IN VIVO AND IN VITRO SENSITIV1TY Of PLASMODIUM FALCIPARUM TO CHLOROQUINE IN ZARIA, NIGERIA

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This is to certify that I carried out the work reported in this thesis at Ahmadu Bello University Teaching Hospital Zaria, under the joint supervision of Drs L. Lege-Oguntoye, B. Werblinska, W. Ogala and P. F. Clurinola. The work of other investigators is acknowledged and referred to accordingly. I solemnly declare that no part of this thesis has been submitted elsewhere for a degree.

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DEDICATION

TO

GOD ALMIGHTY

WHO MADE ALL THINGS POSSIBLE

TO

THE MEMORY OF

MY DEAR MOTHER CELINA UYA

AND TO

ALL THOSE WHO IN VARIOUS WAYS

HAVE DEVOTED THEIR LIVES FOR THE SERVICE OF

GOD AND HUMANITY

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May GOD bless you all.

ABSTRACT

102 Children with Plasmodium Falciparum malaria, aged between 6 months and 7 years were enrolled in the study and the WHO Extended Field test was used to assess the in vivo sensitivity of P.Falciparum to chloroquine. Also the WHO Standard Microtest was used to determine the in vitro susceptibility of P. Falciparum isolated from the ablve patients to chloroquine, mefloquine and quinine.

Of the 59 patients that completed the in vivo study, 53 (90%) were fully sensitive to chloroquine, ie. parasitaemia cleared within 7 days and did not reappear. The mean parasite clearance time for the sensitive cases was 3.47 ± 1.04 days. 6 cases (10%) were resistant to the drug in vivo, 3 at the R1 and 3 at the R II level. In those cases, parasitaemia either cleared and reappeared or did not clear at all.

Of the 36 cases with in vitro results for chloroquine, 24 P. Falciparum isolates (66.7%) were fully sensitive to the drug in vitro. The schizont maturation of those isolates was completely inhibited at 8 pmol/well (1.6×10^{-6} mol/L) chloroquine and below, while 12 isolates (33.3%) were resistant to chloroquine in vitro ie there was schizont growth at 8 pmol/well (1.6×10^{-6} mol/L) chloroquine and above. The EC 50 and EC 99 for the 36 isolates were 0.4×10^{-6} and 4.52×10^{-6} mol/L chloroquine, respectively.

Also 35 cases had *in vitro* results for mefloquine. schizont maturation of 26 isolates (72.2%) was completely inhibited at 16 pmol/well (3.2×10^{-6} mol/L) mefloquine and below, while 10 isolates (27.7%) grew at 16 pmol/well mefloquine. The EC 50 and EC 99 for the 36 isolates were 0.5×10^{-6} and 6.4×10^{-6} mol/L mefloquine, respectively.

13 cases had *in vitro* results for quinine. There was complete inhibition of schizont maturation Of 12 *P. Falciparum* isolates (92.3%) at 256 pmol/well (51.2×10^{-6} mol/L) quinine and below, while one isolate (7.7%) grew at 256 pmol/well. The EC 50 and EC 99 for the 13 isolates were $3.2. \times 10^{-6}$ and 36.2×10^{-6} mol/L quinine, respectively.

33 (55.9%) of the 59 patients that completed the extended field test had corresponding *in vitro* results for chloroquine. A comparison of the *in vivo*/*in vitro* sensitivities shows that 21 cases (63.6%) were fully sensitive to chloroquine both *in vivo* and *in vitro* while 8 cases were sensitive *in vivo* and resistant to the drug *in vitro*. 4 cases were resistant to chloroquine both *in vivo* and *in vitro*.

Our data indicate that there is both *in vivo* and *in vitro* resistance to chloroquine in children with *P. Falciparum* malaria in Zaria urban area. Our results also suggest a decreased *in vitro* sensitivity of *P. Falciparum* to mefloquine and quinine in this area.

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CHAPTER ONE

Introduction

1.1 HISTORICAL BACKGROUND

Malaria is a disease characterized by intermittent fever, anaemia and splenic enlargement. It is caused by parasites assigned to the genus plasmodium, class sporozoa, of which four species, Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae are known to infect man naturally. The name malaria is derived from the Italian word for "bad air", it does not directly relate to the cause of infection but reflects earlier opinion that the disease was spread by mist arising from marshes.

The earliest indication of human disease suggestive of malaria came from ancient Egypt and is found in the Edwin Smith surgical Papyrus 1,600 B.C. (Breasted, 1930). Also the Papyrus Ebers, 1550 B.C, refers to the association of rigors, fever, and splenomegaly to malaria (Garnham, 1966). However, the first exact description of the disease was by Hippocrates 400 B.C, who studied in Egypt and recognised quotidian (daily) tertian (every 48 hours) and quartan (every 72 hours) fevers with the enlargement of the spleen (Boyd, 1949).

The aetiology of malaria remained a mystery until modern times, and no major progress was made except for the discovery of the curative value of cinchona bark by Amerindians and its introduction into Europe in the middle of the seventeenth century. Hippocrates first regarded bile as the immediate cause of fever, but this was soon disregarded and effuvia from swamps or marsh became firmly established as the cause of malaria. It was not until the nineteenth century, when work carried out by many workers like Laveran (1845 - 1922), Patric Manson, Ronald Ross and others, succeeded in showing that malaria is caused by parasites and transmitted by mosquitoes (Garnham 1966).

1.2. Plasmodia

1.2.1 Taxonomy of Malaria Parasites

Malaria parasites belongs to the Phylum protozoa, subphylum sporozoa, class telosporea and subclass coccidia (Honigberg et al 1964). There is uncertainty regarding the taxonomy of malaria parasites below the level of suborder. However, according to the society of proto-zoologists, they belong to the order Eucoccidia and suborder Haemosporina (Baker 1977, Levine 1973), Garnham (1966), classified the suborder Haemosporina into the family Plasmodiidae and genus Plasmodium. The family plasmodiidae shows blood schizogony, they have mosquitoes as their arthropod host and their

gametocytes are restricted to mature erythrocytes. The genus plasmodium represents the malaria parasites of man and animals and is divided into ten subgenera, three comprising the mammalian, four the avian and three the saurian plasmodia.

Out of the three subgenera of the mammalian malaria parasites, only four species are known to infect man naturally, these are P. ovale, P. vivax and P. malariae from the subgenera plasmodium (Plasmodium) and P. falciparum from the subgenera plasmodium (Laverania) (Garnham 1966).

The following simian malaria species are known to occasionally infect man either naturally or experimentally: P. knowlesi, P. siminum, P. schwetz, P. brasilianum, P. cynomolgi bastianellii, P. cynomolgi cynomolgi and P. inui (Chin et al 1965, Deane et al 1966a, and Coatney 1971).

1.2.2 The Vector and Its Life Cycle

Malaria is transmitted by mosquitoes and only the female anophelines are vectors of human malaria, since male anophelines do not feed on blood but almost exclusively on vegetable juices and nectar. In addition to transmitting malaria, anophelines also transmit filariasis and some viral diseases (Bruce-Chwatt 1980).

Malaria can also be transmitted through transfusion of blood containing malaria parasites, or by using unsterilized syringes and needles as commonly seen in drug addicts.

The anopheles belong to the Phylum Arthropoda, Class Hexapoda, order Diptera, suborder nematocera and family culicidae also known as mosquitoes. The subfamily anophelinae consists of three genera namely Anopheles, Bironella and Chagasia (Paul et al 1963).

There are about four hundred species of Anopheles mosquitoes throughout the world, but only some sixty species are important vectors of malaria under natural conditions (Bruce Chwatt 1980). In West Africa, three main anopheles vectors are known to transmit malaria; they are A. gambiae, A. arabiensis and A. funestus. A. melas is involved but to a lesser extent. (Ekanem 1978). Anopheles and culex species are nocturnal in their feeding habits, while some Aedes species are diurnal.

Although Anopheles mosquitoes are most frequent in tropical or sub-tropical regions, they may also be found in temperate climates and even in the Artic during summer. As a rule, Anopheles are not found at altitudes above 2000-2500 meters above sea level.

They may be dispersed or transported to great distances by wind, in ships, railway carriages or air planes.

The mosquito life cycle consists of four stages : egg, larva, pupa and adult. Those stages are always divided between two different habitats, an aquatic and a terrestrial one; the larva and pupa are always aquatic and the adult always terrestrial. The egg and pupa may be considered transitional forms between two separate organisms. The larva and adult each occupying a totally different ecological niche. Larva in general are specialized for growth and the storage of nutrients, whereas adults are specialized for dispersal and reproduction (Foster 1980).

(i) Eggs: The Anopheles mosquito eggs are laid on the water surface or under water and embryonation is usually completed in about 2-3 days at 25-27°C. Hatching of the eggs occurs in response to low oxygen tension.

(ii) Larva: Mosquito larva of all species pass through four intermoult developmental stages. The larval period depends on the species, temperature and availability of food. At 25°C-27°C, and optimum feeding conditions, the period last for about 5-10 days.

(iii) Pupae: After the third moult, the fourth instar Anopheles larva develops into a comma-shaped still aquatic pupa. Pupae have much of the external forms of adults. They do not feed but are extremely active. The pupal stage lasts for about 2-3 days at 25-27°C after which its skin splits dorsally and the adult insect or imago emerges.

(iv) Adults: The time of emergence of adults from the pupal cuticle is set by the time of pupation. Males pupate earlier than females and therefore tend to emerge first. After emergence, they may mate at the breeding site.

Adult females of most species require at least two blood meals before the first batch of eggs can develop. In subsequent cycles, a batch of eggs develops from each blood meal. The blood protein is converted primarily into mosquito egg protein and to some extent into lipid and glycogen which can be used for energy as well as egg development (Foster 1980).

1.2.3 The Life Cycle of Malaria Parasites

Malaria parasites undergo a complex life cycle alternating between a vertibrate and an arthropod host. The life cycle consists of a sexual and

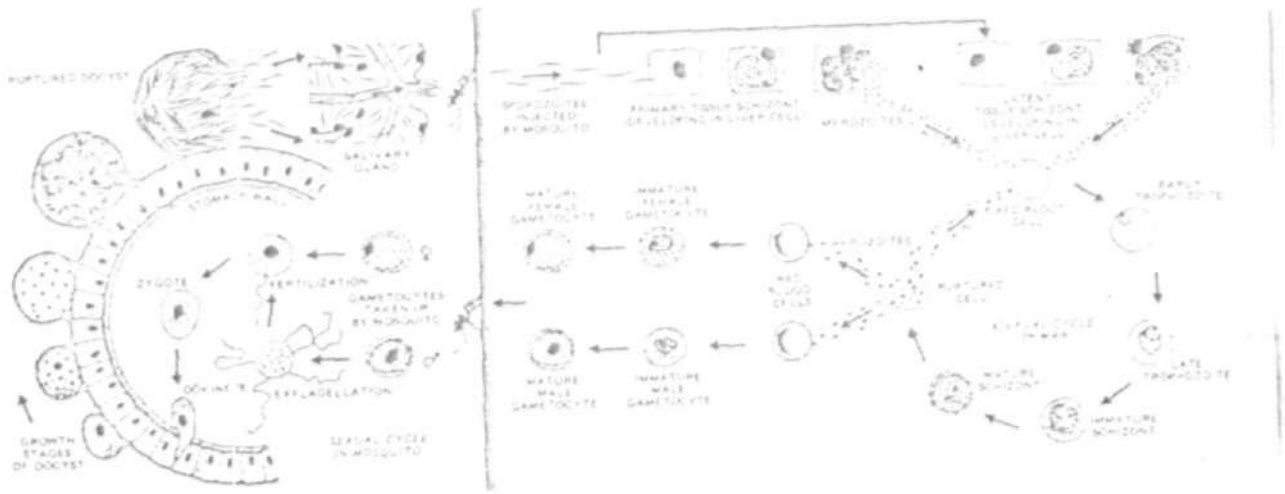
sporogonic mode of reproduction occuring in the arthropod host and an asexual reproductive mode occuring in the vertibrate. The asexual phase is divided into two, the first occuring in the parenchymal cells of the liver where massive numbers of merozoites are produced and the second occuring in erythrocytes (Wernsdorfer 1980).

The sporozoite, upon inoculation by an infected mosquito reaches the blood circulation where it is transported guided by chemotaxis to its target site. On reaching its vicinity, it leaves the capillary lumen and enters the host cell. In mammalian plasmodia, the latter is a hepatocyte. In no case do sporozoite enter red blood cells directly. The sporozoite's entry into the hepatocyte results in a drastic morphological change in the parasite which then appears round or oval and contains a chromatin nucleus surrounded by cytoplasm. The nucleus divides and the cytoplasmic mass grows and exoerythrocytic schizonts are formed. After completion of nuclear division, the cytoplasm segregates and merozoites are formed consisting of a single nucleus and cytoplasm.

In constrast to the erythrocytic stages, exoerythrocytic schizonts do not contain pigment. The number of merozoites produced by one exoerythrocytic

schizont is estimated to be approximately 2,000 in P. malariae, 10,000 in P. vivax, 15,000 in P. ovale and over 30,000 in P. falciparum, roughly corresponding to 12, 14, 15, and 16 nuclear divisions (Wernsdorfer 1980). The duration of the exoerythrocytic development of primate plasmodia roughly corresponds to the pre-patent period and in P. falciparum it is about 5 to 7 days. The cycles of development of malaria parasites in the mosquito and in man is shown in figure 1.1.

Fig 1.1 Cycles of development of malaria parasites in the anopheline mosquito and in man.



From World Health Organization (1963) *Terminology of malaria and of malaria eradication*. Geneva

In mammalian plasmodia, the merozoites originating from oryptozoic schizonts (i.e. schizogony arising directly from sporozoites) can only invade red blood cells thus marking the beginning of the erythrocytic phase or schizogony. In some plasmodia infection (i.e. P. vivax and P. ovale) some sporozoites may remain dormant in the liver and after sometime become reactivated by an unknown trigger. These latent sporozoites or hypnozoites are responsible for relapses in those infection. (Coatney 1976, Garnham 1977a and Knotoski et al 1980).

The infected erythrocytes may retain their normal size as in P. falciparum and P. malariae or become enlarged and round as in P. vivax or enlarged and deformed as in P. ovale. On invading new erythrocytes, the merozoites can either initiate renewed blood schizogony as shown in Figure 1.1 or develop into a female or a male gametocyte (i.e. a macrogametocyte or a microgametocyte).

The mature macrogametocytes usually show a compact nucleus and an accumulation of pigment near the nucleus. In microgametocytes the nucleus is larger and less compact, often with a spongy appearance. The gametocytes are still surrounded by the host erythrocytes membrane. In a number of

mammalian plasmodia, gametocytogony may be initiated directly from cryptozoic merozoites and may have a duration of about 8 days in P. falciparum (Wernsdorfer 1980).

When taken up by a suitable arthropod, the gametocytes transform into gametes. Macrogametocytes shed the erythrocyte membrane and become mature macrogametes without any evident morphological change. In contrast microgametocytes undergo a complete transformation: The nucleus divides three times, forming usually eight new nuclei which combine with cytoplasm to form microgametes having a very specific organellar structure. The exflagellated microgametes then tear free and move actively toward the macrogametes and invade them. After entry, the cytoplasmic materials of the two combine to form the zygote. Exaflagellation usually takes 10-15 minutes and microgamete entry 1 minute.

The zygote is at first a motionless globular body but within 18 to 24 hours of its formation it elongates and becomes mobile; this worm-like stage measuring 18 to 24 μ in length is known as OOkinete. The OOkinete soon forces its way to the stomach wall, passes between the epithelia cells and settles down.

It becomes rounded up into a small sphere with an elastic membrane and is now called an OOcyst.

The OOcyst gradually increases in size. The cytoplasm maintains its syncytial structure while there is intensive nuclear division the speed of which is largely dependant on the environmental temperature. The pigment carried along by the macrogamete still remains in the OOcyst. Ultimately the cytoplasm divides to form sporozoites. Later, the OOcyst bursts liberating thousands of mobile sporozoites into the body cavity of the mosquito where many penetrates the granular cells and ultimately reach the lumina of the salivary ducts, thus making the mosquito infective. When she feeds on blood after piercing the human skin, the sporozoites are injected into the wound and pass into the blood stream of the vertebrate host, thus completing the cycle.

Sporogony, i.e. the period from gametocyte maturation until the development of infective sporozoites in the salivary glands, takes between 8 days and 8 weeks. The number of sporozoites produced by an OOcyst varies according to the plasmodium species. In P.falciparum it is estimated to be 10,000, indicating a 12 hour cycle of nuclear division in the oocyst (Pingle 1965).

Table 1:1 Duration of Developmental Stages in Human Malaria Parasites.

Species	Exoerythrocytic schizogony (days)	Erythrocytic schizogony (days)	Sporogony minimum (days)
P. vivax	8	2	8
P. ovale	9	2	12
P. falciparum	5½	2	9
P. malariae	15	3	16

1.2.4 Distribution

Malaria plasmodia survive only in areas where the mosquito and the infected human populations remain above a critical density for each other. These two critical densities are interdependent but either may fluctuate in a given area (Plorde 1985). The plasmodia are said to be endemic in a particular area where there is a constant measurable incidence of natural transmission over a succession of years. The frequency of splenomegaly and the degree of spleen enlargement in a community are usually good indicators of its contact with plasmodia unless there are other diseases which could cause splenomegaly.

The higher the spleen rate and the larger the average enlarged spleen, the higher the degree of endemicity and the intensity of transmission of malaria in the area. Based on the spleen rates of children and adults endemicity can be classified as follow (Wernsdorfer 1980).

Hypoendemic: Spleen rate (children 2-9 years) 0-10%

Mesoendemic: Spleen rate (children 2-9 years) 11-49%

Hyperendemic: Spleen rate (children 2-9 years) constantly over 50%; adult spleen rate also high

Holoendemic: Spleen rate (children 2-9 years) constantly over 75%; adult spleen rate low.

Malaria in a community may also be regarded as stable or unstable; stable malaria occurs in regions where there is constantly repeated infection (i.e. transmission is perennial and meteorological conditions vary little over the years), the population has a high degree of immunity and epidemics do not occur. P. falciparum is usually the predominant species. Unstable malaria occurs in regions where transmission is intermittent for example where it is seasonal. In such areas, the population has varying degree of

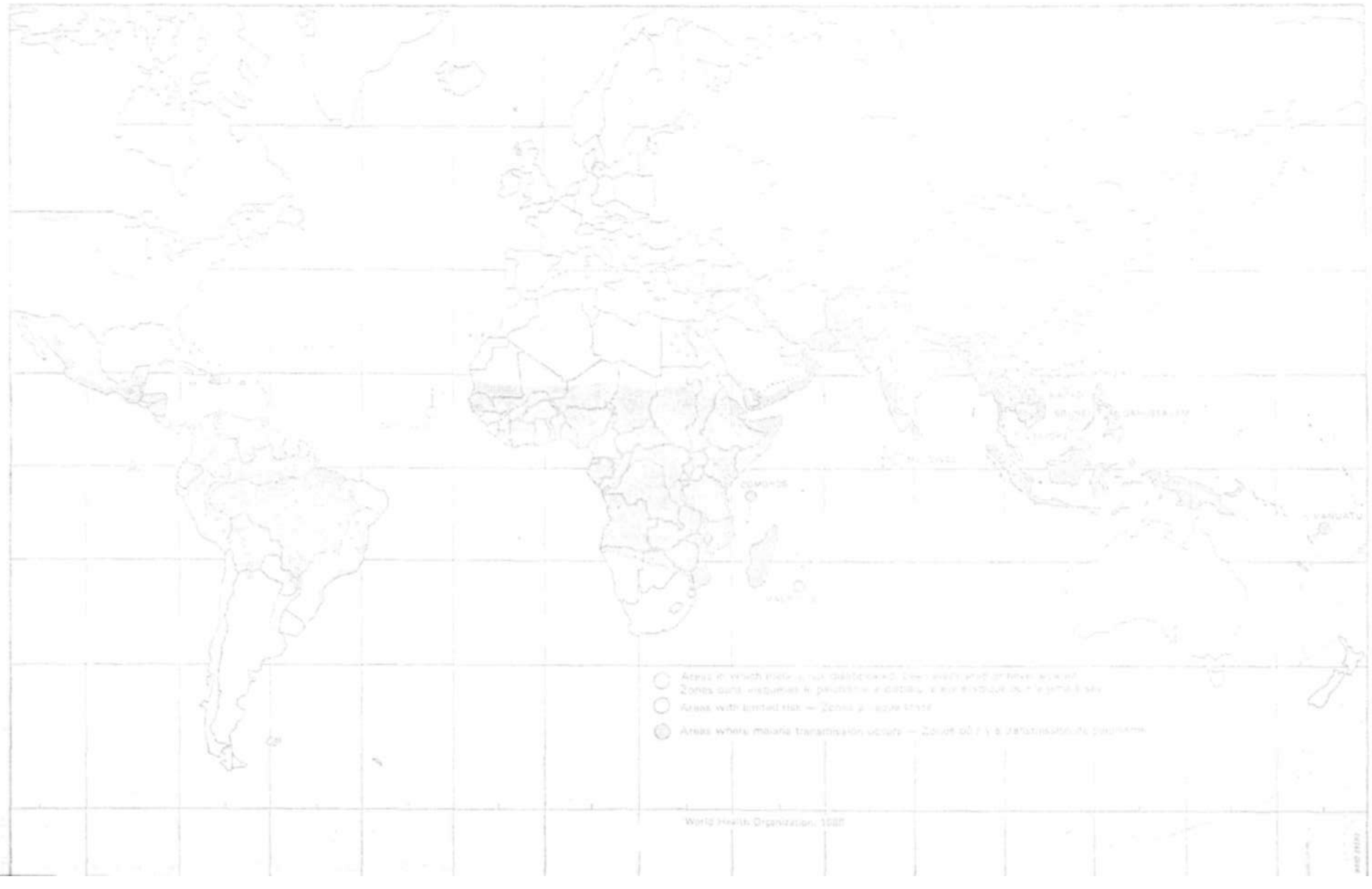
immunity and epidemics are liable to occur (Wernsdorfer 1980).

Africa

Parts of northern and Southern Africa were naturally non-malarious. However, the total number of cases north of the Sahara continued to rise due to some focal outbrakes from 543 in 1983 to 666 in 1984 and 921 in 1985 most of them P. vivax (Weekly Epidemiological Record 1987) 713 local cases of P. vivax were reported in Morocco alone, 3 in Algeria and 194 in Egypt including 10 faciparum infections in 1985. Malaria free areas south of the Sahara are, Capeverde, Lesotho, Reunion, Saint Helena, The Seychelles, most of South Africa republic and some areas above certain altitudes (Weekly epidemiological record 1987).

All countries of tropical continental Africa as well as Madagascar are malarious with P. falciparum representing between 80-99% of the parasite formula followed by P. malariae 1-3% and P. ovale 0-1% P. vivax is practically absent in West and Central Africa. Malaria in tropical Africa is predominantly hyper and holoendemic and the main vectors are A. gambiae and A. funestus. They are responsible for maintaining a high degree of stability of malaria in this region (Weekly Epidemiological Record 1987). The distribution of human malaria in the world is shown in Figure 1.2.

Fig. 1.2: Map showing the distribution of human malaria in the world.



Americas

On the American continent, malarial is endemic in tropical South America except for high altitude areas, also in central America and some parts of Mexico. In Argentina, Costarica and Panama transmission has been considerably reduced, while there is no evidence of continued transmission in Chile, Cuba, Dominica, Grenada, Guadeloupe, Jamaica, Martinique, Peuerto Rica, Saint Lucia, Trinidad and Tobago. The U.S.A. and Canada have succeeded in eradicating malaria and are no longer endemic (Weekly Epidemiological Record 1987).

Asia

In Asia West of India, Bahrain Cyprus, Israel, Jordan, Kuwait, Lebanon and Qatar are free of indigenous malaria, while a reduction in the number of cases has been reported from other countries, they are however on increase in Pakistan and Afghanistan. All countries in middle South Asia are malarious and about 96% of the total population are exposed to malaria risk in varying degrees (Weekly Epidemiological Record 1987).

In East Asia and Oceania; Australia, Brumei, Dar-el-salam, North and South Korea, Hong Kong, Japan Macao, Mongolia, Singapore, large areas of China and most of Oceania are considered malaria free. While

there is improvement in the number of cases in other countries, there is no significant change in Vietnam, Papua new Guinea, the Philippines and Vanuatu. (Weekly Epidemiological Record 1987).

Europe, Including Turkey and the USSR

With the exception of Turkey where endemic malaria is limited to some areas, the above regions are free from malaria, only imported cases have been reported (Weekly Epidemiological Record 1987).

1.3 Malaria

1.3.1 Pathology of Malaria

(a) General Pathology: The plasmodia invading erythrocytes initiate the pathological process and the consequences of this infection influence the hosts other tissues and organs. Factors contributing to the development of the pathological process are (i) parasitaemia, (ii) destruction of damaged erythrocytes and (iii) the defense response of the host against the infection, including Phagocytosis and the development of immunity. P. vivax and P. ovale tend to invade the youngest erythrocytes and P. malariae the older ones while P. falciparum invades red blood cells of any age (Aikawa et al 1980, Bruce-Chwatt, 1980).

During malaria infection, the destruction of host red blood cells occurs not only when plasmodia rupture the erythrocytes at the end of schizogony but also through Phagocytosis of both parasitised and non-parasitised erythrocytes especially in the liver and spleen. These result in some degree of anaemia and contribute to anoxia (Devakul et al 1969).

It has been found that changes in red cells especially on their surface occur during malaria infection e.g. there is a reduction in the electrical charges on the surface of both parasitised and unparasitised erythrocytes in bird malaria. Also in severe malaria, just as in other severe infections, substances such as Fibrin are secreted and appear as fine precipitate around the erythrocytes (Edington and Gilles 1981). These substances apart from aiding Phagocytic desctruction of both infected and non-infected erythrocytes, also cause them to sludge, thus interfering with the passage of blood cells through finer blood vessels. It has also been found that the parasitised red blood cells lose their deformability and thereby log-jam the microcirculation (Edington and Gilles 1981).

(b) Pathological Changes in Individual Organs

The following changes refer mainly to infections with P. falciparum except for P. malariae that may cause nephrotic syndrome (Aikawa et al 1980, Bruce-Chwatt 1980).

(i) Central Nervous System:

There are severe lesions in cerebral malaria due to P. falciparum infection. Three main types of changes can be seen on autopsy. These are:

(1) Gross congestion of meninges and the brain itself, with the small vessels of the gray matter being packed with erythrocytes containing pigmented parasites in all stages of development.

(2) Occlusion of the capillaries and precapillaries of the cortex, ring haemorrhages around blocked arterioles and numerous petechial haemorrhages in in the sub-cortical white matter of cerebrum, brain-stem and cerebellum

(3) Necrotic lesions in midzonal brain tissue with a peripheral reaction of small glial cells (malaria granuloma) around an occluded capillary.

(ii) Spleen: This organ is always affected in malaria. The main change is that of congestion, but later the organ becomes dark from the accumulation of pigment in the parasitized cells contained in capillaries and sinusoids. Parasitised and apparently normal cells and also haemozoin granules are seen in the pulp histiocytes and sinusoidal lining cells. Pigment is also seen in the free form or in giant phagocytic cells. Other findings are diffuse cellular hyperplasia, dilated sinuses and sometimes capillary thrombi. In long standing infections, the connective tissue is increased.

(iii) Liver: It appears swollen and dark in colour. The Kupffer cells are large and numerous and contain pigment. The capillaries are distended with macrophages, parasitised cells and pigment. Degeneration and necrosis in the centro-lobular regions are commonly seen. This may be due to anoxia as a result of stagnation of local circulation.

(iv) Kidneys: In severe P. falciparum infection, there is congestion and punctate haemorrhages in the cortex and medulla. Transient acute

diffuse glomerulomephritis has been described. In the nephrotic syndrome associated with P. malariae there are focal hyalinising lesions of the tuft of the glomerulus and endothelial cell proliferation. Thickening of capillary walls of the basal membrane is due to the deposition of antigen/antibody complexes at this site.

(v) Bone Marrow: Changes here are similar to those seen in the spleen though less marked. There is erythroblastic hyperplasia with large eosinophilic normoblastic cells.

(vi) Placenta: There is gross enlargement and colour change to slate gray. Microscopically abundant parasites are seen in the material circulation in the intervillous spaces, also pigment deposits are seen in macrophages located in the intervillous spaces. The infection of the placenta with plasmodia often results in low-birth weight of the baby and sometimes premature births. Also congenital transmission of malaria to the foetus has been reported (Archibald 1956, Aikawa et al 1980).

Sometimes there may be localisation of malaria parasites in any part of the gastrointestinal tract, leading to local oedema, haemorrhages and even ulceration.

Death from P. falciparum infection in individuals living in areas of stable malaria is either due to cerebral malaria or malaria anaemia while death in the non-immune is associated with acute renal insufficiency, cerebral malaria and pulmonary oedema (Canfied 1969). Other causes of death are medical shock (algid malaria), renal failure, severe diarrhoea, hepatic failure and hyperpyrexia (Edington and Gilles 1981).

1.3.2 Clinical Course of Malaria

(a) Definitions (Adams and Maegraith 1980, Bruce Chwatt 1980).

(i) Incubation Period: This is divided into Intrinsic and Extrinsic incubation periods. The former is the time elapsing between the inoculation of sporozoites i.e. the effective bite, and the appearance of clinical signs. The length of which varies between 10-15 days or more depending on the species. The latter is the time elapsing between the ingestion of infected blood by the mosquito and the appearance of sporozoites in the salivary glands.

(ii) Prepatent Period is the time elapsing between the infection and the first appearance of plasmodia in the peripheral blood.

(iii) Primary Attack is the attack immediately succeeding the intrinsic incubation period.

(iv) Relapse is the recurrence of the clinical signs and symptoms of malaria and the reappearance of parasites in the peripheral blood after the end of a primary attack. These parasites originate from the dormant or latent hypnozoite persisting in the liver, seen mostly in P. vivax and P. ovale infections.

(v) Recrudescence is the persistent of asexual forms mainly of P. falciparum in the erythrocytes usually due to incomplete treatment.

(vi) Reinfection is a fresh infection in an individual who has previously had malaria.

(vii) Periodicity: The primary attack is composed of a number of paroxysms occuring roughly at the same time with the sporulation of the parasites. The interval at which they recur

is known as the periodicity of the fever and it is determined by the length of the erythrocytic schizogony where the life cycle of the plasmodia is synchronized.

In tertian periodicity, the paroxysms may occur every 48 hours (seen in P. vivax and some strains of P. falciparum infections) and in the quartan type, every 72 hours (seen in P. malariae infections). In all cases, of infections, sporulation may occur daily with paroxysms i.e. quotidian periodicity.

A Paroxysm comprises of the following three successive stages (Adams and Macgraith 1980, Bruce Chwatt 1980) (1) The cold stage:- This starts with shivering (rigor) and a feeling of intense cold; vomiting may occur and children often have convulsive seizures. This stage lasts between 15 minutes and 1 hour.

The hot stage:- This follows when the feeling of intense cold gives way to one of distressing heat. The face is flushed, the skin dry and there is intense burning headache. Nausea and vomiting are common. There is often intense thirst while the temperature may rise to $41^{o}C$ or more. This stage lasts from 2 to 6 hours.

The sweating stage: The patient breakes out in profuse sweating and the temperature falls rapidly often below the normal level. He may fall into a deep sleep and on waking feels weak but otherwise normal. This stage lasts from 2 to 4 hours.

(b) Clinical Manifestations of Malaria

The most severe form of malaria is that caused by P. falciparum infection and when neglected or complicated, the disease may become fatal in non-immunes. Malaria caused by other plasmodia i.e. P. vivax, P. malariae and P. ovale may be severe and unpleasant in non-immunes but is very rarely fatal (Adams and Maegraith 1980). Here, the clinical manifestation of P. falciparum malaria will be discussed in detail.

Attacks by P. falciparum malaria may be uncomplicated or associated with serious and often fatal complications.

(c) The Uncomplicated Attack (Adams and Maegraith 1980)

(i) Prodromal Symptoms:- The incubation period varies from 8 to 15 days. Prodromal symptoms occur during the last few days of the incubation period. The patient is depressed and unwell. He complains of severe headache, bone and muscle pains and a nagging backache

in the lumbar or sacro-iliac region. Shivering, nausea and vomiting or diarrhoea are frequent.

(ii) Onset:- The parasites may exist in the blood for long periods before the onset of severe symptoms especially in those with acquired immunity or who are misusing suppressive drugs. Here, the onset is insidious with symptoms such as diarrhoea, persistent dyspepsia, malaise and backache which may not at first suggest malaria.

(iii) The attack (Adams and Maegratith 1980)
The patient usually presents with fever, flushed dry skin and bright eyes. He looks like a case of moderately severe influenza and may not appear very ill. This appearance is deceptive, since serious complications may arise at any stage of either a primary attack or a recrudescence. The headache, bone and muscle pains and malaise of the prodromal period persist and increase as the attack develops.

Anxiety, mental confusion and delirium are common. The patient is asthenic and prostrated. In cases where periodic fever is established, the feeling of wellbeing which normally accompanies the interval between the paroxysms is absent.

(iv) Fever:- In falciparum malaria, the fever is usually irregular at first and shows no sign of periodicity, in about a third of the cases periodicity becomes evident after the first few days and is commonly tertian or quotidian. The paroxysms are often not as regular as in vivax infections and the temperature during the intervals may not fall to normal. In many cases, there is no periodicity and the fever becomes remittent, intermittent or continuous. Even in severe attacks the fever may not be high. It may be absent until towards the end of the attack or appearance of complications. Sometimes there may be no fever at any stage even in the presence of heavy blood infection.

(v) Sweating:- Where paroxysms are present, sweating follows the hot stage when the fever is persistent or intermittent but without paroxysms sweating is common and is often persistent. In cases in which the temperature is high sweating may be absent.

(vi) Anaemia:- Because of the invasive properties of the parasites and the ease with which unparasitized erythrocytes are lysed, severe anaemia is common in falciparum malaria. In mild cases there may be little or no obvious anaemia. The anaemia when

present is normocytic and bone marrow reaction is normoblastic but may be depressed. In very rapid and severe cases, leucopenia is usually present. The degree of anaemia is not always an indication of the severity of the attack. Fatal complications sometimes occur in cases with apparently little red cell destruction.

(vii) Other Findings:- There may be a fall in blood pressure. Epigastric discomfort, anorexia and dyspepsia are common. Watery diarrhoea is frequent and particularly notable in infections with certain strains of parasites prevalent in West Africa (Adams and Maegraith 1980).

There is splenomegaly and deviations of liver and renal function tests. Also cough and other respiratory symptoms may be seen. Herpes labialis is present in about one-third of all cases. In the untreated cases, the parasitaemia increases as the disease progresses, but there may be appreciable daily variations in the parasite density (Adams and Maegraith 1980).

(viii) Course and Prognosis:- If the acute attack is adequately treated and no pernicious symptoms develop, there is little risk to life. Recrudescences are not uncommon in inadequately treated cases.

(d) <u>The Complicated Attack</u> (Adams and Maegraith 1980, Garnham 1980).

Clinical complications also called pernicious malaria may develop without warning at any stage in falciparum malaria. Complications appear most commonly in individuals who have suffered repeated attacks especially those inadequately treated.

The syndromes of pernicious malaria are usually grouped according to the organs which are principally involved. The major forms are: cerebral malaria, hyperpyrexia, gastroinestinal malaria, algid malaria and black water fever. Acute renal failure may develop in complicated or uncomplicated falciparum malaria.

(i) <u>Cerebral Malaria</u>: This syndrome occurs in two clinical forms, true and false. The later is seen when there is a generalized fulminating septicaemic infection with delirium, toxemia, high fever and early death but there is no particular localization in the brain. True cerebral malaria occurs when there is extensive blockade of the cerebral capillaries (Garnham 1980). The patient sinks into a gradually deepening coma. Breathing becomes difficult,

the pupils are contracted, knee jerks may be absent and incontinence is common.

Marsden and Bruce-Chwatt (1975) refer to the alterations in the level of consciousness as beginning with a disturbance in normal behaviour and mental confusion and progressing to delirium and eventual loss of consciousness. Less often the impact is felt in particular regions of the brain and different varieties of cerebral malaria have been described in relation to the area affected.

(ii) Hyperpyrexia:- This may appear early or may develop during the course of an apparently mild attack. It sometimes occurs with cerebral malaria. The skin is hot and dry due to inhibition of sweating and there may be some peripheral cyanosis. The patient is disoriented, delirious or comatous. Incontinence of urine and faeces is common.

(iii) Algid Malaria:- In this condition the patient rapidly passes into medical shock frequently associated with coma. The eyes are sunken, the skin cold and clammy, breathing is shallow and fast and the blood pressure sinks rapidly.

(iv) Gastrointestinal Syndromes (Adams and Maegraith 1980)

Several gastrointestinal syndromes are observed. These are: Bilious remittent fever :- which arises from severe liver damage. There is epigastric discomfort, nausea, vomiting and diarrhoea in its severest form, it passes into a typhoid state with characteristic remittent fever.

Dysenteric Malaria:- It is clinically indistinguishable from acute bacillary dysentery. There is passage of frequent stool containing blood, mucus, epithelia cell and in some cases pus cells.

Choleraic Malaria:- Here, the patient suffers from profuse watery diarrhoea, nausea and vomiting, muscular cramps in the limbs and abdomen and progressive dehydration.

(v) Black Water Fever: This is a state of acute intravascular haemolysis accompanied by haemoglobinuria occuring as a complication of P. falciparum infection. Black water fever was shown to be associated with irregular and inadequate treatment of attacks of malignant tertian malaria with quinine since 1901 (Stephens and Christophers 1901). Few cases have been reported after the administration of chloroquine and also in infections due to P. vivax (Maegraith 1970).

The apparent explanation of this phenomenon may be as follows (Garnham 1980). The quininized and parasitized erythrocytes act as an antigen against which are formed hemolysins. When a dose of quinine is next administered, intravascular haemolysis takes place, methemoglobin is produced and the kidney is damaged first in the cortex and later as a result of ischemia and blockage of the capillaries in the tubules.

The signs for blackwater fever are as follows:- The patient is usually one who has taken quinine before and after a few months or years, the swallowing of the drug is almost immediately followed by malaise, apprehension, pyrexia and the sudden passage of black urine. The patient is jaundiced and prostrated. Spleen and liver are enlarged and anaemia is pronounced. In treating black water fever, quinine should be avoided.

(e) Malaria in Children (Adams and Maegraith 1980)

Malaris is one of the great causes of morbidity and mortality in children in tropical countries. The clinical attack is relatively rare in the first few months of life due to acquired passive immunity from the mother

(ie. congenital or neonatal immunity) but there after for several years children are prone to pernicious forms of malaria.

The clinical picture of malaria in children depends on whether they are suffering from acute recent infection or from the effects of frequent reinfection or long-continued infection or both.

The acute form is seen mostly in children who have not had the full benefit of protective measures or who live in areas where malaria is seasonal. The infected child is dull, restless and miserable with no appetite and food given is often vomited back. There is frequently abdominal pain, wind and diarrhoea, also the abdomen is distended and tender especially in the hepatic and splenic areas. The child may be anaemic and convulsions are common and the temperature may range between 38-40.6^{o}C. If untreated, the child may die or recover temporarily with the abatement of most of the signs and symptoms and gradually slip into the chronic phase.

Chronic malaria is commonest in falciparum areas and is seen in children of all ages up to adolescence. Normal growth and development of the

child is retarded. The patient is listless, wasted with thin skinny flaccid limbs and huge protuberant abdomen. There is chronic dyspepsia, flatulence, anorexia and complicating factors due to malnourishment. The mucous membranes are pale and there is sometimes jaundice. Fever is common and is usually of the remittent type, occasionally intermittent but not periodic.

Often there is severe anaemia and as a rule parasitaemia is low but may rise in exacerbations or fresh infections. Normally only ring forms and gametocytes are seen in the peripheral blood but in severe cases trophozoites and schizonts are present. Spleen and liver are enlarged and tender. The enlarged spleen may rapture following external violence, causing an acute abdominal crisis.

The condition if untreated becomes progressively worse and passes into the state of malaria cachexia which ends fatally.

(f) Malaria in Pregnancy

In tropical Africa, P. falciparum accounts for the majority of malaria cases during pregnancy. Malaria infections in pregnancy is associated with prematurity, low birth weight and rarely congenital

malaria (McGregor et al 1983). Also malaria infection of the placenta has been associated with adverse outcome for the foetus and newborn. There is evidence that the mean birth weight of infants born of infected placenta are depressed (McGregor et al 1983, Watkinson and Rushton, 1983).

While maternal parasitaemia leads directly to placenta infection, it also cause maternal haemolysis which leads to anaemia and this also indirectly affects foetal growth (Gilles et al 1969; Fleming et al 1986).

1.4 Drugs Used In the Treatment of Malaria

Quinine and other cinchona alkaloids were the first and only antimalarial drug for about three centuries (1600 to 1924). The development of synthetic antimalarial drugs was initiated by German workers out of necessity during world war I due to lack of access to quinine. Thus pamaquine was synthesised in 1924 followed by mepacrine in 1930 and chloroquine in 1934, all by German Scientists. Other synthetic antimalarial drugs like proguanil, pyrimethamine, and primaquine were introduced later mainly by British and American Scientists (Pinder 1973, Bruce-Chwatt 1980). The introduction of synthetic antimalarial drugs and especially chloroquine superseded quinine and

chloroquine was placed second to none in the chemotherapy of human malaria. Increasing resistance to chloroquine has however brought quinine back into the main stream of malaria chemotherapy (Pinder 1973, Bruce-Chwatt 1980).

1.4.1 Definitions and Classification of Antimalarial Drugs (Bruce-Chwatt et al 1986)

(i) Definitions

(a) Clinical cure:- Relief of symptoms of malaria by elimination of the asexual blood forms but without complete elimination of the infection.

(b) Radical cure:- Complete elimination of malaria parasites from the body so that relapses can not occur.

(c) Suppressive cure:- Complete elimination of malarial parasites from the body by means of continuous suppressive treatment.

(d) Prophylaxis:- Any method of protection from or prevention of disease.

(e) Chemoprophylaxis:- Protection from or prevention of disease by chemotherapeutic means.

(f) Causal prophylaxis:- Complete prevention of erythrocytic infection by the administration of drugs that destroy either the

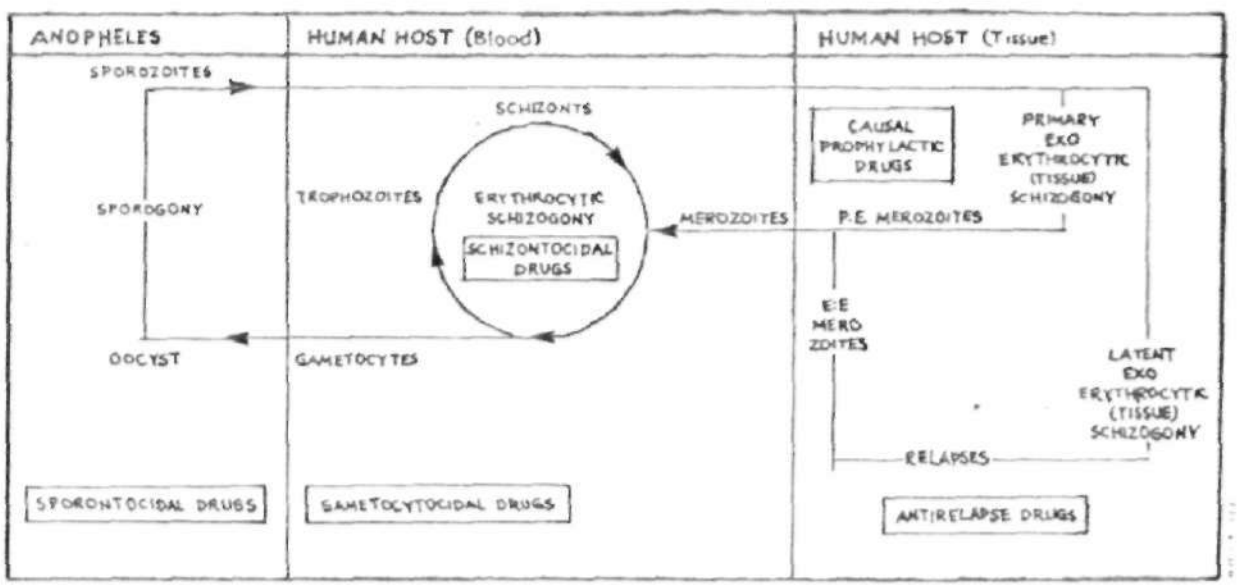
sporozoites or the tissue forms of the malaria parasite.

(g) Clinical prophylaxis:- Synonym of suppressive treatment.

(ii) Classification

Antimalarial drugs can be classified either (i) according to the various stages of the life cycle of the malaria parasite on which they act as shown below or (ii) according to their chemical structures.

Fig. 1.3. Simplified diagram of action of antimalarial compounds on different parts of the cycle of development of P. vivax and P. ovale in the mosquito and in man.



From chemotherapy of malaria (Bruce-Chwatt 1986)

(a) Tissue schizontocides (used for causal prophylaxis):- They act on the primary tissue forms of the parasite and are able to prevent invasion of the red blood cells e.g. proguanil, pyrimethamine.

(b) Latent tissue schizontocides (used as antirelapse drugs):- They act on the hypnozoites or latent tissue forms of P. vivax and P. ovale, thus they are able to achieve radical cure of these infections, e.g. primaquine.

(c) Blood schizontocides (used for clinical and suppressive cure):- They act on the erythrocytic stages of the parasite commonly associated with acute disease and may achieve clinical cure or suppression to subpatent level of all four species of human malaria. Some also act on the gametocytes of P. vivax, P. ovale and P. malariae but not directly on the mature gametocytes of P. falciparum, e.g. chloroquine, quinine, pyrimethamine.

Blood schizontocides may be subdivided into (i) rapidly acting blood schizontocides e.g. chloroquine, amodiaquine and quinine and (ii) slow acting blood schizontocides

e.g. pyrimethamine, proguanil and the sulphonamides.

(d) Gametocytocides:- They destroy all sexual forms including those of P. falciparum e.g. primaquine.

(e) Sporontocides:- May prevent or inhibit the formation of oocysts and sporozoites in anophelines that have fed on carriers of gametocytes e.g. proguanil, pyrimaquine.

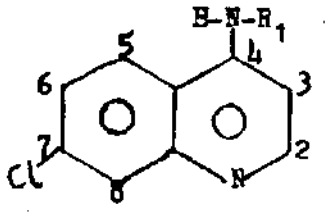
1.4.2 Chloroquine and its Congeners

(a) History and Source

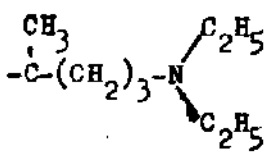
Chloroquine is a 4-aminoquinoline derivative. Other members of this family used clinically although less popular are, amodiaquine, hydroxychloroquine and amopyroquine. Chloroquine was first synthesised in Germany by Andersag in 1934 (Bergqvist 1983) but was rejected on the ground of toxicity. Informations of the 4-aminoquinolines reaching America stimulated the synthesis of a large number of this group of compounds during world war II, out of which chloroquine emerged as the most effective drug and was accepted for clinical use in 1947 (Coatney et al, 1953, Coatney 1963 and Wiselogle 1946).

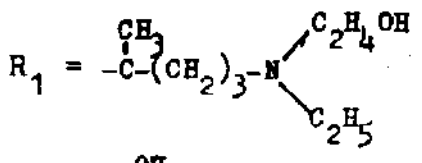
Amodiaquine, hydroxychloroquine and amopyroquine were introduced later (Hill 1963, 1966, Pinder 1970).

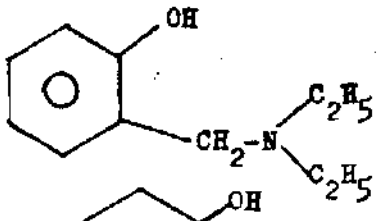
(b) <u>Structure Activity Relationship</u>

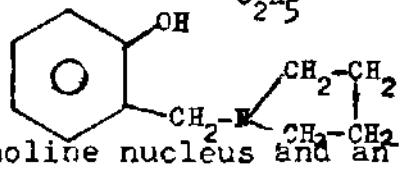


quinoline nucleus

chloroquine R_1 = 

hydroxychloroquine R_1 = 

amodiaquine R_1 = 

amopyroquine R_1 = 

Chloroquine has a quinoline nucleus and an alkyl side chain. Introduction of a methyl group in C-3 position reduces antimalarial activity. Also substituting a methyl group in C-8 causes a complete loss of antimalarial activity. The C-7 position was found to be best for halogen substitution.

On the 4-amino side chain, the 4-diethylamino-1-methyl butylamino group present in chloroquine was optimal for activity but more recently introduction of hydroxy groups on the side chain particularly on an ethyl group of the terminal nitrogen as seen in hydroxychloroquine tends to reduce toxicity and to produce higher blood concentrations (McChesney R.W. and McAuliff, J.P; 1961).

The substituted anilo compounds i.e. amodiaquine and amopyroquine were introduced to combine the antimalarial effect found in some . -dialkylamino-o-cresols with the 4-aminoquinolines. They are claimed to have low toxicity with slightly less activity than chloroquine and are less bitter. (Burckhalter et al, 1946). The 4-aminoquinolines share similar pharmacological properties and on the whole, have little or no advantage over chloroquine (Allen 1977, Coatney et al 1953).

(c) Pharmacological Effects

(i) Antimalarial actions:- Chloroquine is inactive against the sporozoites, primary excerythrocytic stages and latent excerythrocytic stages of P. vivax and P. ovale. It is therefore not used as a

causal prophylactic agent and it does not produce radical cure of infections caused by P. vivax or P. ovale but substantially lengthens the interval between relapses of these two infections.

Chloroquine and other 4-amino-quinolines are highly effective against the asexual blood stages of all four species of human malaria parasites except in areas where drug resistant strains occur. They produce clinical cure of all types of human malaria and radical cure of falciparum and quartan infections. They are excellent suppressive agents against all species and their continued administration for 4-6 weeks results in suppressive cure of falciparum and quartan malaria.

They also act as gametocytocidal agents against P. vivax, P. ovale and P. malariae and are effective against the inmature but ineffective against the mature gametocytes of P. falciparum.

In general, chloroquine and amodiaquine have replaced other blood schizontocides as

the drug of choice for the treatment of acute malaria. Their action is rapid, fever is usually absent after 24 hours and patient parasitaemia being eliminated within 48 - 72 hours following the standard therapeutic regimen of 25mg/kilogram chloroquine base. If the response in falciparum infection is slow or incomplete, drug resistance should be suspected.

Amodiaquine and amopyroquine may have a slight advantage over chloroquine in such cases (Bruce-Chwatt 1986, Leslie and Webster 1985).

(ii) Other actions:- Although developed primarily as an antimalarial agent, chloroquine possesses other pharmacological properties some of which are useful.

It is used in treating extraintestinal amoebiasis and also has some activity against Clonorchiasis and Paragonimiasis.

The drug has anti-inflammatory properties and has been used occasionally in treating rheumatoid arthritis and discoid lupus erythematodes.

Chloroquine is also used with success to treat porphyria cutanea tarda, solar urticaria and polymorphous light eruption.

It also has antiarrythymic properties and is said to be a cardiodepressant. Treatment of these conditions requires much larger doses than are used for malaria. (Leslie and Webster 1985).

(d) Mechanism of antimalarial action

The mechanism of action of chloroquine is not yet clear. However, it causes a number of effects which singly or in combination may relate to its plasmodicidal action.

It was hypothesized from early work that chloroquine might exert its effect at least in part by an interaction with DNA. The drug was found to inhibit the incorporation of ^{36}P-labeled phosphate into RNA and DNA of P. berghei berghei in vitro and P. gallinaceum in vitro and in vivo (Schellenberg and Coatney 1960). It was also reported to inhibit DNA and RNA polymerase enzymes. Changes in several physical parameters were consistent with an intercalation of the drug with guanine and other purine bases of the DNA (Allison et al, 1965; Allison et al 1966; Cohen and Yielding, 1965).

The second possible mechanism is that of interference with the digestion of haemoglobin by the malaria parasite. It has been observed that haematin (ferriprotoporphyrin IX), a transient break-down product of haemoglobin within the parasite can readily disrupt plasmodial and host membranes and can also act as high affinity receptors for chloroquine (Macomber 1967; Chou et al, 1980). It has been suggested that the parasite synthesizes a segregating protein which aggregates with haematin to form the inert malaria pigment haemazoin. It is claimed that chloroquine competes with the segregating protein for ferriprotoporphyrin IX and readily binds with it to form a more effective membranolytic complex (Fitch 1983, Leslie and Webster 1985).

The third possible and most recent mechanism of action is by producing acid vesicle dysfunction. It is claimed that chloroquine, a weak base is transported across cell membranes probably by carrier systems and also by pinocytosis in case of the malaria parasite (Warhurst and Thomas 1975, Yayon and Ginsburg 1982). Within the parasite, according to those authors, chloroquine accumulate in acidic food vacuoles or acid vesicles

probably due to difference in the pH gradient (Yayon 1985; WHO 1987). As a weak diprotic base, it raises the pH of the parasite acid vesicles. This rise in pH causes inhibition of lysosomal proteolysis and also interferes with other cellular functions, thus retarding or inhibiting parasite growth and maturation (Krogstad and Schlesinger 1987).

(e) Pharmacokinetics of Chloroquine

(i) Absorption:- Chloroquine is rapidly and almost completely absorbed from the intestinal tract so that less than 10% of the administered dose is found in the stool. Gustafsson et al (1983) administered 300mg of chloroquine base orally in form of tablets or solution to healthy male adults weighing between 65 and 91kg and obtained peak plasma concentrations of 56-102 mg/l (mean 75 mg/l) in 1-6 hours (mean 3 hours) with a bioavailability of 75%. In another study, Adelusi et al (1982) gave 10mg of chloroquine base per kg body weight in form of tablets to children with malaria. Peak plasma concentrations of about 250 µg/litre were reached after 2 hours with an absorption half-life of 0.56 hours. Intramuscular

administrations are said to produce effective plasma concentrations within 15 minutes. The therapeutic level of chloroquine for sensitive P. falciparum is 30 µg/litre (WHO 1984).

(ii) Distribution: After absorption, chloroquine is widely distributed throughout the body. The drug is extensively deposited in various tissues. The concentrations in spleen, kidney, lungs, heart and liver have been found to be 300 - 500 times higher than those in the Plasma (Berliner 1948, McChesney and Mcauliff 1961, McChesney 1966). The drug has a particular high affinity for the melanin-containing tissues of the skin and eyes. About 50% of chloroquine is bound to plasma proteins namely albumin and α-acid glycoproteins. The binding of the drug to plasma proteins is much less when compared to the extensive binding to tissue. Also the percentage of chloroquine binding to protein is not affected by aspirin which is often given at the same time during the treatment of acute malaria (Adelusi and Salako 1982, Walker 1983).

Because of the extensive tissue distribution and binding of chloroquine,

its apparent volume of distribution is large. According to Gustafsson et al (1983), it is 204 litres per kg after intravenous dosing.

Chloroquine and other 4-aminoquinolines are preferentially concentrated in erythrocytes. The erythrocyte/plasma chloroquine concentration ratio was shown to be approximately 100% in mice infected with P. berghei and only 10% in non-infected mice. The ratio was also less in mice infected with chloroquine resistant parasites when compared to those infected with sensitive strains (Macomber 1966, Warhurst and Hockley 1967). The ratio of chloroquine concentrations in erythrocytes and plasma has been investigated in children with acute malaria (Adelusi et al 1982). The erythrocyte concentration was found to be higher than the plasma concentration during the sampling period of 7 days. At the height of the parasitaemia, the ratio was 21 and this fell to a steady value of about 5.3 after 4 days when parasitaemia had completely disappeared. This study confirms that in man, there is preferential concentration of chloroquine in

red blood cells and that this concentration is further increased by the presence of malaria parasites in the red cells. Other cellular elements of blood i.e. leukocytes and thrombocytes have also been shown to concentrate the drug (Bergqvist 1983).

In all studies on the distribution of chloroquine in different tissues, the peak concentrations were found to occur in erythrocytes and plasma at the same time (Adelusi et al 1982, Gustafsson et al 1983). In contrast peak concentrations in different organs were reached at different times which also differed from the peak concentration times in the plasma and red blood cells (Adelusi and Salako 1982).

(iii) Metabolism

The drug is metabolised by the liver microsomal enzyme system. The major metabolic pathway is by side chain daethylation leading successively to first desethyl and then bisdesethyl chloroquine. The latter can be deaminated to form an alcohol which is oxidised to form carboxylic acid derivatives. Successive dealkylation

of the side chain produces the 7-chloro, 4-aminoquinoline which is resistant to degradation (Kuroda 1962, McChesney 1966). Metabolism of chloroquine occurs slowly and the main metabolite varies in different species. In man it is desethylchloroquine. Studies by GustafssOn et al (1983) and Walker (1983b) show that the concentration of the metabolite is between 25-40% of that of the parent drug chloroquine. The desethyl metabolite has the same distribution profile and tissue binding as the parent drug. Also the concentrations of chloroquine and desethylchloroquine in plasma and skin of two groups of African subjects i.e. those who itched after chloroquine intake and those who did not has been studied and the plasma concentrations were found to be similar in the two groups (Olatunde 1971). However the skin from subjects prone to chloroquine-induced pruritus contained higher concentrations of chloroquine and lower concentrations of the metabolite than skin from other subjects (Olatunde 1971). Recent studies have shown that desethylchloroquine has a similar activity against chloroquine sensitive plasmodia as

the parent drug (Aderounmu and Fleckenstein, 1983).

(iv) Elimination: Chloroquine is eliminated from the body very slowly, the kidney being the main route of elimination. The decline in the concentration of chloroquine in the plasma with time after a single dose is polyexponential, suggesting a multi-compartmental distribution. The decline of the erythrocyte concentration after the peak level has been reached parallels that of the plasma concentration, showing that equilibrium is reached quickly and is steadily maintained between these two media, which can therefore be regarded as behaving pharmaco-kinetically as a single compartment with regard to chloroquine (Adelusi 1982, Gustafsson et al 1983). In constrast the tissues, however, behave as separate compartments with slower elimination rate constants than the plasma. Estimation of the plasma half life of chloroquine depends on the duration of sampling. The longer the sampling period, the longer the half life (WHO 1984).

The total plasma clearance of chloroquine varies between 750 and 1050 ml/minute and the renal clearance is between 400 and 450 ml/minute. Since the value for renal clearance is more than the glomerular filtration rate, the drug is probably excreted by both glomerular filtration and tubular secretion. Maximum excretion was found to occur in the first 24 hours about 10% of the administered dose being excreted in that interval (Gustafsson et al, 1983). Chloroquine is predominantly excreted unchanged while the desethylchloroquine metabolite is accounting for about 25% and the other metabolites for about 3% (WHO 1984).

(v) Toxicity and side effects of Chloroquine

At doses used for chemotherapy of malaria side effects that are often reported include gastrointestinal upset, nausea and vomiting, pruritus, headache, blurring vision, dizziness and fatigue. Those symptoms usually disappear when the medication is discontinued.

The more severe but rare adverse reactions are:- itching in Africans,

cardiovascular abnormalities, ocular damage, neuromuscular disorders, extrapyramidal dysfunction, photoallergic dermatitis and pigmentation of the skin (Bruce-Chwatt 1986).

Itching with or without rash occurs in 10-30% of individuals who take chloroquine (Salako 1984, Spencer et al 1987). It begins within a few hours of taking the drug and continues for 48-72 hours and occurs less frequently in children. The itching occurs only when chloroquine is used therapeutically in malaria suggesting that it results from a reaction between chloroquine and malaria parasite (Salako 1984). It has been observed that the skin from subjects prone to itching contained higher concentrations of chloroquine and lower concentrations of the metabolite than skin from other subjects (Olatunde 1971).

Cardiotoxic effects are most commonly observed after parenteral administration or in toxic over dosage and may take the form of hypotension or cardiac arrest and death (Ratliff et al 1987). The chronic cardiotoxic effects of chloroquine may present clinically as congestive cardiac failure, heart block or as a restrictive cardiomyopathy (Ratliff et al 1987).

Intravenous or intramuscular injections of chloroquine in the usual dose range of 200 to 300 mg are said to be generally well tolerated by adult patients. However parenteral administration of the drug to children may produce serious effects on the cardiovascular and central nervous system(Bruce-Chwatt 1986). Therefore intramuscular injections must not be given to children unless absolutely necessary and intravenous injections are less advisable.

Ocular damage usually follows long term treatment of connective tissue disease with high dosage of chloroquine and long term suppressive therapy. It has also been described in workers exposed to chloroquine phosphate dust in the manufacture of chloroquine tablets (Salako 1984). Such damage consists of lesions to the cornea (Punctate Keratitis), lens opacities, optic nerve neuritis or more seriously the deposition of chloroquine on the retina. The latter type of retinopathy appears slowly and insidiously and may not be detected until sometime after administration of the drug has stopped. It was reported mainly in persons over 50 years of age whose cumulative dosage of chloroquine over several years of treatment was over 100g (Bruce-Chwatt 1986).

Neuromyopathy results from the effects of the drug on the muscle, nerve or neuromuscular junction. The muscle fibres undergo autophagocytosis followed by segmental necrosis (Salako 1984; Estes et al, 1987). The neural damage is characterized by myeloid bodies present in the cytoplasm of Schwann cells (Estes et al, 1987). These neuromyotoxic effects of chloroquine are dependant on daily dosages, duration of therapy and individual susceptibility. They are readily reversed on withdrawal of the drug (Estes et al 1987).

Extrapyramidal features similar to those occuring in parkinsonism have been described in patients treated with chloroquine. In Nigeria, Eronini et al 1977 have reported an incidence of 1 in 5000 persons. The symptoms usually start within 24 hours of taking the drug. It remits spontaneously within 48 hours if left untreated but can be rapidly terminated by giving anticholinergic agents.

(vi) Dosages of Chloroquine

In the tropical environment, chloroquine is used mainly in the treatment of malaria. It produces clinical cure of all types of human

malaria and radical cure of falciparum and quartan infections.

For the treatment of acute malaria attacks in adults, a total of 1500mg chloroquine base is given orally within 3 days; in children, 10 mg/kg is given on the first and second days and 5 mg/kg on the third day (Bruce-Chwatt 1986).

In severe attack, necessitating parenteral therapy, not more than 900 mg base is given to adults in 24 hours and in children 5mg/kg/24 hours - is adequate (WHO 1984) and oral therapy is started as soon as it is feasible. For suppressive therapy, the weekly adult and paediatric doses are 300mg and 5mg/kg base respectively (Bruce-Chwatt 1986).

1.4.3 Summary of Other Antimalaria Drugs

(a) Quinine:- Quinine and other cinchona alkaloids are the oldest known antimalarial agents. Quinine is inactive against the sporozoites and exoerythrocytic stages of malaria parasite. It is a highly active blood schizontocide in all forms of human malaria and hence very effective for clinical cure. With a prolonged administration radical cure is possible in falciparum infection but seldom in vivax infection. It is gametocytocidal in P. vivax, P. ovale and P. malariae and has some activity

against the immature but not against mature gamestocytes of P. falciparum. For suppression and suppressive cure, quinine is less effective than the 4-aminoquinolines or mepacrine (Bowman and Rand 1980).

As a general therapeutic agent, Quinine is more toxic and less effective than chloroquine. However, it is especially valuable for the treatment of chloroquine resistant and multidrug resistant strains of P. falciparum where it is used alone or in combination with other drugs (Leslie and Webster 1985).

In addition to its action in malaria, quinine exerts a number of other actions of some therapeutic value. It has a weak antipyretic action and may be used to lower body temperature in all types of fevers. It also possesses a weak analgesic action which resembles that of aspirin except that it appears to be exerted centrally (Bowman and Rand 1980).

Quinine passes through the stomach unchanged and is quickly and almost completely absorbed from the upper intestinal tract to circulate as a base. Peak concentrations in the plasma are reached 1-3 hours after a single oral dose, the amount in the

red cells being about one-fifth of that in the plasma. With both species, the effective plasma level may depend on the strain of parasite. While the plasma level is affected by the route of administration, the drug appears in the urine within an hour or less irrespective of the route of administration. Quinine is quickly metabolised by the tissues or excreted unchanged in the urine and little remains in the body 48 hours after the last dose. Its half-life is about 10 hours (WHO 1984).

Side effects of quinine include tinnitus, impaired hearing, blurred vision, nausea and headache, which comprise a syndrom termed cinchonism. Permanent damage to vision, balance and hearing may follow the persistent use of high doses in patient experiencing cinchonism. Large doses produce severe depression of the myocardium and pronounced vasodilation; those effects may result in prolonged hypotension and shock. When given intravenously, the drug must be injected slowly to avoid a precipitous fall in blood pressure (Bowman and Rand 1980).

(b) Mefloquine: Mefloquine is a 4-quinolinemethanol derivative, it is a new antimalarial drug developed

mainly to combat proliferation of multidrug resistant strains of P. falciparum. Mefloquine is indicated only for the treatment and prevention of chloroquine resistant falciparum malaria however strains of P. falciparum resistant to the drug have been noted in some regions (Jacobs et al 1987, WHO 1984), Also reduced in vitro susceptibility to the drug has been reported in Nigeria and other West African countries where the drug is yet to be introduced (Salako and Aderounmu 1987, Oduola et al 1987).

The mechanism of action of mefloquine is unknown. In some respects, mefloquine behaves like quinine but it does not intercalete with DNA (Davidson et al 1977). Mefloquine may affect membranes of malaria parasites (Brown et al 1979) and like quinine and chloroquine, it interferes with the clumping of pigment in the parasite (Warhurst and Thomas 1978). Mefloquine also competes for accumulation with chloroquine by infected erythrocytes (Fitch et al, 1979).

Studies in animals have shown that mefloquine is well absorbed after oral administration and is extensively bound to plasma proteins. Peak concentrations in plasma are attained in a few hours and decline slowly over

a period of several days. The gastrointestinal system serves as an important compartment for the drug as it undergoes continuous enterohepatic and enterogastric circulation (Mu et al, 1975). Concentrations in tissues particularly liver and lungs are relatively high for extended periods of time. Excretion is mainly in the feces, and only very small amounts of the drug appear in the urine. Several metabolites are formed and after a single dose of 1g the half-life in blood averages 17 days (Desjardins et al, 1979b; Leslie and Webster 1985).

Mefloquine given orally in single doses up to 1500 mg or in 500 mg doses each week for 1 year is well tolerated in man. Side effects such as nausea, vomiting and dizziness are dose related, self-limiting and uncommon with single doses of 1g or less. Rarely neuropsychiatric disturbances such as disorientation, hallucinations and depression may occur during the second week after drug administration but these respond to symptomatic therapy. Studies of mutagenicity, carcinogenicity and teratogenicity have been negative to date. Because of lack of adequate information, the use of mefloquine in women of child bearing age, infants and children is not yet recommended (Leslie and Webster 1985).

(c) Primaquine. It is a 8-aminoquinoline derivative and acts probably by inhibiting parasite mitochondrial respiration (Bruce-Chwatt 1986). Primaquine is highly active against the gametocytes of all human malaria species and also against the primary exoerythrocytic forms including the latent tissue forms. Primaquine has less activity against the other stages of the life cycle of malaria parasites. Clinically it is used together with blood schizontocides to achieve radical cure of relapsing malaria and to interrupt transmission of P. falciparum in some areas.

After oral administration, the 8-aminoquinoline antimalarial compounds including primaquine are promptly absorbed. They are however rapidly metabolised and only a small proportion of the administered dose is excreted as the parent drug. After a single oral dose, the plasma concentration reaches a maximum in about 1 to 2 hours and then falls with an apparent half-life of 3 to 6 hours. Three oxidative metabolites of primaquine have been identified to date. These metabolites have appreciably less antimalarial activity than does primaquine. However, their hemolytic

activity as assessed by formation of methaemoglobin *in vitro* is greater than that of the parent compound (WHO 1984, Leslie and Webster 1985).

At the recommended dosage, no symptoms of toxicity are likely. When the dosage is increased, toxic manifestations may include anorexia, nausea, epigastric distress, abdominal pain and cramps, cyanosis, the passage of dark urine and occasionally vomiting, Vague chest pain and weakness. In addition, there may be striking effects on the formed elements of the blood and the bone marrow, marked by leukopenia, anaemia, methaemoglobinaemia and suppression of myeloid activity with lesser effects on the heart and circulation. Also there is haemolytic anaemia in patients with glucose -6-phosphate dehydrogenase deficiency. Those manifestations disappear when the drug is withdrawn (Leslie and Webster 1983).

(d) Pyrimethamine and Trimethoprim

These are diaminopyrimidine derivatives and they act by interfering with the synthesis of folinic acid of plasmodia and bacteria. The synthesis of folinic acid requires the incorporation of paraaminobenzoic acid (PABA)

into Folate followed by enzymatic reduction of Dihydrofolate by dihydrofolate reductase to Folinic acid.

The incorporation of PABA is inhibited by sulphonamides and sulphones, while the enzymatic reduction of folate by the diaminopyrimidines and biguanides. Combinations of sulphonamides or sulphones with the diaminopyrimidines offer special advantages in that they are synergistic in effect, low doses of both drugs are needed and the development of resistance is delayed.

The antimalarial effects of pyrimethamine are similar to those of proguanil. It is however more potent because it acts directly and has a longer half-life than proguanil. In general pyrimethamine is a potent drug of extraordinary effectiveness against erythrocytic parasites. Its chief characteristics are:- small once-weekly doses can effect complete suppression of quartan malaria and suppressive cure of falciparum malaria; by inhibiting sporogony, it prevents the transmission of malaria by mosquitoes; the drug is tasteless and therefore easy to administer to children and it is inexpensive. Its limitations is its slowness of action and its tendency to develop resistance in the field.

Pyrimethamine is used alone or in combination with the sulphonamides e.g. pyrimethamine and sulphadoxine (Fansidar), pyrimethamine and sulfalene (metakelfin) and with quinine in the treatment of chloroquine resistant strains of P. falciparum.

Pyrimethamine is slowly but completely absorbed after oral administration. The compound accumulates mainly in kidneys, lungs, liver and spleen and is eliminated slowly with a half-life in plasma of about 4 days. Concentrations that are suppressive for drug sensitive strains remain in the blood for about 2 weeks. At the recommended dosage of 25 mg once weekly, the drug alone causes no significant clinical toxicity except occasional skin rashes and depression of hematopioesis. Excessive doses produce a megaloblastic anaemia resembling that of folic acid deficiency; this reverses readily on discontinuation of treatment or on administration of folic acid (Leslie and Webster 1985, Bowman and Rand 1980, Bruce-Chwatt 1986).

(e) Proguanil and Analogues

They are biguanide derivatives and act mainly by inhibiting plasmodial dihydrofolate reductase enzyme.

Proguanil:- It is highly active against the primary tissue stages and also inhibit gametocyte development in the mosquitoes. It is also an effective blood schizontocide but is not used for clinical cure because it acts very slowly. Proguanil and its analogue chlorproguanil are valuable drugs for causal prophylaxis in falciparum malaria. Their major draw back is their tendency to provoke resistance.

Proguanil is well but slowly absorbed from the gastrointestinal tract peak plasma concentrations being reached in about 3 hours. About 75% of the drug is bound to blood proteins and high concentrations occur in the erythrocytes. The blood concentration rapidly diminishes and daily dosage is necessary. About 60% is excreted unchanged in the urine and about 30% is excreted as the active metabolite cycloguanil (Leslie and Webster 1985, Bowman and Rand 1980).

Toxic effects of proguanil are rare. Large doses may cause vomiting epigastric discomfort and renal irritation.

Chlorproguanil: It has similar actions and uses to proguanil, but is more active and has a longer duration of action. As a prophylactic agent, once weekly dosage is all that is required.

Cycloguanil embonate: It also has actions and uses similar to proguanil. Cycloguanil is injected intramuscularly as a suspension of fine particles in oil. The active drug is slowly released from the depot at the site of injection. A single intramuscular injection protects against sensitive strains of P. falciparum and P. vivax for several months. Unfortunately, the use of this compound often leads to the rapid emergence of resistant strains. Toxic effects include tendences at the site of injection and allergic-like reactions of the skin after a second injection. Prolonged use may cause a slight depression of haemopoiesis due to interference with folic acid metabolism. This effect may be corrected by giving folic acid tablets (Leslie and Webster 1985, Bowman and Rand 1980).

(f) Tetracyclines

The tetracyclines are highly effective against the exoerythrocytic and asexual blood stages of P. falciparum. Their activity against other species of human plasmodia has not been elucidated. Their relative slowness of action makes concurrent treatment with quinine mandatory. Tetracyclines are not recommended for prophylaxis

or general treatment of falciparum malaria. They should be reserved for use in the treatment of chloroquine and sulfonamide/pyrimethamine resistant strains of P. falciparum.

Tetracyclines interfere with protein synthesis in bacteria probably by preventing the formation of peptide linkages. Their prime mode of action against malaria parasites has not been determined.

Most of the tetracyclines are crystalline substances, amphoteric and of low solubility. The hydrochlorides are more soluble and are the compounds chiefly used in therapeutics. Their absorption takes place from all levels of the alimentary canal but only a proportion of the compounds is absorbed. Tetracyclines are freely excreted in both the bile and urine. Urinary excretion accounts for about 20% of an average oral dose.

The oral dosage occasionally used for the treatment of malaria (i.e. tetracycline 250mg, 4 times a day for up to 7 days) may cause nausea, vomiting and diarrhoea. The newer derivatives such as doxycycline and minocycline are given in smaller doses, their intestinal absorption is

increased and they are generally better tolerated. Tetracyclines sometimes have more serious adverse effect on the skin, mucous membranes and gastrointestinal tract. Tetracycline complexes may also be deposited in growing teeth and bones, consequently they should be avoided in women after the fourth month of pregnancy and in infants or young children.

(g) Qinghaosu. It is the antimalarial constituent extracted from the plant Artemisia annual in 1972 at the Chinese Institute of Material Medica. The medicinal herb has been used for centuries against malaria in China. Qinghaosu has been formulated and tested widely in China in both oral and parenteral forms. The drug is effective against asexual blood forms. There is no evidence of activity against tissue stages. Studies in both chloroquine sensitive and chloroquine resistant strains of P. berghei in mice suggest some degree of cross resistance with chloroquine (Bruce-Chwatt et al 1986). However studies in patients with P. falciparum do not show any evidence of chloroquine cross resistance (Bruce-Chwatt 1986).

The drug appears to be remarkably well tolerated in man. Occasionally, slight pain has been recorded at the site of parenteral injections.

Qinghaosu appears to be rapidly absorbed in animals. Using radiolabelled drug, peak concentrations are reached in 1 hour and the half life of the radioactive drug is about 4 hours (WHO 1984).

Results of clinical studies administering Qinghaosu have been reported on over 2000 malaria cases of which over 1500 were P. vivax and more than 500 P. falciparum infections (Bruce-Chwatt 1986). In addition 143 cases of chloroquine-resistant P. falciparum and 141 cases of cerebral malaria have been treated by Qinghaosu (Bruce-Chwatt 1986). For those clinical trials, the drug was formulated in the form of tablets, oil suspension and water suspension. The total dosage administered per adult was tablet 2.5 - 3.2 g; oil suspension 0.8 - 1.2g and watery suspension 1.2 g.

The conclusion made by several research groups was that Qinghaosu is a schizontocide with very low toxicity. It is effective against P. vivax, and both chloroquine-sensitive and resistant strains of P. falciparum respond well to the drug. The rate of recrudescence (up to 30%) occuring 15-30 days after the initial

treatment, is reduced when the drug is administered intramuscularly in the form of oil suspension (WHO 1984).

(h) Halofantrine: It is a phenanthrene-methanol derivative. Like mefloquine, the drug was very active in mouse screening tests, was not photo-toxic, and was effective in single doses in Aotus monkeys with falciparum malaria. Early studies indicated that the compound showed cross-resistance with mefloquine but recent studies in a rodent model and with clones of P. falciparum indicate that cross-resistance is not absolute (WHO 1984). Comparative in vitro studies carried out on P. falciparum isolates from Nigeria and other West African countries show a decreased sensitivity to mefloquine but full sensitivity to halofantrine (Oduola et al 1987). Both drugs are yet to be introduced for clinical use in West Africa.

1.5.1 Drug Resistance in Human Malaria

Drug resistance in malaria can be defined as the ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject (WHO 1965).

Although drug resistance embraces all species of malaria parasites and all acceptable dosages of blood or tissue schizontocides, gametocytocides and sporontocides, in practice it most commonly relates to the effects of the blood schizontocide, the 4-aminoquinolines especially chloroquine on falciparum malaria. This is so because all the other species of human malaria parasites are still fully sensitive to chloroquine (Bruce-Chwatt 1986).

Resistance of P. falciparum to chloroquine was first suspected in South America in the late 1950s and confirmed in 1959 in Thailand (Harinsuta et al 1962; WHO 1984). In the following years more countries in Eastern Asia and South America became affected. In Eastern Asia, resistance has continued to spread outwards from the IndoChina Penisula, and in South America countries like Brazil, Colombia, French Guinea, Guyana Suriname, Bolivia, Ecuador, Venezuela and Southern Panama are involved. Areas where chloroquine resistant P. falciparum has been reported so far is shown in figure 1.4.

In 1978, chloroquine resistant falciparum infections was first reported in non-immune visitors to Tanzania and Kenya (C.D.C. 1978, Spencer 1985) and have continued to be found among non-immune persons visiting East Africa. However, it was as from 1981 that chloroquine resistant falciparum malaria was found

Fig. 1.4: Areas where chloroquine resistant Plasmodium falciparum has been reported.

among semi-immune persons in this part of Africa (Onori 1984, Spencer 1985). Resistance has also been reported in Central Africa and is now spreading to West Africa. So far chloroquine resistant P. falciparum has been reported in ten West Africa countries namely Nigeria, Benin Republic, Togo, Ghana, Ivory Coast, Burkina Fasso, Gambia, Senegal, Portuguese guinea and Guinea (World Health Satistics 1988).

Olatunde (1977) and Eke (1979) were the first to report probable in vivo cases of chloroquine resistant P. falciparum in Nigeria. This was followed in 1985 by a report of treatment failure with chloroquine by Ekanem and recently a resistant case has been reported in a non-immune visitor to Nigeria by the Centers For Disease Control, Altanta, Georgia (C.D.C. 1987).

In vitro resistance to chloroquine and mefloquine has been reported at Ibadan (Salako and Aderounmu 1987).

1.5.2 Mechanisms of Drug Resistance in Plasmodia

In plasmodia, resistance to drugs appears to be a stable and transmissable character of the parasite. Although little is known about the interference of the host (i.e. man or mosquito) on the expression of such genes (WHO 1987).

Several mechanisms have been proposed to explain drug resistance of plasmodia (Beale 1980). However,

spontaneous multiple mutations at one or more gene loci, and selection of mutants due to drug pressure appears to be the important phenomenon involved. The resistance is transferred to the progeny by classical mendelian inheritance during sexual reproduction of the parasite in the anopheline vector. At present, there is no evidence of either the mutagenic effect of any clinically used anti-malarial drug on the plasmodia or confirmed evidence of any extranuclear transmission of resistance factors in the genus plasmodium (WHO 1984, WHO 1987).

There are differences in the rate of spontaneous mutation of different Plasmodium falciparum strains (Wernsdorfer 1981) and immunity can delay the appearance of resistance in a population. The level of immunity to P. falciparum in indigenous Africans is higher than that in people living in other high malarious area like South East Asia, South West Pacific and South America (WHO 1987). This may contribute to the late appearance of resistance to chloroquine in Africa and its initial detection in non-immune subjects in East and West Africa (Onori 1984, Ansdell et al 1974).

Several authors have reported that pre-existing resistance to chloroquine can facilitate the acquisation of resistance to other unrelated compounds

such as mefloquine (Peters and Robinson 1984, Merkli 1980). Also pre-existing pyrimethamine resistance may facilitates the production of chloroquine resistance (Powers and Jacobs 1969).

Chloroquine appears to be beneficial to the survival of chloroquine resistant parasites in that it enhances the infectivity of resistant parasites to vector anophelines. Also the resistant organisms appear to be biologically tougher than their drug sensitive parents (Ramkaran and Peters 1969, Wilkinson 1976, WHO 1984).

1.5.3 Methods of Assessing the Drug Sensitivity of P. falciparum

Both in-vivo and in-vitro methods can be used in assessing the response of P. falciparum to antimalarial drugs. Evaluating the sensitivity of P. falciparum to drugs helps in finding out if resistance is present in a particular area or group of people.

(a) The in-vivo Methods

In the in-vivo method, a standard radical cure treatment with the test drug is given. In case of chloroquine, 25mg of the base per kilogram body weight is administered over three days and the response of the plasmodial infection

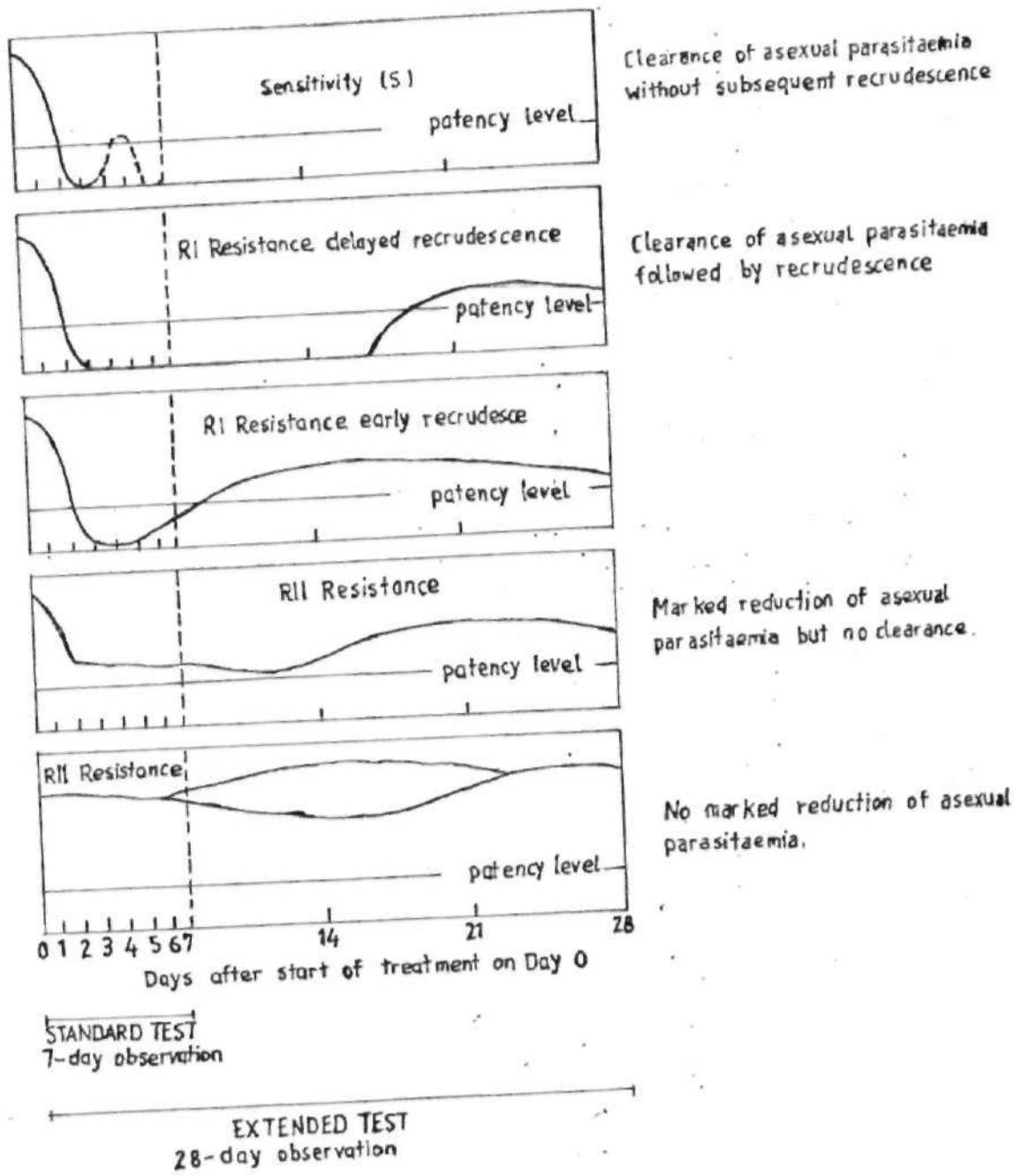
is assessed by determining the level of asexual parasitaemia over a period of seven days when using the "WHO Standard Field Test" or preferably over 28 days with the "WHO Extended Field Test" (WHO 1973, Wernsdorfer and Kouznetsov 1980). A modification of the Extended Field Test has been introduced by the African CCCD programme recently (Breman et al 1987; Khoromana et al 1986).

The Standard Field Test permits a quick observation of the occurence of RII and RIII response but the Extended Field Test is required to differentiate between S and RI responses. The interpretation of results may be summarized as follows and as shown in Figure 1.5 (WHO 1973):-

(i) If no asexual parasites are found by day 6 and parasites do not reappear by day 28, the parasites are sensitive (S).

(ii) If asexual parasites disappear for at least two consecutive days but return within the period of the test, reinfection having been excluded, the parasites are resistant at the Rl level.

Fig. 1.5: In vivo response to field test for sensitivity of falciparum malaria to chloroquine.



From "Chemotherapy of malaria(Bruce-chwatt 1986)

(iii) If the asexual parasitaemia does not clear but is reduced to 25% or less of the original pretest level during the first 48 hours of treatment, the parasites are resistant at the RII level.

(iv) If asexual parasitaemia is reduced by less than 75% during the first 48 hours or if it continues to rise, the parasites are resistant at the RIII level.

The performance and evaluation of the in-vivo test under field conditions is limited by factors such as the following (Spencer 1985):-

(i) Simple methods for the estimation under field conditions of the blood concentration of antimalarial drugs are lacking.

(ii) Self medication may occur either with the antimalarial being investigated or with another drug.

(iii) In endemic areas, the defaulter rate may be high due to logistic, cultural, economic migratory or other obstacles.

(iv) The patient's immune response contributes to the result thus the same parasite may appear sensitive in a semi-immune but resistant in a non-immune patient.

(v) Also in malarious areas, reinfection is possible in the Extended Field Test. Therefore it is difficult to distinguish reinfection from recrudescence (Spencer 1985).

(b) The In-vitro Methods

The in-vitro methods may help in overcoming some of the above limitations and offer a better means of comparism with the in-vivo results. It is also a convenient method for detecting the emergence of drug resistance.

The in-vitro methods may consist of the macrotechnique, the microtechnique in short term culture or the microtechnique in long term culture.

The macrotechnique was developed by Rieckman et al in 1968, it's major shortcoming is the need for venopuncture to collect larger amounts of venous blood. It has been replaced by the short term culture microtechnique for wild field use (WHO 1987, WHO 1984).

The microtechnique in short term culture is a modification of the macrotechnique that requires only a 100ul specimen of blood. It measures the extent to which the maturation of ring forms to normal schizonts

is inhibited after incubation of parasitized blood cells in a suitable medium at various drug concentrations in a candle jar for a period of 24 to 30 hours. It uses the inhibition of schizont maturation as the indicator of drug effect (Wernsdorfer 1980, WHO 1984, WHO 1987, Rieckman et al 1978).

The microtechnique in long term culture uses the inhibition of parasite multiplication and reinvasion as the indicator of drug effect and it involves the continuous incubation of the parasites in a suitable medium at various drug concentrations for about 48 hours or up to 72 hours (Thaithong, 1983; WHO 1984 Trager and Jensen 1976).

All of the above methods make use of either the microscopical analysis to determine the effect of the drug on the parasite or a rapid semi-automatic microdilution method that measures the incorporation of radioactive precursors into specific parasite molecules (WHO 1984, Desjardins, 1979).

1.5.4 Treatment of Chloroquine resistant Falciparum malaria.

If P. falciparum malaria is resistant to chloroquine, recourse must be made to alternative compounds. The choice of drug depends largely on the absence or presence of resistance to the possible alternatives.

Amodiaquine, though a 4-aminoquinoline is more effective than chloroquine against certain strains of resistant P. falciparum (Spencer 1985, Bruce-Chwatt 1986). It is presently used in the treatment of chloroquine resistant falciparum malaria in East Africa (Watkins et al 1988).

The following sulfonamide/antifolate combinations have been used alone in the treatment of a mild to moderate acute attack of chloroquine resistant falciparum malaria in semi-immune persons:- Sulfadoxine 1500mg and Pyrimethamine 75mg given as a single dose. Another combination is that of Sulfalene 1500mg and Pyrimethamine 75mg also to be given as a single dose. The above drugs are available under the trade names of Fansidar and Metakelfin respectively (a tablet of each contains 500mg of the sulfonamide and 25mg of Pyrimethamine). Since they are slow acting blood schizontocides, Quinine is to be preferred in case of severe falciparum malaria. The sulfonamide/ antifolate combinations can also be used together with Quinine (Spencer 1985, Bruce-Chwatt 1986).

Quinine alone or in combination with other drugs is widely used particularly in severe infections. A course consisting of 1800 mg Quinine orally in 3 divided doses daily for 1 week may be adequate . The

above may be followed by 50mg Pyrimethamine daily for 2 days and 25mg Dapsone daily for 3 weeks (Bruce-Chwatt 1986). Best results have been obtained recently by using a short regimen consisting of oral Quinine 600mg 3 times a day for 2 or 3 days followed by 3 tablets of Fansidar or Metakelfin given on the first day or at the end of the Quinine course. Intravenous Quinine may be used on the first day in case of severe malaria (WHO 1984; Bruce-Chwatt 1986). Quinidine, another cinchona alkaloid is 2-3 times as potent as Quinine as a blood schizontocide and can be used at the same dose in place of quinine. However cardiac function must be monitored (Were 1987).

P. falciparum infections resistant to chloroquine and sulfonamide/pyrimethamine combinations may be treated with oral Quinine 600mg three times daily for 5-7 days simultaneously with or followed by Tetracycline 250mg four times daily for 7 days. Doxycycline (0.2g daily) and minocyclin (0.1 to 0.4g daily) are equally effective (Bruce-Chwatt 1986, WHO 1984, Rieckmann 1987).

Qinghaosu, a new drug alone or in combination with other drugs can also be used in the treatment of severe chloroquine resistant falciparum malaria (WHO 1984). Mefloquine may be used alone or in combination with other drugs for the treatment of chloroquine resistant

or multidrug resistant falciparum malaira. Resistance to the drug has been reported in some regions (Jacobs et al 1987, Spencer 1985) also in vitro resistance to Mefloquine has been reported in Ibadan, Nigeria (Salako and Aderounmu 1987). The drug is yet to be introduced for medical practice in West Africa. Fansimef (a combination of 280 mg Mefloquine base, 500mg sulfadoxine and 25mg pyrimethamine) is presently on clinical trial with the hope that it will be effective against multidrug resistant P. falciparum and also delay the development of resistance to Mefloquine (Desouza et al 1987).

Attempts of prolonging the usefulness of chloroquine in areas of resistance by combining it with other drugs were proved abortive with tetracycline and erythromycin (Watkins et al, 1988; Phillips et al 1984). Similar attempts with a calcium antagonist (Verapamil) and a microsomal mono-oxygenase inhibitor (copperlysine complex) though still at the experimental stage are showing good promise (Farago 1987, Rabinovich et al 1987).

Halofantrine, a new drug may also be used in treating chloroquine resistant P. falciparum malaria. In-vitro test carried out on West Africa isolates of P. falciparum show Halofantrine to be more effective than Mefloquine in the region (Oduola et al 1987).

AIMS AND OBJECTIVES OF THE STUDY

Malaria remains a formidable global problem. It is still one of the major killing disease of the tropics especially in children. The effects of its morbidity upon social and economic development are staggering (Pinder 1973).

Chemotherapy has been of great importance in the treatment of malaria. However this was somewhat briefly over-shadowed by the developments of residual insecticides which were thought to eradicate the mosquito vector (Bruce-Chwatt 1986). This was not feasible especially in tropical Africa because of socio-economic factors, the emergence of resistance to residual insecticides and the high stability of transmission in this area. The current interest in tropical Africa is therefore aimed at reducing the burden of the disease by judicious use of chemotherapeutic agents. This again is on the verge of being hampared by the development of parasite resistance to anti-malarial drugs (Bruce-Chwatt 1980).

In human malaria, resistance of other plasmodia specie is not yet a problem since they are still fully sensitive to chloroquine (Bruce-Chwatt 1986). The resistance of P. falciparum to chloroquine has, however,

become a global concern mainly because of the virulent nature of this organism and because of the importance or efficacy of chloroquine in treating falciparum malaria. Hence there is need for continuous evaluation of the status of chloroquine in falciparum malaria. This is not only of Academic importance but will also help in directing government policies relating to malaria control.

Resistance of P. falciparum to chloroquine which was first reported in South America and East Asia in the late 1950s (Harinasuta et al 1962, WHO 1984) has spread from those continents to Africa. It was first reported in East Africa then in Central Africa and now West Africa (Spencer 1985, Onori 1984, World Health Statistics 1988). In-vivo and in-vitro resistance of P. falciparum to chloroquine has been confirmed in Ghana (WHO 1987), Cameroon (Sansonetti et al 1985) and Benin Republic (Le Bras et al 1986).

Earlier reports of chloroquine resistant P. falciparum in Nigeria (Olatunde 1977, Eke 1979) were not accepted as definite proofs because the criteria established by WHO for confirmation of resistance had not been fully respected (Onori 1984). Recently, Ekanem (1985) had reported another case of

treatment failure. Also in-vitro resistance has been reported from Ibadan (Salako and Aderounmu 1987). So far all cases of chloroquine resistance reported in Nigeria had been in the Southern part of the country.

Midala et al (1988) in an in-vivo study of 1985 reported a delayed clearance of P. falciparum to chloroquine in Zaria. The present work is therefore aimed at determining the in-vitro and in-vivo sensitivity of P. falciparum to chloroquine in Zaria, an area in the Northern part of Nigeria, using WHO Standard Procedures (1973). In addition, this study presents data on the comparative in-vitro sensitivity of P. falciparum to chloroquine, Mefloquine and Quinine in Zaria Urban area.

CHAPTER TWO

MATERIALS AND METHODS

2.1. Materials

(a) Chloroquine sulphate tablets (Nivaquine ^{R}), each containing 150 mg of chloroquine base. They were supplied by May and Baker (Nigeria) Limited. All had been recently manufactured and were from the same batch No. 047, due to expire in 1990.

(b) Sulphadoxine/Pyrimethamine tablets, (Fansidar^{R}) each contains 500mg of sulphadoxine and 25 of Pyrimethamine, Manufactured by Roche Pharmaceutical Company, Switzerland. They were bought from Dasauki Chemist, Samaru Zaria. All had been recently manufactured and were from the same batch No. B5006 MFD 03, 86, due to expire in 1992.

(c) Giemsa Stock Solution was obtained from the National Malaria and Vector Control Division, Federal Ministry of Health, Lagos.

(d) Chloroform, manufactured by May and Baker.

(e) Eosin powder obtained from Sigma Company(Nigeria) Limited.

(f) Hydrochloric acid, obtained from BDH Chemicals Ltd. England

(g) Dill-Glazko Reagent (see appendix 1)

(h) KH_2PO_4 ;- Potassium dihydrogen orthophosphate, obtained from BDH Chemical Ltd., Poole England.

(i) $Na_2 HPO_4$:- Disodium Hydrogen orthophosphate, obtained from BDH Chemical Ltd, England.

(j) Phosphate buffer PH 7.2 (see appendix 2)

(k) Methanol R-grade (Re sublimed), manufactured by May and Baker Ltd., Dagenham,England.

(l) Xylene (sulphur free) manufactured by May and Baker Ltd.

(m) Ethanol absolute, manufactured by BDH Chemical Ltd., England.

(n) Microscope, Reichert U.S.A. Model 150/160 obtained from C.D.C. Atlanta Georgia, U.S.A.

(o) Microscope slides 76 x 26mm, obtained from the National Malaria and Vector Control Division, Federal Ministry of Health Lagos.

(p) WHO Standard Micro-test Kits (1 Kit A, 3 Kits B) were obtained from The WHO Regional Office, Manila, Phillipines. The Batch numbers and manufacturing dated of the in vitro test plates were chloroquine, C-92 31/6/1986, C-17 A 20/5/87, and C-18A 4/6/1987. Mefloquine M-79 25/6/1986. M-08A 4/6/1987, M-07A 20/5/87 and Quinine Q-21 3/6/1987.

(q) RPMI-1640 Powdered Media, Batch No. 6101166, manufactured by Gibco Limited, P.O. Box 35, Paisley, Scotland. It was obtained from WHO Geneva. see appendix 3 for the preparation.

(r) Incubator

(s) Electric Shaker.

2.2 Methods

(a) Screening of Patients

The study was conducted at the Paediatric Out-Patient Department of the Ahmadu Bello University Teaching Hospital Zaria, between February 1987 to August 1988.

Patients between the ages of 6 months and 7 years with clinical features suggestive of malaria were selected from the above department and referred to the malaria clinic that had previously been established within the same hospital. The protocol of the study was approved by the Medical Ethical Committee of the Faculty of Medicine, A.B.U. Teaching Hospital, Zaria.

At the Malaria Clinic, thick blood films were made for screening by finger prick and stained with 4% Giemsa solution for 10 minutes. Also the urine of all referred patients was tested for 4-amino-quinolines, using the Dill-Glazko urine test method (see appendix 1). The stained slides were dried and examined for malaria parasites.

Children were taken into the study if they satisfied the following criteria for enrolment:-

(i) If they were found to have pure P. falciparum

infection , with parasitaemia between 1000 and 95,000 parasites per mm^3 of blood.

(ii) If their urine was negative for 4-aminoquinolines (using the Dill-Glazko test).

(iii) If they had denied taking any antimalarial drug for the past 2 weeks

(iv) and, if they were residents of Zaria Urban area.

All excluded children were sent back to the Paediatric Out-patient Department for treatment, with a note indicating the outcome of the screening test. On final selection for inclusion in the study, the aim of the study was carefully explained to the mothers of the children and their consent sought.

(b) In-vivo Methods

The serial number, name, age, weight, sex and exact address including the state of origin of all children taken into the study were recorded (see appendix 4 for the in-vivo record form). All children were assessed clinically by taking the body temperature and enquiring from each mother of the child about presence or absence of diarrhoea, pruritus or vomiting. Also parents of the children were questioned about the presence (1) or absence (0) of fever ; loss of appetite and physical activities over the past 48 hours. The findings

were recorded as follows 0 + 0 if there was no fever and the child was well, 1 + 0, if the child had fever and was well etc (see appendix 4).

After recording the above on day 0, duplicate thick and thin blood films were obtained by finger prick just prior to the administration of chloroquine. The children were then given 25mg/kg of chloroquine base over three days as follow:- 10mg/kg on days 0 and 1, and 5mg/kg on day 2. The drug was personally given by a member of the research team and each child was observed for 30 minutes to ensure that the drug was not vomited. Children who vomited were given a second dose, and those who vomited more than twice were excluded from the study. To reduce vomiting, each child was given a small meal of biscuit and milk prior to drug administration.

The children were followed up daily for seven days and once weekly thereafter up to day 28 (i.e. on day 0 to day 7, and on days 14, 21 and 28). Patients who had parasitaemia after day 7 were treated with Fansidar (i.e. sulphadoxin-pyrimethamine combinations) as 35mg/kg of the sulphadoxine, and were followed up as described above for 28 days.

On each follow-up visit, the patients were assessed clinically and duplicate thick and thin blood films were made for each child. Also the urine test for

4-aminoquinolines were repeated using the Dill Glazko method.

Thick and thin blood films were prepared as follows:- The pulp of the finger was thoroughly cleaned with a disposable swab and allowed to dry. It was then pricked with a sterile lancet and the finger was gently squeezed until a drop of blood exuded. This was gently removed and the second drop transferred to the centre of a well cleaned microscope slide and a thin film was made by spreading the drop with the parallel edge of another clean slide set at an angle of 45^{o} along the first slide.

At the free end of the same first slide, another larger drop of blood was placed and a thick film was prepared by evenly spreading the drop with the corner of another slide to form a circular patch of moderate thickness. Each slide so prepared was labelled, indicating the patients serial number and date, and allowed to air dry.

(i) Staining for definitive parasite counting

The slides were stained with 2% Giemsa solution for 30 minutes. The 2% Giemsa solution was prepared by diluting 4 ml of Giemsa stock solution in a 500 ml measuring cylinder with phosphate buffer (PH 7.2) to give 200 ml. This was then transferred to the staining jar.

The thin films were fixed in methanol. The blood slides were arranged on a staining rack and immersed in 2% Giemsa solution and allowed to stain for 30 minutes. After this, the slides were taken out, gently washed twice in phosphate buffer solution (PH 7.2) and spread out to air dry.

(ii) Examination of blood films and counting of parasites

Species identification was confirmed from both thick and thin blood films of day 0 and patients with mixed infections were excluded from the study. The slides were examined using a standard X 100 oil immersion objective and X 10 eye pieces, achieving a total magnification of X 1000. For the counting of parasites on thick blood films: "the farmer ploughing his field" technique was used while the "battlement" technique was applied to examine the thin films (WHO 1984). A slide was considered negative or the possibility of mixed infection ruled out only after about 100 microscope fields of both the thick and thin films had been examined.

To determine the parasite density, white blood cells and asexual parasites were counted simultaneously from the thick films on two hand tally counters. Counting was stopped when the parasite count reached 500 or the leucocyte count

1000 which ever figure was reached first. However, if either of the above figure was reached before the count of a particular field was completed. Counting continued until all the parasites and leucocytes in that particular field had been counted.

(iii) Determination of Parasite Density (PD)

For the definitive count, the parasite density of each patient for each day was determined by dividing the product of the parasite count and 6,000 by the leucocyte count as follows:-

$$\frac{\text{No. of parasites counted} \times 6000}{\text{No. of leucocytes counted}} = \text{parasite count per } mm^3 \text{ of blood.}$$

(iv) Determination of Parasite density Index (PDI)

The parasite density of each patient on each day was rounded off to the first two digits and was classified as parasite density index (PDI) according to Bruce-Chwatts criteria of classification as follows:- (WHO 1984)

Parasite Count per mm^3 of blood	Class
100 or less	1
101 - 200	2
201 - 400	3
401 - 800	4
801 - 1600	5
1601 - 3200	6
3201 - 6400	7
6401 -12800	8
12,801 -25,600	9
25,601 or more	10

(v) Determination of Mean Parasite Density

The mean parasite density for each day was determined by dividing the sum of the parasite densities of all the patients on that day by the total number of patients studied. The mean parasite density on day 0 was taken as 100% and the mean counts on subsequent days were expressed as percentages of this figure (see table 3.1).

(vi) Determination of Mean Parasite Clearance Time (MPCT)

The MPCT for the total number of in-vivo cases (excluding those with chloroquine resistance) was determined as follows:- For each day, the number of patients whose parasitaemia had cleared was multiplied by the number of days needed for the infection to clear. The products obtained was summed up and divided by the total number of cases to give the MPCT.

For the 53 cases in our study whose parasitaemia cleared within the first seven days and did not reappear, the products were obtained as follows (see table 3.1). On day 1, no patient was cleared of parasitaemia i.e. the product is 1 x 0 = 0.

On day 2, 4 patients were cleared of their parasitaemia i.e. the product is 2 x 4 = 8

On day 3, 32 patients were cleared, and the product is 3 x 32 = 96

On day 4, 9 patients were cleared, and the product is 4 x 9 = 36

On day 5, 6 patients were cleared of their parasitaemia therefore the product is 5 x 6 = 30

On day 6, no patient was cleared of parasitaemia, the product is 6 x 0 = 0

On day 7, 2 patients were cleared of their parasitaemia, the product is 7 x 2 = 14

The sum of the products obtained for the 7 days is 0+8+96+36+30+0+14 = 184. When divided by the total number of cases, the MPCT will be 184/53 = 3.47

The standard deviation was calculted to be ± 1.04; therefore the MPCT for the study is = 3.47 ± 1.04 days.

(c) The In Vitro Method

The microtechnique involving the short term culture of parasitized blood at different drug concentrations was used for the in vitro sensitivity test (WHO 1984, Bruce-Chwatt 1986). The instructions for the use of the WHO test kit A and B were followed (Bull. Wld. Hlth. Org. 1971) and the following items were kept under refrigeration at 4°C until required for use (i) HEPES solution (ii) $NaHCO_3$ solution and (iii) predosed plates. The RPMI 1640 liquid media was prepared from the powder as described in Appendix 3 and kept in the deep freezer at -20°C until required for use.

The whole in vitro procedure from the begining to the stage of incubation was executed under strict aseptic conditions. Fresh growth medium was prepared each day just prior to incubation as follows:- 1ml of 7.2% HEPES solutions and 1ml of 2.4% $NaHCO_3$ solution were added to 10ml of the liquid RPMI 1640 media and the mixture was filtered through a millipore filter.

A sterile 2ml pipette was used to transfer 1.8ml of the so prepared growth medium into a sterile falcon tube. To these, were added 200µL of fresh heparanized capillary blood obtained by finger prick prior to drug administration on day 0. Thus 2ml of medium/blood mixture were finally available from each patient. The falcon tube was closed with a sterile cap and gently agitated to suspend the blood cells.

The seal strips of the appropriate columns of the predosed plates were removed, and 50µL of the medium blood mixture was transferred into each well using a 50µL Eppendorf Pipette with a sterile tip in a descending order, starting from well A (which is the control) and ending at well H with the highest concentration of the test drug. One row of 8 wells (A to H) of each test drug (ie chloroquine, mefloquine and quinine) were allotted to the individual blood specimen of a child. A new pipette tip was used whenever a new row of wells (A to H) was to be filled with the medium/blood mixture

in order to avoid drug contamination of the control well A. For each drug, the concentrations of wells A to H were distributed as follows:-

Chloroquine

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
	PMOL											
A	0											
B	1											
C	2											
D	4											
E	5.7											
F	8											
G	16											
H	32											

Well A is the control; Wells B-H represent a chloroquine concentration line based on a geometrical progression of 2^0; 2^1; 2^2; 2^3; 2^4; and 2^5 Pmol, with an intermediate concentration at $2^{2.5}$ (Well E).

Mefloquine

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
	PMOL											
A	0											
B	0.5											
C	1											
D	2											
E	4											
F	5.7											
G	8											
H	16											

Well A is the control; Wells B-H represent a mefloquine concentration line based on a geometrical progression of 2^{-1}; 2^{0}; 2^{1}; 2^{2}; 2^{3}; and 2^{4}pmol, with an intermediate at $2^{2.5}$ (Well F)

Quinine

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
	PMOL											
A	0											
B	4											
C	8											
D	16											
E	32											
F	64											
G	128											
H	256											

Well A is the control; Well B-H represent a quinine concentration line based on a geometrical progression of 2^2; 2^3; 2^4; 2^5; 2^6; 2^7; and 2^8.

After filling the predosed wells for all the patients enrolled on a particular day, the lids were replaced on each plate and the patient's serial number was inscribed on the lids directly over the appropriate column. The culture plates were then gently shaken on an automatic shaker to dissolve the drug deposit at the bottom of the wells.

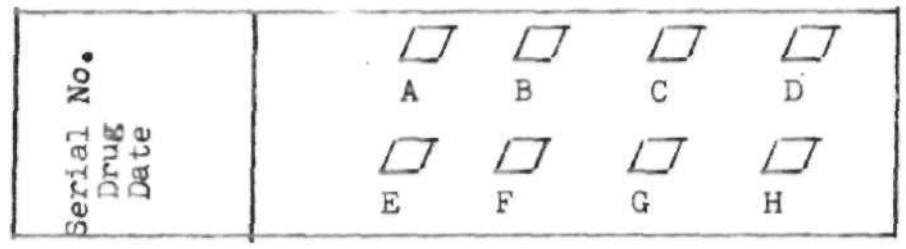
Finally, the plates were placed in a desiccator jar containing 2 paraffin candles. The candles were lit and the lid of the jar was partially closed leaving only a small opening. The jar was tightly closed just before one flame went off. The air tight jar was then placed in an incubator at a temperature of $37.5 \pm 0.5^{o}C$ for 24 hours.

At the end of 24 hours, the jar was taken out and about 30UL of supernatant culture medium from one of the control wells (i.e. Well A) was removed and a thick film was made from the blood deposit and allowed to air dry. The thick film was stained in 1.5% giesma solution for 30 minutes and examined for schizont maturation. The samples were said to be ready for harvest if there were up to 20 schizonts per 200 asexual parasites (ie. 10% schizont maturation) in the control well. If however, the schizont maturation was less than 10%, the plates were placed back into the

air tight jar and incubated for a longer period of time. However, all samples were harvested at the end of 30 hours.

Harvest of Cultured Blood Samples

After incubation, about 30-40 UL of the supernatant culture medium were removed from each well with a capillary tube fitted with a bulb, a fresh capillery tube was used for each single well. Thick blood films were made from the sediment of each well on a microscope slide. A row of 8 blood films (from wells A to H) of each test drug from a patient was prepared on a single slide and labelled with the patients serial number, name of drug and date as shown below.



The prepared thick films were left to air dry at room temperature for about 2 days so as to ensure adequate adherence to the slides. They were then stained in 1.5% Giemsa solution for 30 minutes after which they were taken out and allowed to air dry.

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Counting of Schizonts and Presentation of Results

After drying the stained slides, the number of schizonts containing 3 nuclei or more were counted against 200 asexual parasites in all the test wells.

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The schizont maturation in the individual wells were expressed as a percentage of maturation occuring in the control well A using the following formula.

Control	Samples with drug under Test (was repeated for each test well)	
No. of schizont per 200 parasites after incubation	No. of schizonts per 200 parasites after incubation	% of schizonts relative to control sample (control sample 100%)
M	Z	a = Z/M x 100

Also the mean number of schizonts occuring in each test well for the total number of cases was calculated and the mean percentages of schizont maturation and inhibition in the individual wells were expressed as a percentage of that occuring in the control wells A. (see tables 3.5, 3.6 and 3.7). On a graph, schizont inhibition was plotted against the concentration of drug in the test wells and the log-probit regression line was obtained. The EC99 and EC50 i.e. the concentration of drug required to inhibit 99% and 50% of schizont maturation was calculated from the graph (see figures 3.2, 3.3 and 3.4).

According to WHO (1987), there is satisfactory response to chloroquine if there is complete schizont inhibition at 4Pmol/well (i.e. 0.8 x 10^{-6} mol/L blood) or below. Schizont growth at 8 Pmol/well (1.6 x 10^{-6} mol/L blood) or more is an indication of resistance to

chloroquine. For mefloquine, there is satisfactory response if there is complete schizont inhibition at 32 Pmol (6.4×10^{-6} mol/L blood) or below. Schizont growth at 64 Pmol (12.8×10^{-6} mol/L blood) or more is an indication of resistance. For quinine, Schizont growth at 256 Pmol/well (51.2×10^{-6} mol/L blood) or more is an indication of resistance, while complete schizont inhibition at 128 Pmol/well (25.6×10^{-6} mol/L blood) or below indicates satisfactory response to quinine.

CHAPTER 3

RESULTS

3.1 Screening

The study started in February 1987 and lasted up to August 1988. A total of 2,557 patients were screened for malaria parasites within the period. However due to political unrests in March and April 1987, many patients got lost to follow-up and useful results started coming in as from July 1987. Between July 1987 and August 1988, a total of 2,068 patients were screened. Of these, 178 (8.5%) were found to be positive for malaria parasites, and considered for enrollment. However, of the 175 patients, 20 (11.4%) were dropped because of the presence of 4-amino-quinolines in their urine and 49 (28%) due to either too low or too high levels of parasitaemia. 4 patients (2.3%) had mixed infections with P. malariae. A total of 102 (58.2%) cases of P. falciparum infections were finally enrolled in the study.

3.2 The Patients

Of the 102 cases that were finally taken into the study, only 65 (63.7%) completed the 28 day study protocol and of these, 6 had blood smears of poor quality at one time of the follow-up or the other, thus only 59 cases (57.8%) had complete *in vivo* results. Of the other 37 patients, 34 (33.3%) had defaulted and 3 (2.9%) died. One died on day 0 due to malaria with severe anaemia. She was admitted for close observation and died while on admission. The other two died of kwashiokor between day 14 and 21.

Of the 59 cases that completed the study, 29 (49.2%) were male and 30 (50.8%) female. The children were aged between 6 months and 7 years with a mean age of 3.5 ± 1.25 years. Their weight ranged from 6.8 kg to 24 kg with a mean of 12.16 ± 3.93 kg.

3.3 In vivo Results

(i) Parasitological response:-

The distribution of parasite densities (expressed arithmetically and by Bruce-Chwatt Parasite Density Index (PDI) for the 59 cases that completed the in vivo study is shown in table 3.1. In none of the cases was the parasitaemia on days 2 and 3,25% or above that of day 0. Table 3.2 shows that there was no correlation between the distribution of mean parasite densities on day 0 and the various age groups.

Of the 59 cases that completed the in vivo study, 53 (90%) were fully sensitive to chloroquine. The parasitaemia of the 53 cases disappeared within 7 days and did not reappear throughout the observation period. Cases No. 2225 and 2469 had parasitaemia on day 28 but they were taken as reinfections since their isolates were fully sensitive to chloroquine in vitro (see table 3.5). The parasite density range, mean parasite density and Parasite Density Index for the 53 sensitive cases are shown at the end of table 3.1 and figure 3.1. The Mean Parasite Clearance time

Table 3.1 Parasite Densities (Expressed Arithmetically And By Bruce-Chwatt Parasite Density Index) of 59 Cases Followed up in an *In vivo* Chloroquine Sensitivity Trial of *P. falciparum* in Doka Urban Area, June 1987 - August 1988.

Serial No.	Sex	Age months	Wt kg	Parasite Densities of asexual forms of P. falciparum per mm^3 of blood																																Outcome
				Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 10		Day 14		Day 21		Day 28										
				PS	PDI	PS	PDI	PS	PDI	PS	PDI	PS	PDI	PS	PDI	PS	PDI	PS	PDI	PS	PDI	PS	PDI	PS	PDI										
625	F	60	17	61,000	10	37000	10	840	6	180	3	24	1																							Sensitive
643	M	15	7.2	71,000	8	12500	8	72	1	12	1																									Sensitive
664	M	48	15.3	50,000	10	10000	8	350	3	260	3	48	1																							Sensitive
681	F	18	7.5	15,600	9	4800	7	3600	7	3500	7	3300	7	-		-		2570	4	72	1	1700	1	-												Resistant RII
683	M	84	24	21,000	10	2200	6	350	3	170	2	140	2	12	1	6	1																			Sensitive
685	M	84	16	12,000	9	2400	6	1700	6	180	2	110	2	12	1	6	1																			Sensitive
736	M	36	10.2	16,000	9	3100	6	1900	6																											Sensitive
744	M	48	10.5	34,000	10	10000	8	48	1																											Sensitive
837	F	84	13.5	46,000	10	19000	9	640	4	190	2	36	1																							Sensitive
866	M	10	7.5	18,000	9	10000	8	42	1																											Sensitive
894	M	12	8	5,000	6	3200	7	1900	6	12	1																									Sensitive
902	M	24	11	2,400	6	820	4	120	2	12	1																									Sensitive
903	F	12	8	14,000	8	3600	7	220	3																											Sensitive
907	F	36	9	2,400	7	1400	6	560	4																											Sensitive
920	M	27	12	103,000	10	36000	9	350	4																											Sensitive
951	F	30	9	34,000	8	470	5	23	1	00		00		6	1	12	1	12	1	00		00														Resistant RII
959	M	24	12	21,000	9	520	4	140	2																											Sensitive
974	F	36	24	67,000	10	44000	10																													Sensitive
982	F	48	10	76,000	10	1,80	9	280	3																											Sensitive
984	F	72	18	38,000	10	6500	8	24	1																											Sensitive
1014	F	72	18	57,000	10	3900	7	440	4																											Sensitive
1053	F	36	14.5	1,200	5	620	4	24	1																											Sensitive
1057	F	60	18	85,000	10	9500	8	1600	5	24	1																									Sensitive
1095	F	24	10.5	1,800	6	78	1																													Sensitive
1096	M	36	10	45,000	10	7800	8	3500	7	760	4																									Sensitive
1098	F	60	14	50,000	10	45000	10	1700	6	83	1																									Sensitive
1104	M	6	7	9,800	8	2600	6	130	2																											Sensitive
1183	F	18	8	15,000	9	1800	6																													Sensitive
1255	M	60	13.8	2,300	6	760	4	65	1																											Sensitive

Table 3.1 (Cont'd.)

Serial No.	Sex	Age months	Wt kg	Day 0 PD	Day 0 PDI	Day 1 PD	Day 1 PDI	Day 2 PD	Day 2 PDI	Day 3 PD	Day 3 PDI	Day 4 PD	Day 4 PDI	Day 5 PD	Day 5 PDI	Day 6 PD	Day 6 PDI	Day 7 PD	Day 7 PDI	Day 14 PD	Day 14 PDI	Day 21 PD	Day 21 PDI	Day 28 PD	Day 28 PDI	COMMENTS
1302	M	30	9	4500	7	2300	6	130	2	12	1	00		00		130	2	840	5	FDR		FDR		0		(early recruit-ascaris
1326	F	20	9.5	2800	6	580	4	6	1																	Resistant RI
1354	F	7	7	13000	9	11200	8	3200	5	290	4	370	3	72	1	230	2	300	3	1600	5	FDR		00		Sensitive
1390	F	72	19	11000	8	32200	10	130	2																	Resistance RII
1419	M	72	17	9500	8	3600	7	42	1																	Sensitive
1423	M	48	12	5200	7	2600	7	160	2																	Sensitive
1504	M	34	17	20000	10	12000	8	42	1																	Sensitive
1587	F	24	10.5	6400	7	4400	6	43	1																	Sensitive
1627	F	60	17	9600	7	2400	6	43																		Sensitive
1631	M	48	13	20000	8	4200	6	410	4	47	1	13	1													Sensitive
1651	F	48	14	18000	8	480	3	16	1																	Sensitive
1653	M	30	9.2	5700	7	160	2	13	1	3	1															Sensitive
1830	M	24	10.5	18000	9	12000	8	36	1																	Sensitive
1935	M	11	8.4	35000	10	20000	8	170	3	24	1	12	1													Sensitive
2141	M	12	7.5	35000	10	11000	8	2100	5	290	3	100	2													Resistance RII
2171	M	36	10	64000	10	7400	8	3700	7	120	3	77	2	10	1											Sensitive
2225	F	48	16	37000	10	27000	9	420	3																	(Late relapse-ascaris)
2230	F	54	18	2700	5	470	3																			Resistance RI
2243	F	48	14	96000	10	150000	10	76	1																	Sensitive (Reinfection)
2298	M	36	6.3	41000	10	38000	10	510	4																	Sensitive
2381	M	24	9	7100	5	250	3	6	1																	Sensitive
2436	F	72	17	40000	10	16000	9	620	4																	Sensitive
2457	F	24	11	15000	9	2200	6	16	1																	Sensitive
2469	F	36	11	30000	10	7700	8	1200	7	48	1															Sensitive
2481	M	60	16	9800	8	1400	5	16	1																	Sensitive (Reinfection)
2482	F	72	18	58000	10	26000	10	450	4																	Sensitive
2516	M	72	16	15800	9	3500	7	470	4																	Sensitive
2525	M	37	8	61000	10	52000	10	360	3	24	1															Sensitive
RANGE OF PD				2800 TO 64,000		26 TO 52,000		6 to 1,200		6 to 750		12 to 140		12-12		6 to 6										
MEAN PD				23,100		11,700		570		130		59		12		6										For the Sensitive cases only
MEAN PDI				9.6		6.6		2.3		1.4		1.4		1		1										
MEAN PD AS % OF DAY 0				100%		50.6%		2%		0.6%		0.2%		0.05%		0.02%										
MEAN PARASITE CLEARANCE								1.17 - 1.96 days																		

FDR = Retreated with chloroquine

Table 3.2 Distribution of Mean parasite densities on Day 0 According to age groups of Cases of P. falciparum in an in vivo investigation of chloroquine sensitivity In Zaria Urban Area, 1987/88

Age Group (Years)	Frequency	Mean Weight (kg)	Mean Parasite Densities (mm^3)	Standard Deviation	Parasite Density Range
0.5– 1	11	7.5	22,530	± 14,400	6,960 to 49,000
1.05-2	14	9.7	8,600	± 7,380	1,114 to 43,000
2.05-3	20	10.8	22,660	± 21,270	1,200 to 64,000
3.05-4	13	13.5	35,500	± 29,870	3,890 to 90,000
4.05-5	8	15.8	30,870	± 14,300	2,250 to 95,100
5.05-6	8	17	24,200	± 23,910	6,530 to 58,000
6.05-7	4	17.1	46,030	± 30,780	22,880 to 90,950

Fig. 3.1: Mean Parasite Densities per mm^3 (log scale) of 53 sensitive cases of P. falciparum after treatment with 25mg/kg chloroquine in Zaria urban area 1987/88.

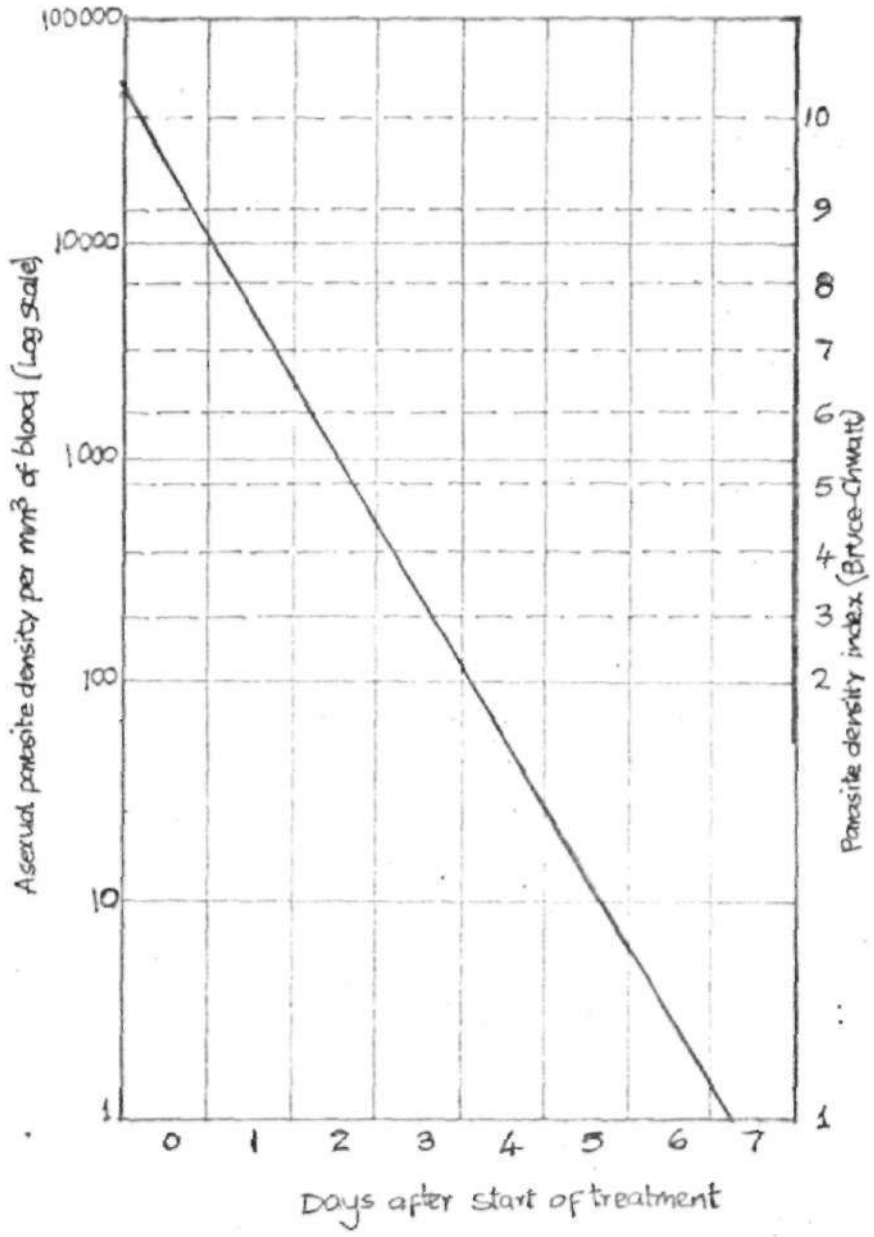


Table 3.3 Distribution of Parasite Densities of 6 Cases of P. falciparum malaria, resistant to chloroquine (25mg/kg) followed up for 28 days in an in vivo investigation in Zaria Urban Area, 1987/88.

Serial No.	Sex	Age months	Wt. kg	Parasite Densities of P. falciparum per mm^3 of Blood									
				DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 14	DAY 21
668	F	13	7.5	15,600	4,800	3,600	3,500	3300	-	-	2,500	72	1,200
950	F	30	9	3,100	400	23	00	00	6	12	12	CQ	00
1302	M	30	9	4,500	2,300	130	12	00	00	130	840	FDR	00
1354	F	7	7	13,000	11,000	3,200	590	370	72	200	300	1600	FDR
1939	M	36	12.4	15,000	1,500	3,400	370	110	42	52	100	190	FDR
2171	M	26	10	64,000	7,100	3,700	120	72	12	00	00	00	6

CQ = Patient treated with a second course of chloroquine (25mg/kg)

FDR = Patient treated with Fansidar (35mg/kg) sulphadoxine)

(MPCT' for the sensitive cases was calculated to be 3.47 ± 1.04 days.

6 patients (10.0%) were resistant to chloroquine in vivo. The distribution of parasite densities per mm^3 of the resistant cases is shown in table 3.3. It can be seen that three (5.1%) cases (No.950, 1302 and 2171) were resistant at RI level. Cases number 950 and 1302 had early recrudesance, while case number 2171 had parasitaemia on days 21 and 28, and was taken as late recrudescence since the corresponding parasite isolate was resistant to chloroquine in vitro (see tables 3.3. and 3.5). Cases No 668, 1354 and 1939 were resistant at the RII level. Two of the resistant cases (No. 1302 and 1939) were visitors from Benue and Imo States respectively, while the remaining 4 cases i.e. case number 668, 950, 1354 and 2171 were residents of Zaria Urban Area.

Cases No. 1302, 1354 and 1939 were retreated with Fansidar (i.e. Sulphadoxin-pyrimethamine combinations) as 35mg/kg of the sulphadoxine and were found to be fully sensitive to it. Case No. 950 was retreated with a second course of chloroquine (i.e. 25mg/kg over three days) and was also found to be sensitive to the second course (see table 3.3.). In both cases, parasitaemia cleared within 7 days of retreatment and did not reappear throughout the 28 days observation period.

(ii) Clinical Response

The clinical response after treatment with 25mg/kg chloroquine base for 3 days is shown in table 3.4.

Table 3.4 Clinical Response of 59 In Vivo cases of P. falciparum to chloroquine in Zaria Urban Area 1987/88.

Clinical symptoms	No. of Cases on each follow-up Day n = 59							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Fever $>$ 37.5°C or History of Fever	57 (96.6%)	28 (47.5%)	6 (10.2%)	5 (8.5%)	0	0	1 (1.7%)	1 (1.7%)
Diarrhoea	25 (42.4%)	9 (15.3%)	3 (5.1%)	2 (3.4%)	0	0	0	0
Vomiting	30 (50.8%)	6 (10.2%)	1 (1.7%)	1 (1.7%)	0	0	0	0
Decreased activity and mental alertness	53 (90%)	20 (34%)	4 (6.8%)	1 (1.7%)	0	0	0	0

On day 0.57 patients (96.6%) had fever $\geq 37.5^{o}C$ or a history of fever. This number decreased to 28 (47.5%) on day 1, 6 (10.2%) on day 2,5 (8.5%) on day 3 and 0 on day 4 and 5. One patient (Case No. 950) had fever and parasitaemia on days 6 and 7, however both disappeared after retreatment with a second course of chloroquine see table 3.4.

53 patients (90%) had a decrease in activity and mental alertness on day 0. These reduced to 20 (34%) on day 1, 4 (6.8%) on day 2, and 1 (1.7%) on day 3. As from day 4, all children were normal.

30 patients (50.8%) were vomiting on day 0, this decreased to 6 (10.2%) on day 1 and 1 (1.7%) on day 2 and 3. However, no child vomited as from day 4 onwards (see table 3.4).

25 patients (42.4%) had diarrhoea on day 0, 9 (15.3%) on day 1, 3 (5.1%) on day 2, 2 (3.4%) on day 3 and none had diarrhoea as from day 4 onwards.

On the whole, all clinical symptoms subsided on day 4, after treatment with 25mg/kg of chloroquine over three days.. Except Case No. 950 who had fever on days 6 and 7, but this also disappears on retreatment with a second course of chloroquine (25mg/kg).

The *in vivo* results show that there was a good correlation between parasitological and clinical responses for the sensitive cases since both parasitaemia and clinical symptoms disappear within 7 days. However, there was no correlation between parasitological and clinical responses for the resistant cases since parasitaemia persisted after day 7.

3.4 *In vitro* Results

(a) *Chloroquine*

Of the 102 *P. falciparum* isolates that were cultured, 36 were successful for chloroquine, that is a success rate of 35.3%. After incubation, the mean number of schizonts per 200 parasites in the control wells was 69 ± 33.9. The *in vitro* results for chloroquine are shown in table 3.5 and 3.8. It can be seen from table 3.5 that there was no correlation between the severity of parasitaemia and the *in vitro* response.

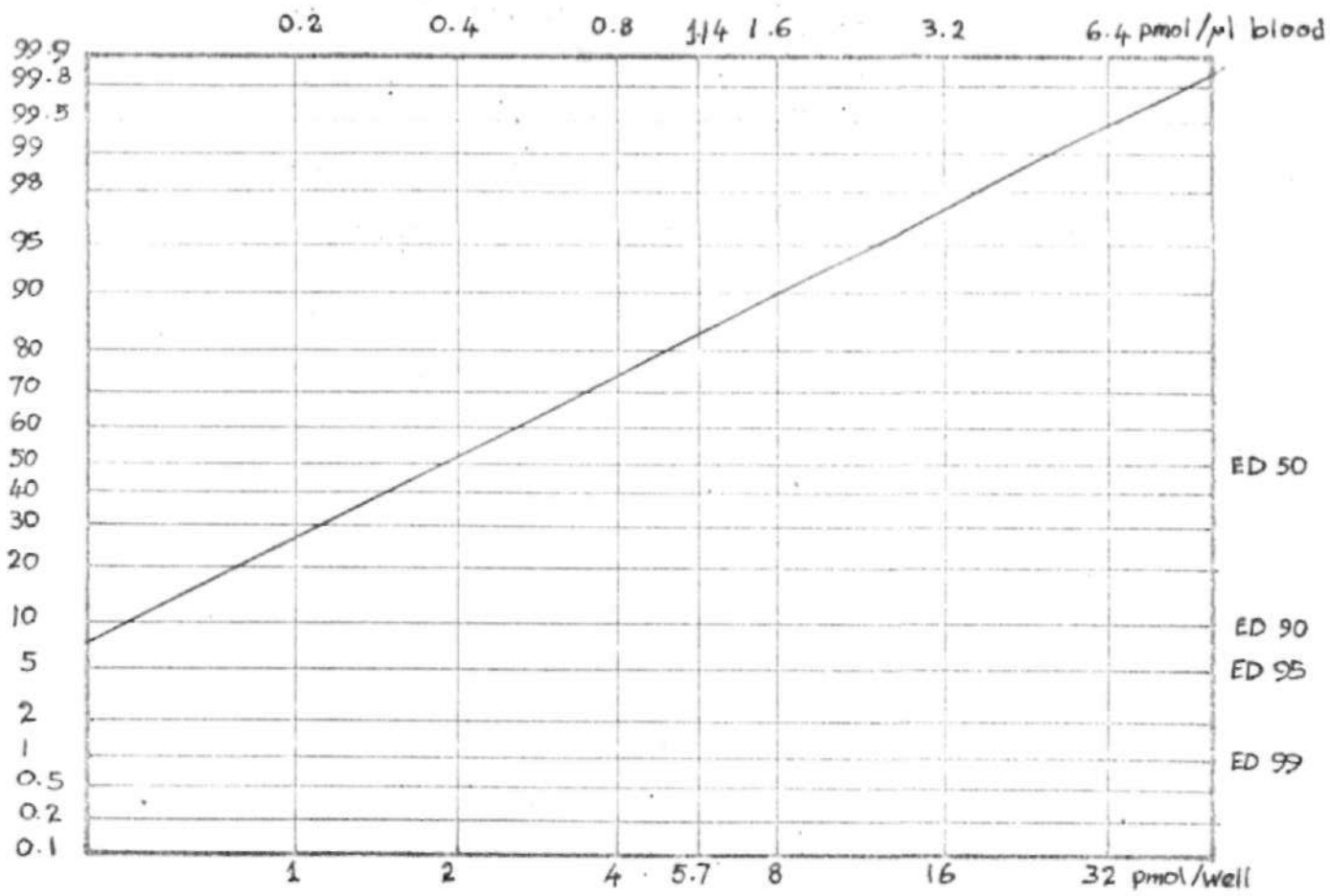
It can also be seen from table 3.5 that 24 isolates (66.7%) were fully sensitive to chloroquine *in vitro* i.e. there was complete inhibition of schizont maturation for those isolates at 8 Pmol/well (1.6×10^{-6} mol/litre) chloroquine and below. 12 isolates (33.3%) were resistant to the drug, i.e. there was schizont growth at 8 pmol/well (1.6×10^{-6} mol/litre) chloroquine and above.

The regression line obtained by probit analysis of the log dose response for the 36 isolates is illustrated in figure 3.2. The EC 50 and EC 99

Table 3.5 In vitro Response of 30 Isolates of P. falciparum to chloroquine in Zaria, 1987/88

S/N	Sex	Age months	Parasite Density on Day 0	Schizont Count Per 200 Parasite In Each Test Well							
				A	B	C	D	E	F	G	H
				Control	1 Pmol	2 Pmol	4 Pmol	5.7 Pmol	8 Pmol	16 Pmol	32 Pmol
643	M	10	11,600	50(100%)	19(38%)	12(24%)	1 (15%)	1 (5%)			
664	M	48	90,000	70(100)	63(90)	65(93)	45(64)	35(50)	12(17)		
668	F	13	5,600	50(100)	51(102)	53(106)	24(48)	4 (8)	1 (2)		
682	M	84	91,100	59(100)	51(86)	50(85)	12(20)	10(31)	10(17)	5 (8)	
725	M	13	1,700	50(100)	10(20)	1 (4)					
738	M	36	16,000	90(100)	81(90)	57(63)					
783	M	30	6,960	51(100)	43(58)	17(33)	1 (3)				
903	F	12	18,000	59(100)	52(88)						
912	M	24	84,000	70(100)	51(73)						
921	F	48	4,000	50(100)	1(10)	2 (4)					
950	F	50	3,100	82(100)	112(136)	22(27)	2 (2)	2 (2)	1 (1)	1 (1)	
958	M	48	5,600	111(100)	100(90)	80(72)	22(20)				
974	F	36	62,000	52(100)	29(56)	2 (4)					
992	F	48	73,000	53(100)	12(23)						
1041	F	72	5,700	104(100)	116(78)	51(34)					
1053	F	36	1,200	50(100)	2(04)	1 (2)					
1096	M	36	45,000	74(100)	69(93)	11(15)	1 (4)	1 (1)			
1354	F	7	13,000	135(100)	121(115)	99(91)	48(90)	82(61)	-	47(45)	4 (4)
1390	F	72	11,000	70(100)	61(87)	1 (4)	1 (1)				
1415	M	72	6,500	103(100)	98(92)	1 (1)					
1651	F	48	18,000	70(100)	60(85)	40(61)	11(41)	10(24)	5 (7)	1 (1)	
1653	M	30	5,300	87(100)	94(108)	60(28)	63(72)	73(84)	18(21)	14(16)	9 (10
1830	M	24	18,000	57(100)	37(65)	53(92)	43(72)	40(84)	37(65)	8 (14)	
1935	M	11	35,000	12(100)	6(50)	6(50)	5(42)	4(33)	1(8)	1 (8)	
2141	M	12	35,000	97(100)	94(97)	81(84)	73(75)	5(5)			
2171	M	36	64,000	135(100)	80(70)	53(86)	96(84)	110(96)	54(47)	3 (3)	2 (2)
2225	F	48	37,000	77(100)	65(84)	2(3)					
2230	F	48	2,700	35(100)	33(94)	6(17)	1 (3)				
2243	F	48	86,000	20(100)	15(75)	14(70)	1 (5)				
2295	M	26	41,000	73(100)	52(71)	60(82)	58(79)	52(71)	51(70)	1 (1)	
2436	F	72	40,000	65(100)	60(92)	50(77)	2 (3)				
2457	F	24	15,000	55(100)	27(49)	22(40)	24(43)	43(78)	12(22)		
2469	F	30	30,000	90(100)	80(89)	11(12)					
2481	M	60	9,000	113(100)	74(66)	9(8)	2(2)				
2482	F	72	58,000	91(100)	61(67)	50(55)	7(8)				
2516	M	72	15,000	80(100)	64(74)	45(50)					
Total Schizonts				2487	1993	1161	627	499	202	81	15
Mean No. of Schizonts				69	55	32	17	14	6	2	0.4
% of Control				100	79.7	46.4	24.6	20.3	8.7	2.9	0.6
% of Maturation				100	79.7	46.4	24.4	20.3	8.7	2.9	0.6
% of Inhibition				0	20.3	53.6	75.4	79.7	91.3	97.1	99.4

Fig. 3.2: Probit regression line for chloroquine of 36 P. falciparum isolates in an in vitro study in Zaria urban area 1987/88.



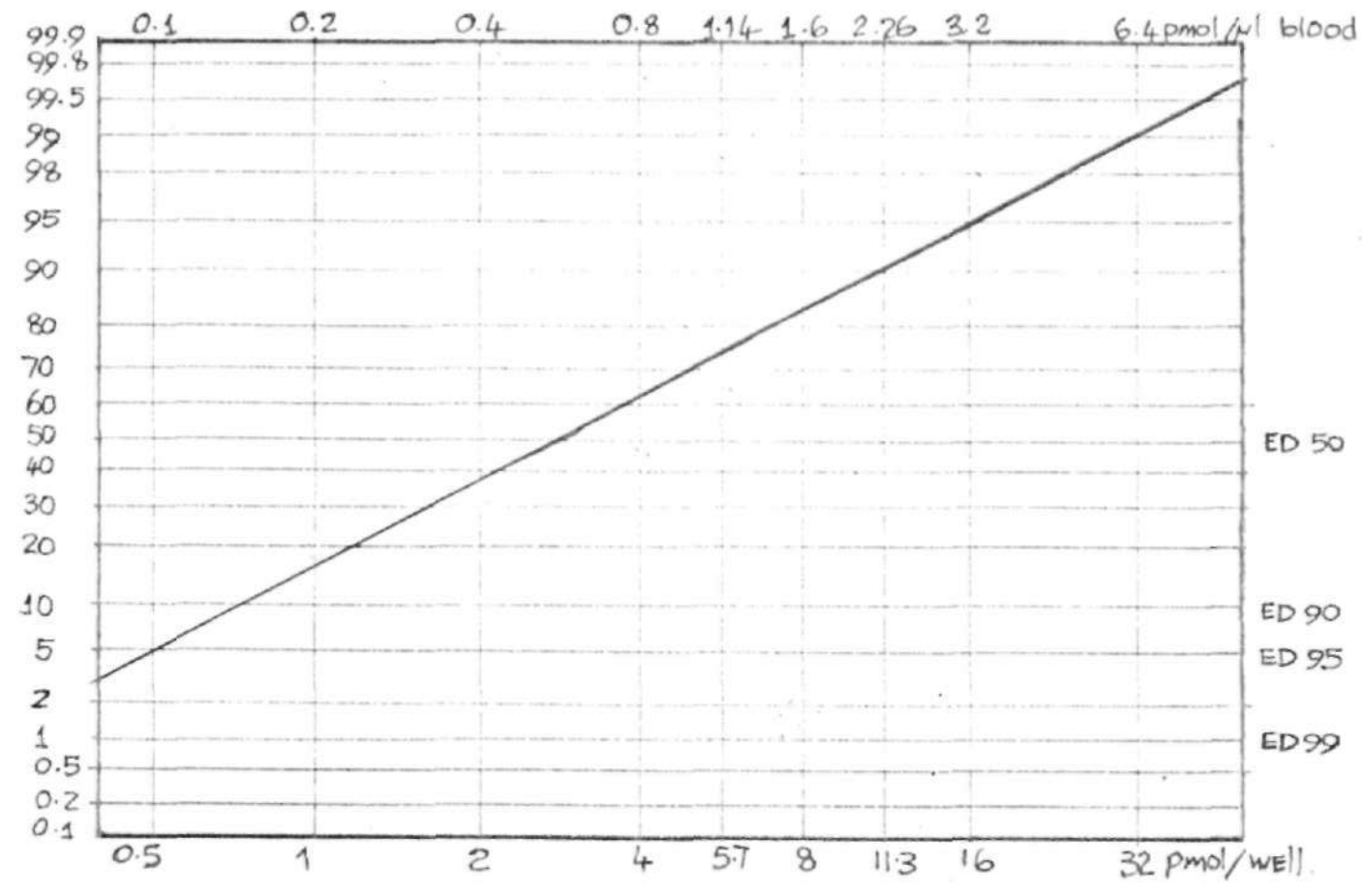
EC 50 = 2 Pmol/well or 0.4 x 10^{-6} mol/L chloroquine

EC 99 = 22.6 Pmol/well or 4.52 x 10^{-6} mol/L chloroquine.

Table 3.6 In vitro Response of 36 Isolates of P. falciparum to Mefloquine in Zaria, 1987/88

S/N	Sex	Age months	Parasite Densities on Day 0	Schizont Count per 200 Parasites In Each Test Well							
				A	B	C	D	E	F	G	H
				Control	0.5 Pmol	1 Pmol	2 Pmol	4 Pmol	5.7 Pmol	8 Pmol	16 Pmol
643	M	10	11,000	20(100)	14(70)	14(70)	13(65)	9 (45)	7 (35)		
664	M	48	90,000	59(100)	45(76)	52(88)	48(81)	39(66)	36(61)	1 (2)	
668	F	13	15,600	47(100)	37(79)	24(51)	24(51)	10(21)	3(6)	1 (2)	
682	M	84	91,000	59(100)	20(34)	62(105)	24(41)	14(24)	20(34)	45(76)	9(15)
725	M	13	1,700	26(100)	12(46)	14(54)	16(62)	14(54)	15(58)	7 (27)	
738	M	36	16,000	94(100)	45(48)	40(43)	68(72)	61(65)	35(37)	27(29)	4(4)
783	M	30	6,900	31(100)	46(148)	25(81)	36(116)	14(45)	15(48)	5 (16)	
903	F	12	18,000	22(100)	21(96)	32(146)	24(109)	30(136)	13(59)	3 (14)	
912	M	24	14,000	70(100)	80(114)	43(61)	22(31)	2 (3)	2 (3)	1 (1)	1(1)
921	F	38	4,000	35(100)	34(97)	25(71)	25(71)	20(57)	4 (11)		
950	F	30	3,100	107(100)	101(94)	105(98)	33(31)	63(59)	8 (8)	2 (2)	
958	M	18	3,800	131(100)	120(92)	139(106)	122(93)	98(75)	102(78)	86(66)	6(5)
974	F	36	67,000	32(100)	27(84)	38(119)	20(63)	17(53)	16(50)	2 (6)	
992	F	48	79,000	62(100)	82(132)	60(97)	34(55)	20(32)	2 (3)		
1041	F	72	57,000	144(100)	96(67)	134(93)	138(96)	77(54)	28(18)	6 (4)	1(0.7)
1053	F	36	1,200	50(100)	20(40)	23(46)	30(60)	6 (12)	3 (6)		
1096	M	36	45,000	74(100)	45(61)	51(69)	75(101)	70(95)	66(89)	42(57)	
1354	F	7	13,000	105(100)	92(88)	79(74)	17(16)	4 (4)	1 (1)		
1390	F	72	11,000	70(100)	74(104)	68(97)	62(89)	9 (13)			
1415	M	72	6,500	98(100)	95(97)	82(84)	88(90)	60(61)	11(11)		
1651	F	48	18,000	92(100)	58(63)	72(78)	58(63)	67(73)	42(46)	30(33)	1 (1)
1653	M	30	5,300	71(100)	59(83)	80(113)	65(92)	85(120)	19(27)	30(42)	1 (1)
1830	M	24	18,000	57(100)	50(88)	44(77)	45(79)	24(37)	3 (5)		
1935	M	11	35,000	12(100)	15(125)	19(158)	12(100)	6(50)	4 (33)	1 (8)	
2141	M	12	35,000	97(100)	94(97)	57(59)	42(43)	35(36)	53(55)	4 (4)	
2171	M	26	64,000	115(100)	98(85)	93(81)	111(97)	107(93)	92(80)	98(85)	
2225	F	48	37,000	77(100)	69(90)	57(74)	58(75)	21(27)	4 (5)		
2230	F	48	2,700	86(100)	76(88)	14(16)	13(15)	25(29)	15(17)	1 (1)	
2243	F	48	86,000	24(100)	15(63)	18(75)	14(58)	14(58)	15(63)	12(50)	1 (4)
2298	M	26	41,000	51(100)	42(82)	43(84)	36(71)	36(71)	35(69)	15(30)	6(12)
2436	F	72	40,000	70(100)	54(77)	83(119)	104(149)	52(74)	30(43)	3(4)	
2457	F	24	15,000	55(100)	39(71)	35(64)	18(33)	8 (15)	5 (9)	1 (2)	
2469	F	36	30,000	97(100)	56(58)	59(61)	60(62)	71(73)	57(59)	46(47)	2(2)
2481	M	60	9,800	113(100)	97(86)	96(85)	92(81)	39(35)	18(16)	7(6)	
2482	F	72	58,000	114(100)	103(90)	73(64)	61(54)	22(19)			
2516	M	72	15,000	114(100)	108(95)	121(106)	73(64)	89(78)	73(64)	15(13)	
Total Schizonts				2581	2136	2670	1781	1332	852	491	32
Mean No. of Schizonts				72	59	58	49	37	24	14	1
% of Control				100	81.9	80.6	68.1	51.4	33.3	19.4	1.4
% of Maturation				100	81.9	80.6	68.1	51.4	33.3	19.4	1.4
% of Inhibition				0	18.1	19.4	31.9	48.6	66.7	80.6	98.6

Fig. 3.3: Probit regression line for Mefloquine of 36 P. falciparum isolates in an in vitro study in Zaria urban area, 1987/88.



EC50 = 2.8 Pmol/well or 0.56 X 10^{-6} mol/L Mefloquine
EC99 = 32 Pmol/well or 6.4 x 10^{-6} mol/L Mefloquine.

Table 3.7 In Vitro Response of 13 Isolates of P. falciparum to Quinine in Zaria 1987/88

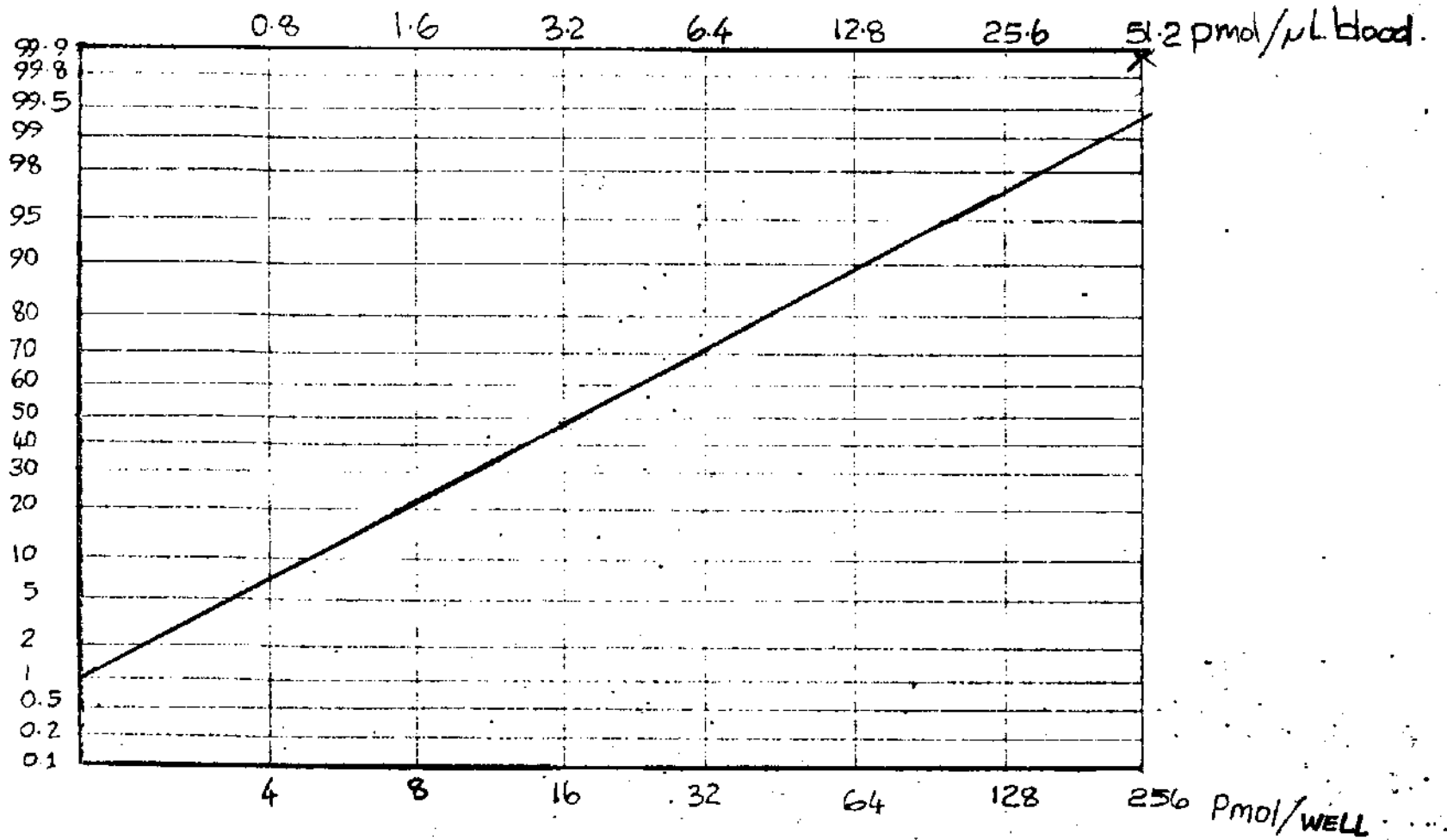
S/No.	Sex	Age (months)	Parasite Densities on Day 0	Schizont count per 200 parasites in each test well							
				A	B	C	D	E	F	G	H
				Control	4 Pmol	8 Pmol	16 Pmol	32 Pmol	64 Pmol	128 Pmol	256 Pmol
1096	M	36	45,000	74 (100)	59(80)	49(66)	51(69)	61(82)	23(31)	4 (5)	
1354	F	7	13,000	105 (100)	93(89)	83(79)	62(59)	41(39)	5(5)		
1390	F	72	11,000	70 (100)	57(81)	56(80)	77(110)	42(60)			
1415	M	72	6,500	83 (100)	76(92)	89(107)	61(73)	40(48)	7(8)		
1651	F	48	18,000	76 (100)	75(99)	83(109)	89(117)	60(79)	42(55)	12(16)	
1653	M	30	5,300	74 (100)	48(65)	96(130)	34(46)	55(74)	45(61)	28(38)	1(1)
2225	F	48	37,000	70 (100)	58(83)	46(66)	32(46)	19(27)			
2230	F	48	2,700	48 (100)	42(88)	26(54)	17(35)	12(25)	11(23)		
2243	F	48	86,000	24 (100)	15(63)	19(79)	7(29)	14(58)	14(58)		
2436	F	72	40,000	75 (100)	56(75)	43(57)	50(67)	36(48)	11(15)		
2457	F	24	15,000	55 (100)	20(36)	13(24)	10(18)	7(13)	4(7)		
2481	M	60	9,800	113 (100)	64(57)	54(48)	67(59)	77(68)	36(32)		
2482	F	72	58,000	91 (100)	39(43)	41(45)	34(37)	5(6)			
Total Schizonts				958	702	698	591	469	198	44	1
Mean No. of Schizonts				73.7	54	53.7	45.5	36.1	15.2	3.4	0.1
% of Control				100	73.3	72.9	61.7	49	20.6	4.6	0.1
% of Maturation				100	73.3	72.9	61.7	49	20.6	4.6	0.1
% of Inhibition				0	26.7	27.1	38.3	51	79.4	95.4	99.9

Table 3.8 Comparative *in vitro* sensitivity of *P. falciparum* isolates to chloroquine, mefloquine and quinine at Zaria 1987/88

DRUG CONCENTRATION X 10^{-6} mol/L	ISOLATES UNDERGOING SCHIZOGONY IN THE PRESENCE OF		
	Chloroquine (n = 36)	Mefloquine (n = 36)	Quinine (n = 13)
0	100%	100%	100%
0.1	-	100%	-
0.2	100%	100%	-
0.4	91.7%	100%	-
0.8	63.9%	100%	100%
1.14	41.7%	94.4%	-
1.6	* 33.3%	72.2%	100%
3.2	25%	27.8%	100%
6.4	8.3%		100%
12.8			84.6%
25.6			23.1%
51.2			* 7.7%

* Schizogony at those concentrations and above indicates *in vitro* resistant

Fig. 3.4: Probit regression line for Quinine of 13 P. **falciparum** isolates in An in vitro study in Zaria urban area, 1987/88



EC 50 = 16 Pmol/well or 3.2×10^{-6} mol/L Quinine

EC 99 = 181 Pmol/well or 36.2×10^{-6} mol/L Quinine.

(i.e. the effective concentrations required for 50% and 99% schizont inhibition) were estimated as 2 pmol/well or 0.4×10^{-6} mol/litre and 22.6 pmol/well or 4.52×10^{-6} mol/litre chloroquine respectively. It has been observed that sensitive isolates normally produce steep regression lines with a EC 50 well below 0.5×10^{-6} mol/litre chloroquine, and that the flatter the regression line and the higher the EC 99 (greater than 1.0×10^{-6} mol/litre) are, the more resistant is the isolate (WHO 1984). Since the EC 99 for chloroquine in this study is greater than 1.0×10^{-6} mol/litre, this indicates that the isolates in this area are resistant to chloroquine in vitro.

(b) Mefloquine

Of the 102 P. falciparum isolates that were cultured on mefloquine plates, 36 (35.3%) were successful i.e. the success rate was the same as for chloroquine. The mean number of schizonts per 200 parasites in the control wells after incubation was 72 ± 34.4. The range of preculture parasite densities of the successful isolates was the same as for chloroquine. The results of the in vitro tests of the 36 isolates are shown in table 3.6 and 3.8. It can be seen from table 3.6 that the schizont maturation of 26 isolates was completely inhibited at 16 pmol/well ($3.2 \times 10^{-}$ mol/litre) mefloquine and below while 10 isolates grew at 16pmol/well mefloquine. According to WHO(1987), Schizont growth at 64 pmol/well (12.8×10^{-6} mol/L) mefloquine and above is an indication of resistance to the drug.

The regression line obtained by probit analysis of the log dose/response for the 36 isolated to mefloquine

is illustrated in figure 3.3. The EC50 and EC 99 for the isolates were estimated to be 2.8pmol/well or 0.5×10^{-6} mol/litre and 32 pmol/well or 6.4×10^{-6} mol/litre mefloquine respectively.

(c) Quinine

25 isolates of P. falciparum were cultured on Quinine plates. 13 (53%) of these were successful. The mean number of schizonts per 200 parasites in the control well after incubation was 74 ± 23. The preculture parasite densities of the successful isolates ranged between 2,700 and 86,000 per mm^3 of blood. The results of the in vitro sensitivity test of the 13 isolates are shown in tables 3.7 and 3.8. From table 3.7, there was complete inhibition of schizont maturation of 12 isolates at 256 pmol/well (51.2×10^{-6} mol/litre) quinine and below, while 1 isolate grew at 256pmol/well quinine.

The regression line obtained by probit analysis of the log dose response for the 13 isolates is illustrated in figure 3.4. The EC 50 and EC 99 of the isolates were estimated to be 16pmol/well (3.2×10^{-6} mol/litre) quinine and 181 pmol/well (36.2×10^{-6} mol/litre) quinine respectively.

3.5 Comparative in vivo/in vitro Results for chloroquine

33 (55.9%) of the 59 patients that completed the Extended Field Test, had corresponding in vitro results for chloroquine, whereas the P. falciparum isolates of the remaining 26 patients either did not grow sufficiently or

not at all. The correlation between the in vitro response to chloroquine of the 33 isolates with the in vivo response is shown in table 3.9 and table 3.10.

Depending on the outcome of the comparative in vivo/ in vitro sensitivity of P. falciparum to chloroquine, the 33 patients may be divided into the following 4 groups.
Group I:- Cases that were sensitive to chloroquine both in vivo and in vitro (See table 3.11).
Group II:- Cases that were sensitive in vivo but resistant in vitro (See table 3.12).
Group III:- Cases that had delayed clearance in vivo but were resistant in vitro (See table 3.13).
Group IV:- Cases that were resistant both in vivo and in vitro (See table 3.14).

The results obtained for these 4 groups of patients are summarized in table 3.9. Also the mean parasite densities per mm^3 (log scale) for the 4 groups is shown in figure 3.5 and the regression lines of the probit analysis of the log dose response for the 4 groups is illustrated in figure 3.6.

(a) Group One:- Cases that are sensitive to chloroquine both in vivo and in vitro

21 (63.6%) cases with corresponding in vivo/ in vitro results were sensitive to chloroquine both in the in vivo and in vitro test. The parasitaemia of all of the patients cleared on day 4 and below, except case number 2141 who had parasitaemia on day 4 but this cleared on day 5. In none of the cases was

Table 3.9 Correlation between the in vivo response of P. falciparum malaria to chloroquine (25mg/kg) in 33 children and the sensitivity of the corresponding isolates in vitro (WHO standard microtest) in Zaria urban area 1978/88

GROUP	DESIGNATION	S/NO.	SEX	WT KG	AGE MONTH	IN VIVO RESPONSE: TYPE OF SENSITIVITY OR DAY OF PARASITE CLEARANCE	IN VITRO RESPONSE: WELL OF GOMEZ'S INHIBITION
I	SENSITIVE BOTH IN VIVO AND IN VITRO	643	M	7.2	10	DAY 4	WELL F
		738	M	10.2	16	" 2	" G
		901	F		11	" 3	" H
		924	F	10	20	" 3	" H
		966	M	10	20	" 3	" H
		1074	F		12	" 2	" G
		1092	F	11	24	" 3	" H
		1096	M	11.5	36	" 3	" H
		1098	M	11	24	" 3	" H
		1390	F	12	24	" 3	" H
		1435	F	10	15	" 3	" H
		2230	F	16	48	" 3	" H
		2330	F	12	24	" 3	" H
		2343	F	11	24	" 3	" H
		2436	F	10	18	" 3	" H
		2452	F	11	24	" 3	" H
		2471	M	16	60	" 3	" H
		2472	F	18	72	" 3	" H
		2516	M	16	72	" 3	" H
II	IN VIVO SENSITIVE, IN VITRO RESISTANT	1651	F	14	48	DAY 3	WELL H
		1830	M	10.5	24	" 3	" H
		2296	M	9.8	36	" 3	" H
		2457	F	"	24	" 3	" G
III	DELAYED CLEARANCE IN VIVO RESISTANT IN VITRO	661	M	15.5	48	DAY 5	WELL G
		682	M	24	84	" 7	" H
		1653	M	9.2	30	" 4	NO INHIBITION
		1935	M	8.4	11	" 3	WELL H
IV	RESISTANT BOTH IN VIVO AND IN VITRO	668	F	7.5	12	R II	WELL G
		953	F	9	30	R I	" H
		1354	F	7	7	R II	NO INHIBITION
		2171	M	10	26	R I	NO INHIBITION

Table 3.10 Correlation between the in vitro response to chloroquine (WHO standard micro test) of 33 P. falciparum isolates with the in vivo response of children infected by the same isolates

In vitro response		In vivo response		
Minimum inhibitory concentration (Pmol/well)		Absence of parasites at D7 and D14	Presence of parasites at D7 and or D14	Total
D	4 and below	10	0	10
E	5.7	8	0	8
F	8	3	0	3
G	16	2	1	3
H	32	5	1	6
>H	>32	1	2	3
Total		29	4	33

Table 3.11 Parasite Densities And Schizont Counts of 21 Sensitive Cases of P. falciparum to Chloroquine in an In vivo/In vitro Sensitivity Study, Zaria Urban Area, 1987/88

Serial No.	Sex	Age	Wt (kg)	In-Vivo Results: Parasite Densities Per mm³ of Blood											In-Vitro Results: Schizont Count Per 200 Parasite in Each Test Well							
															A	B	C	D	E	F	G	H
				Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 12	Day 21	Day 28	Con-trol	1 Pmol	2 Pmol	4 Pmol	5.7 Pmol	8 Pmol	16 Pmol	32 Pmol
643	M	10	7.0	11,200	12,500	72	12								10	19	12	1	1			
738	M	36	10.2	18,000	1,200	4800									94	81	97					
903	F	12	8	18,000	1,600	200									20	5						
921	F	28	15	1,000	4,400	880									20	17	3					
958	M	20	10	3,700	740	148									131	120	85	15				
974	F	36	14	57,000	44,000										30	20	12					
1041	F	72	18	57,000	3,800	440									144	115	24					
1053	F	36	14.5	1,200	600	24									50	7	3					
1096	M	36	10	45,000	7,200	5520	72								74	69	14	3	1			
1390	F	72	19	11,200	32,000	120									70	61	3	1				
1415	M	72	17	6,500	3,600	48									102	94	1					
2141	M	12	7.5	35,000	3,120	1200	600	120							97	94	81	72	5			
2225	F	48	16	37,000	47,000	120								720	77	95	2					
2230	F	54	15	2,700	172										25	23	6	1				
2243	F	48	14	86,000	35,000	76									20	15	14	1				
2436	F	72	17	40,000	16,000	820									65	60	50	2				
2469	F	36	11	30,000	7,700	14200	48							13160	90	80	14					
2481	M	60	16	9,800	1,400	36									113	74	9	2				
2482	F	72	18	58,000	26,000	480									91	61	50	7				
2516	M	72	16	15,000	3,500	470									86	64	43					
Total				632,900	243,630	16520	366	120							1496	1172	496	118	7			
Mean				30,140	11,600	870	92	120							71	56	24	6	0.3			
% of Schizont Maturation															100	78.9	33.9	8.5	0.4			
% of Schizont Inhibition															0	21.1	66.2	91.5	99.6			

Table 7.10 Parasite Densities And Schizont Counts of 4 *In Vivo* Sensitive and *In Vitro* Resistant cases of *P. falciparum* malaria to chloroquine (25mg/kg) in Zaria Urban Area, 1990/91

Serial No.	Sex	Ages	Wt (Kg)	In-Vivo Results: Parasite Densities Per mm^3 of Blood												In-Vitro Results: Schizont Count Per 100 Parasite in Each Test Well							
				Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 14	Day 21	Day 28		A Control	B 1 Pmol	C 2 Pmol	D 4 Pmol	E 5.7 Pmol	F 8 Pmol	G 16 Pmol	H 32 Pmol
1651	F	48	14	18,000	290	18										76	62	46	31	18	5	1	
1830	M	24	10.5	18,000	12,000	36										57	37	53	41	48	37	8	
2298	M	26	6.8	41,000	28,000	610										71	52	58	56	52	51	1	
2457	F	24	11	15,000	2,300	18										55	27	27	34	43	12		
Total				92,000	52,590	682	0	0	0	0	0	0	0	0		259	178	184	162	161	105	10	
Mean				23,000	13,150	170	0	0	0	0	0	0	0	0		65	45	46	41	40	26	3	
% of Schizont Maturation																100	69.2	70.8	63.1	61.5	40	4.6	
% of Schizont Inhibition																0	30.8	29.2	36.9	38.5	60	95.4	

Table 3.13 Parasite Densities and Schizont counts of 4 cases of P. Falciparum with delayed clearance in vivo and in vitro resistance, to chloroquine, in Zaria Urban Area 1987/88

Serial No.	Sex	Age	Wt kg	IN-VIVO RESULTS: Parasite Densities Per mm^3 of blood											IN-VITRO RESULTS: Schizont count per 200 parasites in each test well							
				DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 14	DAY 21	DAY 28		A CONTROL	B 1 Pmol	C 2 Pmol	D 4 Pmol	E 6.7 Pmol	F 8 Pmol	G 16 Pmol	H 32 Pmol
664	M	48	15.5	90,000	10,000	350	250	48								70	63	65	45	25	12		
682	M	84	24	91,000	2,000	350	170	140	12	6						39	51	50	17	16	10	6	
1653	M	30	9.2	5,300	850	43	6	2								87	94	63	73	63	18	14	9
1935	M	11	8.4	35,000	20,000	230	24	12								12	6	6	5	4	1	1	
Total				221,300	32,850	983	460	210	12	6	0	0	0	0		228	214	189	140	120	41	20	9
Mean				55,330	8,215	246	115	67	12	6	0	0	0	0		57	54	47	35	30	10	5	2
% of Schizont Maturation																100	94.7	82.5	61.4	52.6	17.5	8.8	3.5
% of Schizont Inhibition																0	5.3	17.5	38.6	47.4	82.5	91.2	96.5

Table 3.14 Parasite Densities and Schizont counts of 4 *in - vivo* and *in - vitro* resistance cases of *P. falciparum* to chloroquine in Zaria Urban Area, 1987/88

SERIAL NO.	SEX	AGE	WT KG	*IN - VIVO* RESULTS											*IN - VITRO* RESULTS							
				PARASITE DENSITIES PER mm^3 OF BLOOD											SCHIZONT COUNT PER 200 PARASITE IN EACH TEST WELL							
				DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 10	DAY 21	DAY 28	CONTROL	1 PMOL	2 PMOL	4 PMOL	5.7 PMOL	8 PMOL	16 PMOL	32 PMOL
668	F	13	7.5	15,600	4,800	1,600	1,600	1,300	-	-	1,600	72	1,700	-	50	51	53	24	4	1		
950	F	30	9	3,100	100	00	00	00	6	12	12	00	00	00	107	112	27	2	2	1	1	
1354	F	7	7	13,000	11,000	1,200	520	370	72	800	200	1600	FOR	00	105	121	95	94	65	-	17	4
2171	M	26	10	64,000	7,100	1,700	120	77	12	00	00	00	6	30	115	80	99	96	140	54	1	2
TOTAL				95,700	23,000	10,583	4,810	1,747	90	212	1,812	1672	1706	30	377	364	274	216	201	56	21	6
MEAN				24,425	5,825	2,830	1,400	1,250	20	106	330	336	853	20	94	91	69	54	50	19	13	2
% OF SCHIZONT MATURATION															100	96.8	73.4	57.4	53.2	20.2	13.8	2.1
% OF SCHIZONT INHIBITION															0	3.2	26.6	42.6	46.8	79.8	86.2	97.9

Fig. 3.5: Mean parasite densities per mm^3 (log scale) for 4 groups of cases of P. falciparum malaria after treatment with 25mg/kg chloroquine in an in vivo study in Zaria urban area, 1987/88.

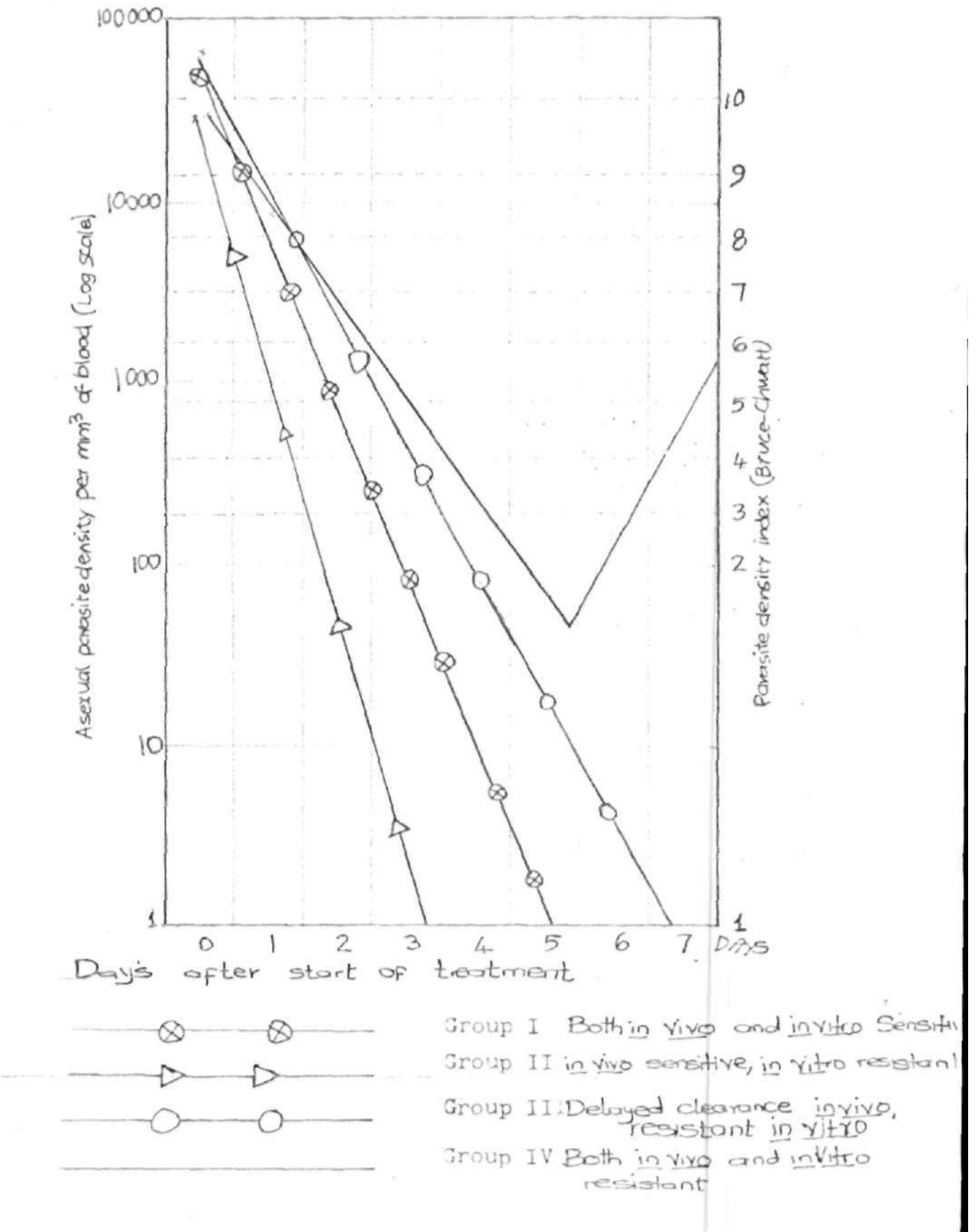
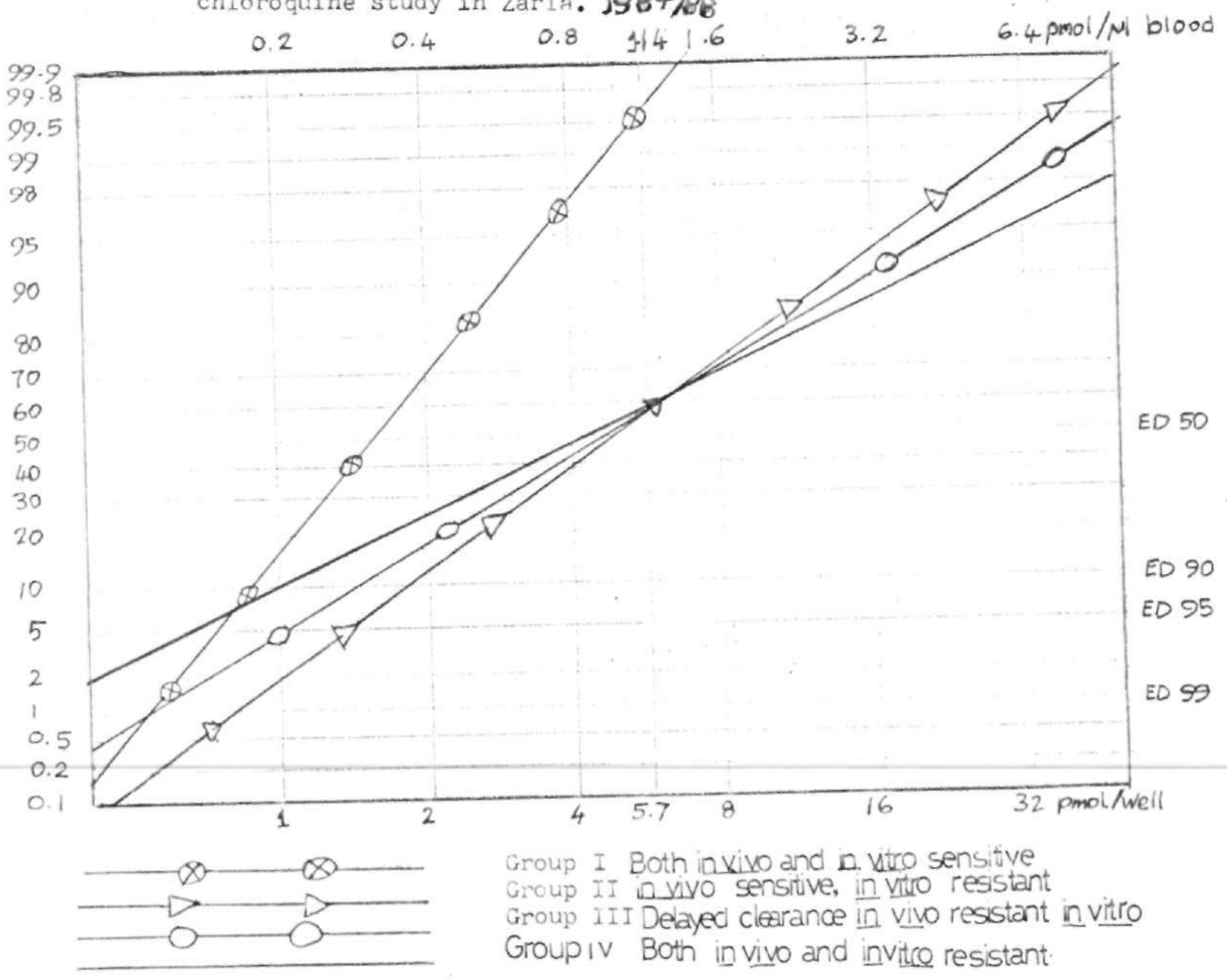


Fig. 3.6: Probit regression lines of 4 groups of P. falciparum isolates in an in vitro chloroquine study in Zaria. 1987/88



the parasitaemia on days 2 or 3, 25% or above that of day 0.

In the in vitro test, there was complete inhibition of schizont maturation in all cases at 8 pmol/well ($1.6x10^{-6}$ mol/litre) chloroquine and below. The distribution of parasite densities and schizont count for the 21 cases is shown in table 3.11.

In two cases (No. 2225 and 2469) parasitaemia reoccured on day 28. They were taken as reinfection since their corresponding in vitro results showed complete inhibition of schizont maturation at 4 pmol/well chloroquine (see table 3.11). The EC 50 and EC 99 for this groupwas $0.3x10^{-6}$mol/litre and $0.914x10^{-6}$mol/litre chloroquine respectively.

(b) Group two:- Cases that were sensitive to chloroquine in vivo but resistant to the drug in vitro

4 (12.1%) cases were sensitive to chloroquine in vivo but resistant to the drug in vitro. In all of 4 cases, parasitaemia cleared on day 3 while in the in vitro test, there was still schizont maturation present at 8 pmol/well and above for all cases (see table 3,12). The EC 50 and EC 99 for this group was estimated to be $1.0x10^{-6}$mol/litre and $6.4x10^{-6}$mol/litre chloroquine, respectively.

Two cases (No. 2298 and 1651) were significantly malnourished and case number 1830 was mistakenly given another dose of chloroquine, apart from the 25mg/kg that was given over three days, while on admission. Also case

number 2457 confess taking an antimalarial drug at home apart from the 25mg/kg that was given. It is therefore likely that this group contains patients which should belong to either group III or IV except for the malnourished cases.

(c) Group III:- Cases with delayed Parasite clearance in vivo but resistant to chloroquinine in vitro

4 cases (12.1%) out of the 33 had delayed parasite clearance in vivo when treated with 25mg/kg chloroquine, while their corresponding P. falciparum isolates were shown to be resistant to the drug in vitro (see table 3.13).

The parasitaemia of the 4 cases cleared on day 7 and below and did not reappear while in the in vitro test, there was schizont maturation present at 8pmol/well ($1.6x10^{-6}$mol/litre) chloroquine and above for all the cases (see table 3.13). The EC 50 for the group was estimated to be $0.9x10^{-6}$mol/litre while the EC99 was above $6.4x10^{-6}$mol/litre chloroquine (see figure 3.6).

(d) Group IV:- Cases that are resistant to chloroquine both in vivo and in vitro

4 (12.1%) cases out of the 33 were resistant to chloroquine both in vivo and in vitro. Case No. 950 and 2171 were resistant at the RI level while case numbers 668 and 1354 were resistant at the RII level. See table 3.14.

In the <u>in vitro</u> study, there was schizont growth in the 8 pmol/well ($1.6x10^{-6}$mol/L) and above for all cases. Case No. 1354 and 2171 had schizont growth at 32 pmol/well (Well H) (see table 3.14). The EC 50 for the group was estimated to be 0.84×10^{-6}mol/litre chloroquine while the EC 99 was well above $6.4x10^{-6}$mol/litre chloroquine (see figure 3.6).

CHAPTER FOUR

DISCUSSION AND CONCLUSION

The study lasted for one and a half years. This long period was mainly due to the fact that the investigators were not used to the methods especially the *in vitro* method at the beginning. Also the political instability that took place in February and May 1987 which led to the closure of the University made it impossible to follow-up some patients as such, many patients were lost.

The screening of 2,068 patients attending the out-patient department provided a suitable number of malaria cases (175) for inclusion in the study. This is in agreement with a similar survey carried out by Midala *et al* in 1985 at the same out-patient department where 129 malaria cases were obtained within 8 months also Olatunde *et al* (1981) working at Ibadan in a six month period follow-up successfully for twenty-eight days, fifty two children with malaria. The present observation attests to the suitability of out-patient clinics as source of selection for malaria cases required for both *in vivo* and *in vitro* drug testing (Fox *et al* 1987).

Children between the ages of 6 months and 7 years were selected for the study because in a holoendemic area like Zaria high parasite counts are more likely to be obtained in children than in adults. This is because at this age, the immunity of the children for malaria has not yet been fully developed (Aderounmu *et al* 1981).

In this study, the Dill-Glazko test used for detecting 4-aminoquinolines in the urine was positive in 20 patients (11.4%) out of the total number of 175 cases with P. falciparum infection, despite the negative history of chloroquine intake in the previous two weeks. This buttresses the fact that some mothers attend the clinic only after self-medication has failed. In a survey on use and misuse of drugs in Lagos, chloroquine was found to be one of the commonest patent drugs used by mothers to treat their children (Oyewole et al 1987). Walker (1983) observed that 50% of children diagnosed as having malaria in a teaching hospital in Nigeria had high plasma chloroquine levels before receiving treatment (WHO 1984). The Haskin's test and the more recent ELISA test which are more sensitive and consistent than the Dill-Glazko test would most probably have picked out more cases with positive urine samples.

The very high prevalence of P. falciparum in Nigeria was evidence in this study as reported by other workers (Midala et al 1988, Olatunde et al 1981). P. malariae was found in only 4 cases (2.3%) out of the total number of 175 cases that were positive for malaria parasites. Though epidemiologically, P. falciparum is the commonest species in the Sudan Savannah belt, it's predominance over other species is further heightened in the wet season (Molineaux and Gramiccia, 1980).

Out of a total of 102 cases of P. falciparum infections that were enrolled in the study, only 65 (63.7%) completed the 28 day study protocol, while 37 (36.3%) defaulted.

This is an improvement over the previous study carried out by Midala et al where 129 cases were enrolled but only 33 (25.6%) cases completed the 28 day study protocol, while 96 (74.4%) had defaulted at various times of the study.

The pre-treatment parasite densitives varied widely between 1100 to 95,000 per mm^3 of blood. Cases with parasite densities below 1000 and above 95,000 were excluded from the study. The distribution of mean parasite densities according to age groups shows that there was no correlation between pretreatment parasite densities and age group.

On clinical response, all clinical symptoms disappeared as from day 4 except one case with early recrudescence who had fever and parasitaemia on days 6 and 7 which both disappeared on retreatment with a second course of chloroquine (25mg/kg). This indicate that there was a satisfactory clinical response observed in this study on oral treatment with 25mg/kg chloroquine base over three days.

Of the 59 cases that had complete in vivo results, 36 (61%) were fully sensitive to the 25mg/kg chloroquine base regimen, In these cases, parasitaemia disappeared within days 2 and 3. 17 (28.8%) cases had delayed parasite clearance i.e. their parasitaemia cleared between days 4 and 7. Two patients (3.4%) had evidence of reinfection on day 28. The mean parasite clearance time for the sensitive cases (ie those whose parasitaemia cleared within 7 days and did not reappear, including those with

reinfection) was 3.47±1.04 days. This is in agreement with the work carried out by Midala et al in Zaria previously where the mean parasite clearance time for 33 sensitive in vivo cases was found to be 3.45 ± 1.23 days.

Six patients were resistant to the drug in vivo, 3 at the RI and 3 at the RII level. The resistant cases were aged between 7 months and 3 years. Two of them were visitors from the middle belt and South Eastern part of the country . This may be expected since cases of treatment failure with chloroquine has been reported in the South Eastern part of the country (Ekanem 1985, Eke 1979). Also a resistant case has been reported in a nonimmune visitor from America to this region (C.D.C. 1987). The remaining 4 cases were residents of Zaria urban area. This would be in agreement with the clinical observation made in this region that a 25mg/kg dose regimen of chloroquine is often not sufficiently effective for the cure of P. falciparum malaria in children (Werblinska personal communication 1986). Midala et al in an in vivo study suggested a probable decreased in sensitivity of P. falciparum to chloroquine in Zaria urban area (Midala et al 1988).

Three of the resistant cases were treated with Fansidar tablets as 35mg/kg sulphadoxine given in a single dose. They were found to be fully sensitive to the drug. It is noteworthy that one of the resistant cases was in error treated with a second course of chloroquine (25mg/kg over tree days) and was also found to be sensitive to the second course.

Although the biological Mechanism by which plasmodia succumb to or resist the actions of chloroquine are not yet clear, spontaneous multiple mutation at one or more gene loci and selection of mutants due to drug pressure appear to be the important phenomena involved in drug resistance (WHO 1987). Also it has been found that there are differences in the rate of spontaneous mutation of different P. falciparum strains (Wernsdorfer 1981). With the high drug pressure in Zaria urban area, it is possible that all the three forces (ie gene mutation, drug pressure and difference in the rate of spontaneous mutation) may be responsible for the development of resistance to chloroquine in this part of West Africa.

The microtechnique involving the cultivation of parasitized blood at different drug concentration was used for the in vitro test.

Of the 102 isolates that were cultured on both chloroquine and Mefloquine plates 36 (35.3%) were successful in both cases. The low success rate was due to the fact that initially, the investigators were not used to the microtechnique. However, higher success rates were achieved towards the end of the study as the investigators became used to the technique. Also to improve success rate, the incubation period was prolonged from the conventional 26 hours to up to 30 hours. This was in agreement with work carried out by Hawort 1987, in Indonesia where increased proportion of successful tests was observed to follow prolongation of the incubation period from 26 hours to

up to 48 hours (Haworth, 1987). For Quinine, 25 isolates were cultured on Quinine plates and 13 were successful, this gives a success rate of 53%.

Of the 36 isolates that were successfully tested for chloroquine, 24 isolates (66.7%) were fully sensitive to the drug in vitro (ie there was complete inhibition of schizont maturation for those isolates at 8 pmol/well (1.6×10^{-6}mol/L) chloroquine and below while 12 isolates grew at 8 pmol/well (1.6×10^{-6}mol/L) and above. The EC 50 and EC 99 for the 36 isolates were estimated as 2 pmol/well (0.4×10^{-6}mol/L) and 22.6 pmol/well (4.52×10^{-6}mol/L) chloroquine respectively. It has been observed that sensitive isolates normally produce steep regressions with EC 50 well below 0.5×10^{-6}mol/litre chloroquine. Also that the flatter the regression line and the higher the EC 99 (greater than 1.0×10^{-6}mol/L) the more resistant is the isolates (WHO 1984). Since the EC 99 for chloroquine in this study is greater than 1.0×10^{-6}mol/litre, this indicates that the isolates in this area are resistant to chloroquine in vitro.

Of the 36 isolates that were successfully tested for mefloquine, the schizont maturation of 26 isolates (72.2%) were completely inhibited at 16pmol/well (3.2×10^{-6}mol/L) mefloquine and below, while 10 isolates grew of 16 pmol/well (3.2×10^{-6}mol/L). The EC 99 was estimated to be 6.4×10^{-6} mol/L mefloquine. Bjorkman and Willcox (1986) obtained an EC 99 of 0.7×10^{-6} mol/L mefloquine for sensitive P. falciparum isolates while working in Liberia. Since

there was schizont growth up to 16 pmol/well mefloquine (well H) in our study and the EC 99 obtained was much greater than that obtained by Bjorkman and Willcox for sensitive isolates of P. falciparum, one may suggest that there is decreased sensitivity to mefloquine in Zaria urban area. This agrees with earlier findings at Ibadan and other West African countries where there was decreased in vitro sensitivity to mefloquine (Salako and Aderounmu, 1987; Oduola et al 1987). Mefloquine has not yet been introduced for clinical use in West Africa.

Of the 25 isolates of P. falciparum that were cultured on quinine plates, 13 (53%) were successful. The schizont maturation of 12 isolates was completely inhibited at 256 pmol/well (51.2×10^{-6} mol/L) quinine and below while one isolate grew at 51.2×10^{-6} mol/L quinine. The EC 99 for the 13 isolates, was estimated to be 3.2×10^{-6} mol/L quinine. Since there was 100% schizont maturation up to 6.4×10^{-6} mol/L (well E) and there was schizont growth at 51.2×10^{-6} mol/L (well H), one may suggest that there is decreased in vitro sensitivity to quinine in this study.

Of the 59 cases that completed the in vivo study, 33 (55.9%) had their corresponding in vitro results for chloroquine. The comparative in vivo/in vitro results showed that 21 cases (63.6%) were fully sensitive to chloroquine both in vivo and in vitro while 4 cases (12.1%) were sensitive in vivo but resistant to the drug in vitro. Also 4 cases (12.1%) had delayed parasite clearance in vivo and were resistant to the drug in vitro while 4 cases (12.1%) were resistant to chloroquine both in vivo and in vitro.

Of the 4 cases that were sensitive to chloroquine *in vivo* but resistant to the drug *in vitro*, two were found to be severely malnourished. It has been observed that malnutrition does not appear to increase susceptibility to severe falciparum malaria, and there is evidence that malnourished children are less likely to develop severe malaria than well-nourished children (WHO 1986). One case from this group was mistakenly given a dose of 15mg/kg chloroquine apart from the 25mg/kg that was given over three days while the last case in the group confessed taking an antimalaria drug at home apart from the 25mg/kg chloroquine course that was given. One may therefore speculate that the immunity of the children coupled with the above reasons may have contributed to the full sensitivity of the 4 cases *in vivo* and resistance *in vitro*.

In conclusion, this study has shown clearly that there is resistance to chloroquine both *in vivo* and *in vitro* in Zaria urban area and possibly in other parts of Nigeria. However, this is more of a problem in children between the ages of 6 months and 3 years, whose immunity to malaria has not yet fully developed. Also one may say that the sensitivity of *P. falciparum* to chloroquine, in Zaria urban area has significantly decreased compared to 1985.

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Appendix 1

Dill and Glazko's Test

Reagents

Eosin (yellowish) 50mg

Chloroform 100ml

Hydrochloric acid 1N Solution 1ml

50mg of eosin (yellowish) was weighed and transferred to a small glass-stoppered separatory funnel. 100ml of chloroform and 1ml of 1N Hydrochloric acid were added to the eosin and the mixture was shaken by hand for a few minutes until the chloroform assumes a light yellow colour due to solution of the eosin. The chloroform layer was allowed to separate after which it was transferred to a brown glass stoppered bottle and stored in the refrigerator at $4^{o}C$

Method: To a small test-tube containing 2ml of urine, ten drops of the Dill-Glazko reagent are added and the contents mixed by vigorous shaking for a few moments. A colour change from yellowish to violet-red in the precipitated chloroform layer was taken as an indication of the presence of amodiaquine or chloroquine in the urine.

Appendix 2

Preparation of Phosphate buffer PH 7.2

Reagents

Potassium dihydrogen phosphate ($K H_2P_{O4}$) 0.7g

Disodium hydrogen phosphate ($Na_2 HPO_4$)..... 1.0g

Distilled water 1000ml

After weighing the two salts separatory, they were put together and dissolved in 1000ml distilled water. The solution was tested for PH using a pH meter and if the PH was found to be less than 7.2, it was adjusted by adding a little more of $Na_2 HPO_4$ as required until the correct PH was obtained.

Appendix 3

Preparation of RPMI-1640 Liquid Media

RPMI-1640 Powder	10.4g
Gentamycin sulphate	q.s
Double distilled water	1000ml

The RPMI-1640 Powder was weighed and dissolved in 1000ml double distilled water, Gentamycin sulphate was added and the solution was sterilized by Filtering through a 0.22 mm millipore Filter. The Filtrate was thereafter aseptically transferred into sterile 250ml bottles and stored in the Deepfreezer at -20^{o}C. The whole process was done under a Laminal Flow Cabinet.