-kd and 35-kd fragments of the Pf 195 protein were completely protected as shown in table 2.1. Immunization with the other fragments of the Pf 195 did not confer protection.

Putarroyo et al. (1987b) immunized another group of Aotus monkeys with the Pf155/RESA protein and fragments of the 83-kd Protein and other merozoite derived proteins in different combinations. Several peptides did not elicit a protective immune response against the experimental infection, regardless of high antibody titres. However, immunization with particular peptides (derivatives of the 83-kd, 55-kd and 35-kd) delayed the onset of disease in some of the vaccinated animals suggesting that these peptides were able to elicit partial protective immunity. Based on this information, a new immunization scheme was developed, using a combination of two or three of the partially protective peptides. When this new group of monkeys were challenged, four of the 8 monkeys immunized with a mixture of two peptides (Spf 31.1 and Spf 55.1) developed a disease similar to controls. The remaining four developed moderate parasitaemia and spontaneously recovered. Of six

Table 2: Resistance to parasitaemia in monkeys immunized with purified proteins

Immunizing molecule	Monkey number	Percentage of parasitaemia on days (after challenge) 6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
155K	87	0	0	0	0	0.88	0.3	2	3	5.35	8.8	0				
	90	0	0	0	0	0	0	0.8	0.2	0.45	0.9	1.5	1.6	2	3.4	10.3
115K	85	0	0.46	0.84	3.48	3	9.93	0								
	150	0	0.05	0.2	1.12	1.67	nd	6.57	0							
105K	165	0	0.33	1.92	3.72	1.09	0									
	168	0	0.81	1.64	3.33	1.15	0									
90K	160	0	0.13	1.2	3.39	4.14	1.36	0								
	170	0	0.1	1.1	1.2	5.09	5.4	1.47	0							
83K	125	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	130	0	0	0	0.6	0.2	0.3	3.63	1.8	1.1	0.6	nd	0	0	0	
	144	0	0	0	0	0	0	0	0.1	0.05	0.5	0.3	0.07	0.03	0.01	0
60K	119	0	0	0	0.1	0.8	0.15	1.5	1.25	1.53	0					
	122	0	0.13	0.25	0.15	0.8	2.5	1.75	0							
55K	81	0	0	0	0	0	0.18	0.24	0.8	1.57	3	3.5	4.4	7.40		
	102	0	0	0	0	0	0.05	0.05	0.5	0	0.1	0.6	0.9	5.4	7.3	0
50K	29	0	0.43	1.5	3.6	4.64	1.0	0								
	56	0	0.66	5.6	4.43	6.42	4.2	11.3	0							
	131	0	0.31	1.5	0.5	3.93	2	1.65	0							
40K	111	0	0	0	0	1.02	0.2	2.7	3.2	1.43	0					
	114	0	0	0	0	0.8	0.5	2	2	2	7.6	0				
35K	135	0	0	0	0	0	0	0.001	0.003	0.03	0.1	0.01	0.01	0	0	0
	159	0	0.25	0.25	1.3	2.23	1.03	2.5	2.1	4	2.1	1.6	0.7	0.08	0.01	0
30K	93	0	0.05	1.52	2.5	4.75	3.45	2.54	4.8	9.40						
	171	0	0.1	2.5	1.6	2.63	2.05	1.3	0							
23K	117	0	0.06	0	1	0	3.6	6.37	6.6	1.2	0					
	137	0	0.05	0.05	0.2	0	1.1	3.2	nd	0						
Controls	501	0.4	0.76	3	2	7.82	9.07	0								
	502	0.03	0.06	0.43	0.47	3.6	3.4	5.9	8.92	0						
	199	1	4.3	3.11	9.8	2.6	0									

nd indicates not determined. Table was obtained from Patarroyo et al (1987)
0 = Beginning of chloroquine therapy

monkeys immunized with a mixture of 3 peptides (Spf 35.1, Spf 55.1 and Spf 83.1), three developed low parasitaemia levels significantly later than in the controls and recovered spontaneously. The remaining three animals never developed parasitaemia.

In order to overcome carrier problems, Patarroyo et al. (1988) synthesized two hybrid polymers: Spf (105) and Spf (66). The spf (105) contained the Spf 83.1, the CSP repeat region (NANP) and the 5' repeat region of Pf 155/RESA while the Spf (66) contained the CSP repeat region, Spf 83.1, Spf 55.1 and Spf 35.1. Thirteen male volunteers were selected to be vaccinated from a total of 109 healthy high school graduate volunteer soldiers from the Colombian Military forces. Antibodies to the merozoites and schizonts were detected in all sera. No correlation was found between antibody levels and malaria protection. After the seventh day of challenge, volunteers who received saline had parasitaemias that rose in 12 hours from very low levels and were promptly treated. Two of four volunteers vaccinated with Spf (105) showed partial control of infection with low parasite counts during days 13 and 14 and received drug therapy with no clinical problems. The other two behaved as controls and were treated similarly. Three of the five volunteers vaccinated with Spf (66) had mild infections with steady increase in parasite counts and total recovery by day 21 (Table 2.2). The fourth volunteer had parasitaemias below 0.41 % and on day 10, decided to withdraw from the study and was treated. The fifth developed parasitaemia similar to the control group.

Table 2.2 Development of Postchallenge Parasitaemia in the vaccinated Volunteers.

	Percentage of Parasitaemia on Days After Challenge																								
	5		6		7		8		9		10		11		12		13		14		15	16	17	18	19	20	21
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM							
Volunteers vaccinated with SPf66/130																											
A.B	0	0	0	0	003	007	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A.C	015	036	040	002	440	285	020	0	171	368	018	006	190	130	015	186	387	094	078	032	001	0	030	030	0	020	0
A.E	0	024	008	0	052	102	0380	002	214	465	011	004	062	116	130	013	330	242	060	00	020	116	044	0	0	0	0
A.A	006	018	028	011	410	100	066	001	208	0	014*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A.C	0	006	007	0	002	031	180	002	070	2 150*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Volunteers vaccinated with SPf66/130																											
A.A	0	005	020	0	018	103	118	004	330	336	092	027	037	660	110	180	488	600	334	876*							
A.S	010	180	025	0	002	220	013	005	603	660	011	020	364	430	060	190	1 050*	—	—	—							
A.O	0	006	002	004	015	112	100	005	1 300*	—	—	—	—	—	—	—	—	—	—	—							
A.B	002	100	054	002	110	400	460	008	2 130*	—	—	—	—	—	—	—	—	—	—	—							
Control volunteers																											
A.C	0	033	022	007	018	270	150	0	115	1 600*																	
J.D	0	033	028	005	008	810	780	017	4 260*	—																	
C.B	0	120	072	0	300	3 300*	—	—	—	—																	
J.E	008	100	115	010	1 700	3 600*	—	—	—	—																	

Time course of parasitaemia in human volunteers immunized with the polymeric synthetic hybrid proteins SPf66/130 and SPf105/130. Control volunteers were immunized with alumina. Challenge was performed by intravenous inoculation of 1×10^5 live sporozoite-infected erythrocytes of *P falciparum* (a wild Colombian strain) derived from a naive volunteer previously infected by transfusion. Parasitaemias were examined every 12 hours after the third day, by thick and thin blood smears stained with Giemsa, field stain, and euchrome orange. Results of microscopic staining are shown.

*Beginning of chloroquine therapy (Patarroyo et al., 1988)

The synthetic hybrid polymer Spf (66) is the first synthetic vaccine for human use against the asexual blood stages of *P. falciparum* malaria. Patarroyo et al. (1992) evaluated the safety and immunogenicity of the synthetic Spf 66 vaccine on Colombian children aged 1 - 14 years. A majority of the children developed high antibody titres against Spf 66. They concluded that the Spf 66 vaccine is safe and highly immunogenic for use in children greater than 1 year old. The efficacy of the Spf 66 vaccine has been put at 82.3% against *P. falciparum* and 60.6% against *P. vivax* (Amador et al., 1992). In a phase III randomised, double blind, placebo-controlled trial in La Tola, Colombia, Valero et al. (1993) showed that the SPf66 malaria vaccine is safe, immunogenic and protective against *P. falciparum* malaria. SPf66 is being taken seriously by the World Health Organization and other trials are under way (Cox, 1993).

2.13.9 Sexual Stage Vaccines

Some target antigens of malaria transmission-blocking antibodies against the sexual stage parasites have been studied most comprehensively in the chicken malaria, *P. gallinaceum* and also in *P. falciparum*.. Three malarial proteins 230Kd, 48/45Kd and 25Kd synthesized by sexual stages and expressed on the surface of gametes and zygotes have been identified as important targets for transmission-blocking immunity (Rener et al., 1983; Vermeulen et al., 1985).

Quakyi et al. (1989) reported that 43% of adult sera reacted with the 230Kd protein and 9% reacted with the 48/45Kd antigen. None of the sera reacted with the 25Kd protein

Antibodies developed in the mammalian host against gametes or zygotes of malaria parasites can block the infectivity of the parasites to mosquitoes. Other antibodies induce complement-dependent lysis of gametes or zygotes (Kaushal et al., 1983).

CHAPTER THREE

3.0 MATERIALS AND METHODS

MATERIALS

3.1 Study Area

The study was carried out at Igbo-Ora, in the Ifeloju Local Government Area of Oyo State, bordering the rain forest belt. It consists of rolling savannah country, with residual patches of forest growing near water courses. Most of the land lies between 400 and 600ft above sea level. The climate is that of the tropical rain-forest zone. There is a warm dry season from November to March, and a cooler rainy season from April to October. The mean annual rainfall is of the order of 1270mm, the wettest month (June) having about 178mm of rain and the driest (December and January) about 80mm (Oguntesi, 1988). Temperatures do not vary greatly, the highest monthly mean minimum being around 34.4°C in February and March, and the lowest monthly mean minimum being around 24.1°C for most of the year. Humidity is fairly high.

Igbo-Ora lies about 97km west of Ibadan and is the largest town (Administrative headquarter) in the Ifeloju Local Government Area with a population of 29,435 inhabitants (1963 population census). It is surrounded by several villages which include Tapa, Aiyete, Idere, Iwagan and Eruwa town. The population belong to the Yoruba tribe. The main occupation of the men are farming and hunting. The occupation of

the women include selling of farm produce (mostly in Lagos), retail trade and brewing pitto (a type of beer) from guinea corn. Malnutrition is not a serious problem.

The health facilities include a hospital (Fig.9) which is run by the University College Hospital (U.C.H.) Ibadan in collaboration with the Oyo State Government. There are two maternity units located at Igbole and Oke-gogo.

Common diseases in Igbo-Ora besides malaria, include schistosomiasis, onchocerciasis, loaisis, dracuntiasis and diarrhoeal infections. Infant mortality rate has been put at 33 to 44 per 1000 live births while child (1-4 years) mortality rate is between 16 to 21 per 1000 children (Oni et al., 1982). Major causes of death was due to malaria (35.6%), measles (19.6%), diarrhoeal diseases (8.1%), prematurity (5.9%) and chest infections (4.5%) (Oni et al., 1982). Birabi et al. (1976) described Igbo-Ora as mesoendemic for malaria. Malaria transmission is perennial although transmission reaches its peak during the rainy season. The malarial species found in Igbo-Ora are *P. falciparum* (about 90%) *P. malariae* (5-8%) and, *P. ovale* (2%). *Anopheles gambiae* and *A. funestus* are the main vectors of malaria parasites (Lawrence, 1965).

3.2 INITIAL SAMPLING OF THE STUDY POPULATION

3.2.1 Mothers and their Newborns

Pregnant women who are long-term residents of Igbo-Ora, reporting for delivery at the Igbole, Oke-gogo and comprehensive

Fig. 3.1 Map of Igbo-Ora town showing major roads and existing medical facilities (+).

maternity units constituted the study population of the mothers. Blood samples (3-5mls) were collected from umbilical cords at delivery and also from parturient women 6-18 hours after delivery into ethylene diaminetetraacetic acid (EDTA) containers. The birthweights, sexes and dates of birth of the newborns were recorded. A questionnaire designed to provide information on the age, parity, use of chemoprophylaxis, occupation and residential address was prepared for each volunteer parturient mother. Mothers were given identification cards and were advised to seek treatment at the Comprehensive Hospital. They were all informed of subsequent visits by a Family Visitor a fortnight later. None of the subjects exhibited signs or complaints of clinical malaria. A total of 116 paired maternal and cord sera were obtained between February and May 1991.

3.2.2 Adult Study Population

The adult study population consisted of students and teachers of the Government Technical College (G.T.C) Igbo-Ora and blood donors at the University College Hospital (U.C.H.) Ibadan blood donor clinic. After careful explanation of the aims, procedures and significance of the study in order to seek their consent, 100 individuals (15 teachers and 85 students) volunteered to participate in the study. A questionnaire designed to provide information on the age, sex, ethnic group, use of chemoprophylaxis and method(s) used to reduce mosquito-man contact was prepared. Volunteer subjects were bled by venepuncture and 5ml of blood collected into EDTA containers in July, 1991, the peak of the rainy

season when malaria transmission is high. They were each given an identification card and advised to seek free medical treatment at the Comprehensive Hospital, Igbo-Ora. Blood samples were transported to Ibadan same day. Thick and thin films were prepared and stained with 5% Giemsa. A small aliquot of blood (1.5ml) was stored (for MNSsU blood grouping) and the remainder was centrifuged. Plasma supernatants were stored at -20°C for the estimation of immunoglobulins and malarial antibodies. The red cell deposits were washed with normal saline and stored at -20°C for haemoglobin genotyping.

Blood samples (5ml) were collected into EDTA sample containers from 224 blood donors at the U.C.H. Ibadan blood donor clinic towards the end of the rainy season and a malaria peak transmission period (October-November,1991). Towards the end of the dry season when malaria transmission is lowest (March,1992) a further 192 blood samples (5ml each) were collected from blood donors at the same blood donor clinic. The age and sex of each donor was recorded during the two sampling periods.

Thick and thin films were prepared from each donor's blood and were stained with Giemsa for malaria parasite examination. A small aliquot of blood collected during the rainy season was stored in EDTA (for MNSsU blood grouping) and the remainder centrifuged at 2,000 r.p.m for 3 minutes. Plasma supernatants were stored at -20°C for the estimation of immunoglobulins and malarial antibodies. The red cell

deposits from the two sample surveys were washed in normal saline and stored at -20°C for haemoglobin genotyping.

3.3 MORBIDITY MONITORING AND SAMPLE SURVEYS

3.3.1 Mothers and Infants

Each mother and her baby were visited fortnightly by a Family Visitor, during which history of recent illness was recorded on a morbidity questionnaire. Mothers and infants who reported ill were brought to the Comprehensive Hospital for diagnosis by the U.C.H. Paediatric Registrars. A thick blood film was prepared from malaria suspected cases and a curative dose of chloroquine given. Mothers were again reminded to use their identification cards in obtaining free treatment at the hospital pharmacy in case of any illness. The fortnightly home visits were carried out for the first ten months and thereafter each mother was visited monthly.

Mothers were requested to visit the Comprehensive Hospital bi-monthly. During such bi-monthly clinics, the rectal temperature of each infant and the oral temperature of each mother was measured. The body weight of each infant was also measured using a portable dish-like beam balance to the nearest 0.1 Kg. Mothers and infants were then referred to the laboratory where the mothers were bled by venepuncture and the infants by finger pricking. A minimum of two heparinized tubes were collected from each infant.

Thick blood films were prepared from both mothers and infants and were stained by Field's rapid staining method (Field, 1942). Subjects positive for malaria parasites and other sick cases were referred to the clinicians for treatment. Plasma from the heparinized tubes obtained by breaking the tubes and plasma from the mothers were transported to Ibadan same day and stored at -20°C for the estimation of immunoglobulins and malarial antibodies. Episodes of clinical malaria were classified into three different categories:

1. Febrile illness with rectal (infants) or oral (mothers) temperature of 37.5°C and above, with the usual malaria symptoms and confirmed parasitaemia. This criteria was adopted because some mothers were incapable of detecting fever in their babies when interviewed.

2. Febrile illness with body temperatures of 37.5°C and above without parasitaemia but with the usual malaria symptoms. This criteria was considered because some mothers administered anti-malarial drugs to their infants on noticing fever before coming to the hospital to seek medical treatment. Likewise, some mothers took anti-malarial drugs or the medicinal herb 'agbo'. In both cases there is an increased likelihood that malaria parasites would not be detected by the thick film method. In the above cases treatment was either continued with the starting anti-malarial drug or an alternative depending on the duration of self medication.

3. Febrile illness with malaria symptoms but for which the malaria parasite test was not done and the patients were treated only for malaria

and they reported well thereafter. These cases include those patients treated by the U.C.H. Ibadan Paediatric Registrars and were not referred for laboratory diagnosis. Information was obtained from case notes.

3.3.2 Adult Study Population

Blood samples were collected by venepuncture (3-5mls) from 33 of the 100 previously sampled volunteers of the GTC, Igbo-Ora in February, 1992; the peak of the dry season when malaria transmission is lowest. Thick smears were prepared and stained with Giemsa. Plasma samples were separated and stored at -20°C.

METHODS

Blood Films and PCV

Thick and thin films were prepared from the cord and maternal blood samples. Thin films were fixed for 3 minutes with methanol. Both slides were stained with 5% Giemsa stain for 30 minutes, air-dried and stored in a slide box until transported to Ibadan for parasitological examination. The packed cell volume (PCV) of both cord and maternal blood samples were measured using a Hawksley microhaematocrit centrifuge. Plasma samples were centrifuged at 2,000 r.p.m for about 3 minutes. Plasma supernatants for the estimation of immunoglobulins and malarial antibodies were separated and stored at -20°C at the Comprehensive Hospital Laboratory. They were later transported in an

ice-packed cooling flask to the Immunology Unit, U.C.H. Ibadan where they were stored at -20°C until analysed.

3.4 PARASITOLOGIC EXAMINATION

Microscopic examination for the detection of malaria parasites was made in 200 high power fields of the thick films, before being considered negative. For parasite positive slides, one hundred high power fields were examined and the malaria parasites counted against leucocytes, assuming a constant leucocyte count of 8,000 per µl of blood (Rooth et al., 1991).

3.5 HAEMOGLOBIN GENOTYPING

Reagents

(a) Preparation of Tris-EDTA-borate buffer Stock Solution.

The following components of the Tris-EDTA-borate buffer stock solution were weighed using a Metler balance:

Tris (hydroxymethyl) aminoethane	51.0g
Ethylene diaminetetraacetic acid	3.0g
Barbitone Sodium	3.2g
Boric acid	16.0g

The above reagents were dissolved in distilled water and the pH adjusted to 8.6 with 0.1N HCl when necessary. The final volume was made up to 1 litre with distilled water. The working Tris-EDTA-borate buffer solution was prepared by diluting the stock solution 1 in 5 with distilled water.

(b) Staining Solution

Ponceau S (0.2g) was dissolved in 50ml distilled water and 3.0g of trichloroacetic acid was added into the solution. The final volume was made up to 100ml with distilled water.

(c) Acetic Acid

7.0ml of Glacial acetic acid was diluted to 100ml with distilled water.

Method

Haemoglobin genotype was determined by electrophoresis on cellulose acetate strips by the method of Marengo-Rowe (1965). Frozen red cells (obtained from students and Teachers of the G.T.C., Igbo-Ora, blood donors at the U.C.H. Ibadan and volunteer mothers and their infants when they were above 6 months of age), were thawed and diluted 1 in 2 with distilled water.

The cellulose acetate membrane was thoroughly soaked by first floating it on the surface of the working Tris-EDTA-borate buffer solution and then immersing when completely wetted for a minimum of 10 minutes. One drop from each of the diluted haemolysed red cells was placed on the raised platforms of the sample applicator plate. The cellulose acetate membrane was removed and placed between clean filter papers to absorb excess fluid.

The test samples were collected from the platforms onto the applicator and applied on to the acetate membrane by vertical contact.

The membrane was then suspended in a two compartment electrophoretic tank filled one-quarter way with the working Tris-EDTA-borate buffer solution. Sufficient tension was applied to maintain the membrane firmly in a horizontal position. The voltage was adjusted to 200mV and the electrophoresis was run for 30 minutes. The cellulose acetate membrane was removed and stained for 2 minutes in the Ponceau S staining solution. Excess stain was removed by rinsing in the 7% acetic acid solution. The haemoglobin bands were read using haemoglobin AS and SC as controls.

3.6 MNSsU(Ge) BLOOD GROUPING

Reagents

(a) Monoclonal antibodies (Mab)

The following Mab (supplied by Prof Geofrey Pasvol, Middlesex) were used to identify the MNSsU blood group antigens:

(i) Anti-M (6A7) Mab was a specific anti-M reagent.

(ii) Anti-N (URIC 157) Mab when used undiluted agglutinates all normal red cells. M+N-S-s-(U-) phenotype red cells are not agglutinated. Trypsin treated N+S-s-(U-) phenotype i.e. glycophorin B deficient cells are also not agglutinated. This anti-N Mab was diluted in phosphate buffered saline (PBS), PH 7.3 containing 3% bovine serum albumin to obtain a specific anti-N reagent. The required dilution (1 volume of anti-N + 10 volumes of PBS, PH 7.3) was determined by titration with normal M+N-, M+N+ and M-N+ red cells.

(iii). Anti-sialoglycoprotein beta (BRIC 10) specific Mab agglutinates normal red cells but not Gerbich negative (Ge-) i.e. glycophorin C negative red cells.

(b) Preparation of Phosphate Buffered Saline (PBS), pH 8.0

The following stock solutions were prepared:

A 0.5M Na_2HPO_4 (70.98 g/L)

B 1.0M KH_2PO_4 (136 g/L)

C 1.5M NaCl (87.75 g/L).

The working PBS solution was prepared by adding 193ml A + 7.0ml B + 100ml C and the pH adjusted to 8.0 when necessary. The final volume was made up to 1 litre with distilled water.

(c) Preparation of Phosphate Buffered Saline (PBS), pH 7.3

The following stock solutions were prepared:

A 0.2M $NaH_2PO_4 . 2H_2O$ (62.401 g/2L)

B 0.2M Na_2HPO_4 (56.8 g/2L)

C 1.5M NaCl (87.75 g/L)

The working PBS, pH 7.3 solution was prepared by adding 23.0ml A + 77ml B + 100ml C and the pH adjusted to 7.3 when necessary. The final volume was made up to 1 litre with distilled water.

(d) Trypsin treated red cells

Bovine pancreatic trypsin (5mg) (BDH Ltd. England) was dissolved in 2.0ml of PBS, pH 8.0. Fresh whole blood samples were centrifuged and the plasma supernatant separated. The red cell deposits were washed three times in PBS, pH 8.0. The washed red cells (0.5ml)

were added to the trypsin solution, mixed gently and, incubated at 37°C for 30 minutes. At the end of the incubation period the red cells were washed three times in PBS, pH 7.3.

Method

The MNSsU(Ge) blood group antigens were identified using the tube agglutination test described by the South Western Regional Transfusion Centre, Bristol, U.K (source of Mab). Trypsin untreated red cells from the study subjects were washed three times in PBS, pH 7.3. A 3% suspension of red cells in PBS, pH 7.3 was prepared from washed normal and trypsin treated red cells. Equal volumes (1 drop) of Mab and red cell suspensions were mixed in a glass tube. The mixture was incubated, undisturbed, for one hour at room temperature. The tubes were gently agitated and the cells inspected for agglutination.

3.7 QUANTITATIVE DETERMINATION OF IMMUNOGLOBULIN CONCENTRATION

Reagents

(a) Anti-sera

Commercial goat anti-human IgG, IgM and IgA (Atlantic Antibodies, Scarborough, U.S.A) were used in the immunoglobulin assay.

(b) Immunoglobulin Standards

Commercially obtained standards of immunoglobulins (IgG, IgM and IgA) were obtained from Behringwerke AG, Germany and used as

constant reference sera in the immunoglobulin assay. The standards were diluted with phosphate buffer, pH 7.2 as follows: 25%, 50%, 75% and 100%.

(c) Preparation of immunodiffusion plates

Noble agar (DIFCO Laboratories, U.S.A) was weighed (3.0g) and added to 99ml of PBS, pH 7.2. Sodium azide (1.0ml of 0.1M solution) was added as a preservative. This mixture was immersed in a boiling water bath and stirred occassionally until the agar was completely dissolved. 1.0ml of goat anti-human IgG, IgM and IgA were pipetted into three labelled tubes containing 8.0ml of phosphate buffer, pH 7.2. The tubes were incubated at 56°C for 10 minutes. An equal volume of the dissolved agar (9.0ml) was added to each labelled tube, mixed quickly and poured immediately on to labelled clean large glass slides measuring 10.0cm x 8.3cm. The agar was allowed to solidify and a series of 72 wells, about 1.0cm apart, were punctured in the agar plates using a gel punch of diameter 0.3cm. The agar from the punctured wells were carefully removed with a pasteur pipette attached to a vacuum pump.

Method

The three major classes of plasma immunoglobins (IgG, IgM and IgA) were quantified by a modification of the single radial immunodiffusion method in agar gel (Salimonu et al., 1978).

The standard immunoglobin diluents were applied into the wells in the order 25%, 50%, 75%, 100% and 200% using a micropipette which

delivers 5µl of solution. The 200% standard was applied by pipetting twice (i.e. 10µl) the undiluted immunoglobulin standard into the same well. The test plasma samples were then applied into each well and the agar plates allowed to stand at room temperature for 3-5 minutes. The agar plates were placed in a moist chamber and diffusion allowed to take place at room temperature for a minimum duration of 6 hours for IgG, 8 hours for IgA and 24 hours for IgM. The diameters of the precipitin rings were read to the nearest 0.1mm using an immunoplate reader. The standard curves were prepared by plotting the ring diameters against concentrations of reference immunoglobulin on a semi-log paper. Using the diameters of the precipitin rings the concentrations of IgG, IgA and IgM in the test samples were read off the standard graph.

3.8 DETERMINATION OF CORD BLOOD TOTAL IgM

Using the single radial immunodiffusion method in agar gel, IgM was not detected in a majority (92%) of the cord blood samples. Consequently the more sensitive ELISA test was employed in the assay of total IgM in these samples. The ELISA test was carried out in the Department of Immunology, University of Stockholm, Sweden.

Reagents

(a) Incubation Buffer

The Incubation buffer consisted of PBS + 0.5% BSA + 0.05% Tween 20.

3.8.1 IgM ELISA

ELISA plates (Nunc. Denmark) were coated with 50µl of affinity purified rabbit anti-human IgM (Miu-chain specific; DAKOPATTS. Denmark) at a concentration of 10µg/ml in coating buffer (pH 9.6). The plates were wrapped in Aluminium foil and left overnight in the cold at 4°C.

Cord blood samples were diluted1:1.000 in incubation buffer. The IgM standard curve was prepared using human cryoglobulin (3.5mg/ml) obtained from a patient with IgM myeloma. The standard solution was diluted with incubation buffer to give 300, 100, 10, 3 and 1ng/ml respectively.

The ELISA plates were washed 4 times with washing buffer (see page 106) and 50µl of the standards and cord samples were added. The plates were incubated for 1hr at 37°C and washed 4 times. Goat anti-human IgM (Miu-chain specific; DAKOPATTS, Denmark) alkaline phosphatase conjugate (Sigma) was diluted 1:1.000 with incubation buffer and 50µl was added per well and incubated for 1hr at 37°C. The plates were washed 4 times and 50µl of substrate added per well. The OD at 405nm of the test samples were recorded using a multiskan ELISA plate reader (Titerlek, U.S.A) when the OD of the highest standard read 1.000. The concentration of IgM in the test samples were read off a standard curve using a Macintosh computer software (Softmax).

The specificity of the Rabbit anti-human IgM was confirmed by adding different concentrations of human IgG instead of test plasma

samples. The OD values were below 0.07. Human IgA could not be used because it was found to cross react with anti-human IgG.

3.9 Antibodies Against the RESA/Pf 155 and Total Blood Stage Antigens of *P. falciparum*

Immunofluorescence assays to detect antibodies against RESA/Pf155 and total blood stage antigens were carried out in the Department of Immunology, University of Stockholm, Sweden.

Reagents

(a) Bicarbonate Coating Buffer (pH 9.6)

Na_2CO_3	-	1.59g
$NaHCO_3$	-	2.93g
NaN_3	-	0.20g

The above reagents were disolved in 1 litre of solution with distilled water.

(b) Phosphate Buffered Saline (PBS)

A stock solution of PBS was prepared with the following reagents:

KH_2PO_4	-	3.06g
Na_2HPO_4	-	24.8g
NaCl	-	48.6g

The above reagents were disolved in 1 litre of solution with distilled water. For use 1ml of stock PBS was diluted with 5ml of distilled water.

(c) Tris-Buffered Hank's (TBH) Solution

Tris buffer (0.15M) pH 7.2 was prepared by dissolving the following reagents in distilled water.

Tris base {Tris(hydroxy methyl) aminoethane} - 1.82g/100ml

Tris HCl {Tris(hydroxymethyl) aminoethane Hydrochloride} - 2.37g/100ml

NaCl - 7.88g/900ml

Tris base solution was added to Tris HCl untill the pH was 7.2. 100ml of the Tris buffer was added to the 900ml solution of NaCl. For immunofluorescence studies, 0.02% NaN_3 was added as a preservative.

TBH solution was prepared by mixing equal volumes of Tris buffer and commercially prepared Hank's balanced salt solution. For malaria parasite cultures 200ml of the TBH solution was dispensed into 300ml reagent bottles and autoclaved.

(d) Malaria Culture Medium (MCM)

One vial of RPMI 1640 medium(Gibco BRL. U.K.) with L-Glutamine was dissolved in double distilled water. Hepes (6.0g), $NaHCO_3$ (2.0g) and 0.5ml gentamicin (50mg/ml) were added to the solution and the final volume made up to 1 litre. The solution was filtered with Nalgene disposable filter (Sybron Corporation, New York). The sterile medium was added (270ml) to 30ml of sterile non-immune human serum obtained from a Swedish donor who had never been exposed to malaria.

(e) *In vitro* culture of *P. falciparum*

(i) Uninfected Erythrocytes (Eo)

Malaria parasite uninfected erythrocytes (Eo) was obtained from a Swedish donor who had never been exposed to malaria. The blood sample was screened by the Central Blood Bank, Sabbabergs, Stockholm.

The blood sample was dispensed aseptically into 50ml falcon tubes and stored at 4oC. Thick film examination was performed to confirm the absence of malaria parasites. The red cells of the donor blood was washed three times with sterile TBH solution and a 5% haematocrit Eo suspension was prepared in warm malaria culture medium.

(ii) Malaria Parasite Infected Erythrocytes (Ei)

The main source of malaria parasites was a Tanzanian strain of *P. falciparum* (F32) isolated in 1978 (Jepsen and Andersen, 1981) and cultured *in vitro* in blood group O+ according to Trager and Jensen (1976). The parasites were stored in liquid Nitrogen.

Liquid Nitrogen frozen *P. falciparum* was thawed in a 37oC water bath and centrifuged at 1800 rpm for 3 minutes in a cold centrifuge. The following solutions were added to the red cell pellet sequentially and centrifuged in a cold centrifuge at 1500 rpm:

1. 1ml 17.5% sorbitol in PBS + 2ml 10% sorbitol + 2ml 7.5% sorbitol
2. 1ml 10% sorbitol + 2ml 7.5% sorbitol + 2ml 5% sorbitol
3. 1ml 7.5% sorbitol + 2ml 5% sorbitol + 2ml 2.5% sorbitol

(e) *In vitro* culture of *P. falciparum*

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2. 1ml 10% sorbitol + 2ml 7.5% sorbitol + 2ml 5% sorbitol
3. 1ml 7.5% sorbitol + 2ml 5% sorbitol + 2ml 2.5% sorbitol

4. 1ml 5% sorbitol + 2ml 2.5% sorbitol + 2ml warm malaria culture medium
5. 1ml 2.5% sorbitol + 2ml warm malaria culture medium

After the last centrifugation the Ei (mostly ring forms) was resuspended in malaria culture medium to 5% haematocrit.

(iii) Culture Procedure

Equal volumes of Ei and Eo suspensions were mixed to yield a final volume of 4ml in a 50ml Nunclon culture flask (Intermed, Denmark). The culture flask was incubated at 37°C in a candle jar with greased edges. Spent medium was replaced every 24hrs with warm malaria culture medium. After 72hrs of culture, one drop of malaria parasite culture was added to one drop of acridine orange (0.001%) and examined under ultraviolet light. The percentage parasitaemia and developmental stages of the parasite were recorded. The culture mixture was washed 3 times with TBH solution and a 5% Ei suspension prepared. The parasite culture was subcultivated at 1% parasitaemia by adding the required volume of 5% Eo to yield a final volume of 4ml.

(iv) Malaria parasite Infected Erythrocyte Monolayers

Parasite growth was monitored after every 72hrs of culture for percentage parasitaemia and parasite developmental stages. When the percentage parasitaemia was about 10% and the parasites were mostly in their late stages (late trophozoites/schizonts) the parasites were harvested

and erythrocyte monolayers prepared for detecting antibodies to total blood stage antigens .When the parasitaemia was between 5-10% and the parasites were in their early developmental stages (ring forms) the parasites were harvested for detecting antibodies to the RESA/Pf 55 antigen. Both the early and late stages of the malaria parasites were washed 3 times with TBH solution and a 1% haematocrit of Ei suspension was prepared.

Erythrocyte monolayers were prepared according to the method described by Perlmann et al. (1984). Fifteen -well multitest slides (Flow Laboratories, Rockville) were treated for 30minutes with one drop (20µl) per well of bicarbonate coating buffer pH 9.6. Immediately after aspiration of the coating buffer , one drop of the 1% Ei suspension was added to each well and the cells were left to settle for 30minutes at room temperature in a humid chamber. Unbound erythrocytes on slides containing late stages of *P. falciparum* were aspirated by suction and the slides were air-dried. Multitest slides with early stages were first immersed in a slide dish filled with PBS to rinse off unbound erythrocytes. The slides were then fixed with 1% glutaraldehyde,washed with distilled water and air-dried. Both the late and early stages monolayers were stored at -50°C.

3.9.1 Immunofluorescence Assay

Antibodies to total blood stage antigens were detected by parasite immunofluorescence (PARIF), a modification of the method described by Voller (1962). Antibodies to the RESA/pf155 antigen were

detected by Erythrocyte Membrane Inununofluorescence (EMIF) as described by Perlmann et al. (1984).

Plasma test samples for PARIF studies were screned at 1:100,000 for adult/cord samples and 1:10,000 for serial samples from infants. Plasma test samples for EMIF were tested at 5-fold dilutions beginning with an initial dilution of 1:10.

PARIF (late stages) and EMIF (early stages) slides were air-dried for 10 minutes and the diluted test samples were added (20µl) to each antigen well and incubated for 30minutes. The slides were washed 3 times with TBH solution. Affinity purified and biotinylated goat anti-human IgG (reacting with both heavy and light chains, 30µg/ml; Vector Laboratories, Inc, CA) was added to each well and incubated for 30 minutes. The slides were washed 3 times with TBH solution. Avidin conjugated with fluorescein isothiocyanate (50µg/ml; Vector Laboratories Inc, CA) was added (20µl) per well and incubated for 30 minutes. The slides were washed 3 times with TBH solution. PARIF slides were mounted with TBH solution while EMIF slides were counterstained with 1 drop/well of ethidium bromide (10µg/ml; Sigma Co., St Louis). All incubations were done at room temperature in a humid chamber.

The slides were scored with a 100x oil immersion lens in incident ultraviolet light in a Zeiss Universal Research microscope (Carl Zeiss Co., Stockholm, Sweden) equiped for simultaneous observation of immunofluorescence(green) and nuclear staining(orange) (band filter 450

- 490, beam splitter FT 510 and barrier filter LP 520). Endpoint titre for antibodies to the RESA/Pf 155 antigen were determined as the last titre giving visible smooth staining of the entire surface of erythrocytes containing ring stages or early trophozoites. Test samples with titres ≥ 10 were considered positive.

For each batch of test, negative controls from Swedish donors who had never been exposed to malaria and a positive control from an African who had an attack of malaria while in Sweden were included.

3.10 ANTIBODIES TO SYNTHETIC *P. falciparum* ANTIGENS

Reagents

(a) Antigens

The following synthetic peptides were used as capture antigens in a peptide ELISA:

1. Synthetic peptides of amino acids from the circumsporozoite protein (CSP) repeat region $(NANP)_n$ -- (NANP)6
2. Synthetic peptides of amino acids from the C-terminal retranter $(EENV)_n$ repeat region of the RESA/Pf 155 antigen – (EENV)6
3. Synthetic peptides of 15 amino acids from the N-terminal of the RESA/Pf 155 antigen (MQTLWDEIMDINKRK, positions 192 - 206) -- LJ5
4. Synthetic peptides of 11 amino acids from the repeat region of Ag332 (positions 2 - 12) coupled to lysine $(SVTEEIAEEDK)_8$ - $(Lys)_7$ — Multiple Antigen Peptide 2 (MAP2)

The first three peptides were obtained from BACHEM (Bubendorf, Switzerland). The fourth peptide was synthesized and purified to homogeneity by high-pressure liquid chromatography in the Department of Immunology, Stockholm University. The ELISA tests were carried out in the Department of Immunology, University of Stockholm, Sweden.

(b) Incubation Buffer

PBS stock solution	-	150ml
Tween 20	-	0.45ml
Sodium azide	-	0.9ml of 20% stock solution.

The above reagents were dissolved in 900ml of distilled water. Prior to use, 0.5g of Bovine serum albumin (BSA) was dissolved in 100ml of the incubation buffer.

(c) Washing Buffer

NaCl	-	45g
Tween 20	-	2.5ml

The above reagents were dissolved in 500ml of distilled water

(d) Enzyme Substrate Buffer pH 9.8

Diethanolomine	-	97ml
$MgCl_2 . 6H_2O$	-	101mg
Distilled water	-	800ml
Sodium Azide	-	1ml of 20% stock solution.

$MgCl_2 . 6H_2O$ was added last. 1M HCl (~100ml) was added to adjust the pH.

(e) Enzyme Substrate

Alkaline phosphatase substrate tablets (Sigma) were used. Each tablet contained 5mg disodium p-nitrophenyl phosphate. One tablet was dissolved in 5ml of enzyme substrate buffer prior to use.

(f) Coupling of Synthetic Peptides to BSA

The (NANP)6, (EENV)6 and LJ5 synthetic peptides were coupled to BSA. Double distilled water (0.5ml) was added to 4mg of each peptide in 10ml tubes. Ammonium hydroxide (0.3M) was added (150µl/tube) to solubilize the peptides. The solubilized peptides were each poured into 10ml tubes containing 2mg BSA. The volume of each tube was made up to 2ml with PBS. Glutaraldehyde (2ml, 0.25%) was added dropwise into each tube while mixing on a touch plate. The tubes were sealed and placed on a roller drum overnight in a cold room. After 24 - 36hrs the BSA coupled peptides were dialized in PBS with 0.02% sodium azide. After 12hrs of dialysis the PBS solution was changed and the dialysis continued overnight. The final concentration of the peptides was 1mg/ml.

3.10.1 Peptide ELISA

The coupled synthetic peptides were diluted to 10µg/ml ((NANP)6, (EENV)6 and LJ5) and 1µg/ml (MAP2) with PBS pH 7.2. 50µl of each peptide was added to duplicate wells of a Nunc immunoplate (Denmark). The plate was wrapped in Aluminium foil and left in the cold room overnight.

The ELISA plates were emptied the next day and 100µl of PBS + 0.5% BSA was added to each well (except for the blank columns) and the plates were incubated for 3hrs at 37°C. All the test samples were diluted 1:1,000 with incubation buffer. Swedish negative controls and a positive control serum sample from a malaria immune African donor were diluted 1:1,000.

The ELISA plates were washed 3 times with washing buffer and 50µl of the test samples/negative and positive controls were added in duplicates. The plates were incubated for 1hr at 37°C and washed 3 times with washing buffer.

Rabbit anti-human IgG conjugated to alkaline phosphatase (DAKOPATTS. Denmark) was diluted 1:1,000 with incubation buffer. The enzyme conjugate was added (50µl/well) and the plates incubated at 37°C for 1hr. The plates were washed as above and 50µl of substrate added per well and incubated at room temperature in the dark. The OD of the test samples were read with a multiskan ELISA plate reader (Titertek. U.S.A) at 405nm against the plate blank when the positive control OD value was 1.000.

Test samples with an OD_{405} less than the mean + 2 standard deviations of the values from 25 Swedish donors never exposed to malaria were considered as negative.

3.11 DETECTION OF MALARIA - SPECIFIC IgM IN CORD BLOOD

Malaria specific IgM was detected in cord blood samples by PARIF using total blood stage antigens (late stages) of *P. falciparum*. Total blood stage antigen slides were air-dried and cord plasma (1:10 dilution) were added to each well and incubated for 30 minutes. The slides were washed 3 times with TBH solution and a drop of rabbit anti-human IgM (Miu-chain specific) conjugated to rhodamine (diluted 1:50 with TBH solution: DAKOPATTS, Denmark) was added per well. The slides were washed 3 times and mounted with TBH solution. Fluorescence was scored with a 100x oil immersion lens in Incident green light in a Zeiss Microscope. The malaria parasites were stained red for positive cord samples.

CHAPTER FOUR

4.0 RESULTS

4.1 Birthweights

The mean birthweight of the study infant population at Igbo-Ora, Oyo State is shown in Table 4.1. There was no significant difference ($t = 1.94$, $P > 0.05$) between the mean birthweights of male and female newborns. The mean birthweights of newborns was significantly different between the different parity groups of the mothers ($F = 9.30$, $P < 0.001$). Mean birthweight of the newborns increased with increasing parity (Fig. 4.1) till parity 3 and thereafter decreased with increasing parity with a marked significant decrease at parity 4 ($P < 0.005$) probably due to the small sample size. The mean birthweight of newborns of primiparae was significantly lower ($t = 5.361$, $P < 0.001$) than those of multiparae. Generally there was a positive correlation between the birthweights of newborn and mother's parity ($r = 0.26$, $P < 0.005$). There was no correlation between birthweight and duration of onset of primary clinical malaria in the infants ($r = 0.133$, $P > 0.20$). The difference in the mean (±S.E) birthweights (Kg) of newborns of malaria positive (3.13 ± 0.05) and malaria negative (3.21 ± 0.04) mothers was not statistically significant ($t = 1.109$, $P < 0.30$). However, a negative correlation was observed between maternal parasite density at delivery and birthweight of newborn ($r = -0.48$, $P < 0.02$).

Table 4.1 Mean (± S.E) birthweights of newborns at Igbo-Ora, Oyo State.

	n	Birthweight(Kg)	Range(Kg)*
Males	55	3.25 ± 0.05	1.1 (2.8 - 3.9)
Females	62	3.14 ± 0.04	1.5 (2.5 - 4.0)
Sexes combined	117	3.20 ± 0.03	1.5 (2.5 - 4.0)

*Values in parentheses indicate minimum and maximum birthweight values

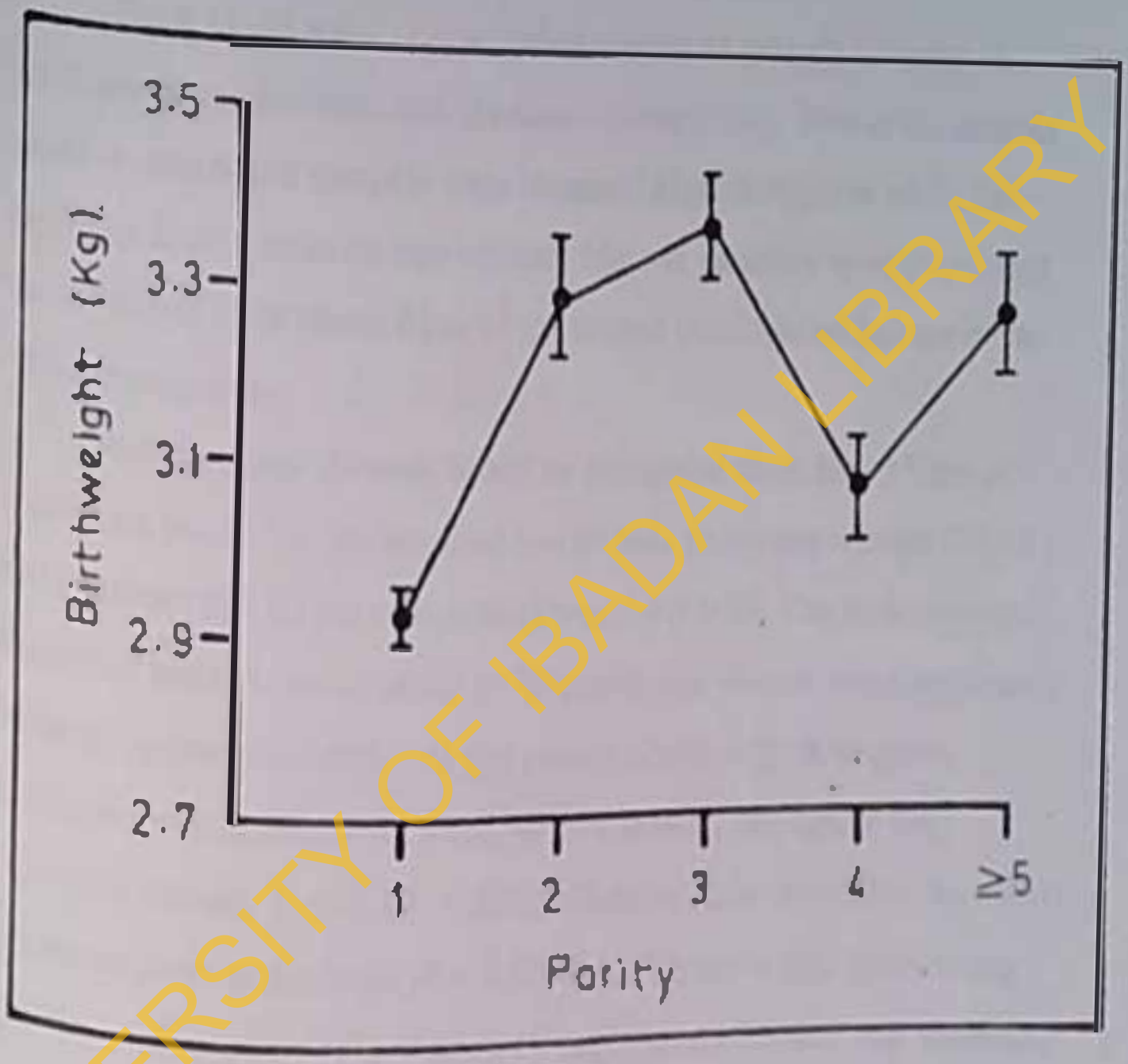

Fig. 4.1 Mean birthweight (± S.E) of newborns at Igbo-Ora, Oyo State according to Parity.

4.2 MALARIA IN THE STUDY POPULATION

(a) Malaria at delivery

Thick blood films of cord blood contained malaria parasites in three out of one hundred and seventeen cases(2.6%). Two of the malaria positive cord blood samples were obtained from multiparae while the third was from a primiparous woman. Malaria parasites were also found on peripheral thick blood films of the former (multiparae) but not in the latter (primiparae).

Malaria parasites were found on peripheral thick blood films of twenty-six out of the one hundred and sixteen parturient women (22.4%) and the mean (±S.E) parasite density was 2.4 ± 0.08. The incidence and density of malaria parasitaemia in the parturient women was significantly different between the different age groups (Table 4.2). A negative correlation was obtained between parasite density and age of the parturient women ($r = -0.47$, $P<0.02$). Parasite rates ($x^2 = 27.8$, $P< 0.001$) and mean parasite densities ($F = 4.53$; $P< 0.02$) decreased significantly with increasing parity (Fig. 4.2) and a negative correlation was obtained between parasite density and parity of the parturient women ($r = -0.54$, $P< 0.005$).

(b) Malaria in Infants and their Mothers

(i) Parasite rates and parasite densities

Table 4.3 shows the number of infant/mother pairs sampled at different time intervals from delivery till one year during the longitudinal studies at Igbo-Ora. Malaria parasite rates increased rapidly

Table 4.2 Malaria parasite rates and mean (± S.E) parasite densities in different age groups of Nigerian parturient women.

	Age Group (Years)			Significance of difference
	≤ 22	23 - 29	≥ 26	
Parasite rate	16/43(62%)	4/31(15%)	6/42(23%)	χ^2=8.62, P<0.02
Parasite density	2.5 ± 0.23	2.6 ± 0.35	2.1 ± 0.21	F=3.56, P<0.05

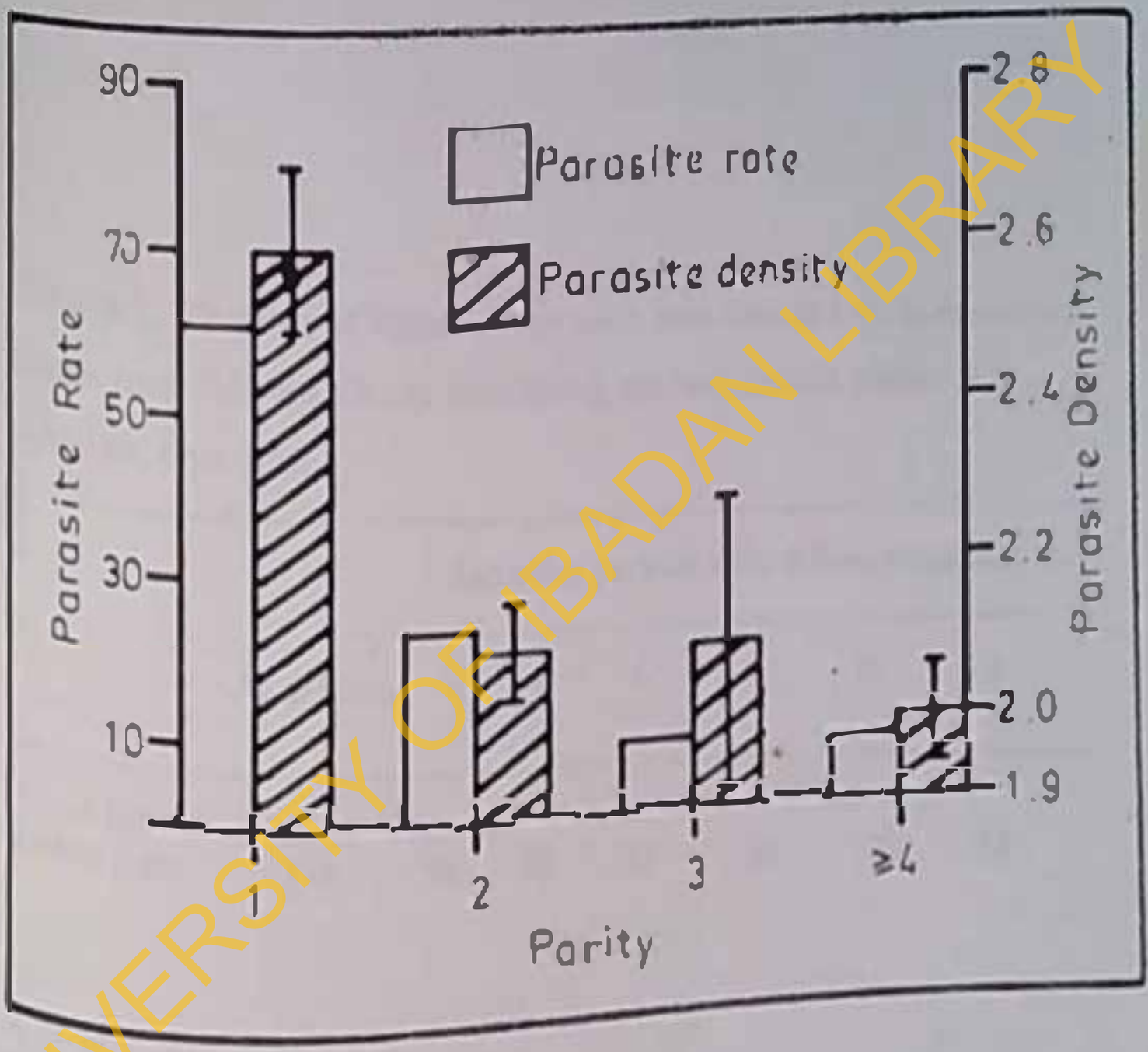

Fig. 4.2 Parasite rates and parasite densities (Mean ± S.E) by parity of 116 Parturient women at Igbo-Ora, Oyo State.

Table 4.3 Number of infant/mother pairs who attended the bi-monthly clinics from delivery till one year during the longitudinal studies at Igbo-Ora, Oyo State.

	Sampling periods after delivery(months)					
At delivery	2	4	6	8	10	12
No. of infant/ mother pairs	116	91	58	45	32	23	35
% of original study population	-	78.5	50.0	38.7	27.6	19.8	30.2

with increasing age of the study infants till 6 months of age and thereafter decreased gradually till one year of age (Fig. 4.3). The number of infants positive for malaria parasites during the bi-monthly clinics increased significantly ($\chi^2 = 37.5$, $P < 0.001$) with increasing age.

Parasite densities were also found to increase with age till 8 months of age and thereafter a gradual fall in parasite densities was observed till one year of age (Fig. 4.3). The mean parasite density at 4 months of age was significantly higher than at two months of age ($P < 0.001$). However, no significant increase in parasite densities with age was observed in subsequent ages of the infants. Generally, there was a positive correlation between parasite density and age of infant ($r = 0.21$, $P < 0.025$).

Figure 4.4 shows the parasite rates and densities of the study mothers on 6 consecutive bi-monthly surveys after delivery. A gradual increase in parasite rates was observed during the first three consecutive surveys after delivery and thereafter parasite rates decreased in the subsequent three consecutive bi-monthly surveys. There was no statistically significant difference in the parasite rates during the 6 consecutive surveys ($\chi^2 = 10.949$, $P < 0.10$). Parasite density increased at the second survey, dropped significantly ($P < 0.005$) at the third survey and increased significantly ($P < 0.005$) at the fourth survey. There was no significant difference in the decrease in parasite density after the fourth survey. The two peaks at survey 2 (4 months after delivery) and

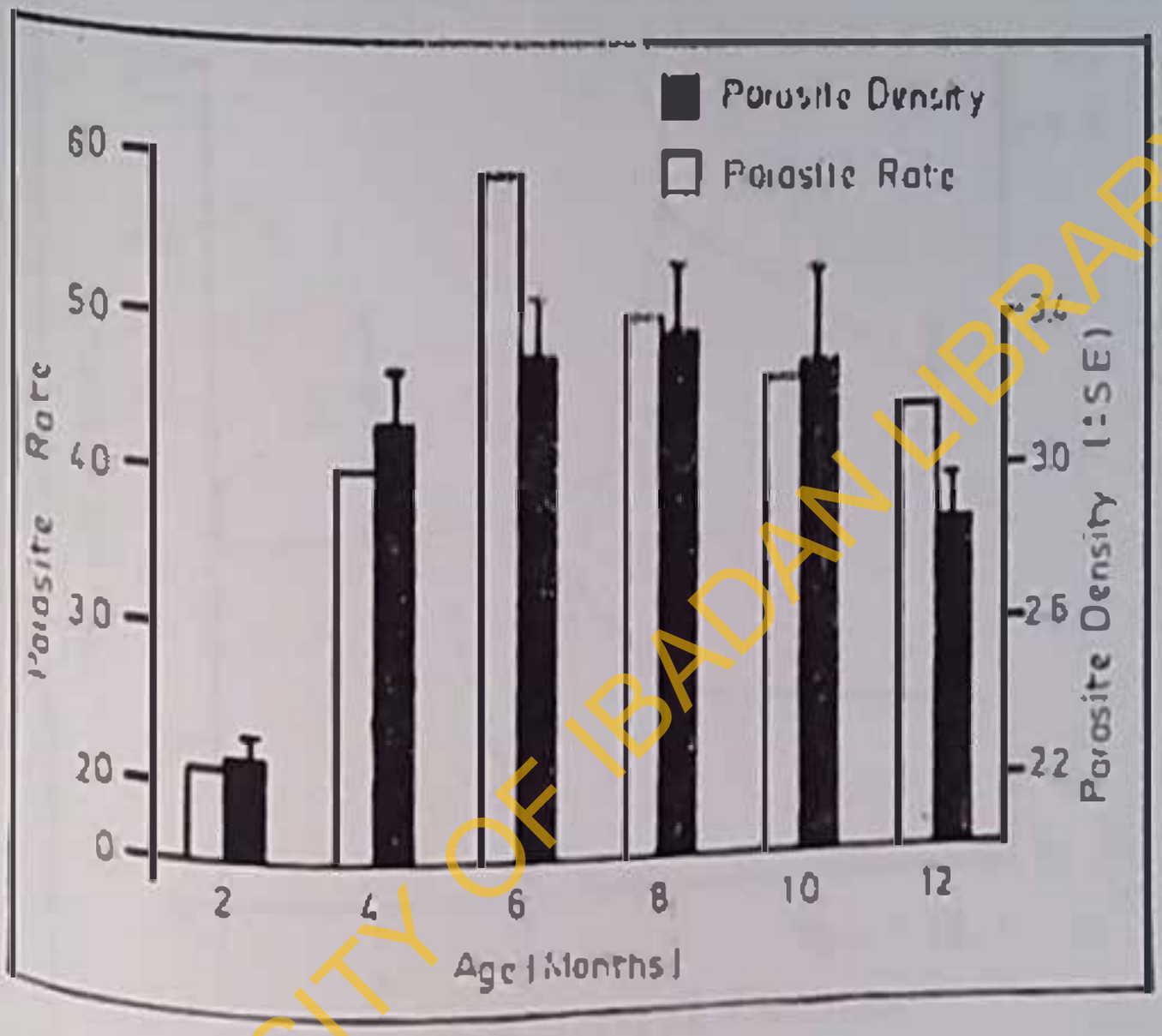

Fig. 4.3 Parasite rates (percentage of infants with any asexual *P. falciparum* parasites detected by the thick film method) and mean (± S.E) parasite densities of Nigerian infants at Igbo-Ora during the first year of life.

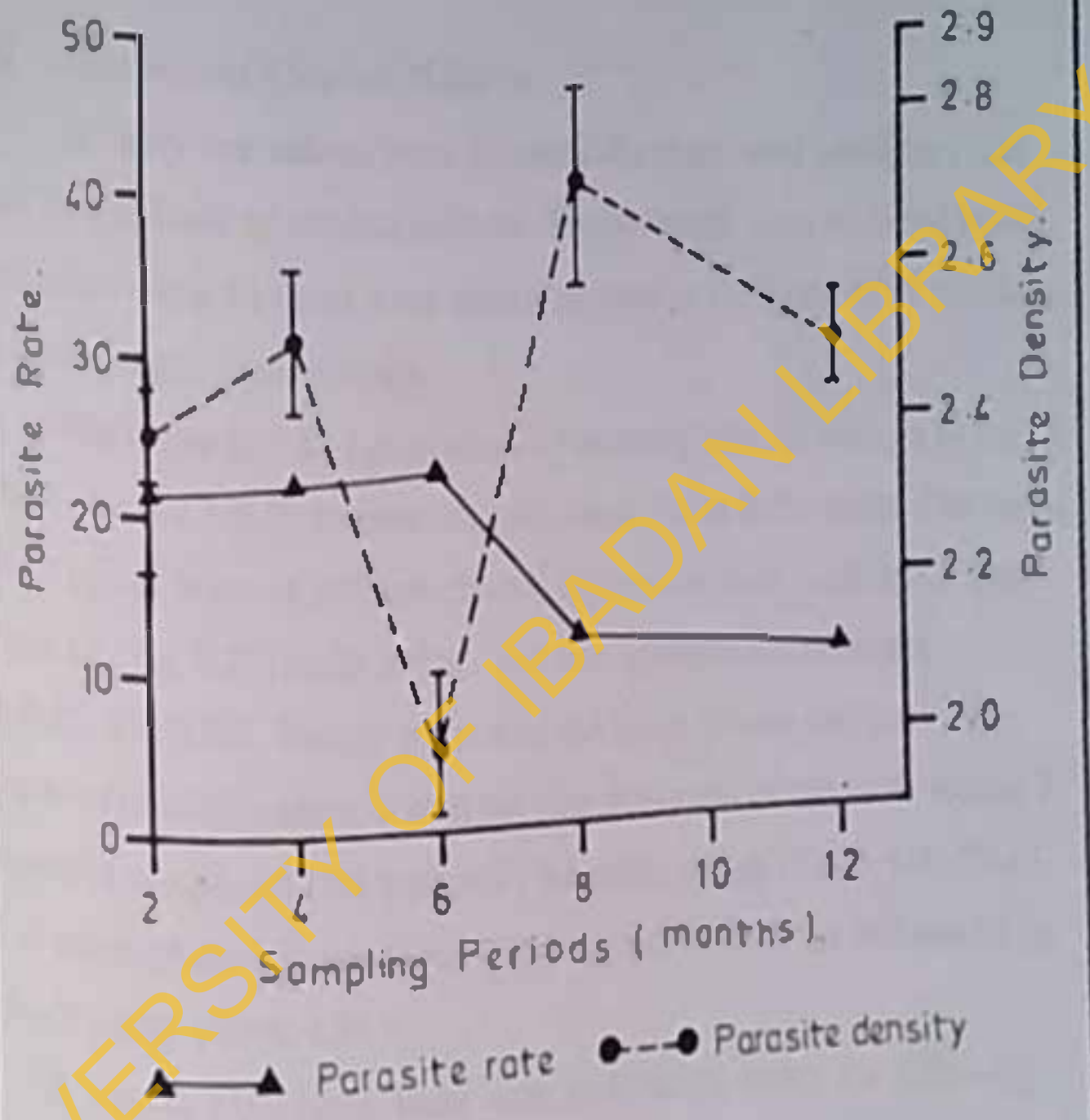

Fig 4.4 Malaria parasite rates and parasite densities (Mean ± SE) of Nigerian mothers at Igbo-Ora during 6 consecutive bi-monthly surveys after delivery.

survey 4 (8 months after delivery) corresponds to the months of June-August and October - December respectively.

(ii) Episodes of Clinical Malaria

Seventy-one infants were successfully monitored untill they had their first episode of clinical malaria. Twenty cases were detected from case notes while 51 cases were either detected at the bi-monthly clinics or by the fortnightly home visits.

The mean (± S.E) age of onset of primary clinical malaria in the 71 infants was 4.2 ± 0.20 months ranging from 2.0 to 8.2 months. The mean (±S.E) age of onset of primary clinical malaria in male (4.27 ± 0.26) and female (4.18 ± 0.27) study infants was not significantly different ($t = 0.23$, $P > 0.50$). Twenty percent of the study infants had their first episode of clinical malaria within the first 3 months of life, 67% within 3 - 6 months of age and 12% within 6 - 9 months of age (Table 4.4). The age of onset of clinical malaria in the study infants was not influenced by mother's parity (Table 4.5).

A total of 2108 home visits were undertaken during the follow-up studies. The mean (± S.E) number of episodes of clinical malaria per infant during the one year follow-up visits was 2.36 ± 0.13. Out of the 44 infants monitored successfully for the first one year of life, 10 had one episode of malaria, 10 had two episodes of malaria while 22 had three episodes of malaria. Two infants had 4 episodes of clinical malaria. Only one incident of severe malaria (from the group of infants

Table 4.4 Mean age of onset of primary clinical malaria in 71 Nigerian infants at Igbo-Ora, Oyo State.

Age Group(months)[a]	n	Mean age[b]	S.E[c]
≤ 3.0	14	2.2	0.07
3.1 - 6.0	48	4.2	0.12
6.1 - 9.0	9	7.2	0.25

[a]Infants who had primary clinical malaria within the specified age group.

[b]Mean age of onset (months) of primary clinical malaria in each age group.

[c]Standard Error.

Table 4.5 Mean (± S.E) age of onset of clinical malaria in Nigerian infants at Igbo-Ora according to mother's parity group.

Parity	n	Mean Age of Onset
1	14	4.35 ± 0.50
2	14	3.82 ± 0.21
3	17	3.87 ± 0.26
≥4	26	4.35 ± 0.38
Significance of difference		F = 0.59, P > 0.50

who had 2 episodes of malaria) was recorded in the study infants during the study period involving a 6.6Kg female of haemoglobin genotype AA and blood group O Rhesus positive. She had febrile convulsion secondary to malaria at the age of 6.3 months. On admission she had a malaria parasite count of 71,308/µl of blood, rectal temperature of 40.6°C and a PCV of 19%.

(c) Malaria in the Adult Study Population

Malaria parasite rates and densities at the July,1991 and February,1992 surveys undertaken at the G.T.C. Igbo-Ora are shown in table 4.6. There was no significant difference in the parasite rates at the July,1991 and February,1992 surveys. On the contrary, the mean parasite density at the July survey was significantly higher than at the February survey (Table 4.6). The incidence of malaria parasitaemia but not mean parasite density was significantly different between the different age groups of study subjects (Table 4.7). There was no correlation between parasite density and age ($r = 0.31$, $P < 0.30$) of the G.T.C study subjects at the July survey. A similar statistical inference could not be made of the February survey because of the small sample size.

Table 4.8 shows the malaria parasite rates and densities at the 2 cross-sectional surveys undertaken at the U.C.H. Ibadan blood donor clinic towards the end of the rainy season (October-November, 1991) and at the end of the dry season (March, 1992). The incidence of

Table 4.6 Malaria parasite rates and mean (± S.E) parasite densities of the study subjects at the G.T.C. Igbo-Ora in July, 1991 and February, 1992.

	July 1991	February 1992	Significance of difference
Parasite rate[a]	18/100(18%)	5/33(15%)	$\chi^2 = 0.697$; P>0.50
Parasite density[b]	2.41 ± 0.06	2.08 ± 0.07	t = 2.54; P< 0.025

[a]Parasite rate = Percentage of subjects with asexual malaria parasites detected in thick blood films.

[b]Parasite density = Log(x) of the number of asexual malaria parasites per microlitre of blood in thick film of positive subjects (±S.E)

Table 4.7 Malaria parasite rates and mean (± S.E) parasite densities in different age groups of the G.T.C. Igbo-Ora study subjects in July 1991.

	Age Group (Years)			Significance of difference
	≤ 20	21 - 25	≥ 26	
Parasite rate	11/23(48%)	5/55(9%)	2/22(9%)	$P < 0.001$
Parasite density	2.42 ± 0.07	2.31 ± 0.16	2.62 ± 0.24	$P < 0.50$

Table 4.8 Malaria parasite rates and mean (± S.E) parasite densities of blood donors at the U.C.H. Ibadan blood donor clinic at the October-November, 1991 and March, 1992 surveys.

	October-November 1991	March 1992	Significance of difference
Parasite rate	91/224(41%)	36/192(19%)	χ^2= 23.33, P< 0.001
Parasite density	2.28 ± 0.04	2.64 ± 0.09	t = 4.11, P< 0.001

malaria parasitaemia was significantly higher in blood donors towards the end of the rainy season than at the end of the dry season. Parasite densities were however, higher in blood donors during the March,1992 survey ($t = 4.11$, $P < 0.001$). Malaria parasite rates at the March ($\chi^2=11.93$; $P<0.01$) but not October-November survey ($\chi^2=3.22$; $P<0.40$) was significantly different between the different age groups of the study subjects (Fig. 4.5). There was no correlation between parasite density and age of blood donor during the October-November ($r = -0.08$, $P > 0.50$) and March ($r = -0.16$, $P < 0.40$) surveys. However, mean parasite densities between the different age groups was significantly different at the March survey but not at the October-November survey (Table 4.9).

4.3 Haemoglobin Genotype and Protection from Malaria

The prevalence of sickle-cell trait in the infant study population was 25% (18/72). There was no significant difference in the parasite rates of haemoglobin AS and AA infants in the first year of life except in infants aged 10months of age (Fig. 4.6). With the exception of infants aged 6 months, mean parasite densities were not significantly different between infants with haemoglobin AS and AA (Table 4.10).

The mean (± S.E) age of onset (months) of primary clinical malaria in haemoglobin AA (4.1 ± 0.20) infants was not significantly different ($t = 1.99$, $P>0.05$) from those with haemoglobin AS (4.9 ± 0.18). There was no significant difference in the number of episodes of

Fig. 4.5 Malaria parasite rates in different age groups of blood donors at the October-November, 1991 and March, 1992 cross-sectional surveys.

Table 4.9 Mean (± S.E) parasite densities in different age groups of blood donors at the October-November, 1991 and March, 1992 surveys.

Age Groups (Years)	October-November	March
≤ 24	2.41 ± 0.12	3.00 ± 0.16
25 - 31	2.20 ± 0.06	2.46 ± 0.10
32 - 38	2.36 ± 0.10	2.26 ± 0.06
≥ 39	2.22 ± 0.10	2.83 ± 0.01
Significance of difference	$F = 1.36$, $P < 0.30$	$F = 4.40$, $P < 0.02$

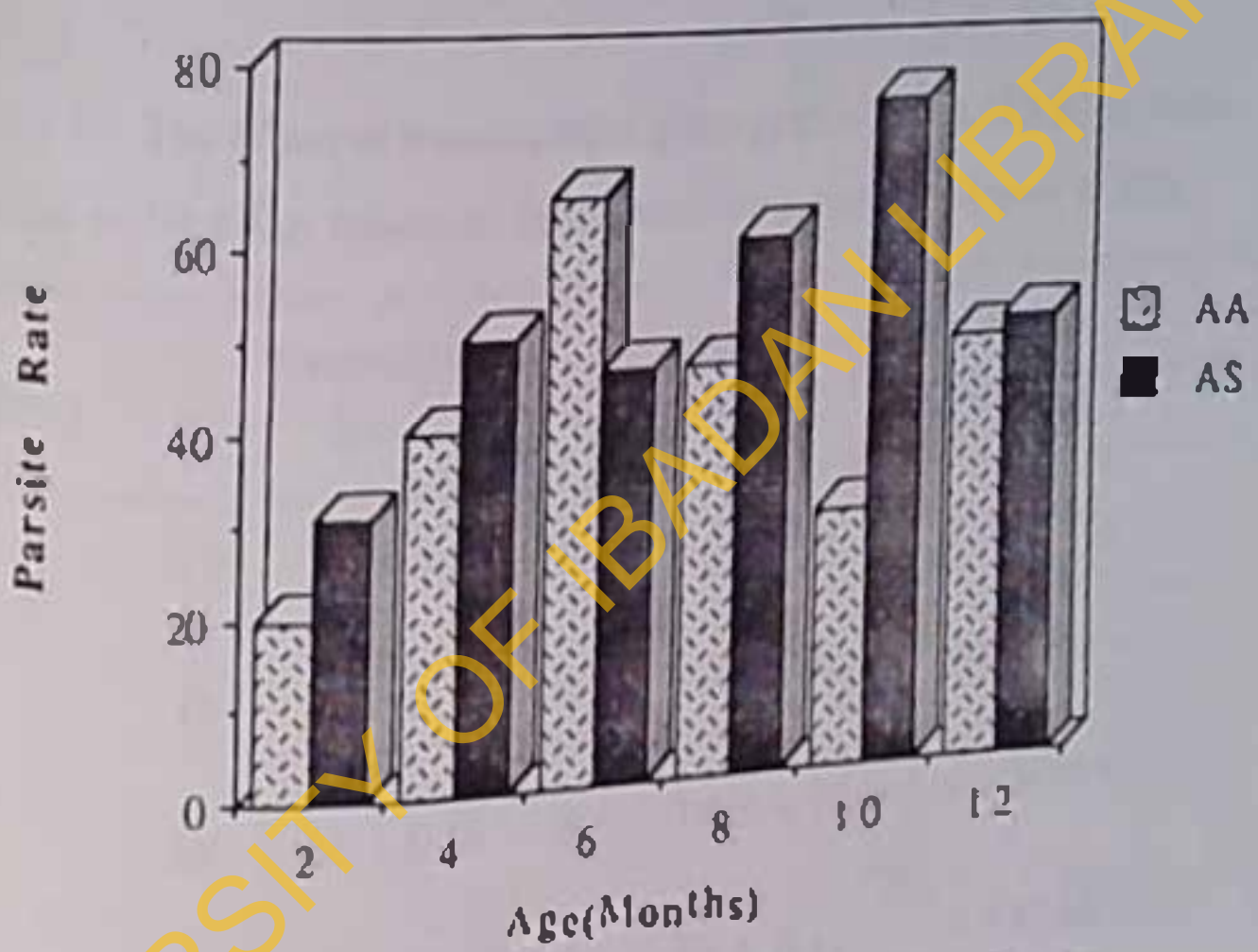

Fig. 4.6 Malaria parasite rates in haemoglobin AA and AS Nigerian infants at Igbo-Ora during their first year of life.

Table 4.10 The effect of haemoglobin genotype on mean (±S.E) parasite densities in Nigerian infants at Igbo-Ora during their first year of life.

Age (Months)	Haemoglobin AA n	AA	Genotype AS n	AS	Significance of difference
2	10	2.1 ± 0.04	5	2.3 ± 0.06	P < 0.20
4	16	3.0 ± 0.14	6	3.2 ± 0.40	P > 0.50
6	20	3.5 ± 0.18	6	2.6 ± 0.12	P < 0.01*
8	10	3.3 ± 0.16	6	3.6 ± 0.32	P < 0.50
10	4	3.2 ± 0.55	6	3.4 ± 0.22	P > 0.50
12	10	2.7 ± 0.10	6	3.1 ± 0.20	P < 0.10

*Statistically significant.

clinical malaria during the first year of life between haemoglobin AA and AS infants (P>0.50).

No significant difference was observed in the parasite rates (χ^2 = 0.18, P> 0.50) and parasite densities(t = 0.48, P > 0.50) between haemoglobin AA and AS mothers at delivery. There was no significant difference in the number of episodes of clinical malaria between haemoglobin AA and AS mothers (P< 0.10).

The prevalence of sickle-cell trait in the G.T.C. Igbo-Ora study subjects was 26% (26/100) while 3.0% (3/100) of the study subjects had haemoglobin AC. Only one subject with haemoglobin SC was detected. There was no significant difference in the parasite rates and parasite densities between haemoglobin AS and AA subjects at the July, 1991 survey (Table 4.11).

The overall prevalence of sickle-cell trait in blood donors at the two cross-sectional surveys in October-November, 1991 and March, 1992 was 27% (113/416). No significant difference in parasite rates was observed between haemoglobin AA and AS donors at the October-November (χ^2 = 0.76, P>0.50) and March (χ^2 = 1.74, P< 0.20) surveys (Fig. 4.7). Mean Parasite densities were significantly lower in blood donors with haemoglobin genotype AS at the March survey, but not at the October-November survey (Table 4.12).

4.4 Chemoprophylaxis in Pregnancy and maluria Parasitaemia

Mothers who were on chemoprophylaxis during pregnancy used a weekly dose of pyrimethamine from the 20th week of pregnancy till

Table 4.11 The relationship between haemoglobin genotype, parasite rates and mean (± S.E) parasite densities in 96 study subjects at the G.T.C. Igbo-Ora in July 1991.

	Haemoglobin Genotype		Significance of difference
	AA	AS	
Parasite rate	14/70(20%)	4/26(15%)	$\chi^2 = 0.26$, $P > 0.50$
Parasite density	2.41 ± 0.08	2.41 ± 0.10	$t = 0.006$, $P > 0.50$

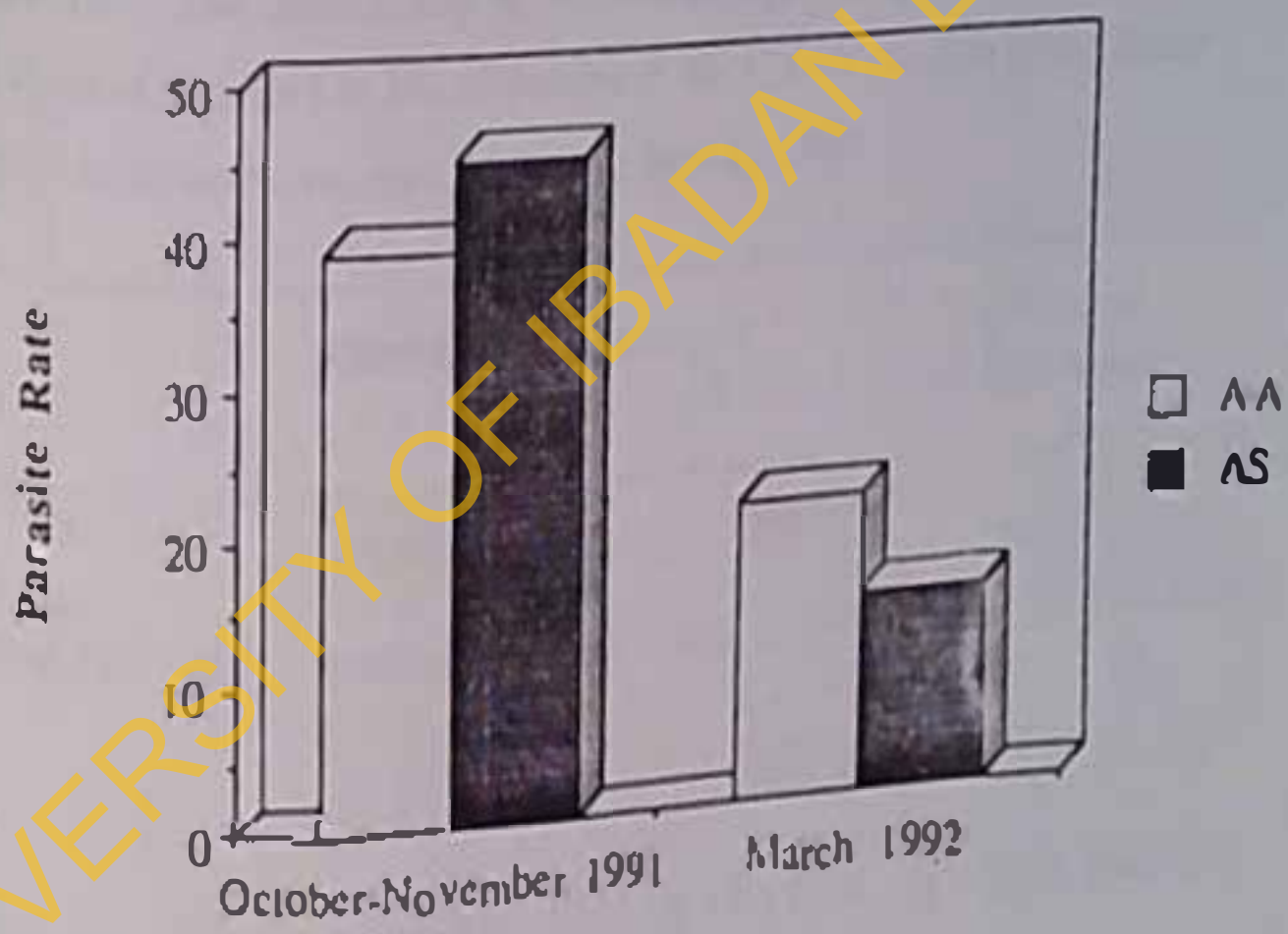

Fig. 4.7 Malaria parasite rates in haemoglobin AA and AS blood donors at the October-November, 1991 and March, 1992 cross-sectional surveys.

Table 4.12 The relationship between haemoglobin genotype and mean (± S.E) parasite densities in blood donors at the U.C.H. Ibadan blood donor clinic in October-November, 1991 and March, 1992.

	Haemoglobin Genotype				Significance of difference
	n	AA	n	AS	
October-November	62	2.28 ± 0.06	29	2.34 ± 0.09	t = 0.59, P > 0.50
March	29	2.73 ± 0.11	7	2.30 ± 0.04	t = 2.02, P=0.050

binh. There was no significant difference in the parasite rates (P< 0.40) and parasite densities (P> 0.50) at delivery between women who were on chemoprophylaxis during pregnancy and those who were not (Table 4.13). There was no significant difference (t=1.17, P< 0.30) in the mean PCV value between mothers on chemoprophylaxis (31.8 ± 0.4) in pregnancy and those who were not (31.4 ± 0.31).

The mean age (months) of onset of primary clinical malaria in study infants born to mothers who were on chemoprophylaxis was 4.29 ± 0.26 and infants born to mothers who were not on chemoprophylaxis was 4.16 ± 0.27. These values are not significantly different (t=0.35, P> 0.50). There was also no significant difference (t=0.71, P<0.50) in the mean (± S.E) birthweights (Kg) of newborns of mothers who were on chemoprophylaxis (3.2 ± 0.04) and those who were not (3.17 ± 0.05).

4.5 Malaria Parasitaemia and PCV Levels

The mean (± S.E) cord blood PCV value was 39% ± 0.25. There was no significant difference (t = 1.822, P< 0.10) in the mean PCV values of males (39.9% ± 0.34) and females (38.9% ± 0.34). There was a positive correlation between cord blood PCV level and mother's parity (r = 0.195, P< 0.05).

Malaria parasitaemia significantly lowered PCV levels between 4 - 10 months of age while the difference in the mean PCV values between malarious and non-malarious infants at 2 and 12 months of age was not significant (Table 4.14). No correlation was observed between cord blood PCV values and duration of onset of primary clinical

Table 4.13 The effect of chemoprophylaxis in pregnancy on parasitaemia at delivery in 116 parturient women at Igbo-Ora, Oyo State.

	Chemoprophylaxis	No Chemo-prophylaxis	Significance of difference
Parasite rate	10/53(19%)	16/63(25%)	$\chi^2 = 0.71$, $P > 0.50$
Parasite density[a]	2.36 ± 0.11	2.42 ± 0.11	$t = 0.38$, $P > 0.50$

[a]Table shows mean ± S.E.

Table 4.14 Correlation between mean (± S.E) PCV levels in malaria positive and negative study infants during the first year of life.

Age (months)	n	Malaria Positive	n	Malaria Negative	Significance[a] of difference	r[b]
2	19	31 ± 0.49	72	32 ± 0.29	P< 0.30	-0.11, P>0.50
4	26	29 ± 3.01	33	32 ± 0.48	P < 0.001	-0.68, P<0.001
6	26	27 ± 0.78	19	31 ± 0.76	P < 0.001	-0.43, P<0.025
8	16	28 ± 1.1	16	31 ± 0.53	P < 0.025	-0.82, P<0.001
10	10	27 ± 1.7	12	34 ± 1.0	P< 0.01	-0.49, P>0.20
12	17	33 ± 0.98	22	34 ± 0.57	P < 0.50	0.17, P>0.50

[a]Significance of difference between mean PCV values of malaria positive and malaria negative infants.

[b]Correlation coefficients between PCV levels and parasite density at different ages of the study infants.

malaria in the infants ($r = 0.09$, $P < 0.50$). There was a positive correlation between birthweight of newborn and cord blood PCV level ($r = 0.57$, $P < 0.001$).

The mean (± S.E) PCV value of the 116 parturient women was 32 ± 0.25% ranging from 26% to 41%. The mean PCV value of primiparous women (30.7% ± 1.98) was significantly lower ($t = 2.084$, $P < 0.05$) than the mean PCV value (31.9% ± 2.8) of multiparous women. A positive correlation was obtained between mothers parity and PCV level at delivery ($r = 0.28$, $P < 0.01$). The mean PCV value of malaria positive (30% ± 0.45) parturient women was significantly ($t = 3.33$, $P < 0.01$) lower than that of malaria negative (32% ± 0.28) parturient women. Chemoprophylaxis in pregnancy had no significant effect on PCV levels at delivery ($t = 0.73$, $P < 0.50$).

4.6 MNSsU Blood Group and Protection against Malaria

Table 4.15 shows the parasite rates and mean parasite densities in different MNSsU blood groups of blood donors at the October-November survey. There was no significant difference in the parasite rates and parasite densities between the different MNSsU blood groups. The duration of onset of primary clinical malaria in the study infants was not significantly different between the different MNSsU blood groups ($F = 0.566$, $P > 0.50$; Table 4.16).

Table 4.15 Parasite rates and mean (±S.E) parasite densities in different MNSsU blood groups of blood donors at the October-November survey.

	Parasite Rate	Parasite Density
MNS_sU	51/131(40%)	2.28 ± 0.05
$M.NS_sU$	22/52(42%)	2.31 ± 0.11
$MN.S_-s.U_-$	9/25(36%)	2.15 ± 0.09
$MNS_-s.U_-$	9/15(60%)	2.34 ± 0.13
Significance of difference	$\chi^2 = 2.77$, $P < 0.50$	$F = 0.39$, $P > 0.50$

Table 4.16 MNSsU blood group and duration of onset of primary clinical malaria in the infant study population at Igbo-Ora, Oyo State.

Blood group	n	Age (months)[a]	± S.E
MNSsU	29	4.26	0.30
M-NSsU	20	4.60	0.40
MN-S-s-U-	4	3.50	0.17
MNS-s-U-	4	3.98	0.98

[a]Mean age of onset of primary clinical malaria; $F = 0.566$, $P > 0.50$

Immunoglobulin Levels and Malaria Parasitaemia

Figure 4.8 shows the standard curves for IgG, IgA and IgM determined by the single radial immunodiffusion method.

(a) Infants and their Mothers

The major fraction of cord blood immunoglobulins was IgG. The mean (± S.E) cord blood IgG at delivery was 1265 ± 34mg/100ml. Mean cord blood IgG was significantly lower than the mean maternal IgG ($t = 5.86$, $P<0.001$). In some cases where the cord blood IgG level was higher than the maternal IgG level, the maternal IgG level was below 1000mg/100ml. There was no significant difference in the mean cord blood IgG level between male and female newborns (Table 4.17). There was no correlation between cord blood IgG level and parity of mother ($r = .0.01$, $P>0.50$) and between cord blood IgG and duration of onset of clinical malaria in the infant ($r = 0.13$, $P< 0.30$). No correlation was obtained between birthweight and cord blood IgG level ($r = 0.06$, $P< 0.50$).

A majority (93%) of the cord blood samples had no detectable IgM using the single radial immunodiffusion technique. However, IgM was detected in all cord sera using the more sensitive ELISA technique. A few (3) cord blood samples had very high levels of IgM comparable with that in adults. The mean cord blood IgM was 44 ± 4.1 mg/100ml. There was no significant difference in the mean cord blood IgM level between male and female newborns (Table 4.17). No correlation was obtained between cord blood IgM and duration of onset of primary clinical malaria

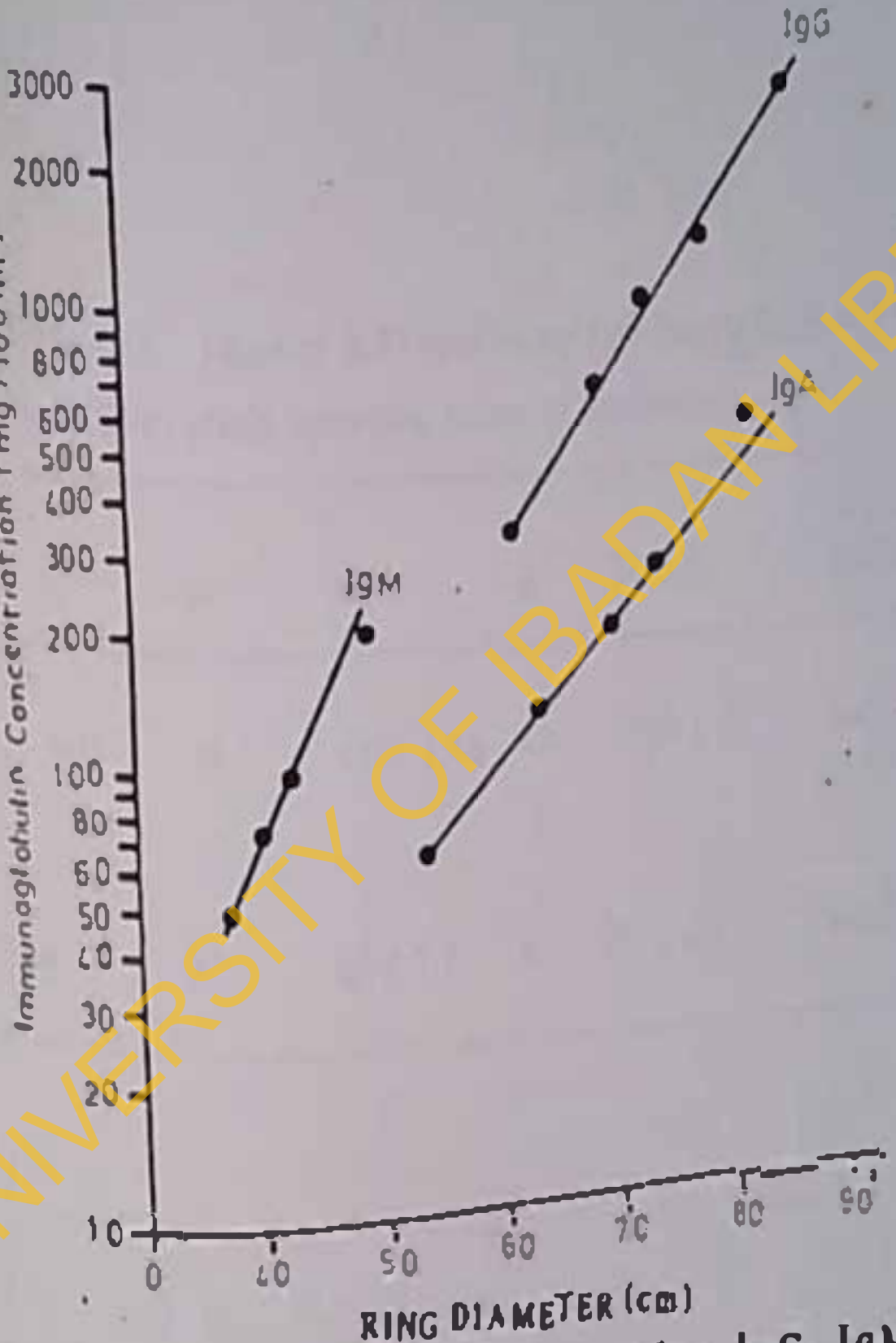

Fig. 4.8 Standard curves for IgG, IgM and IgA.

Table 4.17 Mean (± S.E) cord blood IgG (mg/100ml) and IgM (mg/100ml) levels according to sex of newborn.

	n	Male	n	Female	Significance of difference
IgG	55	1271 ± 46	62	1260 ± 19	t=0.17, P> 0.50
IgM	37	52 ± 7.1	43	37 ± 4.3	t=1.79, P> 0.05

in the infant ($r = -0.28$, $P > 0.05$) and between cord blood IgM and birthweight of newborn ($r = 0.07$, $P > 0.50$).

(i) IgG

Table 4.18 shows mean IgG values in malaria positive and negative infants in the first ten months of life. IgG levels fell significantly ($P < 0.001$) to about half of the birth IgG level at the age of 2 months in both malaria positive and negative infants. Mean IgG values in malaria positive infants rose rapidly at 6 months of age while a gradual rise was observed in malaria negative infants between 6 and 10 months of age. The lowest plasma IgG values were recorded in infants at 4 months of age.

Mean IgG values were significantly lower in malarious infants aged 2 and 8 months than in non-malarious infants of the same age (Table 4.18). There was no significant difference in the mean IgG values of malaria positive and negative infants at 4, 6 and 10 months of age.

(ii) IgM

A steady rise in mean IgM values was observed within the first 8 months of life in both malaria positive and negative infants (Table 4.19). A rapid rise in mean IgM values was observed in malarious infants between the ages of 8 and 10 months. IgM levels were significantly higher in malaria positive infants than in malaria negative infants aged 2, 4, 6 and 10 months.

Table 4.18 Mean (± S.E) plasma IgG levels in Nigerian infants with and without malaria parasites during the first ten months of life.

Age (months)	n	Malaria Positive	n	Malaria Negative	Significance of difference
2	19	562 ± 22	72	691 ± 23	t = 2.82, P<0.01
4	23	555 ± 29	35	609 ± 24	t = 1.44, P<0.20
6	27	717 ± 47	18	696 ± 26	t = 0.33, P>0.50
8	13	1206 ± 72	14	881 ± 95	t =2.71, P<0.02
10	6	1141 ± 73	6	1092 ± 52	t = 0.55, P>0.50

Table 4.19 Mean (± S.E) plasma IgM levels in Nigerian infants at Igbo-Ora with and without malaria parasitaemia during the first ten months of life.

Age (months)	n	Malaria Positive	n	Malaria Negative	Significance of difference
2	19	55 ± 1.3	72	36 ± 0.75	t =11.28, P<0.001
4	23	70 ± 1.8	35	49 ± 1.4	t =9.44, P< 0.001
6	27	93 ± 6.1	18	66 ± 2.4	t =3.59, P< 0.001
8	13	167 ± 15	14	132 ± 13	t =1.77, P< 0.10
10	6	207 ± 36	6	104 ± 9	t =2.78, P< 0.02

(iii) IgA

IgA was not detected at birth in any of the cord blood samples using the single radial immunodiffusion technique. However, IgA was present at 2 months of age in all the study infants. A steady increase in mean IgA levels was observed during the first eight months of life. With the exception of infants aged 4 months, there was no significant difference in the mean IgA values between malaria positive and negative infants during the first eight months of life (Table 4.20).

At delivery, no significant difference in the mean IgG and IgA values was observed between malaria positive and negative parturient women (Table 4.21). However, mean IgM was significantly higher in malaria positive parturient women. Mean plasma IgG, IgM and IgA was similar between the different parity groups (Table 4.22). There was no correlation between IgM ($r = -0.18$, $P > 0.05$), IgG ($r = 0.02$, $P > 0.50$), IgA ($r = 0.09$, $P < 0.40$) and parity of mother. The difference in the mean IgG, IgM and IgA values between mothers who were on chemoprophylaxis during pregnancy and those who were not was not statistically significant (Table 4.23). A positive correlation was obtained between cord blood and maternal IgG levels ($r = 0.65$, $P < 0.001$) but not between maternal and cord blood IgM ($r = 0.44$, $P < 0.50$).

(b) Adult Study Population

Mean plasma IgG and IgM but not IgA values were significantly higher in malaria positive subjects compared with malaria negative

Table 4.20 Mean (± S.E) plasma IgA levels in malaria positive and malaria negative Nigerian infants at Igbo-Ora during the first 8 months of life.

Age (months)	n	Malaria Positive	n	Malaria Negative	Significance of difference
2	19	28 ± 1.0	72	28 ± 0.69	t = 0.22, P> 0.50
4	23	42 ± 1.4	35	38 ± 1.1	t = 2.31, P< 0.025
6	27	66 ± 1.9	18	65 ± 2.1	t = 0.54, P>0.50
8	13	104 ± 8.4	14	92 ± 6.5	t = 1.20, P< 0.30

Table 4.21 Mean (± S.E) plasma immunoglobulin (Ig) levels in malaria positive and negative Nigerian parturient women at Igbo-Ora.

Ig Isotype	n	Malaria Positive	n	Malaria Negative	Significance of difference
IgG	26	1568 ± 79	90	1568 ± 45	t =0.0005, P >0.50
IgM	26	266 ± 11	90	204 ± 9	t = 3.39, P< 0.001
IgA	26	141 ± 10	90	160 ± 8	t = 1.21, P > 0.05

Table 4.22 Mean (± S.E) plasma IgG, IgM and IgA (mg/100ml)levels in different parity groups of Nigerian parturient women at Igbo-Ora.

Parity	n	IgG	IgM	IgA
1	28	1535 ± 83	247 ± 13	155 ± 14
2	22	1526 ± 71	229 ± 21	147 ± 17
3	24	1705 ± 91	185 ± 13	140 ± 11
4	11	1412 ± 119	211 ± 35	160 ± 24
≥5	31	1577 ± 79	210 ± 16	174 ± 13
Significance of difference		F=1.13, P<0.40	F=1.98, P<0.20	F=0.89, P<0.50

Table 4.23 The relationship between plasma immunoglobulin levels and use of chemoprophylaxis during pregnancy in 116 parturient women at Igbo-Ora, Oyo State.

Ig Isotype	Chemoprophylaxis n	Mean ± S.E	No chemoprophylaxis n	Mean ± S.E	Significance of difference
IgG	53	1540 ± 58	63	1591 ± 53	t =0.65, P>0.50
IgM	53	213 ± 12	63	221 ± 11	t =0.49, P>0.50
IgA	53	152 ± 9	63	159 ± 9	t=0.53, P>0.50

subjects in the July, 1991 survey at the G.T.C. Igbo-Ora (Table 4.24). Plasma IgG and IgM levels were found to be significantly higher in malaria positive blood donors than in malaria negative blood donors at the October - November, 1991 survey (Table 4.25). The mean IgA levels between malaria positive and negative blood donors was not significantly different.

4.8. Antibodies Against Total Blood Stage Antigens

Adult and cord blood samples were screened for antibodies against total blood stage antigens of *P. falciparum* at 1:100,000 dilution while serial blood samples collected from infants during their first year of life were screened at 1:10,000 dilution. All test samples showed visible immunofluorescence (Plate 1) at their respective dilutions. Seven out of 121 cord blood samples (5.8%) were positive for malaria-specific IgM antibodies to total blood stage antigens at 1:10 dilution. Three of the seven positive cord blood samples were positive at 1:100 dilution and had the highest IgM values.

4.8.1 Anti-Pf 155/RESA Antibodies

Plate 2 shows membranes immunofluorescence of erythrocytes infected with ring forms of *P. falciparum* (RESA/Pf 155).

(a) Infants and their Mothers

There was a correlation between maternal and cord blood anti-Pf155 antibody titres ($r = 0.64$, $P<0.001$). The prevalence of antibody positivity to the Pf155 antigen was significantly ($\chi^2 = 21.62$, $P< 0.001$) higher in maternal compared with cord blood. However, there was no significant difference ($t = 0.47$; $P> 0.50$) in the mean ($\pm$ S.E) anti-Pf155

Table 4.24 Immunoglobulin levels in malaria negative and malaria positive asymptomatic study subjects of the G.T.C Igbo-Ora at the July survey.

Ig Isotype	Malaria positive n	Mean ± S.E	Malaria negative n	Mean ± S.E	Significance of difference
IgG	18	1309 ± 76	82	1150 ± 29	t = 2.24, P<0.03
IgM	18	171 ± 14	82	138 ± 7	t = 2.17, P<0.05
IgA	18	120 ± 9	82	114 ± 5	t = 0.54, P>0.50

Table 4.25 Mean (± S.E) plasma Immunoglobulin levels in malaria negative and malaria positive asymptomatic blood donors at the October-November, 1991 survey.

Ig Isotype	n	Malaria positive	n	Malaria negative	Significance of difference
IgG	91	1408 ± 52	133	1274 ± 43	t=1.99, P<0.05
IgM	91	174 ± 8	133	144 ± 6	t=2.97, P<0.01
IgA	91	152 ± 7	133	149 ± 5	t=0.39, P>0.50

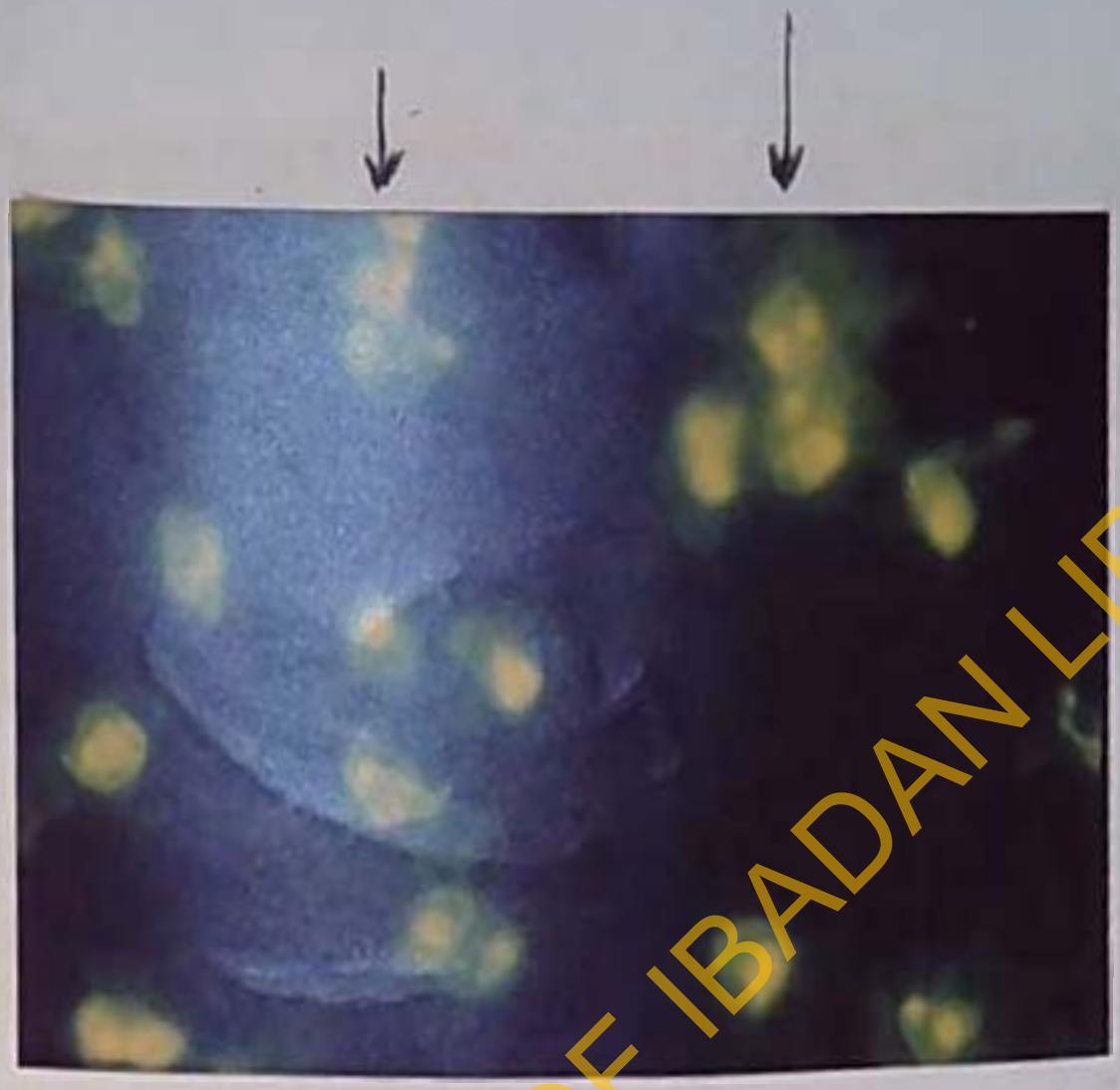

Plate1. Immunofluorescent Staining of blood - stage *P. falciparum* intracellular parasites (mostly schizonts) by immune serum from a blood donor. Serum was diluted 1:25,000.

Plate 2. Immunofluorescent staining of the membranes of erythrocytes infected with ring forms of *P. falciparum* by immune serum from a blood donor. The parasite nuclei were counterstained with ethidium bromide. Serum was diluted 1:50.

antibody titre between maternal (1.72 ± 0.09) and cord blood (1.78 ± 0.09). All anti-Pf155 antibody positive cord blood samples were obtained from anti-Pf155 antibody positive mothers while some positive mothers' cord blood samples were negative for anti-Pf155 antibodies.

The prevalence seropositivity for antibodies to the Pf155 antigen showed an initial fall from birth till 4 months of age (Fig. 4.9). A rapid increase was observed at 6 months of age followed by a gradual rise till one year of life. No correlation was observed between cord blood anti-Pf155 antibody titres and duration of onset of clinical malaria in the infants ($r = 0.32$, $P < 0.10$). There was no significant difference ($t = 0.51$; $P > 0.50$) in the mean (±S.E) age of onset of clinical malaria in the infants between anti-Pf155 antibody positive (4.56 ± 0.33) and negative (4.31 ± 0.34) cord blood samples.

There was no significant difference in the percentage of mothers positive for anti-Pf155 antibodies at delivery and during the six consecutive surveys ($P < 0.50$) after delivery (Fig. 4.10). Similarly, no significant difference was observed in the mean anti-Pf155 antibody titres during the six consecutive surveys (Table 4.26). No correlation was observed between maternal IgG ($r = 0.12$, $P < 0.40$), parasite density ($r = 0.10$, $P > 0.50$) and anti-Pf155 antibody titres at delivery. There was a correlation between mother's age ($r = 0.42$, $P < 0.01$), parity ($r = 0.39$, $P < 0.01$) and anti-Pf155 antibody titres at delivery. Mean anti-Pf155 antibody titres were not similar between the different Parity groups of the mothers (Table 4.27).

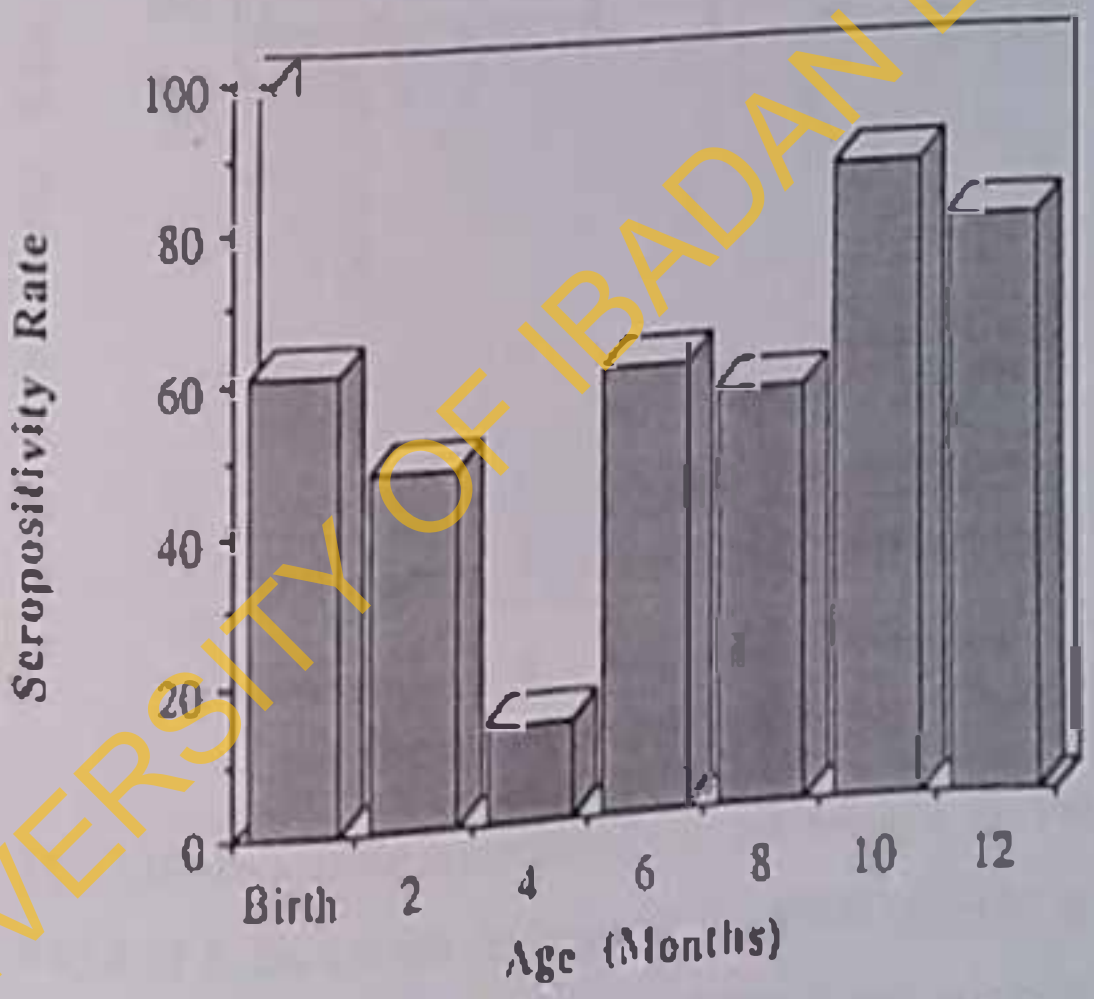

Fig. 4.9 Seropositivity rates for antibodies to the Pf155/RESA in Nigerian infants during their first year of life.

Fig. 4.10 Prevalence scropositivity for antibodies to the Pf155/ RESA of *P. falciparum* in Nigerian Women at Igbo-Ora at delivery and on six bi-monthly consecutive surveys after delivery.

Table 4.26 Mean (± S.E) anti-Pf155 antibody titres of Nigerian women at Igbo-Ora on six consecutive surveys after delivery.

	Sampling Period (Months)					
	At delivery	2	4	6	8	10	12
Mean Antibody Titre	1.72	1.72	1.69	1.73	1.79	1.81	1.83
Standard Error	0.08	0.10	0.15	0.15	0.14	0.08	0.11

$F \approx 0.65$, $P > 0.50$

Table 4.27 Mean (± S.E) anti-PfI55/RESA antibody titres in different parity groups of parturient women at Igbo-Ora.

	Parity					Significance of difference
	1	2	3	4	≥5	
Mean Antibody Titre	1.499	1.489	1.580	1.437	2.214	F = 4.514, P< 0.01
Standard Error	0.114	0.182	0.168	0.226	0.168	
n	14	10	12	8	19	

There was no significant difference in both the number of mothers positive for anti-Pf155 antibodies ($\chi^2 = 0.93$; $P < 0.40$) and in the mean (± S.E) anti-Pf155 antibody titres ($t = 0.45$; $P > 0.50$) between malaria positive (1.64 ± 0.135) and malaria negative (1.74 ± 0.101) mothers at delivery. There was a correlation between maternal and cord blood anti-Pf155 antibody titres ($r = 0.64$, $P < 0.001$).

(b) Adults

There was no significant difference in the Pf155 seropositivity rates ($\chi^2 = 1.84$, $P < 0.20$) and in the mean anti-Pf155 antibody titres ($t = 1.40$, $P < 0.20$) between the July and February surveys of G.T.C study subjects. No significant difference was observed in both the percentage of anti-Pf155 antibody positive ($P < 0.20$) and in the mean anti-Pf155 antibody titres ($t = 0.55$; $P > 0.50$) between malaria positive and malaria negative subjects at the July survey.

There was no correlation between anti-Pf155 antibody titres and parasite densities at both the July ($r = -0.03$, $P > 0.50$) and February ($r = 0.01$, $P > 0.50$) surveys. No correlation was observed between anti-Pf155 antibody titres and age at the July survey ($r = 0.08$, $P < 0.50$). The difference in the mean anti-Pf155 antibody titres in individuals who were sampled consecutively at the July and February surveys was not significant ($t = 1.30$; $P = 0.20$). No significant difference was observed in the mean anti-Pf155 antibody titre and mean ELISA (OD_{405}) value to the $(NANP)_6$ peptide between the different MNSsU blood groups at the July survey (Table 4.28).

Table 4.28 Mean (± S.E) anti-Pf155/RESA antibody titres and mean (± S.E) ELISA (OD_{405}) values to the $(NANP)_6$ peptide in different MNSsU blood groups of G.T.C study subjects at the July survey.

	n	Pf155/RESA	n	$(NANP)_6$
MNSsU	46	2.09 ± 0.13	28	0.16 ± 0.02
M-NSsU	16	2.09 ± 0.22	13	0.21 ± 0.06
MN-S-s-U-	2	2.05 ± 0.35	2	0.21 ± 0.10
MNS-s-U-	10	1.98 ± 0.28	7	0.19 ± 0.04
Significance of difference	F= 0.055, P>0.50		F= 0.506, P>0.50	

The percentage of blood donors positive for anti-Pf155 antibodies at the October-November, 1991 and March, 1992 surveys was not significantly different ($\chi^2 = 0.91$, $P < 0.50$). Similarly, the mean anti-Pf155 antibody titres at the October-November (2.27 ± 0.06) and March (2.33 ± 0.07) surveys was not significantly different ($t = 0.67$, $P > 0.50$).

There was no significant difference in the mean anti-Pf155 antibody titre between malaria positive and negative blood donors during the October-November and March surveys (Table 4.29). In addition no correlation was observed between anti-Pf155 antibody titres and parasite densities at the October-November ($r = -0.18$, $P < 0.20$) and March ($r = -0.19$, $P < 0.40$) surveys.

The mean anti-Pf155 antibody titres of the blood donors could be separated into three groups i.e. "low responders" with EMIF titres between ≥10 and < 250, "medium responders" with EMIF titres between ≥250 and < 7250 and "high responders" with EMIF titres between ≥ 7250 and ≤ 36,250. No significant difference in the mean parasite densities (Table 4.30) and parasite rates at the Oct.-Nov. ($\chi^2 = 0.58$, $P > 0.50$) and March ($\chi^2 = 3.89$, $P < 0.20$) surveys (Fig 4.11) was observed between the three groups. The mean age of low, medium and high responders were not significantly different at the October.-November ($F = 1.52$, $P < 0.30$) and March ($F = 2.66$, $P > 0.05$) surveys.

No correlation was observed between anti-Pf155 antibody titres and age of blood donors at the October.-November ($r = 0.105$, $P < 0.20$) and March ($r = 0.07$, $P < 0.50$) surveys. There was no correlation between

Table 4.29 Mean (± S.E) anti-Pf155 antibody titres in malaria positive and malaria negative blood donors at the October-November and March surveys.

	n	Malaria Positive	n	Malaria Negative	Significance of difference
October-November	80	2.28 ± 0.09	110	2.25 ± 0.08	t=0.26, P>0.50
March	24	2.22 ± 0.15	112	2.21 ± 0.08	t=0.04, P>0.50

Table 4.30 Mean (± S.E) parasite densities in low, medium and high anti-Pf155/RESA antibody responders of blood donors during the October - November and March surveys.

	Low	Medium	High	Significance of difference
October-Nov.	2.27±0.08	2.3 ± 0.07	2.4 ± 0.14	F=0.56, P> 0.50
March	2.5 ± 0.12	2.8 ± 0.15	0	t=1.87, P< 0.20

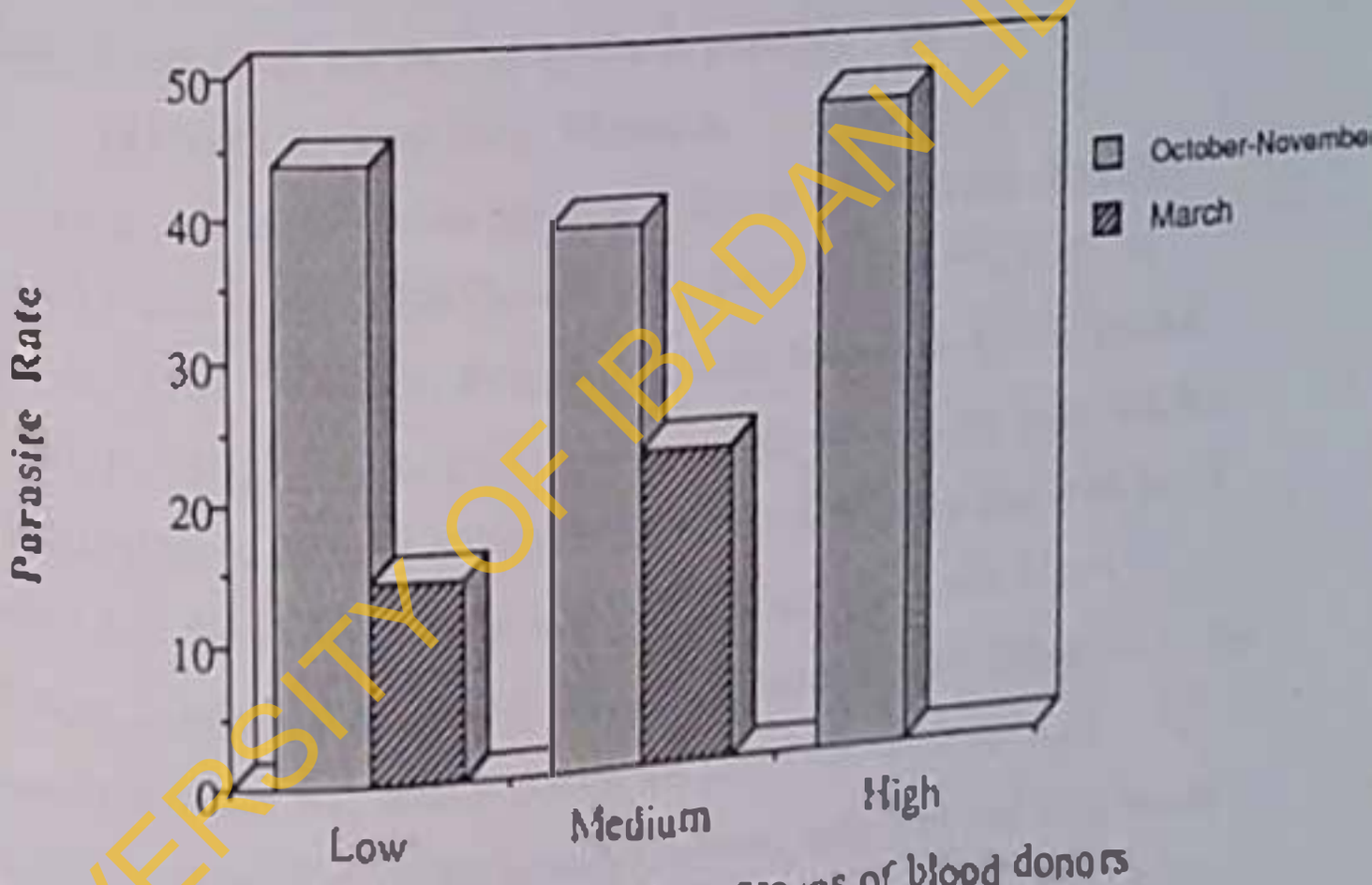

Fig. 4.11 Malaria parasite rates in three groups of blood donors with low (≥ 10 - < 1:250), medium (≥ 250 - < 7250) and high (≥ 7250 - < 1:36,250) anti-Pf155/RESA antibody titres at the October-November, 1991 and March, 1992 surveys.

anti-Pf155 antibody titre and IgG level at the Oct.-Nov. survey (r = 0.18, P> 0.50). The mean anti-Pf155 antibody titres and mean ELISA (OD_{405}) values to the $(NANP)_6$ peptide between the different MNSsU blood groups at the Oct.-Nov. survey was not significantly different (Table 4.31).

4.8.2 ELISA Seroreactivity Against Oligopeptides

Plate 3 shows an ELISA plate with a colour reaction from a malaria immune sera and the test synthetic peptides.

(a) Infants and their Mothers

There was a correlation between maternal and cord blood ELISA (OD_{405}) values to the $(EENV)_6$ (r = 0.32, P<0.01), LJ5 (r = 0.76, P< 0.001) and MAP2 (r = 0.67, P<0.001) peptides but not $(NANP)_6$ peptide (r =0.01, P>0.50). There was no significant difference in the mean ELISA (OD_{405}) values to the four oligopeptides between maternal and cord blood (Table 4.32). Seropositivity rate to the $(NANP)_6$ peptide was higher (P<0.001) in maternal compared with cord blood while no difference in the seropositivity rates was observed with the $(EENV)_6$ (P<0.30), LJ5 (P>0.50) and MAP2 (P >0.50) peptides between maternal and cord blood samples (Fig. 4.12). There was no correlation between cord blood ELISA seroreactivities to the $(EENV)_6$ (r = 0.01, P>0.50), $(NANP)_6$ (r = 0.15, P<0.40), LJ5 (r = 0.02, P >0.50) and MAP2 (r = 0.12, P >0.50) peptides and duration of onset of clinical malaria in the infants.

Infants whose cord blood samples were positive for antibodies to the above antigens showed no significant difference in their duration of onset of clinical malaria compared with those who were seronegative

Table 4.31 Mean (± S.E) anti-Pf155/RESA antibody titres and mean (± S.E) ELISA (OD_{405}) values to the $(NANP)_6$ peptide in different MNSsU blood groups of blood donors at the October-November, 1991 survey.

	n	Pf155/RESA	n	$(NANP)_6$
MNSsU	111	2.33 ± 0.08	72	0.26 ± 0.02
M-NSsU	44	2.16 ± 0.11	30	0.22 ± 0.04
MN-S-s-U-	21	2.24 ± 0.18	19	0.20 ± 0.04
MNS-s-U-	13	1.97 ± 0.21	5	0.18 ± 0.06
Significance of difference		F= 1.03, P<0.40		F= 0.72, P>0.50

Plate 3. Photograph shows an enzyme-linked immunosorbent assay (ELISA) plate with colour reaction resulting from an ELISA employing malaria immune sera and synthetic peptides as capture antigens. Column 1= Plate blank; columns 2+3= Coated with $(EENV)_6$; 4+5= $(NANP)_6$; 6+7= LJ5; 8+9 = MAP2; 10 +12 were coated with bovine serum albumin (BSA) to determine BSA binding for synthetic peptides coupled to BSA ($(EENV)_6$, $(NANP)_6$ and LJ5). Rows A, B, C, D and E contains malaria immune test sera while rows F to H contains malaria non-immune sera (control).

Table 4.32 Mean (± S.E) anti-Pf155/RESA antibody titres and mean (± S.E) ELISA (OD_{405}) values to four oligopeptides in paired maternal-cord blood samples.

Oligopeptides	n	Maternal	n	Cord Blood	Significance of difference
$(EENV)_6$	29	0.08 ± 0.01	20	0.06 ± 0.01	t=1.34, P<0.20
$(NANP)_6$	56	0.11 ± 0.02	45	0.08 ± 0.01	t=1.26, P>0.20
LJ5	18	0.10 ± 0.02	20	0.09 ± 0.02	t=0.36, P>0.50
MAP2	12	0.06 ± 0.02	14	0.05 ± 0.01	t=0.49, P>0.50

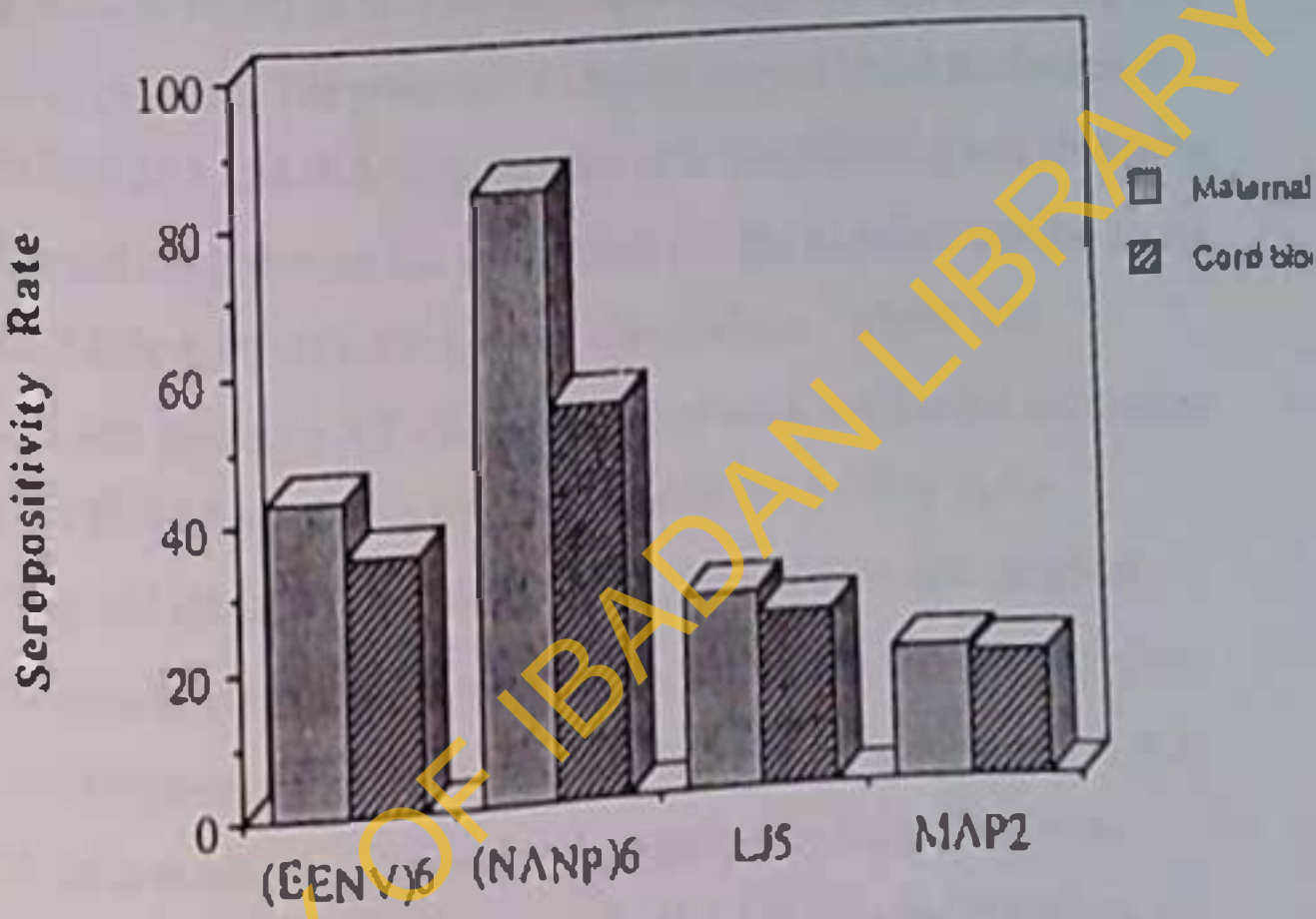

Fig. 4.12 Seropositivity rates for antibodies to the $(EENV)_6$, $(NANP)_6$, LJ5 and MAP2 peptides in paired maternal-cord serum samples from Igbo-Ora, Oyo State.

(Table 4.33). We evaluated the possibility of an enhanced protection against malaria in infants whose cord blood was either positive for antibodies to the Pf155/RESA and (NANP)6 antigens (from the blood and sporozoite stages of the parasite respectively) or to the (EENV)6 and (NANP)6 peptides. The presence or absence of cord blood antibodies to the Pf155/RESA and (NANP)6 antigens or to the (EENV)6 and (NANP)6 peptides did not influence the age of onset of clinical malaria in the study infants (Table 4.34). However, it was observed that infants with haemoglobin genotype AS whose cord blood were positive for antibodies to the Pf155/RESA and (NANP)6 antigens (Table 4.35) or to the (EENV)6 and (NANP)6 peptides (Table 4.36) had their first attack of malaria later in life compared with Hb AA infants. On the contrary, there was no difference in the age of onset of clinical malaria between Hb AA and AS infants whose cord blood were negative for antibodies to the Pf155/RESA and (NANP)6 antigens (Table 4.35) or to the (EENV)6 and (NANP)6 peptides (Table 4.36).

The prevalence seropositivity for antibodies to four oligopeptides {(EENV)6, (NANP)6, LJ5 and MAP2} in infants during the first year of life are shown in Fig. 4.13. The number of infants positive for antibodies to the (EENV)6 peptide fell rapidly from birth (38%) and by 6 months of age, none of the study infants were seropositive. After 6 months of age seropositivily rates rose rapidly to 48% by one year of age.

Seropositivity rates for antibodies to the (NANP)6 peptide fell from birth to about half of the birth seropositivity rate at the age of 2

Table 4.33 Mean (± S.E) age of onset (months) of primary clinical malaria in infants with and without cord blood antibodies to some *P. falciparum* antigens.

Antigens	n	Antibody Postive	n	Antibody Negative	Significance of difference
$(EENV)_6$	13	4.41 ± 0.56	39	4.48 ± 0.26	t=0.13, P>0.50
$(NANP)_6$	29	4.71 ± 0.31	23	4.16 ± 0.01	t=1.16, P<0.30
TJS	8	3.99 ± 0.29	44	4.55 ± 0.27	t=0.85, P<0.40
MAP2	8	3.98 ± 0.28	44	4.5 ± 0.27	t=0.85, P<0.40

Table 4.34. Age of onset of clinical malaria in Nigerian infants at Igbo-Ora and cord blood seroreactivity to the Pf155/$(NANP)_6$ and $(EENV)_6/(NANP)_6$ antigen pairs.

Antigen Pairs	Antibody Positive[a] n	Mean ± S.E	Antibody Negative[b] n	Mean ± S.E	Significance[c] of difference
Pf155 & $(NANP)_6$	27	4.59 ± 0.38	19	3.94 ± 0.41	1.13. P>0.05
$(EENV)_6$ & $(NANP)_6$	17	4.66 ± 0.59	27	4.23 ± 0.37	0.64. P<0.50

[a]Mean age of onset of clinical malaria in infants whose cord blood was positive for antibodies to the Pf155/$(NANP)_6$ and $(EENV)_6/(NANP)_6$ antigen pairs

[b]Mean age of onset of clinical malaria in infants whose cord blood was negative for antibodies to the above antigen pairs.

[c]Student's t-test values and levels of significance.

Table 4.35. The effect of haemoglobin genotype on the age of onset of clinical malaria in Nigerian infants whose cord blood was positive or negative for antibodies to the Pf155/RESA and $(NANP)_6$ antigens.

Haemoglobin Genotype	Antibody Positive[a] n	Mean ± S.E	Antibody Negative[b] n	Mean ± S.E	Combined[c] n	Mean ±S.E
AA	19	4.19 ± 0.35	11	3.85 ± 0.31	30	4.09 ±0.26
AS	9	6.94 ± 0.74	13	4.00 ± 0.69	22	4.98 ± 0.66
Significance of difference[d]	3.63, P<0.01		0.18, P > 0.50		1.47, P > 0.05	

[a]Mean age of onset of clinical malaria in infants whose cord blood was positive for antibodies to the Pf155/RESA and $(NANP)_6$ antigens.

[b]Mean age of onset of clinical malaria in infants whose cord blood was negative for antibodies to the Pf155/RESA and $(NANP)_6$ antigens.

[c]Mean age of onset of clinical malaria in infants whose cord blood was either positive or negative for antibodies to the Pf155/RESA and $(NANP)_6$ antigens.

[d]Student's t-test values and levels of significance.

Table 4.36. The effect of haemoglobin genotype on the age of onset of clinical malaria in Nigerian infants whose cord blood was positive or negative for antibodies to the $(EENV)_6$ and $(NANP)_6$ peptides.

Haemoglobin Genotype	Antibody Positive[a] n	Mean ± S.E	Antibody Negative[b] n	Mean ± S.E	Combined[c] n	Mean ±S.E
AA	12	4.18 ± 0.42	18	4.53 ± 0.44	30	4.43 ± 0.33
AS	10	7.47 ± 0.73	12	3.54 ± 0.81	22	4.72 ± 0.84
Significance of difference[d]	4.26, P<0.01		1.17, P > 0.05		0.38, P >0.50	

[a]Mean age of onset of clinical malaria in infants whose cord blood was positive for antibodies to the $(EENV)_6$ and $(NANP)_6$ antigens.

[b]Mean age of onset of clinical malaria in infants whose cord blood was negative for antibodies to the $(EENV)_6$ and $(NANP)_6$ antigens.

[c]Mean age of onset of clinical malaria in infants whose cord blood was either positive or negative for antibodies to the $(EENV)_6$ and $(NANP)_6$ antigens.

[d]Student's t-test values and levels of significance.

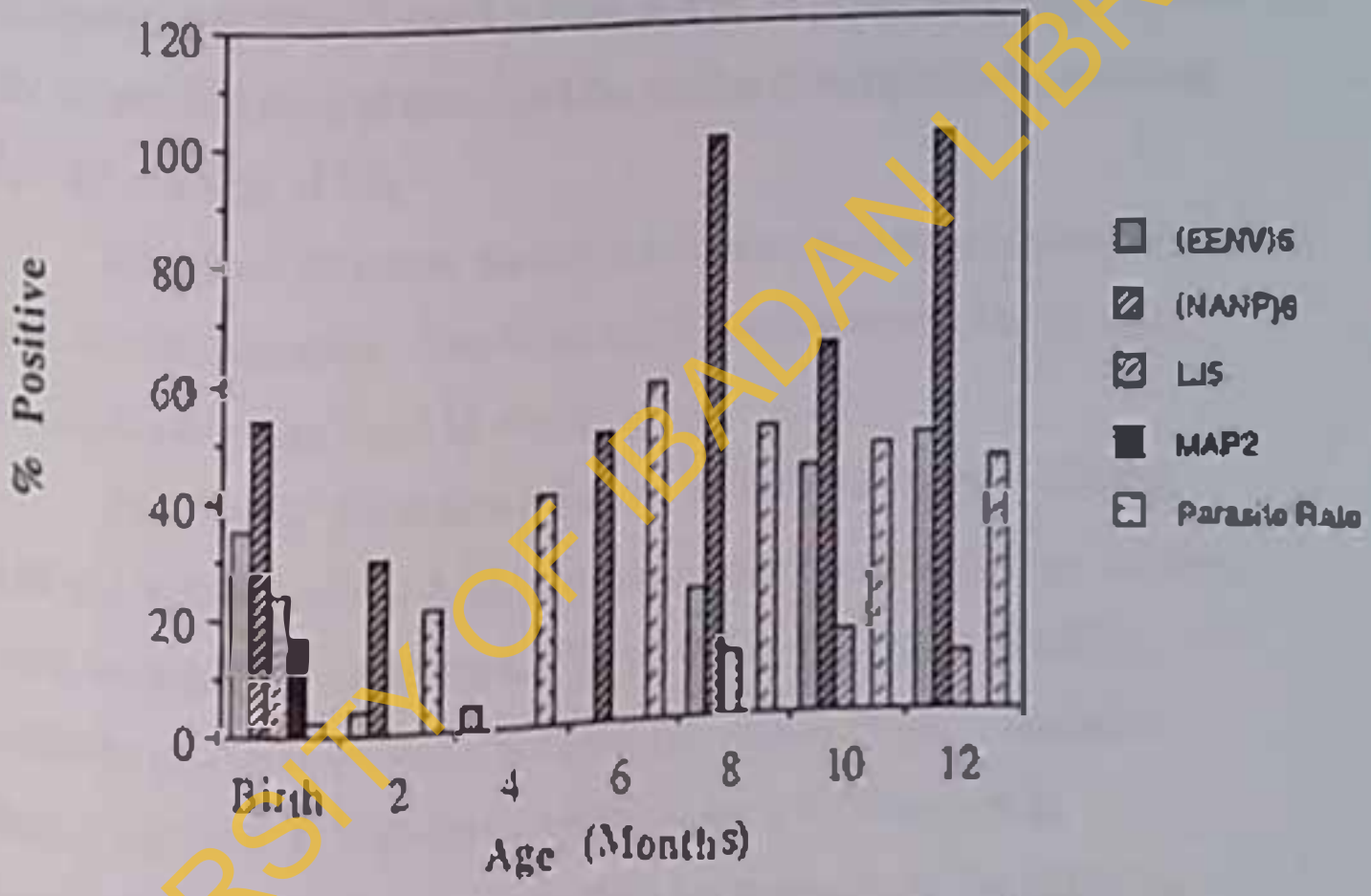

Fig. 4.13. Prevalence of seropositivity for antibodies to the $(EENV)_6$, $(NANP)_6$, LJ5 and MAP2 antigens of *P. falciparum* and malaria parasite rates in Nigerian infants during their first year of life.

months. None of the infants was seropositive at the age of 4 months. However, seropositivity rates rose rapidly at 6 months of age and remained high till one year of age.

Twenty-four percent of the cord blood samples were positive for antibodies to the LJ5 peptide. However, none of the infants were seropositive between 2 and 6 months of age. At 8 months of age, 11% of the infants had seroconverted and the number of seropositives remained low till one year of life.

Seventeen percent of the cord blood samples were positive for anti-MAP2 antibodies. None of the infants were seropositive for these antibodies between 2 and 12 months of age.

The number of mothers positive for antibodies to the $(EENV)_6$, LJ5 and MAP2 peptides during the six consecutive surveys after delivery were not significantly different (Fig. 4.14). However, anti-$(NANP)_6$ antibody positivity showed significant ($\chi^2=18.28$, $P< 0.01$) variation during the surveys. With the exception of the $(NANP)_6$ peptide ($\chi^2=14.22$, $P< 0.01$) seropositivity rates for antibodies to the $(EENV)_6$ ($\chi^2 = 5.19$, $P<0.30$), LJ5 ($\chi^2 = 9.42$, $P> 0.05$) and MAP2 peptides ($\chi^2 = 4.46$, $P<0.40$) were similar between the different parity groups (Fig. 4.15). None of the primiparae was seropositive for antibodies to the LJ5 and MAP2 peptides. Mean ELISA (OD_{405}) values for the $(EENV)_6$, $(NANP)_6$, LJ5 and MAP2 peptides were similar between the different parity groups (Table 4.37).

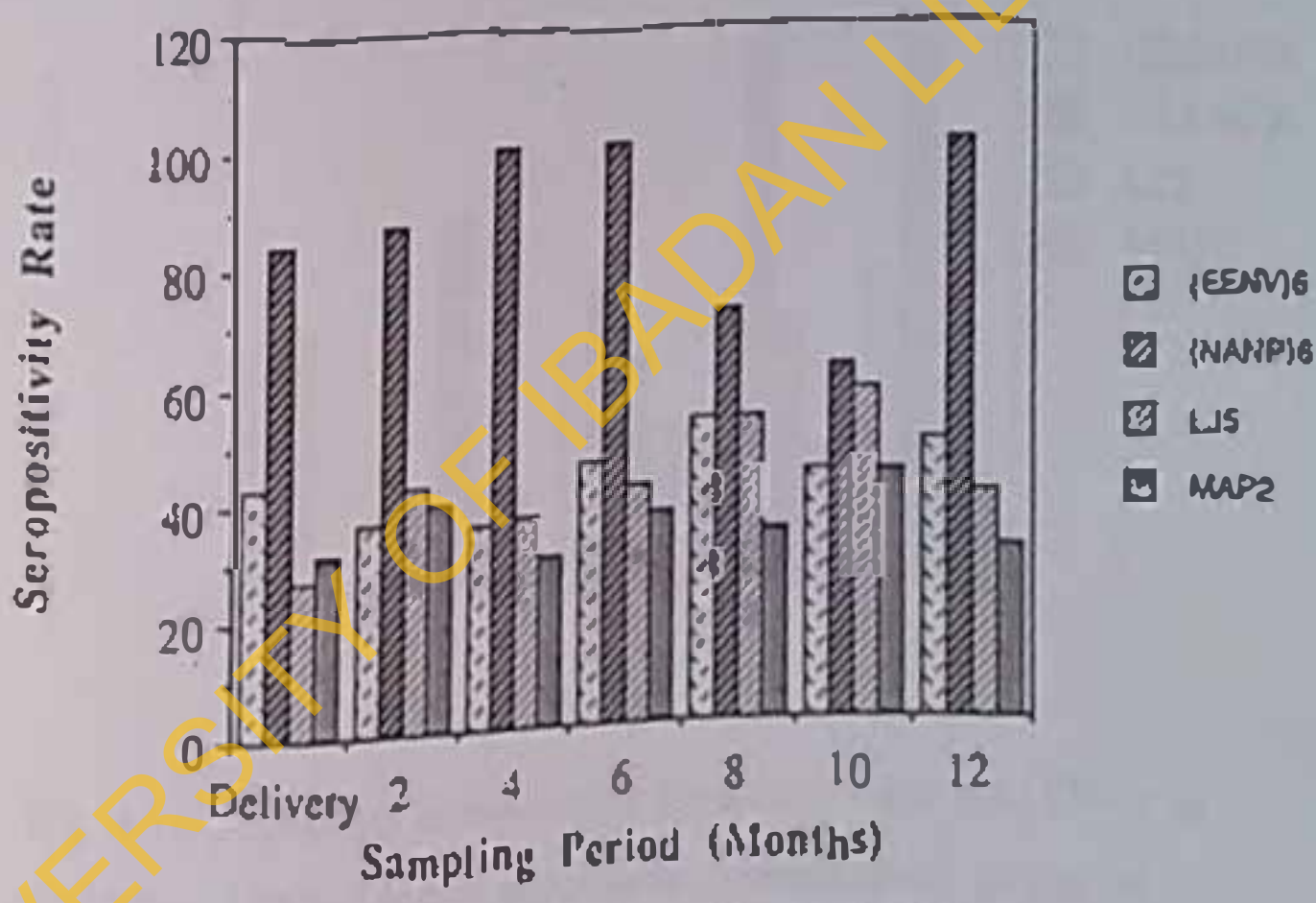

Fig. 4.14 Seropositivity rates for antibodies to $(EENV)_6$, $(NANP)_6$, LJS and MAP2 antigens of *P. falciparum* in Nigerian women at Igbo-Ora at delivery and on six bi-monthly conseculive surveys after delivery.

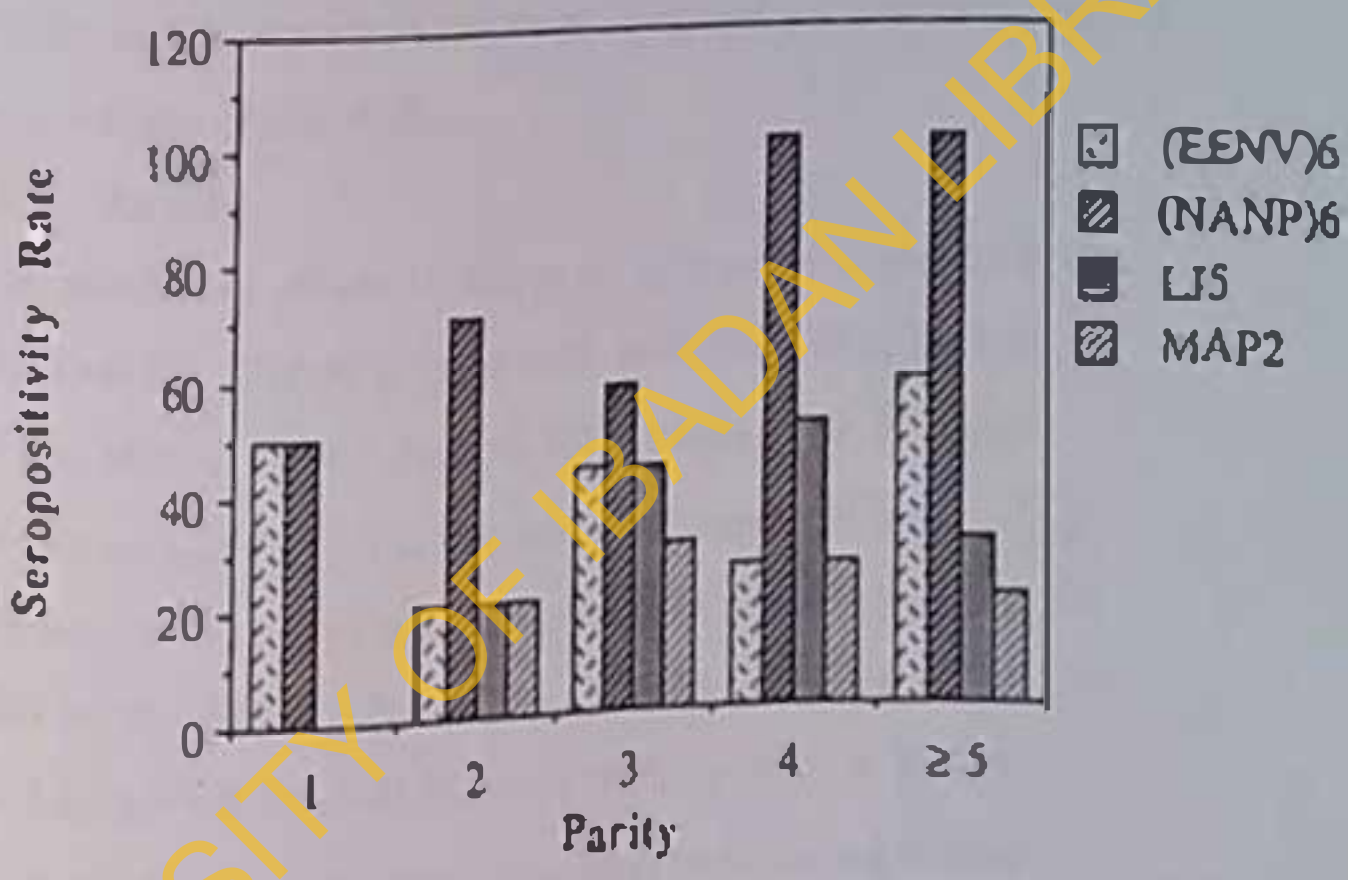

Fig. 4.15 Prevalence seropositivity for antibodies to the $(EENV)_6$, $(NANP)_6$, LJ5 and MAP2 peptides in different parity groups of parturient women at Igbo-Ora.

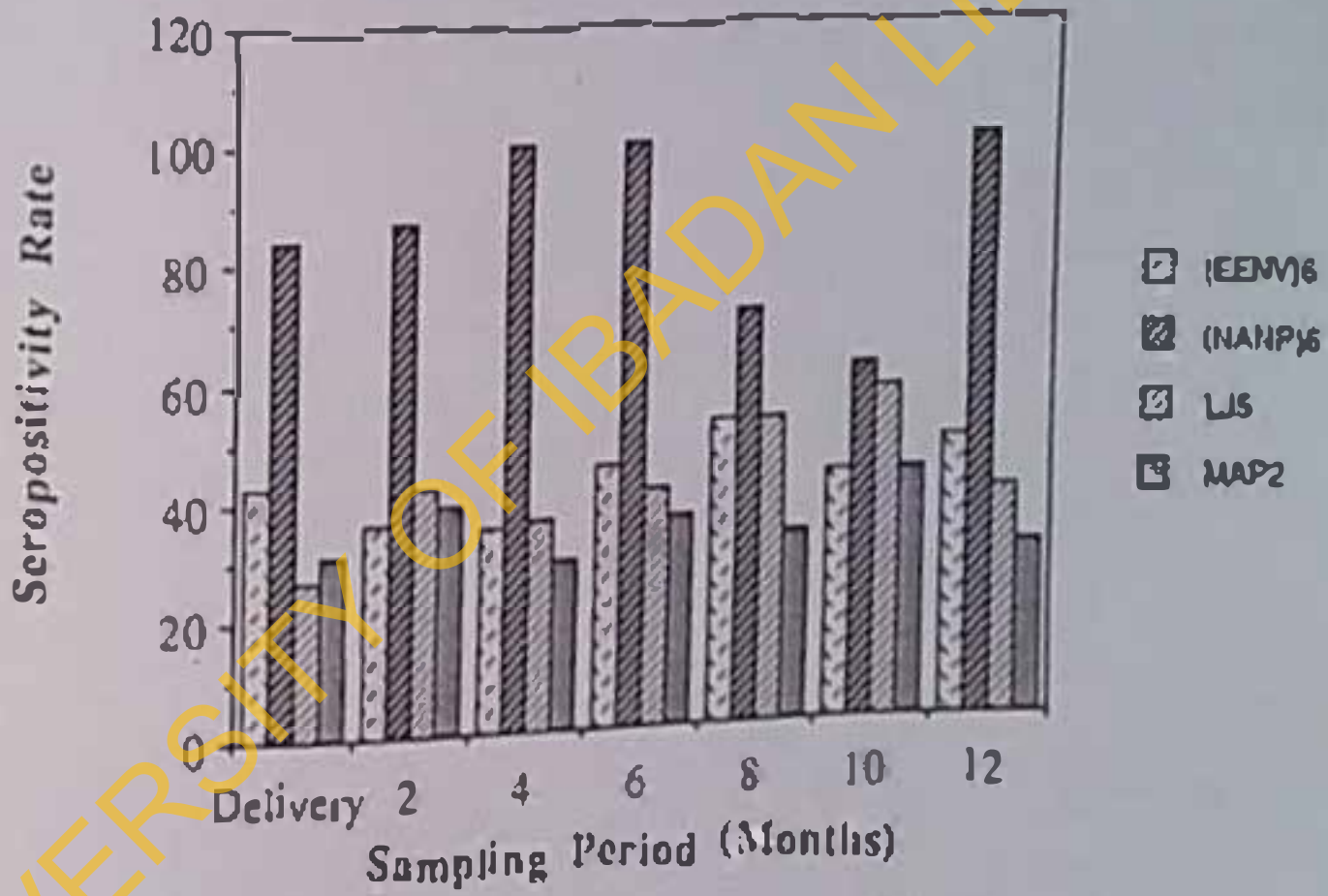

Fig. 4.14 Seropositivity rates for antibodies to $(EENV)_6$, $(NANP)_6$, LJ5 and MAP2 antigens of *P. falciparum* in Nigerian women at Igbo-Ora at delivery and on six bi-monthly consecutive surveys after delivery.

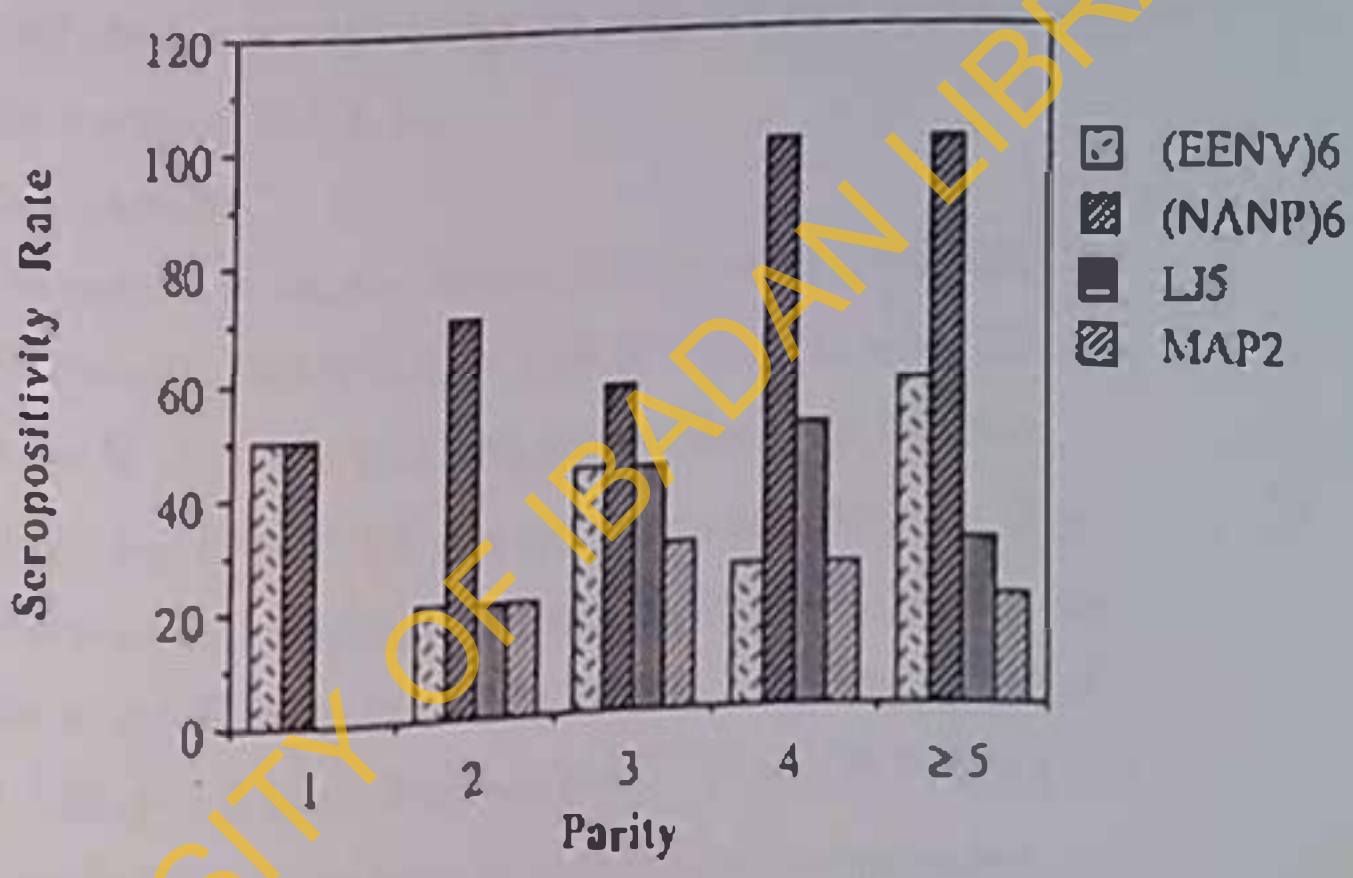

Fig. 4.15 Prevalence seropositivity for antibodies to the $(EENV)_6$, $(NANP)_6$, LJ5 and MAP2 peptides in different parity groups of parturient women at Igbo-Ora.

The prevalence seropositivity for antibodies to the $(EENV)_6$ and $(NANP)_6$ peptides were similar between malaria positive and negative parturient women (Fig. 4.16). None of the malaria positive parturient women was positive for antibodies to the LJ5 and MAP2 peptides. Mean ELISA (OD_{405}) values for the $(EENV)_6$ peptide but not $(NANP)_6$ was significantly higher in malaria negative compared with malaria positive parturient women (Table 4.38).

(b) Adults

The number of subjects positive for antibodies to the $(EENV)_6$ (χ^2=0.66, P<0.50), $(NANP)_6$ (χ^2 = 0.09, P>0.50), LJ5 (χ^2 = 1.86, P<0.20) and MAP2 (χ^2 = 0.22, P>0.50) peptides at the July and February surveys at the G.T.C was not significantly different (Fig. 4.17). There was no significant difference in the positivity rates for antibodies to the $(EENV)_6$ (χ^2 = 0.40, P>0.50), $(NANP)_6$ (χ^2 = 0.16, P>0.50), LJ5 (χ^2 = 0.43, P>0.50) and MAP2 (χ^2 = 0.34, P>0.50) peptides at the July survey between malaria positive and malaria negative subjects (Fig. 4.18). No significant difference in the mean ELISA values to the $(EENV)_6$, $(NANP)_6$, LJ5 and MAP2 peptides between malaria positive and negative G.T.C. subjects (Table 4.39).

Individuals sampled consecutively at the July and February surveys demonstrated similar ELISA (OD_{405}) values for the $(EENV)_6$ and MAP2 peptides while significantly higher ELISA values were recorded for the $(NANP)_6$ and LJ5 peptides at the July survey (Table 4.40). No significant difference was observed in the number of subjects

Table 4.37. Mean (± S.E) ELISA (OD_{405}) values to the $(EENV)_6$ and $(NANP)_6$, LJ5 and MAP2 peptides in Nigerian parturient women of different parities.

Parity	n	$(EENV)_6$	n	$(NANP)_6$	n	LJ5	n	MAP2
1	10	0.02 ± 10^{-2}	15	0.14 ± 0.04	0	0	0	0
2	7	0.14 ± 10^{-3}	10	0.08 ± 0.03	7	0.05 ± 10^{-3}	7	0.01 ± 10^{-3}
3	15	0.08 ± 0.04	11	$0.03 \pm .005$	9	0.11 ± 0.02	11	0.05 ± 0.03
4	7	0.17 ± 10^{-3}	11	0.09 ± 0.04	11	$0.07 \pm .004$	7	0.04 ± 10^{-3}
5	27	0.09 ± 0.02	24	0.14 ± 0.03	15	0.14 ± 0.05	11	0.11 ± 0.02
Significance of difference		$F=2.63$ $P>0.05$		$F=1.61$ $P<0.20$		$F=1.06$ $P<0.40$		$F=2.81$ $P<0.20$

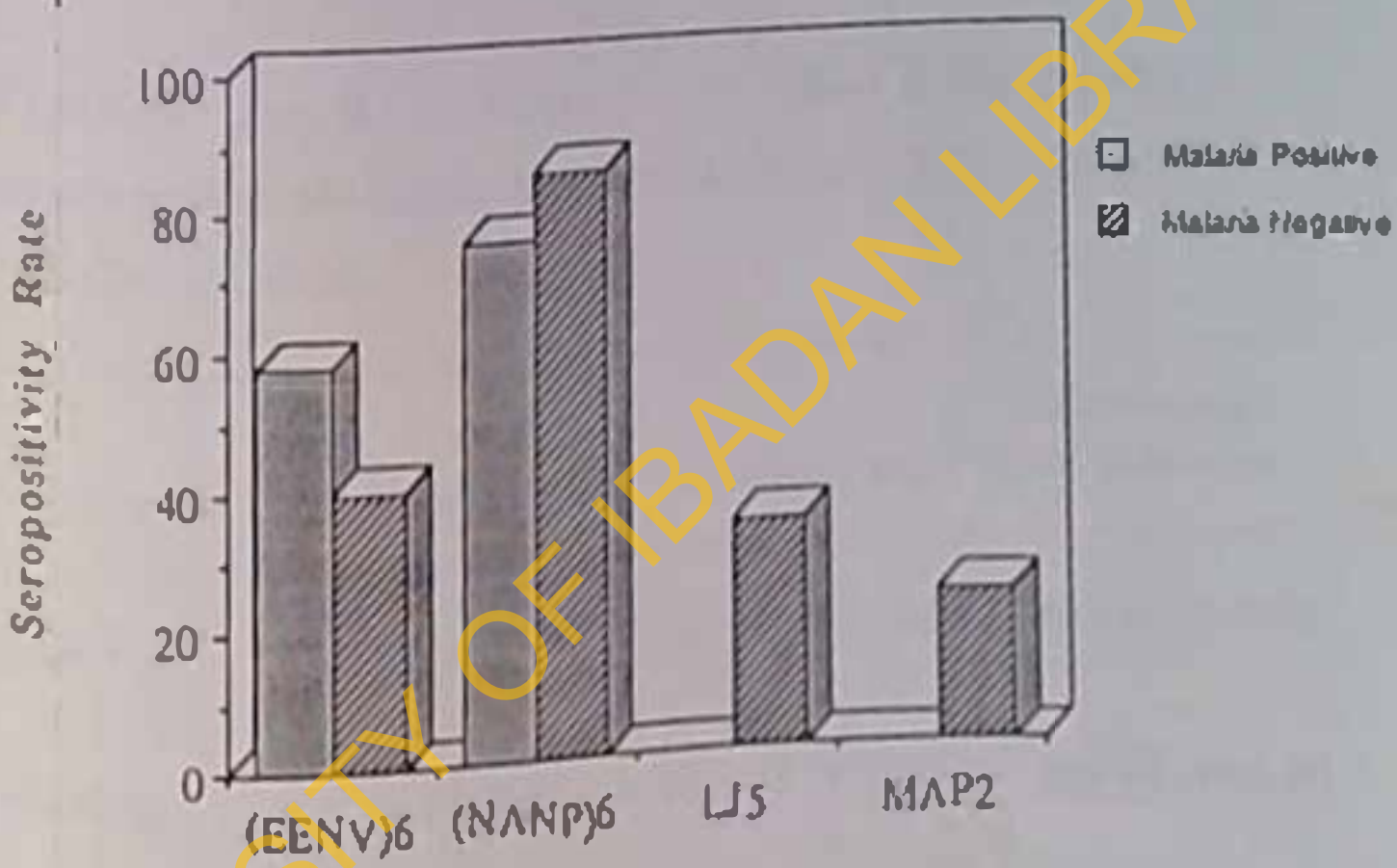

Fig. 4.16 Seropositivity rates for antibodies to the (EENV)6, (NANP)6, LJ5 and MAP2 peptides in malaria positive and negative parturient women at Igbo-Ora, Oyo State.

Table 4.38 Mean (± S.E) ELISA (OD_{405}) values to oligopeptides in malaria positive and malaria negative Nigerian parturient women at Igbo-Ora, Oyo State.

Oligopeptides	n	Malaria positive	n	Malaria negative	Significance of difference
$(EENV)_6$	7	0.02 ± 0.005	22	0.10 ± 0.08	t=2.41, P<0.03
$(NANP)_6$	9	0.18 ± 0.05	47	0.10 ± 0.02	t=1.87, P<0.10
LJS	0	-	18	0.10 ± 0.02	-
MAP2	0	-	12	0.06 ± 0.05	-

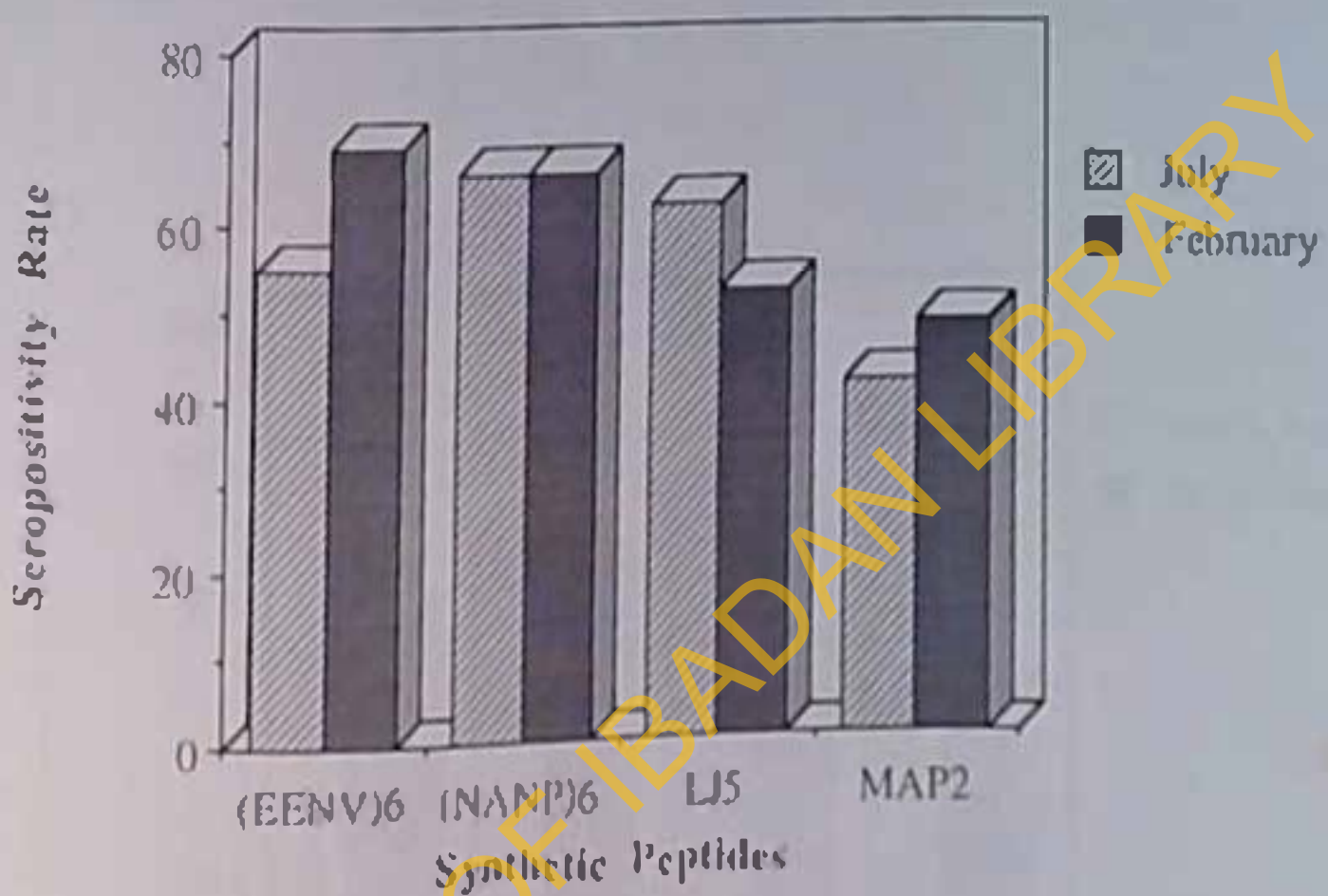

Fig. 4.17 Seropositivity rates for antibodies to the $(EENV)_6$, $(NANP)_6$, LJ5 and MAP2 peptides in G.T.C., Igbo-Ora study subjects at the July, 1991 and February, 1992 cross-sectional surveys.

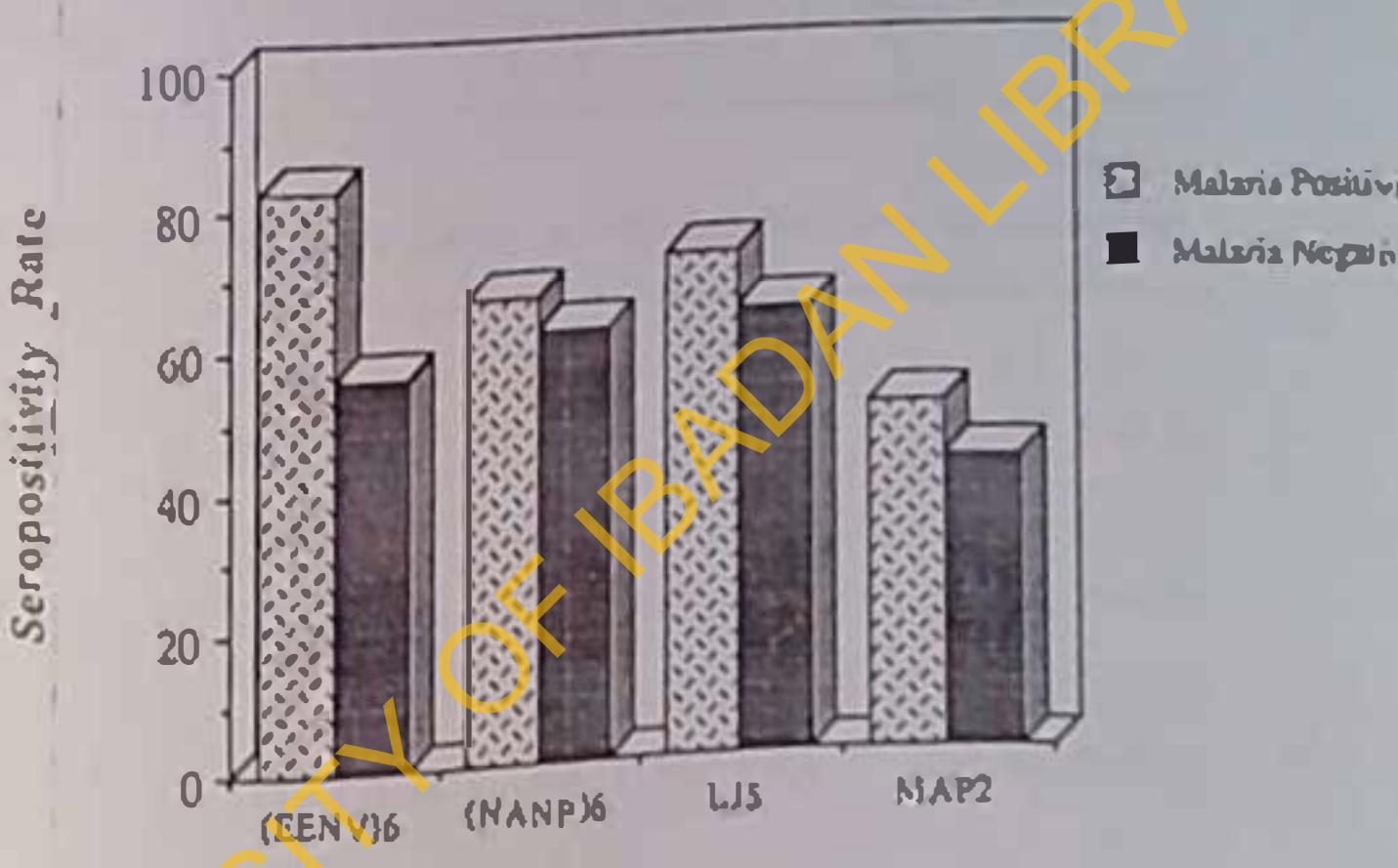

Fig. 4.18 Seropositivity rates for antibodies to the (EENV)6, (NANP)6, LJ5 and MAP2 peptides in malaria positive and negative G.T.C. Igbo-Ora study subjects at the July, 1991 survey.

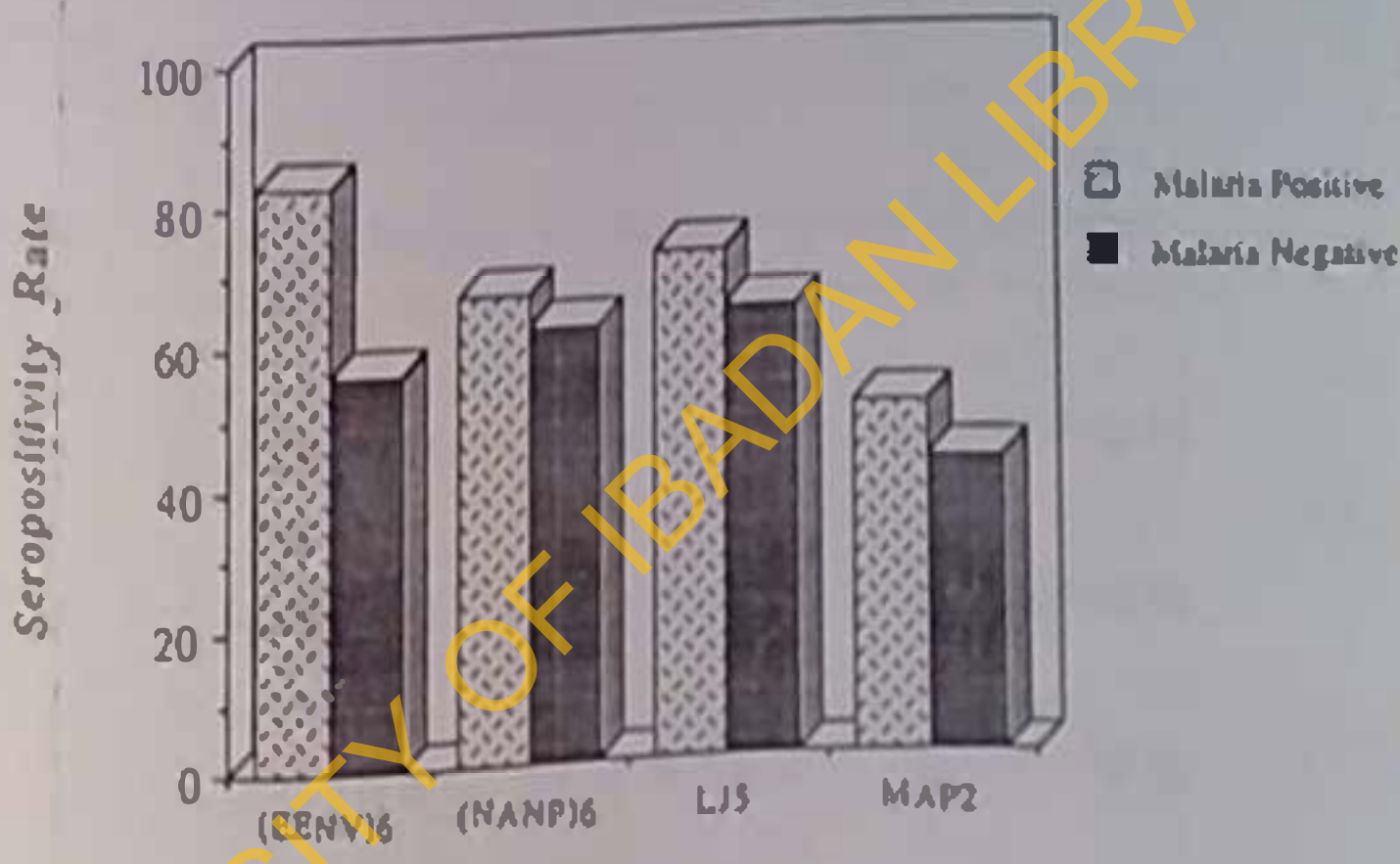

Fig. 4.18 Seropositivity rates for antibodies to the (EENV)6, (NANP)6, LJ5 and MAP2 peptides in malaria positive and negative G.T.C. Igbo-Ora study subjects at the July, 1991 survey.

Table 4.39 Mean (± S.E) ELISA (OD_{405}) values to oligopeptides in malaria positive and malaria negative G.T.C study subjects at the July, 1991 survey.

Oligopeptides	n	Malaria Positive	n	Malaria Negative	Significance of difference
$(EE_{NV})_6$	15	0.29 ± 0.07	45	0.24 ± 0.04	t=0.67, P>0.50
$(NA_{NP})_6$	12	0.27 ± 0.06	48	0.23 ± 0.02	t=0.74, P>0.50
LJ5	13	0.42 ± 0.08	52	0.36 ± 0.03	t=1.47, P>0.30
MAP2	9	0.25 ± 0.04	34	0.22 ± 0.02	t=0.79, P> 0.50

positive for antibodies to the (EENV)6 (P<0.50), (NANP)6 (P>0.50), LJ5 (P<0.20) and MAP2 (P<0.10)during the two consecutive surveys (Fig.4.19).

There was no significant difference in the positivity rates for antibodies to the (EENV)6 (χ^2=0.23, P>0.50), LJ5 (χ^2=1.29, P<0.30) and MAP2 (χ^2=2.65, P<0.20) peptides between the October-November and March surveys of blood donors at the U.C.H, Ibadan. However, the number of seropositives for the (NANP)6 peptide was significantly higher (P< 0.001) at the October-November survey compared with the March survey (Fig. 4.20).

There was no significant difference in the number of seropositives to the $(EENV)_6$ (χ^2=0.02, P>0.50), (NANP)6 (χ^2=3.23, P<0.10), LJ5 (χ^2 = 0.01, P>0.50) and MAP2 (χ^2 = 0.09, P>0.50) peptides (Fig. 4.21) between malaria positive and negative blood donors at the October - November survey. Similarly, at the March survey , no significant difference was observed in the number of seropositives to the $(EENV)_6$ (χ^2 = 0.02, P>0.50), (NANP)6 (χ^2 = 0.05, P>0.50) and LJ5 (χ^2 = 2.07, P<0.20) peptides (Fig. 4.22) between malaria positive and negative blood donors. None of the malaria positive blood donors were seropositive for antibodies to the MAP2 peptide.

Table 4.40 Mean (± S.E) ELISA (OD_{405}) values to four *P. falciparum* peptides in individuals sampled on two consecutive surveys (July, 1991 and February, 1992) at the G.T.C Igbo-Ora.

Peptides	n	July 1991	n	February 1992	Significance of difference
$(EENV)_6$	16	0.28 ± 0.06	20	0.13 ± 0.04	t=1.89, P>0.50
$(NANP)_6$	19	0.15 ± 0.03	19	0.04 ± 0.01	t=3.36, P<0.01
LJ5	18	0.23 ± 0.05	15	0.07 ± 0.02	t=3.44, P<0.01
MAP2	12	0.14 ± 0.03	14	0.07 ± 0.02	t=0.83, P<0.50

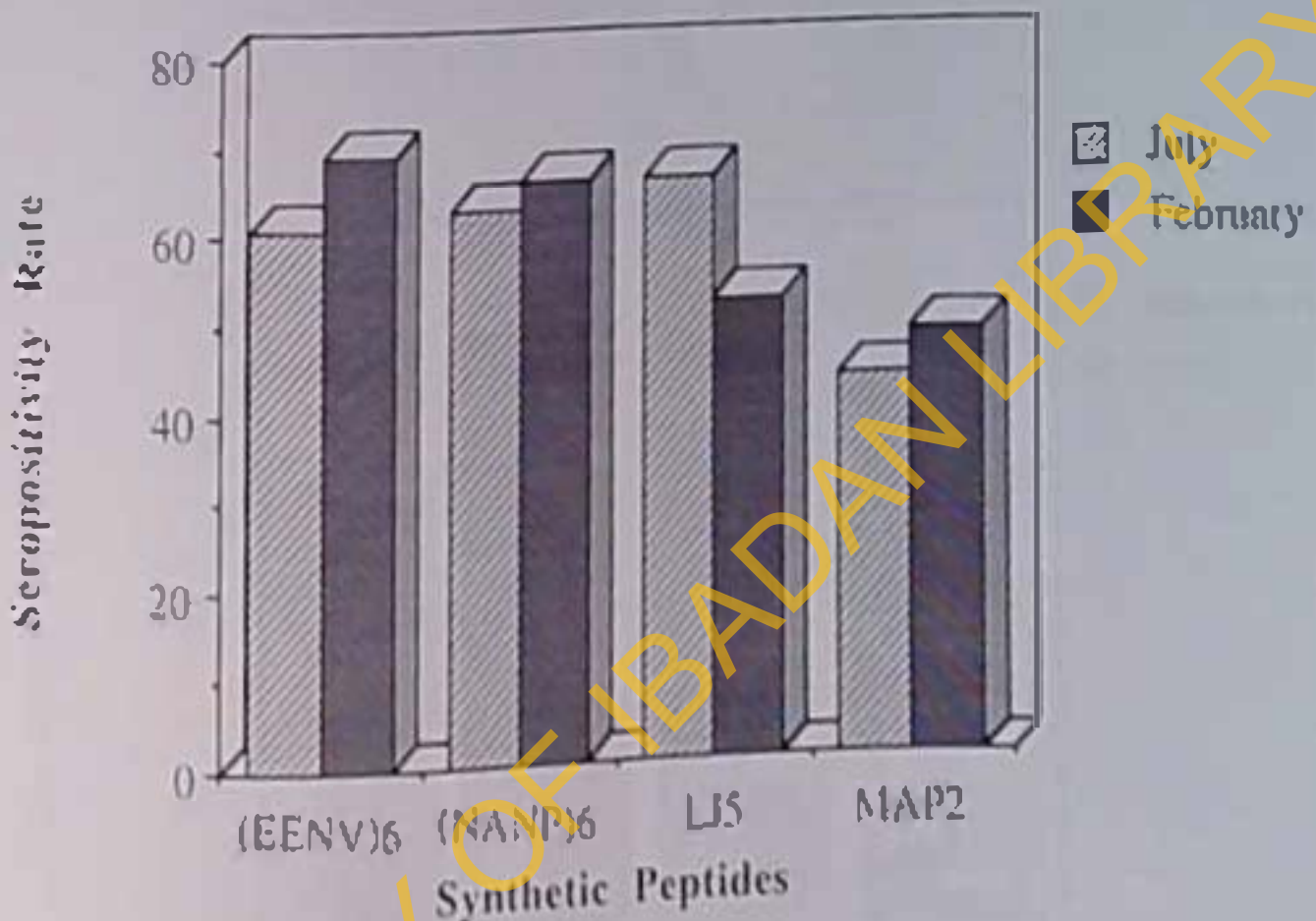

Fig. 4.19 Seropositivity rates for antibodies to the (EENV)6, (NANP)6, LJ5 and MAP2 peptides in G.T.C study subjects sampled consecutively at the July, 1991 and February, 1992 surveys.

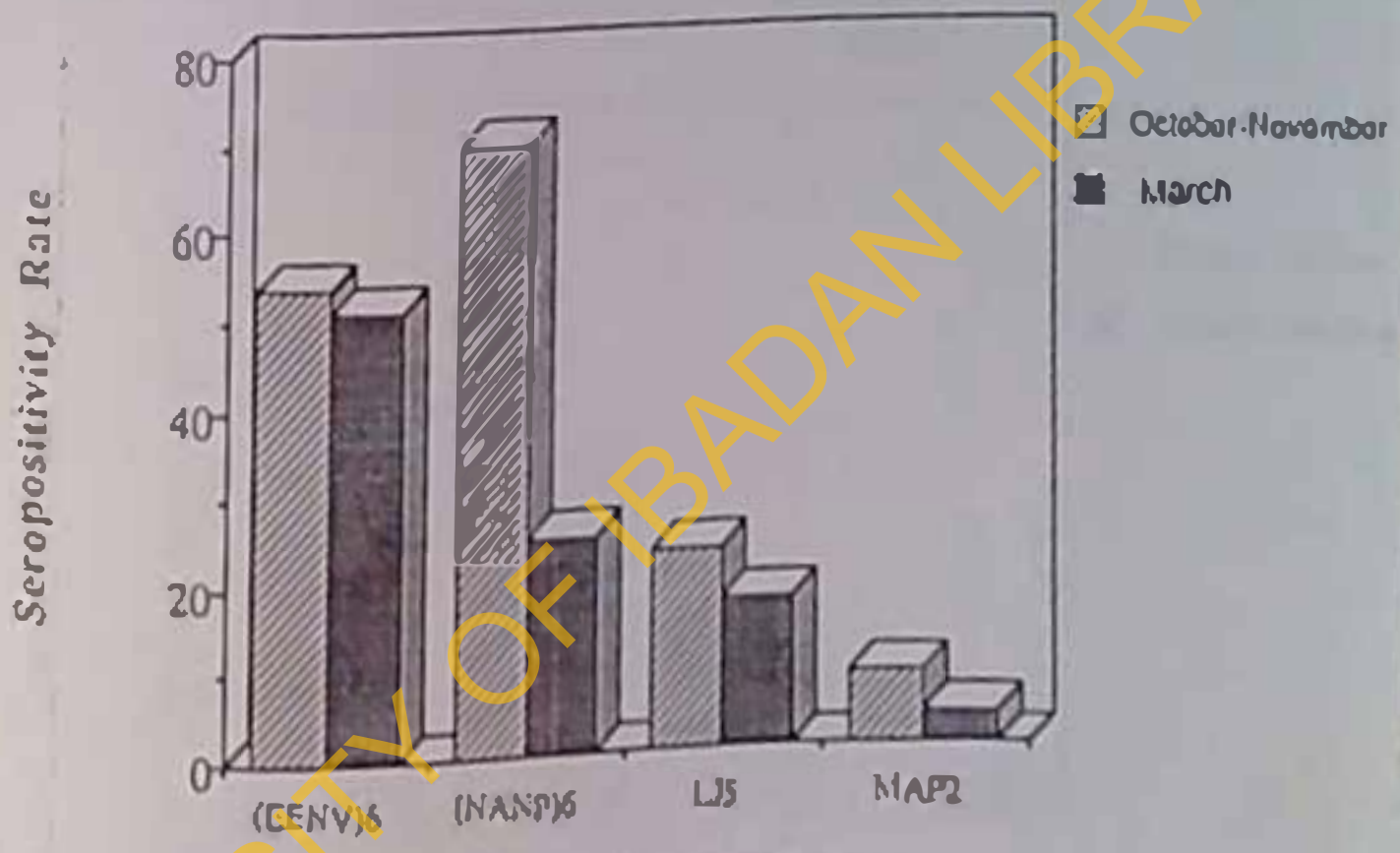

Fig. 4.20 Seropositivity rates for antibodies to the (EENV)6, (NANP)6, LJ5, and MAP2 peptides in blood donors at the October-November, 1991 and March, 1992 cross-sectional surveys.

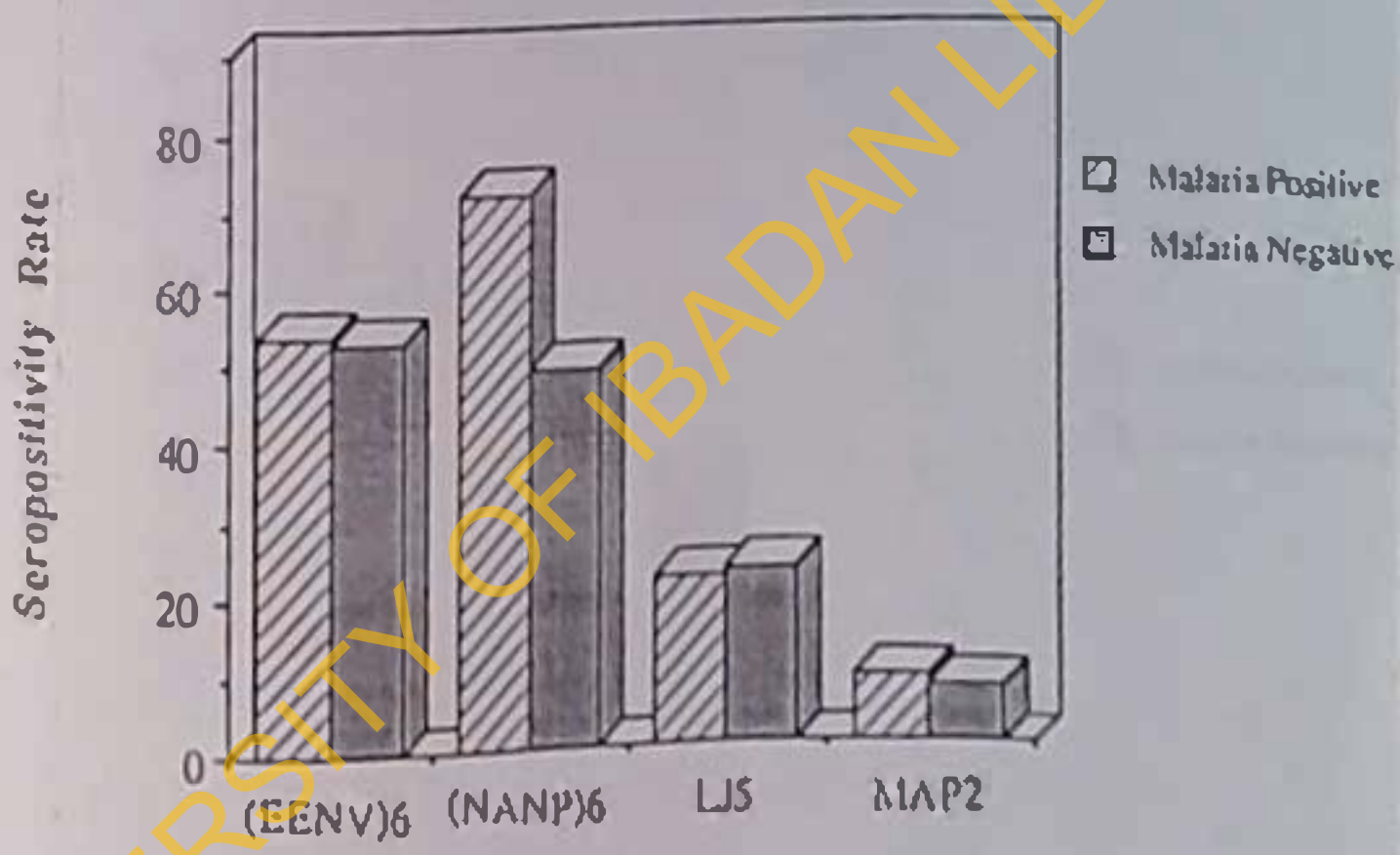

Fig. 4.21 Seropositivity rates for antibodies to the (EENV)6, (NANP)6, LJ5 and MAP2 peptides in malaria positive and negative blood donors at the October-November, 1991 survey.

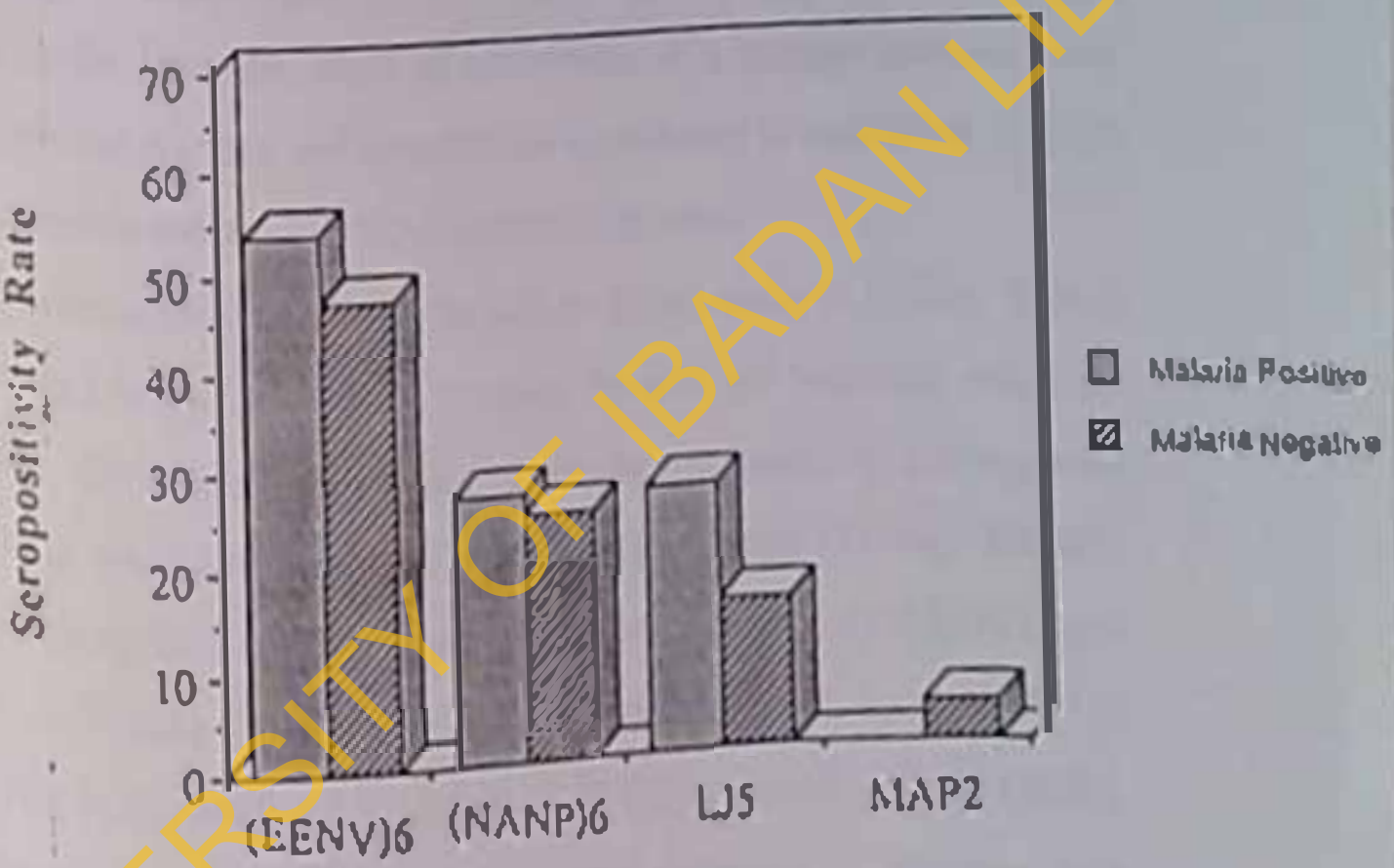

Fig. 4.22 Seropositivity rates for antibodies to the (EENV)6, (NANP)6, LJ5 and MAP2 peptides in malaria positive and negative blood donors at the March, 1992 survey.

CHAPTER FIVE

5.0 DISCUSSION

This study investigates malaria parasite rates and densities in Nigerian infants and adults and also determines which, if any, of a range of assays of the host response to malaria provide useful information concerning the immune status of individuals in a malaria endemic area. Cross-sectional surveys and longitudinal monitoring of individual subjects offers a possible means of achieving these objectives.

The mean birthweight of the infants in this study is 3,200g. This is 200g (± 30g) below the Havard standard birthweight reference value for Caucasians (Jelliffe, 1966). Mean birthweight was similar to that reported by Lawoyin and Oyediran (1992) in Ibadan, Nigeria (3170g). Slightly lower birthweights have been reported by Brice-Chwatt (1952) in Lagos (3048g), Archibald (1958) in Northern Nigeria (3076g), Spitz (1959) in Eastern Nigeria (2940) and Reinhardt et al. (1978) in Ivory Coast (3080). The observed increase in mean birthweight compared to previous data from Nigeria may not be unrelated with improved ante-natal care since Igbo-Ora is the first rural community in Nigeria to adopt the primary health care programme. None of the study infants had a low birthweight (less than 2500g).

The presence of peripheral malaria parasites in Parturient women had no significant effect on the birthweight of their newborns. The difference in the mean birthweight of babies born to infected or non-

infected mothers was 80g. Spitz (1959) observed a slightly higher difference (89g) between babies born to mothers with or without placental parasitaemia. Higher values have been reported by Bruce-Chwatt (1952) in Southern Nigeria (145g) and Archibald (1958) in Northern Nigeria (298g). Birthweight differences between babies born of mothers infected or non-infected at delivery are variable but in all studies (Bruce-Chwatt, 1952; Spitz, 1959; Reinhardt et al., 1978) lower mean birthweights were associated with placental malaria. However, in the present study maternal peripheral parasite density was found to correlate negatively with birthweight of newborns. It is not known if there is any correlation between placental and maternal peripheral parasitaemia.

Our observation that birthweight was not significantly influenced by sex is consistent with the findings of Nhonoli et al. (1975) and Greenwood et al. (1989) in The Gambia. However, Reinhardt et al. (1978), Antia-Obong et al. (1986) in Calabar and Lawoyin and Oyediran (1992) in Ibadan, Nigeria reported a significantly higher mean birthweight in males compared with females.

It has been suggested that the increase in birthweight with parity is progressively reduced at higher parities (Urrabin, 1991) and that birthweights may even decline below values for primiparae at higher parities (Reinhardt et al., 1978). Analysis of 50,000 birthweights in Scotland showed no significant rise in birthweights after the second baby (Thomson et al., 1968). In the present study the mean birthweights of the newborns increased significantly from parity 1 to 3 and decreased slightly

for parities 4 and above. Newborns from primiparous women had the lowest mean birthweights and primiparae had the highest incidence and density of malaria parasitaemia. This observation is in accordance with the findings of Bruce-Chwatt (1952) in Nigeria, Reinhardt et al. (1978) in Ivory Coast, McGregor et al. (1983) and Greenwood et al (1989) in The Gambia. Malaria is the only known specific environmental factor which selectively depresses birthweight to a greater extent in primigravidae than multigravidae, and this relates to the much higher prevalence and density of malaria in primigravidae (Brabin, 1991). It is possible that the generalized immune depression observed in pregnancy is more pronounced in first pregnancy.

Since low birthweight has been attributed to maternal or placental malaria infection (Spitz, 1959; Reinhardt et al., 1978; Brabin, 1991), then chemoprophylaxis intervention that protects the mother throughout pregnancy might be expected to show greater differences in mean birthweight values between prophylactic and non-prophylactic groups. As a matter of policy, all women at Igbo-Ora are given a single dose (3 tablets) of chloroquine at first ante-natal clinic attendance followed by weekly pyrimethamine prophylaxis for those who can afford the cost.

In this study, weekly pyrimethamine prophylaxis from the 20th week of pregnancy resulted in a modest increase in birthweight of 53g. This observation is similar to the findings of Hamilton et al. (1972) in East Africa who reported a birthweight increase of 70g and that of Gilles et al. (1969) in Nigeria who observed no significant difference in

birthweights following weekly pyrimethamine prophylaxis. However, Morley et al. (1964) in Western Nigeria reported an overall increase in birthweight of 157g following monthly pyrimethamine prophylaxis; the highest was observed in primigravidae and women with 4 or more previous pregnancies. A similar observation was reported by Greenwood et al. (1989) in The Gambia following maloprim prophylaxis. The duration of prophylaxis was not stated in both studies.

It is evident from these studies that chemoprophylaxis in pregnancy has maximal effects on birthweights of newborns of primigravidae and multigravidae of higher parities. The result of the present study must however, be interpreted with caution as it might have been influenced by several factors such as the prevalence of pyrimethamine resistant strains of *P. falciparum*. Furthermore, the pregnant women in this study were not closely monitored to ensure compliance with malaria chemoprophylaxis and lastly prophylaxis was not commenced early in pregnancy. Chemoprophylaxis interventions early in pregnancy may have maximal effect on birthweight. None of the previous studies addressed this issue satisfactorily. Further studies are therefore needed to confirm the benefits of early chemoprophylaxis in pregnancy.

No incidence of congenital clinical malaria was recorded amongst the study infants in Igbo-Ora thus supporting the view that congenital malaria must be very rare among indigenous populations of holoendemic countries (Bruce-Chwatt, 1952). Its infrequency does not appear to be due to failure of the parasite to cross the placental barrier or to gain access to

the foetus. Cord blood parasitaemia was detected in 2.6% (3/116) of the deliveries. In these 3 cases, two of the mothers (multiparae) had peripheral parasitaemia while the third mother (primiparous) was negative for malaria parasites. The presence of malaria parasites in cord blood might have been due to a 'spill over' at birth of parasites from mother's blood into cord blood probably due to mechanical trauma of the placenta during uterine contractions as suggested by Nhonoli et al. (1975).

Low density infections of cord blood are frequently recorded in African newborns; prevalences of 3 - 8% (Korinann, 1972) and 9.6% (Vleugels, 1984) have been reported in Tanzanian newborns and 21% in babies in Ivory Coast (Reinhardt et al., 1978). On the other hand peripheral blood parasitaemia is rarely detected in the newborn (Bruce-Chwatt, 1952; Spitz, 1959; Williams and McFarlane, 1970).

In malaria endemic regions, where even adults may have a cumulative parasite rate approaching 100% (Bruce-Chwatt, 1963a), determining whether an illness episode is due to co-existent parasitaemia is extremely difficult. Parasite density alone is not necessarily a good indicator since it was observed in this study that even very low parasitaemias in infants above 6 months of age may be causally related to symptoms. By contrast, apparently asymptomatic individuals with high parasitaemias were encountered in the cross-sectional surveys of the adult study population. Episodes of clinical malaria in infants was almost always accompanied by the presence of malaria parasites in thick blood films.

Longitudinal monitoring of infants proved sensitive in detecting incidences of parasitaemia and episodes of clinical malaria. Malaria parasite rates and densities rose rapidly in the study infants after 2 months of age and remained high throughout the first year of life. This observation suggests that the African infant has a considerable degree of resistance to malaria infection during the first 2 months of life and raises doubts whether any appreciable protection can be claimed beyond 3 months of life. Malaria parasite rate at 2 months of age (21%) was higher than that reported by Gilles (1957) in The Gambia (10%) and Bruce-Chwatt (1952) in Nigeria (3%). In the later study first infections were contracted during the second half of the first year of life. Spencer et al. (1987) in Kenya detected malaria parasites in some infants less than one month of age and almost half of the infants 4 months of age and above were positive for malaria parasites. They suggested that the relatively linear rate of acquisition of infection observed in the first 4-5 months of life indicate that transplacentally acquired immunity does not influence the development of parasitaemia. In a recent study of Kenyan infants, Kramer et al. (1993) reported that the median age at which infants became infected with malaria parasites was 17 weeks (4.25 months).

The pattern of increase in parasite rates during the first 6 months of life are similar to those reported by Bruce-Chwatt (1952) in Lagos, Gilles et al. (1969) in The Gambia and Molineaux and Gramiccia (1980) in Northern Nigeria. However, infants above six months of age were observed in the present study to have similar proportion of parasite rates

while Bruce-Chwatt, (1952), Gilles et al. (1969) and Molineaux and Grammiccia, (1980) found that parasite rates continued to increase up to 80 - 90%. The reason for this difference in findings is unknown. Anti-malarial drugs are now readily available compared to the 1960's and some of the infants may have been on chemoprophylaxis. The most critical period of the studied infants was between 4 and 10 months of life when malaria parasite rates/densities were highest and episodes of clinical malaria was common. This may represent the period when inherited immunity is on the wane.

Data from the present study indicate that the African infant exhibits some degree of 'premunition' (a phenomenon common with African adults) during the first 2-3 months of life. Within this period some malaria positive infants did not present with clinical symptoms of malaria and parasitaemia was mild. On the contrary, infants who experienced an episode of clinical malaria within this period had very high parasite densities and clinical symptoms were relatively mild. As the infant ages, above 4 months of age premunition is gradually lost and by 6 months of age mild infections usually led to episodes of clinical malaria.

The mean age of onset of primary clinical malaria in the study infants was 4.2 ± 0.20 months. The duration of onset of clinical malaria showed a high degree of variability with the earliest onset at 2.0 months and the latest onset at 8.2 months (in heterozygous twins). The reason for the wide variation between individual infants is unclear. This variation in duration of onset of clinical malaria may be explained partially by genetic

factors such as the major histocompactibility complex (MHC) restriction. It has been suggested that possession of some HLA class 1 antigens renders the individual susceptible to clinical malaria (Piazza et al., 1972; Osoba et al., 1979; Hill et al., 1991). On the contrary, some HLA class II haplotypes have been shown to protect against severe malaria (Hill et al., 1991).

Clinical episodes of malaria were most common between 3 and 9 months of age. This was mirrored to some extent by the high parasite rates and densities found within this age group. However, parasite rates and densities remained high after 8 months of age while relatively less infants experienced clinical malaria within this period. One possible explanation for this pattern is that some infants were probably developing immunity against the disease but not against the parasite. In addition some infants might have been on chemoprophylaxis which is commonly available in medicine shops.

Most of the study infants (67%) had their first episode of clinical malaria between three and six months of age. This observation disagrees with the previous assertion that clinical malaria rarely occurs in infants below 6 months of age (Bruce-Chwatt, 1952). This finding is of direct relevance to rural health workers who religiously stick to the previous suggestion that clinical malaria and infants below 6 months of age have nothing in common. During the longitudinal studies in Igbo-Ora, three cases of fever in infants below 6 months of age was diagnosed for septicaemia by the Nursing Sister. Peripheral blood examination however,

showed high densities of *P. falciparum* parasites in the affected infants. This observation suggest that blood film examination of infants presenting with fever is of vital importance in arriving at a conclusive diagnosis of malaria. This simple procedure is very relevant as it was found in this study that almost all the infants presenting with symptoms of clinical malaria had detectable parasitaemia.

Previous longitudinal studies of malaria in infants did not adequately address the issue of clinical malaria. These studies based their investigations on changes in parasite rates and densities during the first year of life (Bruce-Chwatt, 1952; Gilles et al., 1969; Molineaux and Gramniccia, 1980; Spencer et al., 1987).

The protection of the African newborn against clinical malaria has been linked to various factors such as foetal haemoglobin (Allison, 1954; Gilles, 1957), milk diet and the selective biting by mosquitoes (Muirhead-Thomson, 1951). It was beyond the scope of this study to investigate the contribution of the above factors in the protection of the study infants. However, all the infants were breastfed during the first year of life and adult food was gradually introduced at varying times with the earliest mother beginning at 4 months of age. None of the infants was fed with commercial milk.

It was observed in this study that birthweight, haemoglobin genotype, cord blood PCV, MNSsU blood group and chemoprophylaxis in pregnancy had no significant effect on the duration of onset of primary clinical malaria. Pitcher-Wilmott et al. (1980) suggested that low

birthweight infants had lower levels of IgG which may account in part, for their increased susceptibility to infection. However, none of the study infants had a low birthweight (less than 2500g). This may probably explain why birthweight did not significantly influence the duration of onset of clinical malaria in the infants. In addition, no correlation was obtained between birthweight and cord blood IgG levels. Comille-Brogger et al., (1979) suggested that during the first six months of life, there is no significant effect of haemoglobin S on malaria infection, partly because haemoglobin F prevails and partly because of passive inununity and relatively low exposure. If this suggestion is true then haemoglobin S could not signif,cantly affect the duration of onset of clinical malaria in the study infants as majority of them had their first episode of malaria between 3-6 months of life.

Oppenheimer et al. (1986) demonstrated an association between the level of haemoglobin at birth and malaria parasite prevalence at 6 and 12 months of age in infants. Further longitudinal studies are required to investigate the epidemiology of malaria in infants in relation to the pattern of malaria and anaemia in pregnancy.

Genetic variants of erythrocyte sialoglycoproteins which resist merozoite invasion have been described (Miller et al., 1977; Pasvol et al., 1982b). The reason why MNSsU blood group could not influence the duration of onset of primary clinical malaria in the study infants can be partially explained by the discovery of some *P. falciparum* isolates which can invade erythrocytes deficient in both glycophorin A and B or sialic

acid. However, for other *P. falciparum* strains to invade erythrocytes these molecules are essential (Wahlgren et al., 1989). In addition, the frequency of occurence of deficient glycophorins is very low e.g. for gpB deficiency a prevalence rate of about 8% was observed in Igbo-Ora.

In infants there is evidence for an interaction between chemoprophylaxis in pregnancy and risk of malaria infection in the first year of life (Brabin, 1991). Spencer et al. (1987) found no significant difference in parasite rates between infants born to mothers who were on chemoprophylaxis in pregnancy and those who were not. Further studies are required to evaluate the effect of chemoprophylaxis commenced early in pregnancy on the duration of onset of parasitaemia or clinical malaria.

All the study infants had at least one episode of clinical malaria during the first year of life. The mean number of episodes of malaria per infant during the one year follow-up studies was 2.3. Clinical episodes of malaria were most common between 3 and 9 months of age. This was mirrored to some extent by the high parasite rates and densities within this age group. However, parasite rates and densities remained high after nine months of age. One possible explanation for this pattern is that some infants may have been on chemoprophylaxis which might have protected them against clinical malaria but not parasitaemia.

In African children, severe anaemia is a common presenting feature of malaria. WHO (1990b) reported that the degree of anaemia correlates with parasitaemia, schizontaemia and serum total bilirubin. It was

observed in this study that malaria parasitaemia had a significant effect on PCV levels in infants between 4 and 10 months of age. The mean PCV levels of malaria positive infants within this age group was significantly lower than that of malaria negative infants. The difference was so distinct that during the bi-monthly clinics the PCV of the infants within this age group could be reliably used as a diagnostic tool for clinical malaria since almost all of them had PCV values below 25%. These observations confirms previous findings by McGregor et al. (1956), Greenwood et al. (1987) and Snow et al. (1991). McGregor et al. (1956) suggested that significantly reduced PCV levels and erythrocyte sedimentation rates in malarious infants was entirely due to malaria infection. In a study of Kenyan infants, Bloland et al. (1993) reported that malaria parasitaemia was associated with lower haemoglobin concentration as early as the second month of life.

Several mechanisms have been postulated to account for the anaemia seen in association with malaria. Among such mechanisms are:- intravascular haemolysis, extravascular removal of parasitized red cells by phagocytic cells, immune mechanisms, bone marrow hypoplasia, diminished iron incorporation and folate deficiency (Esan, 1975). The first mechanism was most likely responsible for the observed low PCV levels in the parasitized infants. A plausible explanation may be that the reticuloendothelial system of the infant is not yet mature to account for the observed destruction rate of red cells. Secondly all the infants were breastfed within the above stipulated period (4-9 months) and had

adequate nutrient intake as reflected in their normal weight for age. The suggestion that intravascular haemolysis was responsible for the observed low PCV levels in parasitaemic infants is corroborated by the findings of WHO (1990b) which reported that the degree of anaemia correlates with serum total bilirubin. Lastly, that malaria parasitaemia was responsible for the observed low PCV values is evident by the high parasite rates and densities between 4-10 months of age. In addition, a significant negative correlation was observed between PCV levels and parasite densities within 4 - 8 months of age.

The above observations suggest that control of malaria in the study area (IgboOra) would lead to a substantial increase in PCV levels.

The parasite rates and densities observed in the adult study population correspond well with findings from other malaria endemic areas (Petersen et al., 1990; Bjorkman et al., 1990). Parasitological results of the longitudinal studies at the C.T.C. Igbo-Ora show that malaria transmission in Igbo-Ora is perennial although parasite density was higher during the rainy season (July). This finding is confirmed by the observation of high parasite rates and densities in the Igbo-Ora study infants which is indicative of a high level of transmission.

The crude parasite rate of 40.6% in blood donors recorded during the end of the rainy season underlines the well known fact that naturally acquired immunity to malaria takes years to develop and is never absolute. However, it raises the question of blood transfusion malaria

especially in vulnerable recipients such as infants, young children and pregnant women.

Parasitological data confirms previous observations that adults living in malaria endemic environments have a high degree of immunity against malaria (Björkman et al., 1990; Petersen et al., 1990). They are normally asymptomatic although they are carriers of recurrent low grade parasitaemia, and a negative finding does not exclude parasitaemia but may rather describe a subpatent density of parasitaemia.

The prevalence of the sickle-cell trait in the study population was 25 - 27%. A prevalence rate of 25-29% in Nigeria (Molineaux et al., 1979; Adekile et al., 1992), 14% in Zaire (Nagel and Fleming, 1992) have been reported. Haemoglobin genotype had no effect on parasite rates and parasite densities in infants during their first year of life. However, it may be interesting to mention here that the only case of severe malaria recorded involved a haemoglobin AA female infant.

It has been suggested that haemoglobin S does not protect infants below 6 months of age. However, between 6 months and 2-5 years of age, AS heterozygotes have significantly lower malaria morbidity and mortality (Luzzatto, 1979). Marsh et al. (1989) observed significantly lowered parasite densities and episodes of clinical malaria in children aged 1-11 years with haemoglobin AS compared to AA controls. In this study haemoglobin AS infants showed no relative protection against malaria parasitaemia nor against clinical malaria during the first one year of life.

Haemoglobin genotype had no effect on parasite rate and density at delivery in the study mothers. This finding agrees with the observation of Brabin and Perrin (1985) in western Kenya. However, Fleming et al. (1984) in northern Nigeria reported a slight protective effect in Primigravidae.

In the adult study population, haemoglobin genotype had no influence on parasite rates and densities in both the G.T.C. and blood donor study subjects. However, blood donors with haemoglobin AS had significantly lower parasite density during the rainy season compared to AA donors.

It is evident from the present investigation of haemoglobin S polymorphism and susceptibility to malaria that the strongest protection is from severe malaria and death with less protection from mild illness and very little from parasitaemia.

It was observed in this study that the mean maternal IgG level was higher than that in cord blood. This finding agrees with the observations of McFarlane (1966b), McFarlane and Udcozo (1968), Williams and McFarlane (1970) and Salimonu et al. (1978) who found higher levels of IgG in African mothers in the tropics than those of the cord blood sera of their newborns. In Caucasians, Kohler and Farr (1966), Allansmith et al. (1968) and Pitcher-Wilmott et al. (1980) found that maternal IgG was usually lower than cord serum IgG. They concluded that IgG is actively transported from the mother to the foetus through the placenta.

Although it has been demonstrated that IgG is selectively transferred across the placenta, it has been shown that a 12-week- (Hyövarinen et al., 1973) or 20-week-old (van Furth et al., 1965) human foetus can synthesize a considerable amount of IgG which contributes negligibly to the total foetal IgG. McFarlane (1966) detected, in addition to IgG in cord sera, small amounts of IgM but no IgA in the dry season. In the rainy season, he detected increased concentrations of serum IgG, IgM and some IgA in cord sera and suggested that the foetus might respond to antigens and synthesize its own immunoglobulins if adequately stimulated.

McFarlane et al. (1970) suggested that the African foetus may have capacity to synthesize its own immunoglobulin at a much earlier period of intrauterine life than its Caucasian counterpart due presumably to a higher antigenic stimulation of the former by tropical infection, particularly malaria. The African foetus, because of the relatively high concentration of IgG received from its mother, would catabolize its supply of maternal IgG more rapidly than the Caucasian foetus, the earlier it catabolizes its supply of maternal IgG, the lower its total IgG at birth.

A positive correlation was observed between maternal and cord sera IgG. This confirms the previous finding of Williams and McFarlane (1970) that most of the foetal IgG may have been passively acquired. However, in this study a few cord sera had higher IgG values than their maternal IgG level. In some of these cases, maternal levels were relatively low (below 1000mg/100ml). This observation suggests active

placental transport in the presence of low maternal IgG values. Gitlin (1971) reported that placentally transported IgG is not only a passive reflection of the maternal IgG level but that a second enzymatic mechanism may exist which actively transfers IgG between the maternal and foetal circulation. This enzyme is inhibited at high maternal IgG levels and is increasingly activated at low maternal levels.

Cord blood IgM was not detected in a majority of cord blood samples using the single radial immunodiffusion method. However, with the more sensitive ELISA test, most cord blood samples demonstrated relatively low levels of IgM. Since IgM can not cross the placenta, it follows that cord IgM must have been synthesized by the foetus in response to antigenic stimulation. Previous studies of cord-versus-maternal IgM levels detected IgM in all cord blood samples studied (Allansmith et al., 1968; Williams and McFarlane, 1970).

There are conflicting reports as regards the presence of IgA in cord sera. Mohnna (1965) did not detect IgA in Caucasian cord sera. McFarlane and Udeozo (1968), Williams and McFarlane (1970) and Ladipo et al. (1978) detected IgA in African cord sera. Adeniyi and Ayeni (1976) did not detect IgA in Nigerian cord sera. In the present study none of the cord sera had detectable IgA using the single radial immunodiffusion method in agar gel.

The high level of cord sera IgG found in the study infants fell dramatically to about half its value at 2 months of age. This dramatic fall in IgG may be because: (i) most of the cord IgG was of maternal origin

which was in turn being catabolized faster than the infant was synthesizing its own IgG (McFarlane et al., 1970); (ii) high levels of plasma IgG predisposes its rapid catabolism (Fahey and Robinson, 1963); (iii) haemodilution factors are known to occur in the first month of life as a result of rapid blood volume expansion (Adeniyi and Oyeni, 1976).

After the initial rapid fall in IgG, blood levels remained relatively low till about the fourth month of life when a steady rise was observed. The infant at this state appears to have taken up the synthesis of its own IgG. These observations agree with the findings of Allansmith et al. (1968) and Adeniyi and Ayeni (1976). However, in the present study it was found that although majority of infants seroconverted at 6 months of age, some infants seroconverted at 4 months of age. The observation of a significantly lowered IgG level in malaria positive infants compared to negative infants at two months of age suggest antigen consumption of transplacentally acquired malaria-specific IgG. On the contrary, Satimonu et al. (1982) found significantly elevated IgG and IgG1 subclass levels in malaria-infected adult patients compared with non-infected controls. This observation suggest that malaria infection in adults triggers IgG and preferentially IgG1 production.

It is generally thought that many unexplained illnesses are a consequence of some imbalance of immunity. However, it is often difficult to interpret observed serum Ig levels in relation to the disease in which they occur. This may be partly explained by the fact that not all Igs are antibodies. Buckley and Dorsey (1970) observed that maximum

serum Ig concentrations were reached in the third decade of life. Mean IgM levels decreased significantly by the sixth decade while mean IgG levels decreased from the third through the sixth decade. West et al. (1962) reported that small quantities of IgM are often present at birth. They observed that IgM synthesis increased from the second to fourth days of life and by 9 months of age adult levels are attained. Adult levels were maintained for about 2 years and then drops to about 70% of adult levels during the 5th - 9th years of life. IgG synthesis started at about 4th - 6th week of life and adult levels were obtained at about the third year of life. IgA synthesis was observed to start about the third to fourth week of life and increased slowly and uninterruptedly such that adult levels were attained by adolescence (West et al., 1962). Results suggests early synthesis of IgM and late synthesis of IgG and IgA in life.

The initial antibody response to infections generally in the neonatal period is of the IgM class (Stiehm et al., 1966). It may be concluded therefore that the fairly rapid rise in the level of IgM in the first 10 months of life reflects the primary immune response of the study infants to various antigenic stimuli from the common infections known to occur. That malaria parasitaemia may be responsible in part for the observed rapid rise in IgM values is corroborated by the finding of significantly higher IgM levels in malaria positive infants compared with negative infants throughout the first 10 months of life except in infants aged 8 months.

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Mean IgA levels between malaria positive and negative infants was not significant throughout the first 8 months of life except in infants aged 4 months. This observation suggest that malaria parasites do not constitute a strong stimuli in the production of IgA during this period even though malaria specific-IgA has been demonstrated in immune adults.

These results show that the Nigerian infant possesses the innate capacity of producing immunoglobulins required for mounting humoral antibody responses early in life. Activation of this system is an important prerequisite for survival in the tropics. This system is vital for the augmentation of maternal protection against malaria which was found in this study not to exceed the first 2 months of life.

Harte and Playfair (1983) did not observe an immunological response in mice born to immune mothers who were vaccinated with blood stage parasite antigens. The observed failure was attributed to transplacental specific maternal IgG in the progeny (Ajjan, 1988). This maternal antibody while directly inhibiting priming by the vaccine also serves to induce a population of afferent T suppressor (Ts) cells which specifically inhibit the development of memory T helper cells involved in IgG production. Harte and Playfair (1983) observed that Ts cells persist in mice until 8 weeks of age, being maintained by the presence of maternal antibody.

The age at which inununization is performed must therefore take into account the disappearance of passive antibodies of maternal origin. The lowest level of IgG in the infants occured at 4 months of age after

which most of the infants seroconverted. Consequently when eventually a malaria vaccine is found, infant immunization may be most appropriate at 6 months of age and above.

Mean IgG and IgA levels in malaria positive and negative parturient women at Igbo-Ora was not significantly different. However, the mean IgM value for parasite positive women was significantly higher than in negative women. The effect of parasitaemia on Ig levels has yielded variable results. Reinhardt et al. (1978) reported significantly higher IgG and IgM but not IgA levels in parasite positive parturient women compared with negative women while Logie et al. (1973) found only elevated IgG values in parasitaemic women compared to controls.

Chemoprophylaxis in pregnancy did not influence the levels of IgG, IgM and IgA at delivery. There are conflicting reports as regards the effect of chemoprophylaxis on serum Ig levels. While McGregor and Gilles (1960) found a significant decrease in IgG levels in Gambian children on regular chemoprophylaxis, Molineaux et al. (1978) in Northern Nigeria reported that there was no change in total IgG values in infants and adults following combined vectoral control and chemoprophylaxis.

Significantly higher mean IgG and IgM levels was obtained in malaria positive compared with negative adult study subjects at the G.T.C Igbo-Ora in the July, 1991 survey and in blood donors at the U.C.H. Ibadan in the rainy season survey.

Malaria infection rapidly induces an increase in Ig synthesis (Cohen et al., 1961). While McGregor (1968) and Target (1970) recorded a

substantial increase in IgM levels in subjects with acute falciparum malaria, Tobie et al. (1966) and collins et al. (1971) observed that in malaria infected adults IgG, IgM and IgA levels rose simultaneously. Pasay et al. (1993) observed higher levels of malaria-specific IgG and IgM in malaria parasite positive compared with malaria negative adult study subjects. In the present study higher IgG and IgM levels were associated with malaria positive compared with negative subjects. However, unlike in the G.T.C study subjects, mean IgG levels were not different between malaria positivie and negative parturient women. The difference in observation may be partly explained by the different sampling periods. The parturient women were sampled between February and March when malaria transmission is low while the G.T.C subjects were sampled in July when malaria transmission is high.

All test sera were positive for IgG-specific antibodies to *P. falciparum* total blood stage antigens. Although all the cord samples had measurable IgM by the ELISA test, only a small number (5.8%) of cord samples was positive for *P. falciparum* - specific IgM antibodies indicating that majority of the cord IgM were synthesized in response to antigens other than malaria. In Gabon, Chizzolini et al. (1991) reported a slightly higher number (11.9%) of seropositives for *P. falciparum* - specific IgM antibody in cord blood samples. They suggested that IgM production by the foetus was probably facilitated by a placental parasitaemia severe enough to cause histopathological aberations.

Desowitz et al. (1993) in Papua New Guinea using the ELISA test did not detect malaria-specific IgM antibodies in 46 cord sera tested. They suggested that their observation may be partially explained by low malaria transmission in Papua New Guinea since, unlike in tropical Africa, malaria of pregnancy presented as a relatively benign infection with high placental parasitaemia rates of low density in the primiparous group. They reported that 36.9% and 16.6% of cord sera were positive for antimalarial IgG and IgE antibodies respectively. In the present study, malaria parasite rates and densities were highest in primigravidae and *P. falciparum* -specific IgM antibodies was detected in a few samples. The presence of malaria-specific IgM in cord blood suggests intrauterine sensitization of the foetus by malarial antigens. It appears that in endemic areas malaria parasites can stimulate malaria-specific antibodies in utero.

Antibodies to the crude parasite antigen is a measure of exposure and the data indicate heavy exposure of the test population to malaria infection. The use of methods employing crude blood-stage antigens to measure the humoral antimalarial response does not allow the differentiation of protective responses from those that merely reflect cumulative exposure. However, it is generally assumed that if humoral mechanisms are important in protecting against malaria, they must be hidden in the mass response detected by the use of crude antigen preparations. A number of specific aspects of the anti-malarial immune response for which *in vitro* assays are available were examined.

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The prevalence of antibodies to Pf155/RESA was higher in maternal compared with cord blood. This observation agrees with our observation of a higher maternal IgG compared with cord blood IgG. A number of cord blood samples failed to show an antibody response even though their mothers were positive for anti-Pf155 antibodies. In a majority of these anti-Pf155 antibody negative cord blood samples the corresponding maternal samples demonstrated low antibody titres (1:10 - 1:50). Generally cord blood samples had lower anti-Pf155 antibody titres than did the maternal group. Similar findings have been reported by Collins et al. (1977) and Campbell et al. (1980) involving antibodies to total blood stage antigens and anti-sporozoite antibodies (Nardin et al., 1981). Kramer et al. (1993) reported similar mean titres of antibodies to the merozoite surface protein - 1 (MSP-1) in paired maternal and cord serum samples.

EMIF data from parturient women indicate a wide range of response to the Pf 155 antigen, which was, however, fairly consistent for each individual mother at the six bi-monthly consecutive surveys. The reason for the variation between individual mothers of EMIF titres is unclear. As the EMIF titres were consistent on consecutive surveys, whereas some mothers were positive for malaria parasites on one survey and negative on another, antigen consumption of antibodies is not thought to be the cause. The variation may rather be explained by genetic factors such as MHC restriction and allotypic restriction of antibody repertoire.

Anti-Pf155 antibodies were found to correlate with parity and age of the parturient women. Anti-Pf155 antibodies have been shown to increase with age in previous studies (Wahlgren et al., 1986; Deloron and Cot, 1990). Deloron et al. (1989a) in Kenya reported that primigravidae had the lowest anti-Pf155 antibody titres followed by nulligravidae and lastly multigravidae. Both the presence and titre of anti-Pf155 antibodies had no protective effect against malaria infection at delivery in the present study.

The number of infants positive for anti-Pf155 antibodies fell rapidly after birth reaching its lowest prevalence rate at 4 months and rose rapidly at 6 months of age. Prevalence rates remained high till one year of life. Passively acquired malarial antibodies from the mother may persist for 4 - 6 months after birth but any further persistence is masked in this population by a rapid rise of antibodies in response to antigenic stimulation in infants older than 4 months. In The Gambia, McGregor et al. (1965) reported a rapid decline in titres of antibodies to total blood stage antigens during the first 16 weeks of life. However, contrary to observations in the present study as regards the evolution of anti-Pf155 antibodies, antibody levels remained low in the remainder of the first year of life. In El Salvador, Campbell et al. (1980) observed that over 50% of infants lacked detectable antibodies to total blood stage antigens before the age of 3 months. Kramer et al. (1993) in a study of Kenyan infants reported that the median age at which infants lost detectable maternal anti-MSP-1 antibodies was 20 weeks. They suggested that the loss of malaria specific

antibodies is associated with increased risk of infection in infants less than 3 months old.

The EMIF data of the adult study population indicate a high degree of variability in their reactivity to the Pf155 antigen probably due to genetic factors. Both the percentage of positive subjects and mean titres to the Pf155 antigen remained unchanged at the rainy and dry season surveys. Similarly the individual titres of antibodies to the Pf155 antigen were consistent on two consecutive surveys of the G.T.C study subjects. In all the adult populations studied, no correlation was observed between anti-Pf155 antibody titres and age, parasite rates and parasite densities. In previous reports, both the prevalence rates and level of seroreactivity to Pf155 were found to increase with age (Wahlgren et al., 1986; Deloron et al., 1989a; Chizzolini et al., 1989). In these studies, cross-sectional surveys included all age groups while in the present study the youngest subject was 15years old. It therefore follows that by adolescence, individuals in malaria endemic areas have been maximally sensitized to different malarial antigens and demonstrate an appreciable degree of naturally acquired immunity. Continuous exposure to malaria infection into adulthood results in an improvement of the quality but not quantity of antibodies as the individual is exposed to different strains and antigens of the malaria parasite.

The *in vitro* finding that antibodies directed against Pf155 antigen specifically inhibit parasite growth can not therefore be confirmed *in vivo* in the adult study population. However, it may be that by adulthood the

individual has acquired protection through several mechanisms and hence is not dependent on one unique response, e.g. humoral response to Pf155 antigen. Bjorkman et al. (1990) observed that high reactivity to Pf155 in a group of adult Liberians did not relate to any degree of protective immunity as all study subjects were hyperimmune and no correlation was found with the observed parasitaemias.

Parasite rates/densities were not different between low, medium and high responders to the Pf155 antigen in blood donors at both the rainy and dry seasons. However, the absence of malaria parasitaemia in high responders at the march survey could imply that Pf155 antibodies offers protection against infection in situations of low transmission, but other factors are more important when the infection pressure is intense. This finding contrasts with the observation of Petersen et al. (1990) who reported lower parasite densities in Pf155 high responders (≥ 1:250) in the rainy season. They suggested that anti-Pf155 antibodies offer additional protection in situations of intense transmission. However, Petersen et al. (1990) failed to reproduce the lower parasite densities in Pf155 high responders in a subsequent rainy season.

Most naturally occuring antibodies to the Pf155 are directed against epitopes of the 4 amino acid sequence EENV in the 3' repeat region (Collins et al., 1986). Maternal and cord blood serореactivities to the $(EENV)_6$, LJ5 and MAP2 but not $(NANP)_6$ peptides showed a significant correlation. Although there was no difference in the mean ELISA values to the $(EENV)_6$, $(NANP)_6$ LJ5 and MAP2 peptides between

maternal/cord paired sera. a considerable number of cord samples was seronegative for the $(NANP)_6$ peptide. In The Gambia. using the IFA test, Nardin et al. (1981) reported only one case of a sporozoite seronegative child born to a seropostive mother involving 20 maternal/infant pairs.

The seropositivity rates to $(EENV)_6$ as measured by ELISA dropped rapidly after birth and by 6 months none of the infants was positive. The number of seropositives then increased steadily till one year of life. The discrepancy in the pattern of evolution of antibodies to the Pf155 and $(EENV)_6$ may reflect the difference in sensitivity and specificity of the two test methods. Anti-Pf155 antibodies measured by EMIF includes epitopes other than the immunodominating $(EENV)_6$ including epitopes cross reacting with other antigens. and these may be more pronounced during different periods of the development of the immune system. Hogh et al. (1991) in a longitudinal study of Liberian children observed that the seropositivity rates to Pf155 and $(EENV)_6$ were both maximum in infants aged 3- 11months and from 1 - 4 years of age.

The seropositivity rates for anti-$(NANP)_6$ antibodies fell rapidly after birth and by four months of age none of the infants was positive. However, at 6months, half of the infants sampled were seropositive and the response fluctuated till one year of life. This observation suggest that antibodies against the immunodominant region of the CSP develop early in life after exposure to malaria infection. In The Gambia, Nardin et al. (1981) reported that at about 6 weeks of age, anti-sporozoite antibody titres in infants correlated with those of their mothers although the titres

were lower in infants. However, at 6 - 7 months after birth these antibodies could not be detected in any of the infants. In this study, antibodies against the sporozoites were measured by the circumsporozoite precipitation reaction and the IFA technique two methods which lack sensitivity and specificity compared with the $(NANP)_6$ ELISA. In a study of Liberian infants and children, Hogh et al. (1991) reported that $(NANP)_6$ seropositivity rates did not correlate with age. $(NANP)_6$ seropositivity rates fluctuated over the years and in children the highest rate was in the 3-5 years age group.

Cord blood seropositivity rates to the LSA and MAP2 peptides was very low compared with the other peptides tested. At 2 months of age, none of the infants were seropositive for both antigens. While none of the infants was seropositive to the MAP2 peptide throughout the first year of life, a small number of infants seroreacted to the LSA peptide between 8 - 12 months of age. The correlation of maternal and cord malarial antibody titres and the loss of these antibodies during the first 4 to 6 months of life suggest that the infants' malarial antibody responses were passively acquired.

The seroreactivity rates to the different malarial antigens tested in infants showed different patterns of variation during the first year of life. The observed difference in the evolutionary pattern of malarial antibodies in infancy can be partially explained by differences in immunogenicity of the antigens, MHC restriction and degree of exposure to mosquito bites. Generally all the antibodies detected showed rapid decline after birth till 4

to 6 months of age probably due to antigen consumption of antibodies. On the contrary, seropositivity for antibodies to most antigens tested increased after 4 - 6 months of age probably reflecting antigenic stimulation.

Inspite of the different patterns of seroreactivity to the different antigens, it is apparent that between 4 and 6 months of age antibodies to the different antigens tested were either low or not detectable. In addition, parasite rates and densities were highest in the infants within the 4-6 months age group. While parasite rates and densities declined gradually after 6months till one year of age, seroreactivity rates to malarial antigens tested increased rapidly within this period suggesting the development of naturally acquired immunity to malaria. This suggestion is consolidated by the observation that episodes of clinical malaria in the infants were not frequent towards the end of the first year of life. Parasitological and immunological data of the infants in Igbo-Ora demonstrate a high level of exposure to malaria infection early in life which is reflected by their high seroreactivity rates to different malarial antigens especially the CSP antigen. This observation agrees with the behavioural patient of the indigenes of Igbo-Ora as infants spend a greater part of their first year of life with their mothers in the farm where they are maximally exposed to mosquito bites. The finding of an active antibody response to malarial antigens in infancy encourages the hope that a malaria vaccine administered early in life may accelerate the development of naturally acquired immunity and thus protect the population most at risk.

Previous studies in malaria hyperendemic areas have suggested that transplacental transfer of malaria antibodies may provide a significant degree of protection for the newborn during the first few months of life (Bruce-Chwatt, 1952; Biggar et al., 1980). In the present study the presence and level of cord blood IgG and antibodies to four peptides [(EENV)6, (NANP)6, LJ5 and MAP2] tested including the Pf155/RESA did not correlate with the duration of onset of primary clinical malaria in the infant. Furthermore, we observed no difference in the age of onset of clinical malaria in infants whose cord blood was either positive or negative for antibodies to the Pf155/RESA and (NANP)6 antigens which represent antigens from different stages of the parasite: sporozoite and blood stages respectively. A similar finding was recorded for the (EENV)6 and (NANP)6 antigens.

It is evident from the present study that HbAS and seropositivity for antibodies to antigens from two different stages of the malaria parasite (sporozoite and blood stages) delays the age of onset of clinical malaria in the study infants when compared with AA seropositive infants. Although our results indicate that transplacental antibodies offer no significant protection against malaria during the first few months of life, antibodies in concert with other factors such as Hb genotype may be responsible for the protection of the newborn against clinical malaria during the first few months of life. It was observed that parasite rates and densities were very low at 2 months of age and increased by almost a 100% by the age of 4 months while malarial antibodies declined rapidly after birth with the

lowest levels at 4 months of age. This finding suggests some relative protection of the infant during the first 2 - 3months of life.

The effectiveness of malarial antibodies in protecting infants against malaria is unclear. Edozien et al. (1962) and Sabchareon et al. (1991) have shown that γ-globulin and IgG fractions from malaria immune subjects respectively demonstrate antimalarial activity when administered to acutely ill malaria patients. Previous studies have shown contrasting results as regards the protective role of transplacental malarial antibodies. While McGregor et al. (1965) reported that transplacental malarial antibodies provide a significant degree of protection for the newborn. Collins et al. (1977) and Campbell et al. (1980) in El Salvador and Riggar et al. (1980) in Ghana suggested that transplacentally acquired antibody may not be clinically relevant in protecting the infant from malaria.

A prominent feature of humoral immune response in the adult study population is the consistency in antibody seropositivity/titres to some malarial antigens tested on cross-sectional and longitudinal surveys. However, anti-(NANP)6 antibody seropositivity rates were higher at or towards the end of the rainy season when malaria transmission was highest as compared with the dry season or begining of the rainy season. This finding indicates that anti-(NANP)6 seropositivity reflects exposure to infective mosquito bites and consequently transmission intensity. Previous studies have suggested that measurement of antibody responses to the $(NANP)_n$ may serve as a measure of malaria transmission intensity in

seroepidemiological studies (Druilhe et al., 1986; Esposito et al., 1988). How long the antibody response to the $(NANP)_6$ antigen persists after natural exposure is not known. Whether the fluctuating response observed reflects poor immunogenicity or is the result of immune suppression remains speculative.

It was observed in the present study that high seroreactivity to the $(EENV)_6$ was usually followed by high reactivity to the LJ5 peptide in all populations studied. This is not unexpected as both peptides are derived from the same antigen (Pf155/RESA) although the $(EENV)_6$ peptide is immunodominant. However, in a few cases higher reactivity to LJ5 was observed compared with the $(EENV)_6$ peptide. This finding was more common with the IgboOra study subjects who relatively showed higher reactivity to the the LJ5 peptide compared with the blood donors in Ibadan. The observed difference may be explained partially by the fact that humoral immune response to malarial antigens is MHC restricted since a given MHC molecule is able to bind some, but not all, of the peptides derived from a given antigen during processing (Riley et al., 1991).

The presence or absence of malaria parasites in the adult population had no effect on seropositivity rates to malarial antigens tested. Results confirms previous report by Bjorkman et al. (1990) in Liberia who found no association between antibodies to the Pf155 and its repeat sequences and malaria parasitaemia. On the contrary, reports by Nguyen-Dinh et al.(1987) and Marsh et al. (1989) in The Gambia suggest a protective role

for Pf155 antibodies. Similarly while Hoffman et al. (1986) in Indonesia, Del Giudice et al. (1987) in Tanzania and Esposito et al. (1988) in Burkina Faso reported that anti-CSP antibodies are protective, Hoffman et al. (1987) in Kenya, Pang et al. (1988) in Thailand and Marsh et al. (1988) in The Gambia argue that these antibodies do not protect against malaria. So far there are no reported seroepidemiological studies involving the Ag332 repeat region (MAP2). However, in a recent study of 10 adult Gambians (Perlmann et al., 1994) higher levels of IgE and IgG-specific antibodies to the Ag332 of *P. falciparum* repeat sequence was observed compared with the $(NANP)_6$ peptide.

CHAPTER SIX

6.0 CONCLUSIONS AND SUGGESTIONS FOR FURTHER STUDIES

6.1 CONCLUSIONS

Malaria parasitization in endemic areas is an exceedingly complex phenomenon. It is worthy to stress here that the analysis of inter-correlated data generated by studies of this nature requires great caution. Results both positive and negative, are best regarded as indicating directions for further investigation than providing definitive answers. The relationship with age or parity of practically all important variables concerned with malaria provides an example of confounding factors which may be encountered in studies of this nature. It is therefore important to take into consideration such confounding factors when evaluating field data. Against this background of caution a number of conclusions can be drawn from this study.

(1) Pyrimethamine prophylaxis from the 20th week of pregnancy till delivery does not significantly influence birthweight, maternal and cord blood PCV level, maternal immunoglobulin (IgG, IgM and IgA) levels and duration of onset of clinical malaria in the infant.

(2) Parasitological data demonstrate that adults in malaria endemic areas are usually carriers of low grade asymptomatic malaria parasitaemia suggesting that immunity against malaria is not sterile. Unlike in infants,

episodes of clinical malaria in adults may not always be accompanied by the presence of malaria parasites in thick smears.

(3) Transfer of maternal antibodies to the foetus may involve both active and passive transport mechanisms across the placenta and involves principally IgG antibodies. However, the foetus is capable of synthesizing IgM antibodies in response to antigenic stimulation such as malaria.

(4) The rapid increase in both parasite rate and density after 2 months of age and the rapid decline in antibody levels to about half the birth level at 2 months of age suggest that protection of the African infant against clinical malaria is probably limited to the first 2 months of life.

(5) Most of the infants experienced their first episode of clinical malaria between 3 - 6 months of age. Haemoglobin genotype, cord blood PCV, birthweight and MNSs(U) blood group do not alter significantly the duration of onset of clinical malaria in the infant. Similarly cord blood antibodies against the Pf155, $(EENV)_6$, $(NANP)_6$, LJ5 and MAP2 antigens had no influence on the duration of onset of clinical malaria in the infants.

(6) Malaria parasitaemia or clinical malaria in infants is usually accompanied with anaemia due to intravascular haemolysis.

(7) The strongest protection of haemoglobin AS is from severe malaria with less protection from mild illness and very little from malaria parasitaemia.

(8) The African infant's initial immune response to malaria infection involves the production of IgG in the adults however, malaria

parasitaemia or acute malaria results in increased production of IgG and particularly IgM.

(9) This study shows that transplacentally acquired immunity is transient. After 4 months of age the study infants were capable of producing antibodies to the $(NANP)_6$, Pf155/RESA, $(EENV)_6$, and LSA antigens. The relatively earlier production of antibodies against the $(NANP)_6$ and Pf155 antigens in infants, the most susceptible age group, encourages the hope that a sporozoite and blood stage vaccine administered early in life may accelerate the development of immunity and thus protect the population most at risk.

(10) In the studied population there was no correlation between anti-Pf155 antibody titre, ELISA seropositivity to the $(EENV)_6$, $(NANP)_6$, LSA and MAP2 antigens and malaria parasitaemia. Results do not imply that these antigens are of less importance for the development of a malaria cocktail vaccine and subsequently malaria immunity. This observation indicate that unless the humoral response generated by vaccination is qualitatively or quantitatively different from that induced naturally, it will not be possible to link the humoral immune response from vaccination to protective immunity. In addition, antibodies against malarial antigens are not the exclusive mediators of protection against malaria parasitaemia. It is well known that cell-mediated immunity alone or in concert with antibody production is important for maintaining acquired immunity to malaria. In addition both non-specific cellular and

humoral immune responses may play a fundamental role in acquired immunity to malaria.

6.2 SUGGESTIONS FOR FURTHER STUDIES

In the present study, cord blood IgM was detected in all samples tested while malaria-specific IgM was detected in a few cord blood samples. This observation suggest intrauterine sensitization of the foetus by malarial antigens. Sequel to this finding, *in vitro* lymphoproliferative studies on malaria-specific IgM positive cord blood samples using either crude malaria parasite preparations or defined malarial antigens are essential. Results from such a study may confirm the existence of memory cells in neonates capable of responding to malarial antigens and thus consolidate recent observations of malaria-specific IgM and IgE antibodies in cord blood samples.

Although chemoprophylaxis has been recommended to control the exacerbation of malaria associated with pregnancy, results of the present study do not indicate any significant differences of all malariometric indices studied between protected and non-protected groups. However, it is known that malaria parasitaemia in pregnancy is highest during the first trimester (McGregor, 1984). Consequently chemoprophylaxis intervention early in pregnancy may have maximal beneficial effects on outcome of pregnancy. None of the previous studies including the present study addressed this issue satisfactorily. Further studies on early chemoprophylaxis in different parity groups using different drug regimes

to overcome the possible problem of drug resistance are urgently needed to confirm the benefits of early chemoprophylaxis in pregnancy.

There is good evidence that maternal anaemia affects pregnancy outcome (Brabin, 1991). Antimalarial drug efficacy in pregnancy can therefore be quantified in relation to the prevalence of anaemia in the study population. In addition the incidence of severe anaemia in pregnancy cohorts on different drug regimes should be established.

Previous studies have demonstrated an association between highly parasitized placentae and low birthweight (Bruce-Chwatt, 1952; Kortmann, 1972; Reinhardt et al., 1978); none of the studies investigated the relationship between maternal peripheral parasitaemia and birthweight of newborn including other maternal malariometric parameters. Further studies are required to investigate the possible existence of a correlation between maternal peripheral parasitaemia and placental parasitaemia in different parity groups. Furthermore, information on the effects of maternal anaemia on placental weight and birthweight of newborn are required in women with and without placental/peripheral malaria.

Comparative studies on lymphocyte transformation assays and malarial antibody levels between placental and maternal peripheral blood is required. Information from such a study may help explain the phenomenon of malaria exacerbations in pregnancy especially in primigravids.

With the recent development of microassay techniques for lymphoproliferative studies using finger prick samples and cytokine assays using whole blood samples, future studies are required on the development of cell mediated immunity to malaria in infancy with particular reference to variations in the levels of cytokines following first and subsequent malaria infections.

7.0

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