SELECTED GENETIC RED-CELL MARKERS AND THE EPIDEMIOLOGY OF CHLOROQUINE-ASSOCIATED PRURITUS IN NIGERIA

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ABSTRACT

Anecdotal reports suggest that there may be a familial component to chloroquine-associated pruritus (CAP). To test this hypothesis, the epidemiology of CAP was studied with respect to haemoglobin S (HbS) and glucose-6-phosphate dehydrogenase (G6PD) deficiency both of which are recognised genetic red-cell markers for blacks and for malaria. ABO blood groups which are also red-cell markers but not specific for blacks or malaria served as internal control.

The prevalence of CAP was determined prospectively between January 1988 and June 1992 using cross-sectional survey in patients described below. The sample size was determined using EPI INFO 5. Only one individual per family was recruited. Field trips were made and a total of 1,315 patients were recruited into the study. A pretested questionnaire was used to collect information on personal details and family history of CAP from the recruited individuals. All the study patients except the school-children received chloroquine at recruitment time and were observed directly for CAP 24 to 72 h later. Blood samples were then collected into sequestrene bottles (or filter papers for samples from distant areas). ABO blood groups, Hb and G6PD types were determined. Malaria parasite screening and full blood counts were also done on University College Hospital (UCH) samples.

Prevalence of CAP among UCH patients was 124/300 (41.3%), pregnant women 23/55 (42.0%), urban school-children 85/150 (56.7%), rural school-children 61/121 (50.4%), rural preschool-cluldren 33/151 (21.8%), Kano 16/121 (13.3%), Maiduguri 40/165 (24.2%), Potiskum 56/163 (49.6%) and Port-Harcourt 9/108 (8.3%). CAP increased progressively with age. CAP was more common in families of itchers than non-itchers. There was an association between cumulative chloroquine intake and CAP. In all the populations studied, except among pregnant women, the sickle-cell trait was less common but G6PD deficiency was more common among itchers than non-itchers. By contrast however, ABO blood groups distribution was similar among itchers and non-itchers.

A simple, rapid, sensitive and highly reproducible high performance liquid chromatographic (HPLC) method was developed for the assay of chloroquine and its main metabolite, desethylchloroquine. With the method it was demonstrated that the pharmacokinetics and urinary excretory pattern of chloroquine and desethylchloroquine were similar in 7 itchers (4 Hb AA and 3 Hb AS) and 8 non-itchers (4 Hb AA and 4 Hb AS) as well as in the Hb AA and Hb AS individuals. Nevertheless, there was evidence that the itchers were unable to metabolize chloroquine as extensively as the non-itchers, as the ratio of the area under the curve (AUC) of the metabolite to that of the parent drug was lower in the itchers compared to the non-itchers. Also the peak concentration (Cmax) of chloroquine was lower in Hb AS individuals when compared with Hb AA. However, bioavailability problem could not be ruled out in the Hb AS subjects.

Seven of the 9 subjects prone to CAP actually itched, 6 of the 7 had residual chloroquine (26.7±11.2ng/ml) in zero hour plasma, whereas the remaining 1 itcher as well as the 2 who did not itch had no measurable quantity of chloroquine in the zero hour plasma.

It is concluded that CAP may be associated with certain genetic factors which include Hb and G6PD types.

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CERTIFICATION

We certify that this work was carried out by OLUSEGUN GEORGE ADEMOWO in the Department of Phannacology and Therapeutics, University of Ibadan, Ibadan, Nigeria under our supervision.

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DEDICATION

This work is dedicated to the tireless scientists who have continuously waged seemingly unending war against our friendly enemy, the most successful and prolific organism '*Plasmodium*'.

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ABBREVIATIONS

AUC	Area under the curve
CAP	Chloroquine-associated pruritus
Cl_R	Renal clearance
C.V.	Coefficient of variation
CQ	Chloroquine
DCQ	Desethylchloroquine
g	Gramme
G6P	Glucose-6-phosphate
G6PD	Glucose-6-phosphate dehydrogenase
GSSG	Oxidized glutathione
Hb	Haemoglobin
h	Hour
HPLC	High pressure liquid chromatography
Kg	Kilogram
l	Litre
mm	Minutes

mg	Miligramme
ml	Millilitre
mM	Millimolar
NADP	Nicotinamide adenine dinucleotide phosphate
NBT	Nitroblue tetrazolium
ng	Nanogramme
nm	Nanometre
pH	Hydrogen ion concentration
PMS	Phenazine methosulphate
SD	Standard deviation
$t^1/_2$	Half life
ug	Microgramme

CHAPTER 1

1.1 INTRODUCTION

Severe adverse drug reactions are a restraining factor in drug administration, even where the compound is superior therapeutically to others for a particular indication. Adverse drug reactions may be extensions of either pharmacokinetic or pharmacodynamic effects of a drug. Variability in the ability to express adverse drug reactions are in part thought to be due to intersubject variability. On the other hand interindividual differences in drug response are dependent on pharmacogenetic principles. The process of expressing pharmacogenetic variability could be markedly influenced by age, sex, diet, environmental pollutants, co-administration of drugs, underlying disease process and the environment in which the individual lives (Vesell, 1977; Eichelbaum, 1981; Sjoqvist *et al*., 1980; Breimer, 1983 and Vesell and Penno 1983).

Malaria is one of the most prevalent of human diseases. It is a major health hazard which occurs mainly in the tropics. Previously temperate and sub-temperate regions of the world were also affected (Bruce-Chwatt 1986). The total number of patients affected by malaria annually is estimated to be 103 million of which 86% live in Africa south of the Sahara (WHO, 1989). It is estimated that 1-2 million people die of malaria and malaria related causes every year.

In Nigeria, although the risk of malaria exists throughout the country, the endemicity of malaria is mesoendemic in the urban but holoendemic in the rural areas. The disease consistently ranks among the five most common causes of death among all age groups (Federal Ministry of Health 1989). It also represents substantial social costs due to school absenteeism and reduced economic productivity. Pregnant women are at a higher risk of malaria infection relative to their non-pregnant counterparts living in the same endemic condition (Nosten *et al*, 1991; Gilles *et al*, 1969; Brabin *et al*, 1990; Steketee *et al*, 1987, 1988). However, children above 6 months old are most vulnerable (Bruce-Chwatt, 1963). The mortality rate is highest during the first 2-4 years of life (Bruce-Chwatt, 1952;

Greenwood *et al*., 1987). Some individuals have the benefit of genetically controlled protection mechanisms. These protection mechanisms are due to mutant genes especially those causing glucose-6-phosphate dehydrogenase (G6PD) deficiency and sickle cell disease (Luzzatto 1979, Gilles *et al*., 1967). The long association of malaria parasites with man has led to the spread of these mutant genes in the latter (Luzzatto 1979). Haemoglobin S (HbS), and G6PD deficiency which are otherwise deleterious genes have reached polymorphic frequencies in malaria endemic regions. It was suggested that they confer selective advantage in malaria. Allison, (1960) first suggested that the heterozygous state i.e. the sickle cell trait (Hb AS) is responsible for the maintenance of HbS gene in the malarious areas because of the partial resistance it confers against malaria. However, this protection is not present in homozygous children (Hb SS) with malaria in whom the infection can be fatal (Adeloye *et al*, 1971; Luzzatto, 1974). It has also been demonstrated that the thalassaemias may protect against malaria, thus explaining the persistence of this otherwise deleterious haemoglobinopathy (Weatherall and Clegg, 1981). Motulsky and Campbell-Kraut (1961) and Allison (1964) were the first to observe a close correlation between the present and past prevalence of malaria and G6PD deficiency. The heterozygous carriers of the gene are protected against the life threatening form of malaria i.e. severe and complicated malaria caused by *P. falciparum* (Allison, 1964; Livingstone, 1971; Bienzle *et al*, 1972).

Chloroquine is the most widely used antimalarial drug in malaria endemic areas (Bruce-Chwatt 1986, WHO, 1984). Pruritus is a major side effect of chloroquine and it has very important therapeutic consequences. This is because it is so unpleasant that those who have had experience of it are often unwilling to take further dose of the drug for subsequent malaria attacks. It is a major cause for caution in the use of chloroquine (Federal Ministry of Health, 1989).

The clinical course of the chloroquine-associated pruritus (CAP) was first described by Ekpeclu and Okoro, (1964). It was characterised by a biting or pricking sensation affecting all parts of the body including the scalp, the palms of the hands and the sole of the feet. It usually began within a few hours of taking the drug and often continues for between 48 and 72 hours irrespective of treatment with antihistamines. It is usually severe enough to make sleep impossible.

Despite the relentless advance of parasite resistance, chloroquine remains an effective treatment for severe malaria in some parts of the tropics (Salako and Adeyemi, 1987; White *et al.*, 1987, 1988). The drug still remains the first line of drug for the treatment and suppression of malaria because it is cheap, safe and well tolerated at the recommended dose. However, CAP which is experienced by some individuals in the population considerably limits the acceptability of the drug as a suitable antimalarial and this is a cause of concern for both malariologists as well as malaria patients.

The overall objective of the present study is to investigate the prevalence of CAP and test the hypothesis that the population distribution of CAP may be controlled by certain genetic factors.

1.2 PATHOPHYSIOLOGY AND IMMUNOLOGY OF MALARIA

1.2.1. Biology of the Malaria Parasite

The disease in man is carried by four species of plasmodium namely: *Plasmodium falciparum, P. malariae, P. ovale and P. vivax* (Bruce-Chwatt 1986). The plasmodium are protozoan parasites transmitted to man by anopheline mosquitoes. The major vectors of the human malaria are. *A. arabiensis, A. funestus and A. melas. P. falciparum* is the most pathogenic agent of the human malarias (WHO, 1984; Luzzatto 1979). It is strictly species specific preferentially parasitizing human cells (Triggs, 1975). *P. malariae, P. ovale and P. vivax* infections have milder consequences. With the exception of *P. falciparum*, all the other species are classified as causing relapsing malaria. This is because they have secondary exo-erythrocytic stage of development which provides a reservoir of parasites for the re-infection of the erythrocytes. This can give rise to a recrudescence of symptoms months or even years after a clinical cure has been obtained by the destruction of the asexual form in the blood. There is however, evidence of a latent tissue stage (hypnozoites) in the hepatic cells for *P. vivax and P. ovale* and lack of exo-erythrocytic stage for *P. malariae* (Bruce-Chwatt, 1986).

P. falciparum-caused disease is sometimes called malignant tertian malaria, because it produces a fulminating infection in non-immune victims with spikes of fever every third day corresponding to the bursting of the erythrocytes and the release of the merozoites. Untreated falciparum malaria in non-immune individuals may rapidly progress to a fatal conclusion.

P. malariae-caused disease is also referred to as quartan malaria because spikes of fever come every fourth day. In West Africa, quartan malaria is associated with a high incidence of nephrotic syndrome.
P. malariae is unique in that infection can remain dormant for many years.

P. ovale which is common in West Africa causes a rare form of relapsing malaria. Its periodicity is similar to that of *P. vivax*, but it runs a milder course and is more easily treated.

The primary host of the plasmodium parasites is the anopheline mosquito in which the parasite undergoes sporogony - a process of sexual reproduction which results in the formation of motile sporozoites which are inoculated into the circulation. The secondary host are the humans in which schizogony occurs - a process of asexual reproduction. The asexual cycle occurs in two different places, first in the tissues and then in the erythrocytes. They are referred to as exo-erythrocytic and erythrocytic schizogony respectively. These results in the production of mature schizonts which break up to release merozoites (Garnham, 1966; Pratt, 1977; Wyler, 1982). The release of merozoites from infected erythrocytes marks the beginning of the clinical features of malaria, of which fever is invariably a constant feature. The incubation period is shortest for *P. falciparum* (12 days), and longest for *P. malariae* (28 days). *P. vivax* has incubation period of 13-17 days (Bruce-Chwatt, 1971). Some strains show much longer incubation period of up to nine months. Details of life history of the malaria parasite can be seen in Fig. 1.4.1

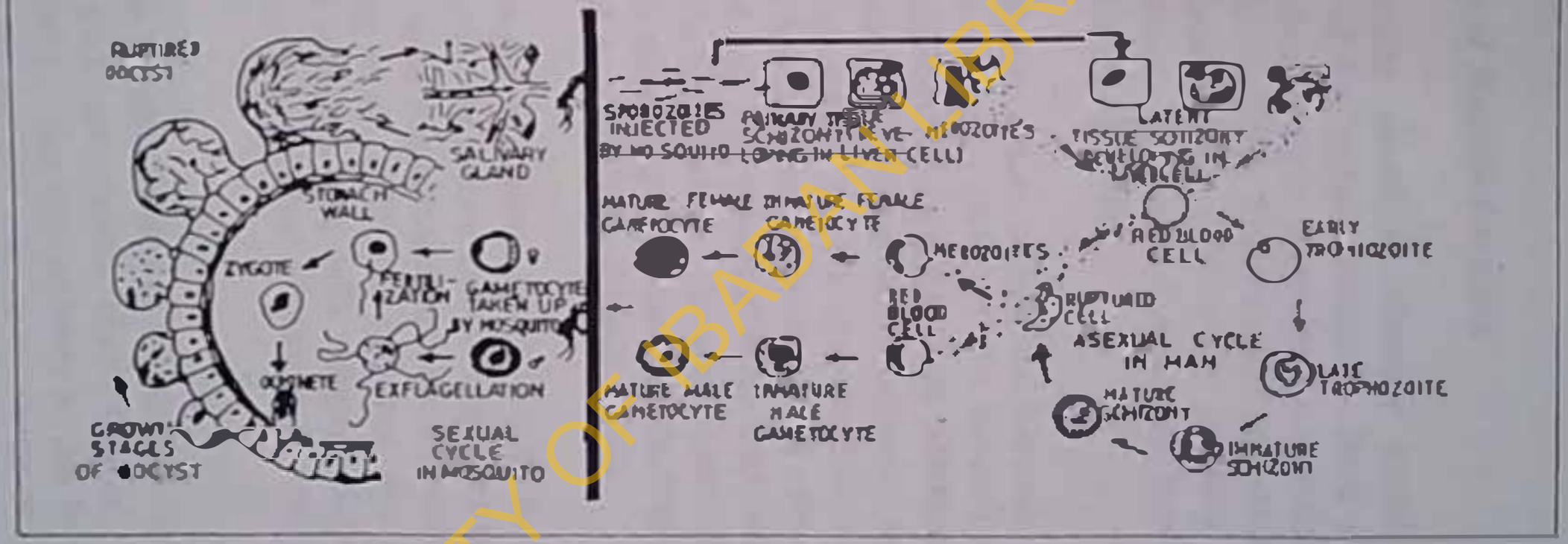


FIG. 2 : CYCLES OF DEVELOPMENT OF MALARIA PARASITES IN THE ANOPHELINE MOSQUITO AND IN MAN

FROM WORLD HEALTH ORGANIZATION (1963) TERMINOLOGY OF MALARIA AND OF MALARIA ERADICATION - GENEVA

1.2.2. Immunology of Malaria Parasites

Malaria immunity develops following invasion of the organism by erythrocytic forms of the malaria parasite. There is however no convincing evidence that the exo-erythrocytic stages have any significant effect on the immune response (Bruce-Chwatt, 1986). The immunity appears to be acquired towards the late stage intracellular asexual erythrocytic parasites or towards the liberated extracellular merozoites. It does not appear to have any obvious effect upon gametocytes (Hawking *et al*., 1966).

Acquired immunity is both cellular and humoral. There is also evidence of complement activation in man (WHO, 1990). Infected red cells are removed from circulation by macrophages and phagocytosis. Human leucocyte antigens (HLA) have also been associated with protective immunity against malaria (Hill, 1992). Antibodies which are species specific opsonize schizonts-laden red cells and also bind to merozoites thus inhibiting invasion into red cells. Antibody activity has been found in IgG, IgM and IgA immunoglobulin fractions of immune sera but most predominantly in the IgG fraction. Serum concentrations of IgG, IgM and IgA-levels are elevated early in the infection but decline rapidly and with prolonged exposure, IgG level is raised (Bruce-Chwatt, 1986).

There is a degree of common antigenicity existing among different species of mammalian plasmodia. Protective immune responses to malaria are usually species specific, although some cross-protection is known especially in rodent system (Cox 1970, Nussenzweig *et al*., 1972) and they may also be strain specific. In West Africa, over 90% indigenes have duffy antigen negative red cells. This has been linked with the resistance of West Africans to *P. vivax* infection (Miller *et al* 1976, 1977).

In infants in the early months of life, the manifestation of malaria are usually mild with low grade parasitaemia (Bruce-Chwatt, 1951; Salako *et al*., 1990; Akindele *et al*., 1993). This may probably be due to the fact that neonates born to immune mothers are partially immune by virtue of the persisting transplacentally acquired maternal antibodies (Sodeinde and Dawodu, 1985; WHO, 1990). The

protection in children decreases in the first few months of life and a partial immunity is acquired by repeated attacks of malaria (Bruce-Chwatt, 1952; Gilles *et al*, 1967).

The continuous protection of non-immune persons with antimalarial drugs is mandatory. Continuous regular chemosuppression in children born in malarious area will not only reduce the high morbidity and mortality rates due to malaria but will also enhance the physical development. There is much indirect evidence to link the effectiveness of chemotherapy with the immune status of the host. Treatment of infected semi-immune individuals is effective with lower dose schedules than those required for infected non-immunes (WHO, 1973).

1.3 GENETICS AND MALARIA

1.3.1. Malaria and Sickle Cell Gene

Sickle cell anaemia (SCA) is a severe, chronic disorder characterised by recurrent episodes of excruciating pains in the limbs, back, abdomen or chest associated with fever, jaundice and anaemia which becomes very severe. Sudden and even life threatening complications such as osteomyelitis, pneumonia, meningitis, cerebrovascular accidents and renal infarcts are common (Barret-Cannor 1971; Portney and Heroson, 1972; Powars, 1975).

Sickle cell anaemia is caused by the homozygous presence of a mutant gene, the S-gene. The gene product, the sickle haemoglobin carries the amino-acid valine instead of glutamic acid at the 6th position from the amino terminal of the β-globin chain. This alteration causes ready polymerisation of the S-haemoglobin into tactoids which are stacked together to form fibres or bundles of S-haemoglobin. This distorts the cell to its characteristic sickle shape. This sequence of events is called the sickling process and is induced or enhanced by hypoxia, acidosis and hyperosmolar states. Though it is generally reversible but, it could become irreversible. The sickled red blood cells are relatively rigid and cannot flow through the capillary bed easily leading to ischaemia and if sufficiently severe, infarction.

Sickle cell disease (SCD) is a broader term which includes SCA as well as haemoglobin S+C disease and S-β-thalassemia. The latter two are clinically less severe. However, SCD excludes the sickle cell trait (i.e. haemoglobin A+S) who are clinically normal apart from a slight reduction in the ability to concentrate urine (Taylor *et al*., 1978).

Most of the studies done in children indicated lower parasite counts and lessened severity of malaria infection in Hb AS when compared with Hb AA subjects (Allison, 1961, Edington and Watson-Williams, 1965, Gilles *et al*., 1967, Walters and Bruce-Chwatt, 1956). Although some workers (Carnevale *et al*., 1981,

Michel *et al*., 1981; Hill, 1992) could not confirm this, Fleming *et al* (1985) reported that it could only be observed in non-immunes, generally between the age of six months and five years.

Several mechanisms have been postulated for protection of Hb AS individuals from malaria. It has been suggested that the utilisation of oxygen by the parasite in the red cell as well as the normal oxygen loss to the tissues could cause hypoxia. This induces increased sickling of the parasitized cell and its consequent removal from circulation by the reticuloendothelial system (Miller *et al*., 1956; Luzzatto *et al*., 1970). The sickling may also be attributed to the lowering of the intracellular pH by *P. falciparum* resulting from loss of potassium ions. (Roth *et al*., 1978; Friedman *et al*., 1979). It has also been shown that sickling children have significantly higher IgG levels than non-sickling children. This may imply that the trait enhances the antibody responses against malaria parasite (Lucas and Gilles 1984).

1.3.2. Malaria and Glucose-6-phosphate dehydrogenase (G6PD: EC 1.1.1.49)

1.3.2.1 G6PD and the Red Cell

The ability to obtain energy by Kreb's cycle or synthesize protein is lost by the mature erythrocyte as it is without mitochondria. It therefore obtains energy from the high energy compound Adenosine triphosphate (ATP) generated by the Ebden-Meyerhoff pathway (Harries, 1963) for the maintenance of its structure and ion transport across its membrane. Under steady state conditions, 95 per cent of the glucose available to the erythrocyte is metabolised in this way and the remaining 5 per cent enters the pentose phosphate pathway which serves as the source of reduced nicotinamide adenine dinucleotide phosphate (NADPH). The reducing potential of NADPH is required for the integrity of the erythrocyte, maintaining an adequate level of reduced gluathione (GSH) which protects sulphhydryl groups against intrinsic and drug-induced oxidative damage. G6PD is the first and also the rate-limiting enzyme of this pathway. There is a substantial decrease in the activity of G6PD with erythrocyte age (Rubinstein *et al*, 1956, Marks *et al*, 1958; Piomelli *et al* 1968, Bienzle 1981). However, despite this loss of enzyme activity, normal old red cells contain sufficient G6PD activity to maintain GSH levels in the face of oxidant stress. About 0.1% of normal G6PD activity is required for the generation of NADPH (Luzzatto and Testa, 1978; Kirkman *et al* , 1980)

G6PD is a ubiquitous enzyme which has been found in every organism and tissue in which it has been sought. This is not surprising in view of its metabolic role (Bienzle 1981; Sodemde 1992). Apart from the production of NADPH in erythrocytes, it is involved also in the synthesis of pentose for incorporation into nucleic acids (Luzzatto and Testa 1978, Sodemde, 1992)

G6PD deficient erythrocytes have decreased enzyme production, as a result of accelerated breakdown, formation of molecules with decreased catalytic activity or production of enzyme molecules with reduced stability (Beutler, 1978, Bienzle, 1981). These abnormalities result in a heterogenous group of G6PD deficiency

ranging from mild enzyme deficiency to almost complete lack of enzyme activity (Bienzle 1981). The enzymatic defect in G6PD mediterranean is due to a greater enzyme instability and red cells of all ages are grossly deficient in the enzyme (Subliran and Glader, 1980). The deficiency of G6PD A-, the commonest G6PD deficient variant is due to accelerated breakdown of G6PD molecules (Bienzle 1981).

G6PD variants of erythrocytes occur world-wide (WHO 1966, 1967; Livingstone 1967; Luzzatto and Battistuzzi 1984). The genetic variability of G6PD is only second to haemoglobin (Luzzatto and Testa 1978). Some are sporadic i.e. only few cases encountered while some are polymorphic i.e. when the frequency in a population is above 1%. The existence of different variant was established on the basis of red cell enzyme activity, electrophoretic mobility, the Michaelis constant for its substrate, heat stability and pH optimum. These are the major types of variants found among the blacks viz:

G6PD B:- Commonest and found all over the world. It shows normal enzyme activity and is not associated with haemolysis (Boyer et al 1962; Yoshida 1966).

G6PD A:- Has a slightly reduced but clinically normal enzyme activity. It does not cause haemolysis. It is found in Africa and populations with African ancestry. It has a faster electrophoretic mobility than the B-type (Yoshida 1967).

G6PD A⁻:- Commonly found in population of African origin (Beutler 1979). It results in a mild enzyme deficiency associated with haemolysis. Its electrophoretic mobility is identical to that of A- type (Beutler 1978).

Under certain dietary conditions like ingestion of Vicia fava beans or after treatment with certain anti-malarial drugs like primaquine, the genetically determined enzyme deficiency impairs the normal metabolic function of the erythrocyte. This causes its premature destruction and thus manifests as a severe

haemolytic anaemia especially in children (Motulsky 1960, 1964, 1965; Motulsky and Campbell-Kraut, 1961; Livingstone, 1971; Allison, 1975). Other factors that could precipitate haemolysis in G6PD deficient persons especially neonates are exposure to menthol or camphor
(Olowe and Ransome-Kuti, 1980). Septicaemia caused by certain bacteria like *E. coli, S. aureus or albus* has also been implicated (Effiong and Laditan 1976).

G6PD deficiency is inherited as an X-linked disorder (Marks *et al*., 1961). There is therefore a lack of father to son transmission (Gross *et al*., 1958; Childs *et al* 1958). Because of the X-linkage, a full expression of the trait occurs in homozygous females and hemizygous males in whom the X-chromosomes present carry the mutant gene. In heterozygote females, two different biochemical types of the enzyme can be seen in two cell populations with an approximately 1:1 ratio. 72 This ratio may vary depending on the time of inactivation (Lyon 1961). Such variations have been reported by Luzzatto *et al* (1979).

I.3.2.2. G6PD and Malaria

Evidence so far shows that all the populations in which G6PD deficiency is frequent have been exposed to malaria over extended periods of time (WHO 1967; Luzzatto 1979). This has led to the suggestion that the deficiency may confer selective advantage under certain conditions. An exception was seen in the indigenous American population who do not have G6PD deficiency in spite of malaria endemicity. This could be because the G6PD gene cannot become polymorphic in a population if it did not get there by migration or arise *in situ* by mutation (Luzzatto and Battistuzzi 1981).

Malaria morbidity and mortality is expected to be lower in carriers of G6PD A⁻ gene either in the heterozygous, homozygous or hemizygous state. Allison and Clyde (1961) and Gilles *et al* (1967) found significantly lower parasite counts in young enzyme-deficient African children. However, Martin *et al* (1979) and Krutrachue *et al* (1962) could not demonstrate this. Bienzle *et al* (1972) reported a significantly lower parasitaemia in heterozygous females with genotype Gd^{A-} Gd^{B}

(but not G^{dA-}/Gd^{A}) than in G6PD normal or deficient subjects, male or female. It was then concluded that like the haemoglobin S heterozygote, the heterozygous G6PD deficient females are partially protected against malarial infection and thus is probably responsible for the maintenance of polymorphic frequencies of G6PD deficient genes in malarious areas of Africa (Bienzle *et al* 1979).

The cellular mechanism by which heterozygous females are protected from the malaria parasite has been well investigated *in-vitro* using the established continuous culture of *P. falciparum* (Trager and Jenson 1976). Friedman (1979) found that *P. falciparum* grows equally well in normal as in G6PD deficient red cells under standard culture conditions but growth was significantly impaired under oxidative stress imposed by higher oxygen tension. Luzzatto *et al* (1983) tried to explain why it is the heterozygous female rather than hemizygous deficient males that are protected from malaria. They showed normal invasion of both normal and deficient red cells but maturation was delayed and impaired in deficient red cells. But after several passages, the parasites adapted and no difference in maturation was obtained. This is why hemizygous males and homozygous females are not protected. But in heterozygotes this adaptation would be frustrated each time a parasite emerging from G6PD normal cell happens to invade a deficient cell. This would likely limit significantly the probability of life threatening levels of parasitaemia being reached in these subjects (Bienzle *et al*, 1972).

1.4 HISTORY OF CHLOROQUINE

Chloroquine, 7-chloro-4-(4-diethylamino-1-methy-butylamino) quinoline is a 4-aminoquinoline antimalarial schizonticidal compound. It has trade names and code designates as follows:

Chloroquine diphosphate: Aralen, Arloctor, Bemaphate, Chinamine, Gontochin, Resochin, Resoquine, Klorokin etc.

Chloroquine sulphate: Nivaquine, Nivaquine B,

Chloroquine was synthesized in 1934 in Germany during a search for antimalarials in preparation for an impending war by Andersag in the laboratory of Bayer Company in Elderfield. It was tested for antimalarial activity but in 1935, it was thought to be too toxic for human use and then discarded (Coatney, 1963). In view of this, during the second world war, in a systematic search for synthetic antimalarial compounds, chloroquine was one of the twenty-five different 4-aminoquinoline derivatives which were obtained by United States of America workers. Finally, a compound designated SN 7618 (later named chloroquine) was selected as the most promising - only to discover a few months later that it was identical with the compound patented in Germany and the U.S.A. under the name of Resoclun. Preliminary clinical studies showed it to be more potent and less toxic than mepacrine. It was also found to control the symptoms of malaria more promptly and to clear the peripheral blood of malaria parasites more rapidly than mepacrine. The latent period for relapse was found to be longer at concentration within a margin of safety much greater than either mepacrine or quinine.

Chloroquine was first prepared in the form of a dihydroxy-benzoic acid derivative, but since then a less toxic 3-methyl derivative (santoquine) had been synthesized in the United States of America. An official pharmacopoeial name of chloroquine was finally adopted for the chemical.

1.5. CHEMISTRY OF CHLOROQUINE

Chloroquine is a synthetic compound having a quinoline ring similar in structure to that of quinine. It is chemically known as 7-chloro-4-(4-diethylamino-1-methylbutylamino) quinoline. It contains an assymetric carbon atom (asterisked) Fig. 1.5.1. Thus it has two isomeric forms 'd' (dextro) and l (laevo). It occurs as white, bitter crystals with melting point between 86 and 87°C. The molecular weight of chloroquine base is 320, chloroquine phosphate 516 and chloroquine sulphate 438. It is soluble in organic solvents and dilute mineral acids but sparingly soluble in water. Chloroquine is a quinoline containing nitrogen atoms in the molecule, the 4-amino-group on the quinoline increases its basic properties hence chloroquine is a strong base (pKa 10).

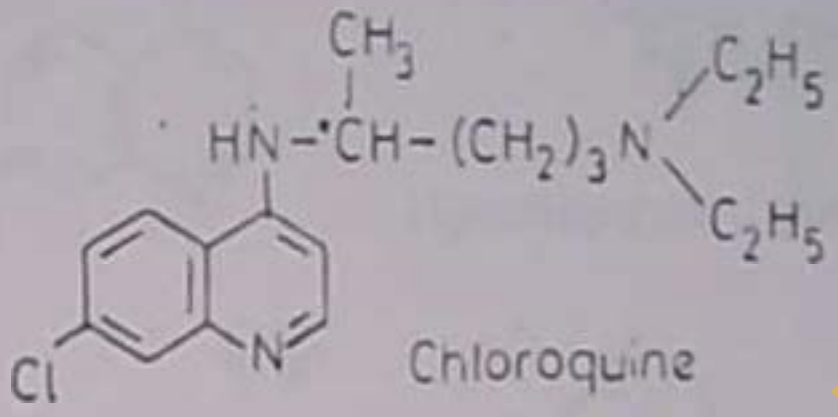
Chloroquine contains the same alkyl side chain as quinacrine but differs from the latter in possessing a quinoline instead of an acridine nucleus and in lacking the methoxy radical. Chloroquine differs from primaquine, pamaquine and pentaquine by the position of the alkyl side chain and in having chlorine instead of a methoxy ring substitution. The introduction of a hydroxy group on the side chain, for example in an ethyl group of the terminal nitrogen (e.g. as in hydroxy-chloroquine) reduces toxicity but produces high blood levels as a result of low volume of distribution.

The dextro (d), laevo (l) and 'dl' forms of chloroquine are indistinguishable in potency tests in duck malaria, but the 'd' isomer was found to be somewhat less toxic than the 'l' isomer in mammals (Goodman and Gilman, 1980). Pamaquine, hydroxychloroquine, santoquine and amodiaquine are all chloroquine derivatives (Fig. 1.5.2).

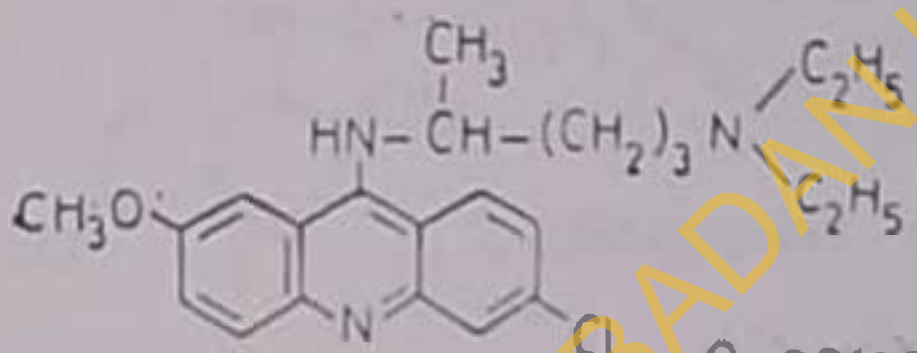
The chlorine atom in the 7th position of the quinoline nucleus appears to be crucial for the antimalarial activity of the 4-aminoquinolines. Methyl substitution in position 3 of the nucleus as in santoquine reduces the activity and further methyl substitution in position 8 completely eliminates the antimalarial activity (Berliner *et al* 1948; Coatney *et al* 1953).

Quinoline Congeners

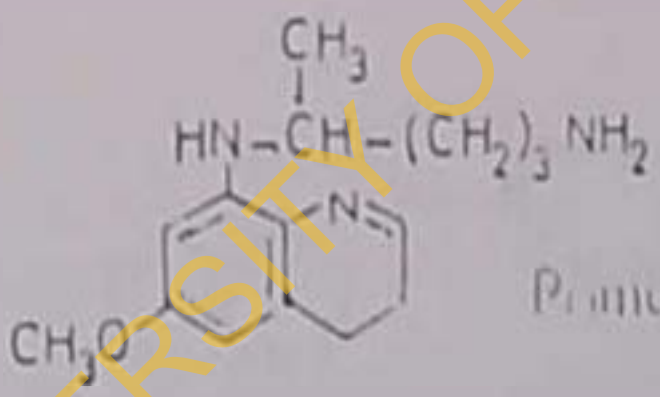
FIG 1.5.1



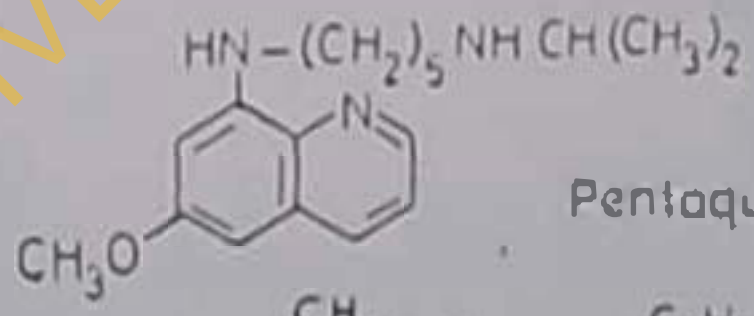
Chloroquine



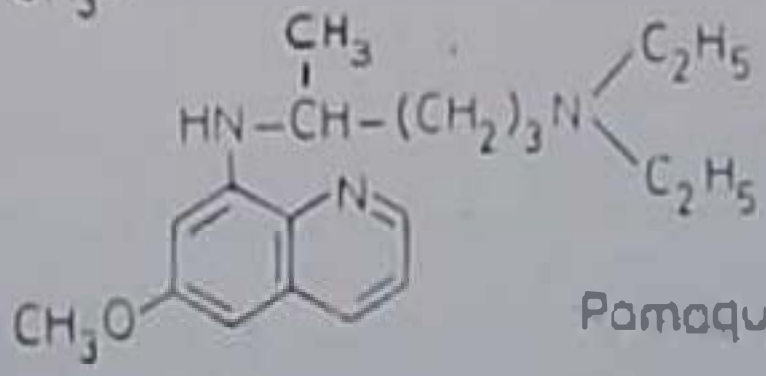
Quinacrine



Primaquine

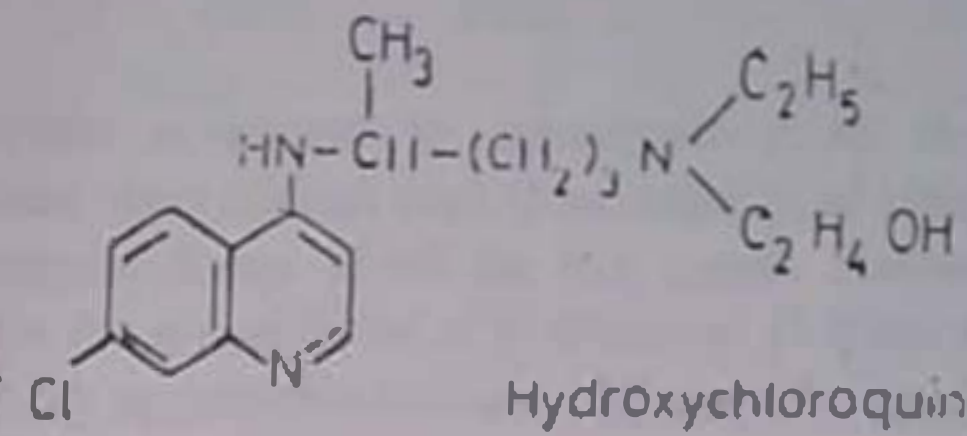


Pentaquine

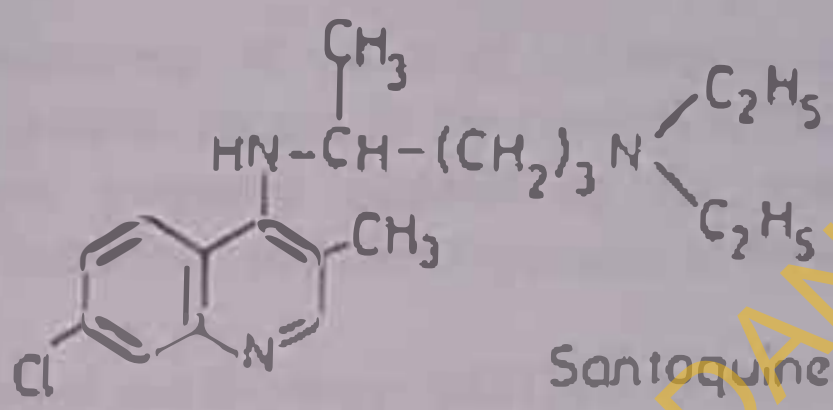


Pamaquine

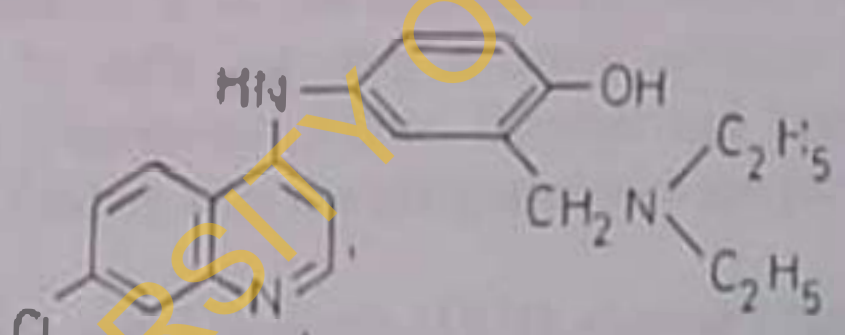
FIG.1.5.2 Chloroquine analogues



Hydroxychloroquine



Santoquine



Amodiaquine

1.6 MECHANISM OF THE ANTIMALARIAL ACTION OF CHLOROQUINE

Chloroquine is known to concentrate more in plasmodium-parasitized erythrocytes than unparasitised ones (Macomber *et al* 1966). It is effective against asexual erythrocytic forms of all the four human plasmodia species. It is also effective against the sexual forms of *P. malariae, P. vivax and P. ovale*. It does not however exert any appreciable biological effect on the pre-erythrocytic parasite.

The mechanism of action of chloroquine has not been fully elucidated. Various mechanisms interact in the eventual schizonticidal action of chloroquine. All hypotheses proposed centered on the observation that chloroquine is concentrated in the food vacuole or lysosome of the parasite.

Homewood and Warhurst (1971) put forward a physico-chemical explanation that chloroquine possesses just the right combination of lipid solubility and ability to become doubly protonated to permit it to pass from the serum into the trophozoite phagosomes which are normally maintained at acid pH which is required for optimum functioning of the parasites proteolytic enzymes. The initial uptake of chloroquine by cells and drug sensitive parasites is thus very rapid and energy independent (Mackenzie, 1970). Krainer and Matusik (1971) suggested that the high affinity binding sites are associated with the parasite membrane.

Fletcher and Macgrath (1972) observed that chloroquine intercalates with plasmodial DNA. It was also observed that chloroquine inhibits the uptake of ^3H adenosine by trophozoites in short culture. Studies have shown that chloroquine and its congener form a complex with DNA that prevents DNA from acting as a template for its own replication or transcription to RNA (Hahn, 1975; Kwalungo-Berlin and Melnizh, 1990). Allison *et al* (1965) and Cohen and Yielding (1965) found that chloroquine inhibits markedly DNA polymerase and RNA polymerase to a lesser extent, by combining with the DNA polymer. It has been postulated that the quinoline ring of chloroquine is inserted between the base pairs of the DNA double helix so that the chlorine atom in position 7 of the quinoline ring lies in close

proximity to the 2-amino group of guanine in a guanine-cytosine base pair. The diaminoaliphatic side chain of chloroquine lying across the minor groove of the DNA helix ties the two strands together by interacting ionically with the phosphoric acid groups of both strands. The elcctronegativity of the substituent at position 7 of the quinoline ring appears to be critical to the stability of the complex. The length of the side chain bridging the minor groove of the DNA helix is also critical. Antimalarial activity is maximal when there are four carbon atoms between the two nitrogen atoms in the side chain, whereas a side-chain with 3 or 5 carbon atoms instead of 4 has only two-thirds of the antimalarial activity of chloroquine and a chain with 2 or 6 carbon atoms exhibits only one third of the activity. The selective toxicity for the malarial parasites must therefore depend on a chloroquine concentration mechanism.

Studies have shown that chloroquine by virtue of its weak base properties accumulates in the food vacuole and phagosomes which is analogous to the mammalian lysosomes. This leads to depletion of the acid radicals with the resultant alkalinisation of the food vacuole (which is normally acidic) by the concentrated drug with concomitant interference with the function of the digestive enzymes thereby leading to impairment of the digestion of haemoglobin and subsequently, a drastically reduced supply of amino acids to the parasites. These would then stimulate autolysis of haemoglobin by depriving the cell of its main amino acid source which in turn results in the formation of haemozoin clumping and death of the parasite (Warhurst and Robson, 1971; Krogstad and Schlesinger, 1987).

Quinine and mefloquine which are effective against chloroquine resistant strains of malaria were found to antagonise the clumping produced by chloroquine in a competitive manner (Peters *et al*, 1975). The phagosomes become labile with higher drug concentrations, thus permitting leakage of the phagosome contents into the general endoplasm of the parasite. This influence on the energetics of the parasites may also be responsible for inhibitory effects of chloroquine, quinine and primaquine on amino acid incorporation and other metabolic processes of the malaria parasite.

The mechanism of preferential accumulation is unclear, a number o hypotheses have been made Fitch (1970) thought that there must be participation of high-affinity receptor sites for the process Fitch (1983) reported that chloroquine binds to ferriprotoporphyrin IX, a breakdown product of haemoglobin which is toxic to the cell The resultant complex is toxic and unable to be sequestered as haematin, the black pigment of the cell and hence lysis of the parasite occurs (Orjih *et al*, 1981; Yayon *et al*, 1985) as the complex impairs the ability of cell membranes to maintain cation gradient (Banyal and Fitch, 1982) Chloroquine is also shown to inhibit the degradation of endocytic vesicles in human malaria parasites, which are then starved (Yayon and Ginsburg, 1983)

The pharmacological and pharmacokinetic properties of antimalarials have been suspected to have a great effect on the development of drug resistance (Peters 1967) Other factors postulated to be responsible for the emergence of resistant strains include the misuse of drugs, the repeated treatment of groups of individuals with infrequent or inadequate dose regimens associated with severity of the parasite challenging the immune status of the host (Peters, 1982; Walker *et al*., 1983) It has been shown that chloroquine is not well concentrated in the food vacuole of resistant cells (Fitch, 1970; Macomber *et al*., 1966; Krogstad *et al*., 1987) Martin *et al* (1987) showed that the chloroquine resistant phenotype could be inhibited by incubating parasites in the presence of verapamil, a calcium channel antagonist

Fitch (1989) postulated the existence of a drug receptor in parasitized erythrocytes He observed that the initial uptake of chloroquine into the erythrocytes produces a steady state distribution within minutes and saturation takes place at a relatively low concentration of the drug in the external medium Substrates have also been suggested to play an important role in the mechanism of drug resistance to chloroquine since they are required for active transport or provision of a drug receptor or some other components involved in the drug binding or accumulation Fitch *et al* (1974) observed that glucose, glycerol, pyruvate and lactate stimulate the drug binding while ionoacetate and dinitrophenol was shown to possess an inhibitory effect The ATP concentration has been shown to increase in both sensitive and resistant parasites when glucose and glycerol are used as substrate

1.7 SOME OTHER PHARMACOLOGICAL ACTIONS OF CHLOROQUINE

Chloroquine has anti-inflammatory effects that have been useful in the treatment of rheumatoid arthritis, intestinal and hepatic amoebiasis and dermatoses like discoid lupus erythematosus (Goldman *et al* , 1953, Mackenzie, 1970, Stillman, 1981). The mechanism of the anti-inflammatory action is not clear. It also has antihistaminic, anticholinesterase and anti-protease properties.

The anti-inflammatory action of chloroquine probably derives from effects on membrane stabilization of lysosomes (Weissmann, 1964). Chloroquine has been found to penetrate the cells and accumulate in the lysosomes (Zvaifler, 1964), leading to the reduction of the activity of several lysosomal enzymes (Weissmann, 1965, Wibo and Poole, 1974). This may partially inhibit the polymorphonuclear leukocyte contribution to rheumatoid inflammation (Mackenzie, 1970). Ward (1966) also showed that chloroquine inhibited both chemotaxis and phagocytosis in the granulocytes. Chloroquine has been demonstrated to inhibit cyclo-oxygenase in guinea-pig skin (Ruzicka and Printz 1982). An anti-inflammatory action would thus be presumed through the blockade of prostaglandin synthesis.

Olatunde (1976) reported that chloroquine appears to inhibit a number of enzymes while stimulating a few and that most of the enzymes affected by the drug are non-microsomal enzymes. Chloroquine inhibits alcohol dehydrogenase and glutamic dehydrogenase competitively. Chloroquine also inhibits imidazole N-methyl transferase by 39% at concentration 10^{-5}M while 10^{-3}M produces complete inhibition of the enzymes. One of the enzyme systems concerned with melanin production - the tyrosine oxidase system is inhibited by chloroquine in tadpoles and an abnormal brown pigment was produced by treated animals (Peters 1970). Thus may be the mechanism by which chloroquine produces achromotrichia i.e. whiteness of the hair after prolonged usage.

Akubue (1975) described the antihistaminic property of chloroquine. He showed that chloroquine at low concentrations is a specific antagonist of

histamine, probably by a competitive antagonism. The pA_2-pA_{10} value for chloroquine using histamine as the agonist in isolated guinea pig ileum was 0.87.

Chloroquine depresses and finally blocks neuromuscular transmission. It also depresses the action potential in the axons without changing their membrane potential (Chinyanga *et al*., 1972). They suggested that the probable mechanism of action was by depressing the sodium conductance mechanism in the axon terminals.

Vartanian (1974) suggested that both direct depressant effect upon the cardiac muscle and smooth muscles of the blood vessels as well as central effect on sympathetic nervous system will account for the membranogenic effect of chloroquine. However, it has been suggested that the cardiovascular collapse due to chloroquine toxicity is mainly due to its direct depressant action on the heart rather than vasodilation because there was no significant change in the mean arterial pressure in this condition (Olatunde 1971).

Chinyanga *et al* (1974) observed that chloroquine depressed the spontaneous contractions of the oestrogen primed uterine muscle as well as inhibits the uterus stimulated with oxytocin and high potassium solution. The inhibition of potassium-depolarisation was dose-dependent and time-dependent, and was rapidly reversed by increasing the calcium concentration in the bathing fluid. They therefore suggested that the action of chloroquine on guinea-pig uterus, in either normal physiological solution or potassium-depolarizing solution, was manifested by antagonism of calcium entry through the cell membranes.

The local anaesthetic action of chloroquine which has been reported in literature (Tanenbaum and Tufanelli, 1980) may be accounted for by the effects of the drug on neurotransmission at the neuromuscular junction as well as the membrane stabilizing effects of the drug.

1.8. PHARMACOKINETICS OF CHLOROQUINE

A lot of painstaking research and review has been done on the pharmacokinetics of chloroquine especially in animals and caucasians, and recently in black Africans. McChesney *et al* (1967a,b,c) showed that chloroquine metabolism differs in different animal species e.g. monkeys differ from man whereas the metabolism is almost similar in rats and man.

Chloroquine is rapidly and almost completely absorbed from the gastrointestinal tract after oral administration in healthy adults (Gustafsson *et al* 1983) and children with uncomplicated malaria (Adelusi *et al*, 1982).

Peak concentration of up to 600ng/ml were reached in 24 hours after drug administration (Adelusi *et al*., 1982; Gustafsson *et al*, 1983; Frisk-Holmberg *et al*, 1984; Walker *et al*., 1987; White *et al*., 1983). The bioavailability of the drug relative to parenteral treatment was 70% in adult malaria patients (White *et al* 1987) and 75% in healthy adults (Gustafsson *et al*, 1983). Identical plasma or whole blood concentration profiles were observed after intramuscular and subcutaneous administration of chloroquine. (White *et al* 1987, 1988). Peak concentrations in severe malaria is usually reached within 20 minutes and can be as short as 5 minutes. This results in transiently high (500-3500 ug/litre) and potentially toxic blood concentrations if doses of 5 mg base/kg or larger are given (White *et al*, 1988). It is presumed that formulations which retard slighly the rate of absorption after intramuscular or subcutaneous administration could be safer (Prakongpan *et al*, 1989). Studies with chloroquine suppositories suggest that rectal bioavailability is less than half that of oral chloroquine but sustained therapeutic blood concentrations may be achieved (WHO 1990).

Chloroquine is extensively distributed into various tissues of the body and that affinity of the drug for different tissues varies (Adelusi and Salako, 1982a,b; Frisk-Holmberg *et al*., 1984; Grundman *et al*., 1972). Berliner *et al* (1948) showed that the concentrations in the tissues far exceed those in the plasma. Chloroquine has a high affinity for melanin producing organs and tissues like those of the eyes

and skin. Ascorbic acid was reported to cause a decrease tissue retention of chloroquine (McChesney *et al* 1965). The reported exceptionally large volume of distribution of chloroquine (about 200 times the total body fluid volume) has been ascribed to its extensive tissue distribution and binding (Gustafsson *et al*., 1983, WHO, 1990). Despite its high tissue affinity, chloroquine is poorly bound to plasma proteins. It is about 55% protein-bound in plasma (Adelusi and Salako 1982c, Walker *et al*., 1983a). It binds weakly to α-1-acid glycoprotein and albumin (Walker *et al* 1983a). However, protein binding is unlikely to be an important determinant of chloroquine pharmacokinetics or response. Chloroquine concentrations in the cerebrospinal fluid are very low with a mean of 2.7% of corresponding whole blood concentrations (White *et al*., 1988).

Erythrocyte concentration of chloroquine and other antimalarials is of great interest since the malaria parasite is intra-erythrocytic during the acute malaria phase of its life cycle. Studies show a markedly higher chloroquine concentration in erythrocytes than plasma (Berliner *et al* 1948, Frisk-Holmberg *et al* 1979, Adelusi *et al* 1982). Peak chloroquine concentrations occured in erythrocytes and plasma at the same time (Adelusi *et al* 1982, Gustafsson *et al*, 1983). By contrast, peak chloroquine concentration was reached at different times in different organs like the heart, lungs, kidney and liver. These times were different from those of plasma and erythrocytes (Adelusi and Salako, 1982a,b).

Bergqvist and Domeji-Nyberg (1983) showed that the leucocytes and thrombocytes concentrate far more chloroquine than the erythrocytes. This is why serum chloroquine concentrations is usually higher than those of plasma since thrombocytes release chloroquine during the clotting process (Rombo 1984).

1.8.1 Chloroquine Metabolism

Gandette and Coatney (1961) demonstrated the presence of an enzyme system that catalyses the metabolism of chloroquine in rabbit liver homogenate. Chloroquine undergoes appreciable metabolism in the body of man and animals

Drugs that inhibit the actions of liver microsomal enzymes prolong the half life of chloroquine (Bowman and Rand, 1980).

Chloroquine is metabolised by side chain de-ethylation leading first to the formation of the major metabolite, desethylchloroquine and later bisdesethlchloroquine which is a primary amine that can undergo deamination to form an alcohol, the 4-hydroxyl compound which then undergoes oxidation to form the 4-carboxylic acid derivative (Fig. 1.8.1). Successive dealkylation of the side chain ultimately leads to the compound 4-amino-7-chloroquinoline (Kuroda 1962, McChesney *et al* 1966). Essien (1978) reported the presence of chloroquine-N-oxides (IV) and (V) as important metabolites in urine of patients 3-24 hours after chloroquine ingestion.

The quinoline nucleus of chloroquine is resistant to degradation. Desethylchloroquine has been shown to be active against *P. falciparum* (Adeloumnu and Fleckeistein, 1983) but less active than the parent compound and also is eliminated more slowly (Gustafsson *et al*. 1987). This suggests that some metabolites of chloroquine might contribute partially to its antimalarial activity.

Desethylchloroquine and chloroquine has been reported to have the same distribution profile and tissue binding (Fletcher *et al*, 1975, Gustafsson *et al*., 1983). Walker *et al* (1983b) reported the detection of desethylchloroquine in plasma 30 minutes after the administration of 10 mg/kg chloroquine. The desethylchloroquine reached peak concentration between 2 and 12 hours which is also the same for the parent drug. Its concentration remained at about 25-40% of that of chloroquine after peak concentration has been reached.

Chloroquine is eliminated from the body very slowly such that the drug and its metabolites can be detected in plasma for 21-60 days after a single dose of 5 mg/kg depending on the sensitivity of the assay method. The decline of plasma chloroquine concentration with time after a single dose is polyexponential suggesting a multicompartmental distribution. The decline erythrocyte and plasma chloroquine concentration after peak level has been reached parallels, indicating that a steady equilibrium is reached quickly between the two media, which are therefore

regarded as a single compartment with respect to chloroquine (Adelusi *et al* 1982, Gustafsson *et al* 1983). However, the decline phase for various tissues diverge away from the plasma curve with time. These tissues are therefore regarded as separate compartments with slower elimination rate constants than plasma.

Chloroquine is associated with a long half life. Various half-lives have been reported by different investigators after oral administration. This is because the determination of the half-life of a drug depends on the identification of the true terminal log linear elimination phase which is difficult to obtain with chloroquine in view of its continuous redistribution from tissues stores to plasma over weeks. It would also depend on the sampling time and sensitivity of the assay method. Half lives of between 7 and 12 days have been reported (McChesney *et al* 1967a; Brohult *et al* 1979; Adelusi *et al* 1982; Gustafsson *et al* 1983; Walker *et al*, 1987).

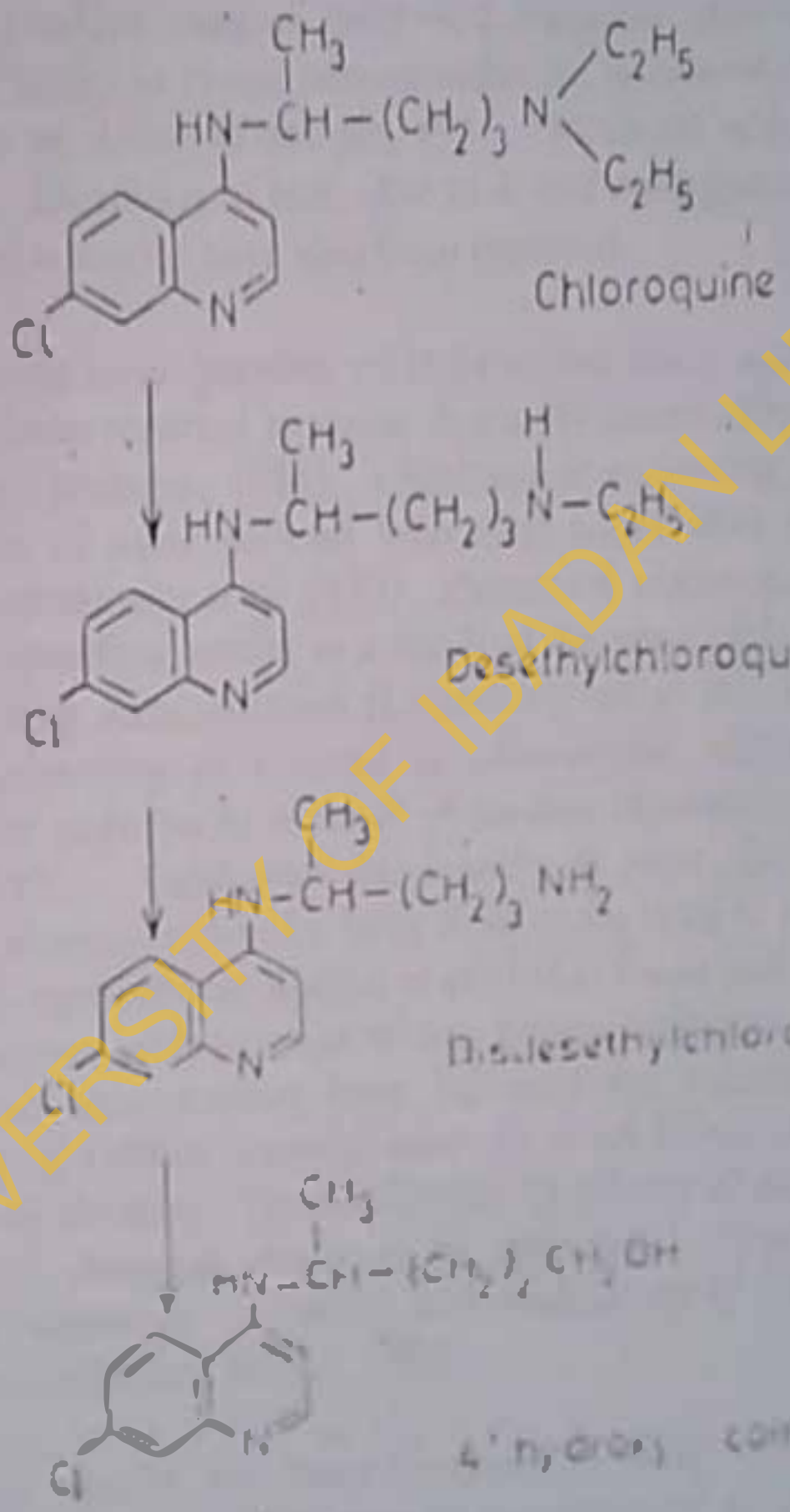
Chloroquine is eliminated both by metabolism and renal excretion (Aderounmu *et al*, 1980; Walker *et al*, 1987). Both chloroquine and desethylchloroquine are removed mainly via the kidney. The renal clearance is substantially greater than the glomerular filtration rate. It is therefore suggested that renal excretion of chloroquine probably takes place by both glomerular and tubular secretion. Aderounmu *et al* (1980), Gustafsson *et al* (1983) and Walker *et al* (1987) showed that urinary excretion of chloroquine and desethylchloroquine after a single oral dose is greatest during the first 24 hours. Berliner *et al* (1948) found that after oral administration of 0.4 g of chloroquine disulphate, daily faecal excretion was 8% and urinary excretion 14% daily. Jailer *et al* (1949) showed that the urinary excretion was 23% of the daily dose and that the urinary excretion was decreased to 13% by concurrent administration of 20 g of sodium carbonate per day whereas 8 g of ammonium chloride given daily increased urinary excretion to 35%. Salako *et al* (1984) reported that the elimination of chloroquine is reduced in renal failure but this is irrelevant in malaria therapy.

Frisk-Holmberg *et al* (1979) reported that chloroquine exhibited a dose-dependent kinetics. However, the studies of Gustafsson *et al* (1983) and Aderounmu and Lindstrom (1983) indicated that the kinetics of chloroquine are linear. Fletcher *et al* (1975) and Price-Evans *et al* (1979) observed differences in

the chloroquine excretion between racial groups following administration of chloroquine. They therefore suggested that the differences might be due to genetic control which might be polygenic. However, Walker *et al* (1987) showed no difference in the pharmacokinetics of chloroquine between caucasians and black subjects.

Metabolic Degradation Pathway for chloroquine

FIG 1.8.1



1.9 SIDE AND TOXIC EFFECTS OF CHLOROQUINE

It has been reported that oral administration of therapeutic doses o chloroquine produce various mild and transient side effects such as headache dizziness, difficulty in visual accommodation, weakness, and itching (Salako, 1984; Gustafsson *et al*, 1983; Wiselogle, 1946; Dornhorst and Robinson 1963; Olatunde, 1967, 1969). Bleaching of hair, skin rash and mild gastro-intestinal symptoms such as nausea and vomiting have also been reported.

Parenteral administration of chloroquine using appropriate dosage in malaria patients has been reported to cause death (Williams, 1966; Gilles, 1966; Olatunde, 1970; Tuboku-Metzger, 1984). Chloroquine poisoning by the oral route has also been reported of some patients who died hours after ingestion of large dose of chloroquine (Robinson *et al* 1970). Parenteral chloroquine is a potent vasodilator, hypotension therefore occurs as a result of the transiently high blood concentrations that follow drug administration (Looareesuwan *et al*., 1986; Wiute *et al*., 1988). The death occurring as a result of chloroquine administration may be due to cardiovascular collapse as a result of cardiac depression and extreme vasodilation (Olatunde 1971). Cardiovascular toxicity is most likely to occur following the inadvertent administration of a large dose of the drug to severely ill children (Wiute *et al*., 1987), especially as Walker *et al* (1983) found substantial plasma chloroquine levels in children with pyrexial illness before treatment. Although most cases of cardiovascular abnormalities have followed the parenteral administration of the drug, Oli *et al* (1980) reported cases of heart block in two patients on chronic treatment with the drug. The cardiovascular effects of chloroquine may probably be related to its chemical similarity to quinidine. Thus it depresses myocardial contractility, depresses excitability and conductivity of cardiac muscle and produces peripheral vasodilation (Salako, 1984).

Ocular toxicity has been reported in those who received high doses of chloroquine for long period of time for the treatment of systemic lupus erythmatosus (Carr, 1986; Henkind and Rothfield, 1963) and malaria prophylaxis (Verdy 1975). The mechanism of chloroquine-induced retinopathy is unclear but it is widely

presumed to be associated with melanin which is abundant in retinal tissue However, Kuhn *et al* (1981) found that flunitrazepam, which is similar to chloroquine and has affinity for melanin in retinal cells did not induce chloroquine-like retinal changes after long-term administration to cats and mice while chloroquine under similar conditions induced changes. It was therefore concluded that the retinotoxic effect of chloroquine may not be the result of its affinity for melanin-containing tissues and that the affinity of a drug for melanin-containing tissues is not a sufficient reason for regarding it as potentially harmful to the eye.

Abnormal involuntary movements similar to those that occur in Parkinsonism have been reported in some patients treated with chloroquine and other 4-aminoquinolines such as amodiaquine (Akindele and Odejide 1976; Umez-Eronini and Eronini 1977; Singhi *et al*, 1977). The mechanism for chloroquine-induced involuntary movements has been suggested by Majumdar, (1977). He postulated that melanin is derived through the pathway, phenylalanine - tyrosine - dopa - quinone - melanin. It is inferred that chloroquine will also bind avidly to tissues rich in dopaminergic receptors because of the structural relationship between dopa and melanin. When this happens in the nigrostriatal system, dopaminergic transmission in this system may be blocked leading to involuntary movements. Osifo (1979) however proposed another mechanism based on the weak but specific adrenergic-neurone blocking action of chloroquine.

Mild neuromuscular disturbances, usually manifested as muscle weakness are common during oral treatment of malaria with chloroquine. A few patients receiving chloroquine parenterally also complain of diplopia and difficulty in accommodation. These reactions are transient and disappear within thirty minutes of administering the drug (Gustafsson *et al*., 1983). Polyneuropathy, which sometimes occurs during long-term treatment with chloroquine genrally resolves on terminating the therapy (Marks 1979). Chloroquine induced neuropathy could be due to action of the drug on the nerve, muscle or neuromuscular junction (Argov and Mastalgia, 1979).

Ototoxic effects have been reported in some patients administered chloroquine (Mukherjee and Mukherjee 1979) and developing foetus when the drug

is taken by the mother during pregnancy daily for up to the sixth week of gestation (Hart and Naunton 1964). It has been shown experimentally that chronic administration of amphophilic drug e.g. chloroquine leads to an accumulation of lysosomes of different types containing phospholipids in the inner ear (Bichler and Sponendlin, 1980). Hence the ototoxic effects of chloroquine may be due to its capacity to produce lipoidosis of the inner ear.

One important side-effect of chloroquine which occurs after the administration of the therapeutic dose of the drug for malaria treatment is 'pruritus' (Berliner *et al*, 1948; Craige *et al*., 1948; Ekpeclu and Okoro, 1964; Olatunde, 1969, 1977; Abila and Ikueze 1989). The reaction is more common among blacks taken chloroquine for the treatment of malaria. The reaction is discussed in detail in the next section.

1.10 PRURITUS

Pruritus (itching) has been described as an unpleasant cutaneous sensation which provokes the desire to scratch or rub the skin (Osifo, 1989). Shelley and Arthur (1957) showed histologically that the itch point is a point of density of fine fibre endings in the sub-epidermal area, and that itching is believed to be mediated by the fine unmyelinated C-fibres. Casual physiological itching has been associated with the concept of spontaneous itch (Rothman, 1954) which can be distinguished from pathological itching which often accompanies skin diseases and has also been associated with itching hyperexcitability.

Conscious perception of itch is subjected to psychic modulation, while mental distraction may depress itch perception, mental alertness and boredom may intensify itch with the same amount of pruritogenic stimulus (Comma, 1952). Progress on the evaluation of the pathophysiology of pruritus has been very slow when compared with pain which employs the same neural mechanism as itch. This is probably because there has been no systhetic or naturally occurring palliative with specificity for severe itch such as morphine for pain relief. Thus there is no specific lead to follow for development of new antipruritic agent (Osifo, 1989). Also empirical screening for antipruritic agents has been difficult because of the absence of any reliable and widely accepted sub-human model of pruritus.

1.10.1 Chloroquine Associated Pruritus

Chloroquine-associated pruritus (CAP) was described by Ekpechi and Okoro (1964) as 'an entirely subjective reaction that is characterized by a widespread pricking sensation affecting mainly the hands, feet and scalp in both male and female adult patients. It is predominant in blacks who had received therapeutic doses of chloroquine for clinical malaria. The pruritus was described as flitting from one part of the hand or foot to the other, each sensation demanding instant attention, not of typical scratching but of rubbing or merely patting the affected area

The reaction usually begins 6-18 hours after oral administration of chloroquine and reaches its maximum intensity between 12-18 hours. It usually subsides by the end of the second or third day with or without treatment with systemic antihistamines or soothing lotion. The reaction is usually severe enough to make sleep impossible for as long as it lasts. The reaction occurs in all age groups but it is unusual for it to be experienced on the first exposure to the drug. Hence it occurs more often in people who use chloroquine relatively frequently for the treatment of malaria (Olatunde and Obih, 1981; Salako, 1984; Spencer *et al*., 1987). However, the reaction usually do not occur in reactors after long time of not taking the drug, but the reaction reappears in such individuals after a fairly frequent administration of the drug (Ekpechi and Okoro, 1964).

The reported prevalence of chloroquine-associated pruritus varies. It ranged between 8 and 28% (Ekpechi and Okoro 1964; Olatunde 1977, 1969; Olatunde and Obih, 1981). Spencer *et al* (1987) reported that the pruritus was commoner in adults (20.3%) than children (12.8%). Osifo (1984) reported 74.3% among adult patients taking chloroquine for malaria treatment. Mnyika and Kihamia (1991) also reported 45% among Tanzanians but only 27% of the patients were current itchers. Olatunde (1969) did not find CAP in all the non-negroe immigrants who had taken chloroquine. However, Osifo, (1984) found one Asian and one white patient among the 109 positive respondents who itched to chloroquine. There was no significant difference in the incidence among both sexes. Spencer *et al* (1987) like Mnyika and Kihamia (1991) reported that the presence or absence of malaria parasitaemia did not affect the development of CAP. The epidemiology of CAP has not been well investigated in pregnancy, however, Steketee *et al* (1987) reported pruritus in 77% of gravid women and only 19% in nulligravids.

The pathogenesis of CAP is still a matter of debate. The fact that the reaction occurs after one or more course of the drug suggests a hypersensitivity reaction even though subsequent course of the reaction is not typical of such reaction. Also the reaction is not associated with erythema urticaria, papules, vesicles or other known lesions which might be expected with cutaneous reactions of such an acute onset. Guderian *et al* (1986) suggested that the pruritus seen after chloroquine ingestion

may be associated with the death of onchocerca volvulus microfilariae, an event that is known to be accompanied by an intense pruritic reaction (Mackenzie and Kron, 1975). However, Burnham *et al*., (1989) found no evidence for an association between CAP and onchocerciasis. Abila and Ikueze (1989) tried to find support for an allergic basis for CAP. They found that antihistammes were ineffective as was the use of ketotifen but prednisolone was found to be beneficial. They suggested that this was due to prednisolone blocking the release of itch sensation, thus supporting an allergic basis for CAP.

Ebong and Okonkwo (1976) investigated the possibility of antigen-antibody reaction being the cause of CAP. Medical students with a history of CAP were studied before and during an episode of CAP. Preliminary result showed normal IgG and IgA and low IgM before a CAP episode. During the reaction, IgA level dropped but IgM levels rose markedly. Also an indirect action through histamine release has been ruled out since experimental and clinical evidence have shown that chloroquine antagonises some histamine responses including bronchial asthma (Ayieh-Smith and Boyc, 1974; Agarwal and Deshmankar, 1963).

Olatunde (1971) investigated chloroquine concentration in the plasma and skin of patients prone to itching and those not prone. He found after an intravenous dose, that the plasma levels of the drug were comparable in both groups. However, in the skin, patients prone to itching had higher unchanged chloroquine and lower chloroquine metabolites than patients not prone to itching. He suggested tentatively that patients prone to develop CAP may have a slower rate of metabolism. However, Essien *et al* (1989) observed that there appears to be an extensive metabolism of chloroquine in individuals who itched to the drug producing a high level of mono-desethylchloroquine which probably determines the degree of pruritus experienced. They also speculated that CAP might probably be due to either a specific action of mono-desethylchloroquine on sensitised receptors or nerve endings in the skin or an interaction of this metabolite with acetyl glyceryl ether phosphoryl chloride (AGEPC), a new class of potent phospholipids implicated in many aspects of allergy and inflammation. Mono-desethylchloroquine like chloroquine has a high affinity for phospholipids. There has been no report of itching in people using chloroquine for prophylaxis despite the 15% incidence in

patients using it therapeutically (Olatunde, 1980). This observation raises the possibility that this reaction may result from some as yet unidentified product of a reaction between chloroquine and the malaria parasites (Salako, 1984). The increased occurrence of CAP among patients with malaria as compared with healthy volunteers suggests that an acute malaria febrile illness creates pathophysiologic conditions that promote the development of CAP (Osifo, 1984). Osifo (1980) suggested that the redistribution of chloroquine, principally into the skin and skeletal muscle of pyrogen treated rats could be due to the known hemodynamic changes associated with the pyrogenic reaction in mammals. He also found a strong correlation between the kinetic profile of chloroquine concentration in the skin of pyrogen treated pigmented rats and the temporal profile of intensity of CAP. By extrapolation, chloroquine kinetics in human skin during malarial fever could follow a similar pattern. It has been suggested that the febrile paroxysms of malaria are associated with peripheral vasoconstriction and partial ishaemic injury to the skin leading to itching hyperexcitability (Osifo, 1989). The increased incidence of CAP among blacks as compared with whites may be related to the amount and distribution of melanin in the skin (Osifo, 1984, 1989).

1.11 AIMS AND OBJECTIVES OF THE STUDY

That genetic factors may determine the population distribution of CAP has been suspected for long. The fact that it occurs mainly in black African people and seems to run in families, lend credence to this.

For successful malaria chemotherapy, there is a triangular interaction between the host, the parasite and the drug (Fig. 1.11.1).

Fig. 1.11.1 Triangular Interaction in Chemotherapy

HbS and G6PD deficiency are polymorphic genes which are recognised genetic red cell markers for malaria and blacks (Sodeinde 1992, Luzzatto 1979) among whom CAP is commonest. These genes are known to influence not only host-parasite interactions (Allison 1954, Martin 1982, Fleming *et al* 1985) but also drug-parasite interactions (Nguyen-Dinh and Parvin 1986). Therefore there is the need to investigate the influence of these genetic markers on host-drug interactions which CAP represents. ABO blood groups have been used as internal control since they are also genetic markers but are not peculiar to blacks or specific for malaria.

Although there is a plethora of data on CAP, its mechanism and pathogenesis are still obscured. It is therefore important to investigate the epidemiology as well as the pharmacogenetic and pharmacokinetic features of this side effect of chloroquine which tends to militate against satisfactory drug compliance in malaria therapy. Thus the specific aims of the study were:

1. To determine the prevalence of CAP among different groups of individuals treated for malaria with chloroquine.

2. To investigate the possible influence of heredo-familial factors on CAP.

3. To investigate the possible influence of the following genetic red cell markers on the prevalence of CAP.

   i. Haemoglobin type.
   ii. Glucose-6-phosphate dehydrogenase (G6PD) status.
   iii. ABO blood group.

4. To investigate the disposition of chloroquine and its metabolite desethylchloroquine, among itchers and non-itchers to chloroquine

CHAPTER 2

2.0 DETERMINATION OF THE PREVALENCE OF CAP AND THE INFLUENCE OF GENETIC FACTORS AMONG PATIENTS TREATED FOR MALARIA

2.1 INTRODUCTION

There have been several reports on the epidemiology and clinical course of CAP in Nigeria and some other tropical countries where chloroquine is used for the treatment of malaria. Racial and familial predisposition to CAP has been reported (Osifo 1989). It is known that most conditions that are associated with genetic factors may also be influenced by certain environmental factors (Emery 1979). Hence the need to investigate the prevalence of CAP among different groups of individuals in different geographical areas.

Due to the peculiarities of the physiological state of pregnancy, with its associated high risk of malaria morbidity, the prevalence of CAP in this group was indicated. It was also desirable to study separately the prevalence of CAP among elderly patients (> 60 years old). This is because of the presumption that the cumulative exposure to chloroquine in this group of individuals may not be considerably more than in younger adults. This is in view of the fact that chemotherapy with chloroquine is just about 40 years old and its widespread use here as an antimalarial is even shorter.

2.2 PATIENTS, MATERIALS AND METHODS

2.2.1 PATIENTS

Patients of both sexes who received chloroquine phosphate for clinically suspected or microscopically proven malaria were recruited into the study. Informed consent was obtained from the patients, or in the case of children, their parents or guardians after explaining the purpose of the study to them.

Inclusion Criteria

1. Children > 1 year old as well as adults who received the therapeutic dose of chloroquine by the oral or parenteral route for the treatment of malaria.
2. Positive Dill-Glazko test carried out on a specimen of urine collected between 24 and 48 hours after drug administration. This test confirms the presence of 4-aminoquinolines in the body.
3. Informed consent.

Exclusion Criteria

1. Children below 1 year old.
2. Children with altered state of consciousness.
3. Infections like measles or any other dermatosis which may cause pruritus on its own.
4. Negative Dill-Glazko test at 24 to 48 hours after drug administration.
5. Transfusion in the preceding 6 months, as this could interfere with results of haematological tests.
6. No informed consent.

2.2.2. STUDY DESIGN

2.2.2.1. Sample Size Consideration

The study was a multicentre cross-sectional survey. The sample size was determined using the computer programme EPI INFO Version 5 for cross-sectional studies written according to Fleiss (1981). The power of the study was set at 80%, confidence interval 95% and an estimated CAP prevalence of 30%. The minimum sample size was calculated to be 112. Since the study was a nationwide survey the investigation was carried out in at least one Local Government Area (L.G.A.) in each of the 3 Primary Health Care (PHC) zonal areas chosen. Ibadan city (centre of the study) as well as some other rural areas were chosen in Zone B, Kano, Maiduguri and Potiskum in Zone D and Port-Harcourt in Zone A. (Fig. 2.2.1.).

2.2.2.2 Sample Selection

Quality control was assured in sampling by making sure that the Hardy-Weinberg rule of constancy of the frequency of genes from one generation to the other was upheld. Consequently only one index person per family was recruited into the study.

A total of 1,315 subjects were recruited into the study. Three hundred and eighty-six patients including 55 pregnant women and 31 elderly patients (> 60 years) were recruited sequentially from U C H. One hundred and fifty urban school-children from Ibadan city, 121 rural school-children from Abanla (20km South of Ibadan) and 151 rural pre-school children from Idere (10km from Igbo-Ora) were recruited. The rural preschool-children were selected by stratification of the village and the children recruited from the chosen compounds. Those recruited were randomly selected from those who had clinical indication for chloroquine and were given the drug.

Patients were also recruited from different geographical areas for the epidemiological mapping of the country. Thus. 121 patients were recruited from Hasiya Bayero Children's Hospital, Kano. 165 from the University Teaching Hospital, Maiduguri, 113 from State Hospital, Potiskum Yobe and 108 from the University of Port-Harcourt Teaching Hospital (Fig. 2.2.1)

MAP OF NIGERIA: SHOWING PRIMARY HEALTH CARE ZONES

FIG. 2.2.1

SOURCE:

FEDERAL MINISTRY OF HEALTH, NIGERIA (1989)

2.2.2.3 Methods

Each patient or parent/guardian was interviewed using a questionnaire that was pretested among medical students. The questionnaire was designed to collect information on patient's personal details, reaction to chloroquine and family history of CAP (see appendix for questionnaire).

At the University College Hospital (UCH), Ibadan, the questionnaire was administered to medical students and staff as well as in and out-patients who met the criteria for recruitment into the study. All the patients received the World Health Organisation (WHO) recommended dose of 25 mg/kg body weight of chloroquine spread over 3 days. They were required to come for daily follow-up and report the exact time that pruritus occurred. In-patients were observed by the Doctors/Nurses who recorded the period of pruritus.

Patients from other geographical areas were also recruited based on the criteria for recruitment into the study. All the patients except the school-children were administered chloroquine and observed directly for CAP. However in the case of the school-children, a history of recent reaction of chloroquine was accepted. This is because of the ethical and logistic problem of administering the drug to healthy school-children.

A separate questionnaire was administered to pregnant women. The questionnaire in addition to the above information also collected information on patient's parity, history of fever and antimalarial drug use with respect to CAP especially before conception. Where CAP was experienced during pregnancy, the patient was asked to compare the intensity with that experienced before conception.

2.2.2.4. Blood Collection

Blood (3-5ml) was withdrawn from each patient by venepuncture between 24 and 48 hours after chloroquine administration. About 2ml was immediately transferred into a sequestrene bottle and the remainder into a bottle containing acid-

citrate-dextrose (ACD). All samples were stored for not more than 24 hours at 4°C before tests were carried out on them.

Blood samples from distant areas were collected through a finger prick which was made with a sterile lancet and 2 drops of blood dropped onto the same spot on a piece of Whatman 3mm chromatography paper. A duplicate of this was made on the same paper which was left on a support to dry. The filter paper was thereafter well labelled and stored in an air-tight container over a desiccant (silica gel). The samples were transported to Ibadan where haemoglobin types and G6PD status were determined.

The samples in sequestrene bottles were used for the following tests:

i. Packed cell volume (PCV).
ii. White blood cell count (WBC), total and differential.
iii. Reticulocyte count (retics).
iv. ABO blood group.
v. Haemoglobin electrophoresis.
vi. Malaria parasite screening.

The sample in ACD was used for the determination of G6PD type.

2.2.2.5 Urine Collection

Urine specimen was collected from each patient at a point in time between 24 and 48 hours after drug administration into universal bottles. It was used for Dill-Glazko test to confirm the presence of chloroquine in the patient's urine and therefore in his/her blood.

2.2.3. MATERIALS

Magnesium Chloride BDH Chemicals England

D-Glucose-6-Phosphate (G6P) Sigma England

Nicotmamide Adenine Dinucleotide Phosphate (NADP) Sigma England

Nitroblue Tetrazolium Salt (NBT) Sigma England

Phenazine Methosulphate (PMS) Sigma England

Saponin Sigma England

Tris (Hydroxymethyl) methylamine Hydrochloride (Tris. HCL) buffer. Sigma

Normal Saline (0.85%) Sodium chloride BDH Chemicals England.

Boric Acid BDH Chemicals England

Acid Citrate Dextrose (ACD) Sigma England

Drabkin's Solution BDH Chemicals England

Glacial Acetic Acid (Turks solution) BDH Chemicals England

New Methylene Blue-Sodium citrate-saline mixture Sigma England

Leishman's stain Sigma England

Antisera (Anti A, Anti B, Anti AB and Anti D) Sigma England

Chloroform BDH Chemicals England

Hydrochloric acid	BDH Chemicals England
Eosin (yellowish)	Sigma England
Capillary tubes	VWR Scientific USA
Cellulose acetate gel	Chemetron, Milno
Cellulose acetate membrane	Oxoid, England
Electrophoretic tank	Shandon
Haematocrit centrifuge	Hawksley
Haemocytometer	Hawksley
Water-bath	Gallenkamp, England
Microscope	Olympus BH2

2.2.4 TESTS

2.2.4.1 Blood Counts

Packed cell volume (PCV) was determined using capillary methods and spinning in a Hawksley micro-haematocrit centrifuge at 11000g for 5 min at room temperature. White blood count (total and differentials) and reticulocyte counts were performed by the standard haematological methods (Dacie and Lewis, 1984). Total white blood count was done manually using haemocytometer after making a 1 in 20 dilution of blood in Turks solution (2% acetic acid with gentian violet). Differential white blood count was done by staining thin blood films with Leishman's stain and Leucocytes counted microscopically based on morphology.

Reticulocyte count was done by adding 1 drop of whole blood to 1 drop of reticulocyte fluid (new methylene blue-sodium citrate-saline mixture), thin film made and reticulocytes counted microscopically.

2.2.4.2. Blood Grouping

ABO blood groups were determined using the agglutination tile method.

2.2.4.3. Malaria Parasite Screening

Thick and thin films were made on the same slide. The thin film was fixed with methanol and slide was then flooded with Leishman's stain diluted 1 in 3 with buffer pH 6.8. Slides were examined microscopically for malaria parasites.

2.2.2.4 Determination of G6PD Types

(a) *Fluorescent Spot Test*

This was done according to the method of Beutler and Mitchel (1968). This test is based on the principle that NADPH generated in red cells in the presence of G6PD, fluoresces under long wave-length ultra-violet (UV) light.

Reagent Mixture

G6P (10 mM) 28.5 mg
NADP (7.5 mM) 57.4 mg
Saponin (1%) 200 mg
GSSG (8 mM) 49 mg

All were dissolved in 30 ml deionised water and 30 ml Tris HCl (75 mM) pH 7.8 added. The mixture was then made up to 100 ml with deionised water.

Method

The blood cells were washed in normal saline, removing the supernatant and buffy coat. A 0.01 ml of the washed packed red cells were added to 0.1 ml of the reagent mixture and incubated at room temperature for 10 min. Spots were then made on Whatman 3 mm chromatography paper. The spots were left for 10 min and then viewed under UV light in a dark room. Control samples of G6PD normal and deficient samples were also set up at the same time.

Fluorescence was produced by NADPH formed from NADP in the presence of G6PD. No appreciable fluorescence was seen when the blood was G6PD

deficient. The drawback of this method however, is that it does not distinguish between heterozygotes and homozygote normals.

(b) *G6PD and Haemoglobin Electrophoresis Using Cellulose Acetate-gel*

Method:

1. *Preparation of Haemolysate*

A 0.5 ml of blood in ACD was washed three times in normal saline. The buffy coat was removed at the end of every wash. The packed cells were resuspended in an equal volume of lysing fluid containing 85 ug/ml NADP. The NADP helps to protect the enzyme. The tube was shaken and spun at 6000g for 5 min. The clear haemoglobin supernatant was separated from the stroma. The final haemoglobin concentration obtained was approximately 10g/100ml.

2. *Preparation of Strip*

The cellogel (Chemetron, Milano) was stored in 30% methanol. For use, a strip was blotted between two sheets of filter paper and soaked in Tris-EDTA-Borate (TEB) buffer pH 8.6 for at least 1 hour. Then soaked in a fresh buffer containing 10mM NADP for at least 30 min. The penetrable surface which is usually the matted dull surface was identified after blotting.

3. *Preparation of Tank*

Shandon electrophoretic tank was used with 250ml of TEB buffer (pH 8.6) put into each of the 2 compartments of the tank. The shoulder gap was adjusted to about 8cm. Filter paper pads were used as wicks. They were impregnated with the buffer solution and placed over the perspex shoulder pieces with one edge immersed in the cathodic and the other in the anodic compartments of the buffer solution. The buffer impregnated gel was blotted and positioned in the tank with the penetrable

surface facing up. The system was equilibrated for 10 min at 4°C at constant current of 1mA per cm width of strip and voltage of about 250V.

4. *Application of the Sample*

The current was broken and each sample was applied on the strip using a monoapplicator which delivers approximately 2ul aliquot of each sample. A gap of 1cm was allowed between samples which were applied parallel to the cathodic shoulder. Standard controls of G6PD type A, A⁻ and B were included in each run.

5. *The Run*

The tank was put into the fridge and current switched on again. The run lasted about 1 hour. The electrophoresis was done at 4°C to protect the enzyme from the heat generated during the run.

6. *The Staining*

The reaction mixture was prepared fresh at the end of each run in a plastic plate. The reaction mixture comprised the following reagents mixed in the order below:

Reagent	Volume	Final Concentration
Tris HCl (pH 8.0)	500ml	
G6P (10mg/ml)	100ul	30mM
NADP (5mg/ml)	100ul	6.5mM
$MgCl_2$ (0.1M)	100ul	18mM
NBT (2mg/ml)	380ul	2.3mM
PMS (3mg/ml)	250ul	6.5mM
Distilled water	4000ml	

At the end of the run, the current was broken and the haemoglobin bands which had separated, were recorded. The strip was then put face down in the

reaction mixture spread in the plastic plate for staining which was done in the dark. The plate was covered and put in the incubator at 37°C for 15 min to allow colour to develop.

The violet coloured bands indicated the positions of the enzyme protein and it appeared at about 4 mm ahead of the haemoglobin A band. The strips were then washed under tap water and preserved in distilled water at 4°C. Fig. 2.2.2. shows a typical G6PD electrophoretic strip.

Principle of the Staining

When the electrophoresed gel is treated with the reaction mixture containing the substrate (G6P) and coenzyme (NADP) together with an electron carrier (PMS) and tetrazolium salt (NBT), a zone of colour due to the formation of diformazan as a result of reduction of tetrazolium salt appears wherever a dehydrogenase occurs.

G6PD types A and A⁻ move at about 110% as type B. But the A⁻ stains faintly compared with A or B.

2.2.4.5. Determination of Haemoglobin Type and G6PD Status from Samples on Filter Paper

Two discs (5mm diameter) were cut from the sample spot on the chromatography paper using perforator. These were dropped into Kahn tubes. To each tube was added 0.1 ml of G6PD screening reagent mixture. The tube and its content was left for about 20 min at room temperature to allow for elution of the enzyme from the paper discs. The sample was then spotted onto Whatman 3mm chromatography paper. After drying in air for 10 min. the spots were screened with U.V. light in the dark room. The remaining lysate was used for haemoglobin electrophoresis.

The tank was prepared as earlier described for G6PD electrophoresis. Cellulose acetate membrane strips were used. The strip was soaked for 5 min in the buffer (pH 8.6) and then blotted. A multi-applicator was used to apply samples onto

the strip. Known controls (containing Hb A+S+C) were included in each batch of electrophoretic run. It was run at a constant current of 8 mA and a voltage of 200-250V for 30 min at room temperature.

The strip was stained for 30 sec in 0.5% malachite green stain. It was washed in three changes of 3% glacial acetic acid in water and then dried at room temperature. The haemoglobin type of each sample was determined using the bands of the control samples as standard references. Fig. 2.2.3 shows a typical Hb electrophoretic strip.

Fig 2.2.2

A Typical G6PD Electrophoretic Strip

a = G6PD Type A
b = G6PD Type B
Arrow = G6PD Type A

Fig. 2.2.3

A Typical Haemoglobin Electrophoretic Strip

2.2.4.6 Qualitative Test for Chloroquine in Urine

Two millilitres of urine sample was put into tapered tube and 10 drops of Dill-Glazko reagent was added. The mixture was mixed well vigorously for a few minutes. A colour change from yellow to violet red in the chloroform indicates the presence of 4-aminoquinoline in urine.

The Dill-Glazko reagent was prepared as follows:

Eosin (yellowish)	50mg
Chloroform	100ml
HCl (IN)	1ml

The mixture was shaken until the chloroform attained a yellowish colour. It was stored in an air-tight brown bottle.

2.2.4.7 Statistical Analysis

Differences in proportions were tested using Chi squared test. Fisher's exact test or Z-test where appropriate. Differences in means were also tested using the Student's t-test. Significance was designated as $P < 0.05$.

2.3 RESULTS

2.3.1. GENERAL U.C.H. PATIENTS

Three hundred patients (196 males and 104 females) were recruited from U.C.H. Those who experienced CAP were referred to as itchers and those who did not react as non-itchers.

One hundred and twenty-four patients (41.3%) developed CAP. All but 7 of the current itchers had had previous experience of itching to chloroquine. CAP only occurred regularly in 108 (87.1%) of the positive respondents.

Pruritus started between 2 and 24 h (mean 13±15 h) after chloroquine administration and increased in intensity to a peak plateau between 12 and 36 h (mean 22±9 h). It subsided between 36 and 96 h (mean 58±12 h).

Twenty-four patients received parenteral chloroquine (10 adults and 14 children). Only 4 (17%) itched, even though 9 (including the 4) of them were habitual itchers to oral chloroquine. This showed a lower rate of itching to chloroquine when administered parenterally than orally.

2.3.1.1. CAP and Sex

Of the 196 males studied, 88 (45%) and 36 (35%) of 104 females, had CAP (Table 2.3.1). The ratio of male to female in the population studied was 1.9 : 1 while the ratio among itchers was 2.4 : 1. There was no significant difference in CAP frequencies among male and female patients ($p > 0.05$).

Table 2.3.1.

Distribution of CAP with Sex

Sex	Itchers	Non-itchers	Total	% itchers
M	88	108	196	45.0
F	36	68	104	35.0
Total	124	176	300	41.3

M = Male F = Female

2.3.1.2. CAP and Socio-economic class

Table 2.3.2 shows the prevalence of CAP among the 3 socio-economic classes studied. There was no significant difference between the classes ($p > 0.05$). The result showed that there was no association between socio-economic class and itching to chloroquine.

Table 2.3.2.

Distribution of CAP and Socio-economic class

Class	Itcher	Non-itcher	Total	% Itcher
Upper	45(36.3)	69(39.2)	114	39.5
Middle	39(31.4)	57(32.4)	96	40.6
Lower	40(32.3)	50(28.4)	90	44.4
Total	124(100)	176(100)	300	41.3

Figures in parenthesis are the percentages of the classes among itchers and non-itchers

2.3.1.3. CAP and Age

The patients were aged 1 to 33 years with a mean age of 10±12.1 years Table 2.3.3 shows the distribution of age with CAP. It shows a progressive increase in incidence of CAP with increasing age ($r = 0.95$) (Fig. 2.3.1). The result also showed that 72.5% of adults (> 15 years) and 32.0% of children (< 15 years) itched to chloroquine. This showed a significantly higher frequency of itching ($p < 0.05$) in adults than children

Table 2.3.3.

Age Distribution with CAP

Age (year)	Itchers	Non-itchers	Total	% Itchers
1 > 5	25	79	104	24.0
5 > 10	46	65	111	40.4
10 > 15	16	18	34	47.1
> 15	37	14	51	72.5
Total	124	176	300	41.3

2.3.1.4 CAP and Family History

Only 197 patients answered the question of family history with respect to CAP. Table 2.3.4 shows that 96% of the itchers have at least a member of the family (first degree relation) prone to CAP. Whereas only 39% of the non-itchers who responded have members of the family prone to CAP ($P < 0.05$). This result shows that itching to chloroquine was significantly more common in the families of most itchers while itching was much less common in the families of non-itchers. Also in 28 of these families, itching reportedly starts at a particular age range, usually between 3 and 18 years (mean 15.2±7.3) years and in 10 families, itching disappears between 12 and 21 years (mean 13±5.8) years.

Table 2.3.4.

CAP and Family History of Itching

Family History of Itching	Itchers	Non-itchers
Yes	77(96%)	46(39%)
No	3(4%)	71(61%)
Total	80(100%)	117(100%)

Figures in parenthesis are the percentages of those with or without family history of itching

2.3.1.5. CAP and Parasitaemia

Result in Table 2.3.5 shows that the parasite rate among itchers was (10.0%) and among non-itchers, 8.1%. There was no difference in the parasite rates ($p > 0.05$). The frequency of itching among those with positive and negative parasitaemia were similar. This showed no association between itching and parasitaemia. The degree of parasitaemia in the patients ranged from 1000 to 750,000/ul of blood.

Table 2.3.5.

Distribution of CAP with Malaria Parasitaemia (MP)

M.P.	Itchers	Non-Itchers	Total	% Itchers
+	8(10.0%)	9(8.1%)	17	47.1
-	72(90.0%)	102(91.9%)	174	41.4
Total	80(100%)	111(100%)	191	41.9

Figures in parenthesis are the percentages of positive and negative parasitaemia among itchers and non-itchers

2.3.1.6 CAP and Frequency of Chloroquine Intake

Tables 2.3.6a and b show the number of chloroquine doses taken in the last 1 year by itchers and non-itchers among non-haemoglobinopathic and haemoglobinopathic patients respectively. Because patients with haemoglobinopathy are known to take chloroquine more frequently than non-haemoglobinopathic patients, the 2 groups were analysed separately. In the last 1 year, among non-haemoglobinopathic patients, none of 29 itchers (0%) but 7 of 75(9%) non-itchers had not taken chloroquine. However, 13 of 29 (45%) itchers and only 17 of 75 (23%) non-itchers had taken the drug more than three times in the preceding 1 year. Similarly among haemoglobinopathic patients, only 1 of 23 (4.3%) itchers and 5 of 49 (10.1%) non-itchers had not taken chloroquine in the last 1 year, whereas 9 of the 23 (39.1%) itchers and only 11 of the 49 (22.4%) non-itchers had taken more than 3 doses of the drug in the same period

This shows a significantly higher cumulative intake of chloroquine in itchers relative to non-itchers ($p > 0.05$) among both haemoglobinopathic and non-haemoglobinopathic patients.

Table 2.3.6

CAP and Chloroquine Intake in the Last 1 Year

(A) Non-Haemoglobinopathies

No of Chloroquine doses	Itchers	Non-Itchers	Total
Nil	0	7	7
1	4	6	10
2	4	28	32
3	8	17	25
> 3	13	17	30
Total	29	75	104
% Nil	0	9.3	6.7
% > 3	44.8	22.7	28.8

(B) Haemoglobinopathies

No of Chloroquine doses	Itchers	Non-Itchers	Total
Nil	1	5	6
1	5	7	12
2	2	15	17
3	6	11	17
> 3	9	11	20
Total	23	49	72
% Nil	4.3	10.2	8.3
% > 3	39.1	22.4	27.8

2.3.1.7 CAP and Haematulogical Indices

For the consideration of haematological indices, sicklers were analysed separately. This is because they are considered haematologically to be a special and unique group of individuals in view of their peculiar pathophysiology.

2.3.1.8. Analysis of Haematological Indices in Non-Haemoglobinopathic Patients

CAP and PCV

The mean PCV was 33.3±10.2% in the itchers and 28.9±8.3% among non-itchers. There was no significant difference between the two groups ($p > 0.05$).

CAP and WBC

The mean total white blood cell (WBC) count was 6,405±6,090/ul of blood in the itchers and 7,698±4,817/ul of blood in the non-itchers. There was no significant difference between the two groups ($p > 0.05$).

Table 3.17 shows the relationship between CAP and white cell differentials with reference to eosinophil counts. There was no difference in the absolute counts of the different leucocytes in itchers and non-itchers ($p > 0.05$).

CAP and Reticulocyte Count

The mean reticulocyte count in the itchers was 1.02±2.0% and 1.6±2.6% in non-itchers. There was no significant difference between the two groups ($p > 0.3$).

Table 2.3.7.

CAP and Absolute WBC with reference to Eosinophil Count

Leucocyte	Itchers	Non-Itchers
Neutrophil	4736±4313	4172±2433
Lymphocyte	5041±2738	4266±1841
Eosinophil	306±437	186±105
Monocyte	198±221	169±100

2.3.1.9. Analysis of Haematological Indices in Sickle Cell Diseases Patients (Hb SS and Hb SC)

One hundred and four sicklers took part in the study. They had a mean age of 7.6±6.2 years. Forty-two (40.4%) itched after taking the drug.

CAP and PCV

The mean PCV was 21.0±5.2% in itchers and 24.6±8.4% in non-itchers. There was no significant difference between the 2 groups ($p > 0.05$).

CAP and WBC

The mean total WBC count was 9300±6665 mm^{-3} of blood in itchers and 10950±10510 mm^{-3} in non-itchers. There was no significant difference between the 2 groups ($p > 0.05$).

CAP and Reticulocyte Count

The mean reticulocyte count was 16.0±12.9% in itchers and 11.9±13.7% in non-itchers. The difference was not statistically significant ($p > 0.2$).

2.3.1.10. Genetic Factors and CAP

CAP and Haemoglobin Types

There were 195 non-haemoglobinopathic patients comprising 51 adults (> 15 years) and 144 children (< 15 years). Among the 82 (42%) patients who itched to chloroquine, the frequency of the sickle cell trait (Hb AS) was 14.6% while the frequency was 24.8% among non-itchers (Table 2.3.8). The difference was statistically significant ($p < 0.05$). However, there was no difference ($p > 0.05$) in the frequency of Hb AA or Hb AC among itchers and non-itchers.

Table 2.3.8.

CAP and Haemoglobin Types

Hb Type	Itchers	Non-itchers	Total	% Itchers
AA	65(79.3%)	78(69.0%)	143	45.5
AS	12(14.6%)	28(24.8%)	40	30.0
AC	5(6.1%)	7(6.2%)	12	41.7
Total	82(100%)	113(100%)	195	42.1

Figures in parenthesis are the percentages of haemoglobin type in itchers and non-itchers

CAP and G6PD Types

For epidemiological studies with G6PD, the males are separated from the females. The males are used in the analysis because G6PD deficiency is fully expressed in males unlike in females where there could be heterozygote G6PD deficiency since the gene for G6PD is X-linked (Sodeinde, 1992)

One hundred and twenty-five males without haemoglobinopathy were considered for G6PD analysis. Table 2.39 shows that out of 57 itchers, there were 12 (21.1%) G6PD deficient patients as compared with 8 out of 68 non itchers (11.8%). This shows a statistically significant higher frequency of G6PD deficiency (A⁻) among itchers than non-itchers ($p < 0.05$). Also a statistically significantly

higher proportion of itchers among G6PD deficient patients than in normal patients ($p < 0.05$).

Table 2.3.10 shows the distribution of G6PD types and CAP in sicklers. There was also a higher percentage of itchers among G6PD deficient (A⁻) individuals than in non-deficient subjects. Likewise, there was a significantly higher frequency of A⁻, 20% among itchers than non-itchers, 11.1% ($p < 0.05$).

Table 2.3.9.

CAP and G6PD Type in Male (Non-Haemoglobinopathy)

G6PD	Itchers	Non-itchers	Total	% Itchers
A	5(14.7%)	10(14.7%)	15	33.3
B	40(70.2%)	50(73.5%)	90	44.4
A⁻	12(21.1%)	8(11.8%)	20	60.0
Total	57(100%)	68(100%)	125	45.6

Figures in parenthesis are the percentages of G6PD types among itchers and non-itchers

Table 2.3.10.

CAP and G6PD Type Distribution Among Sicklers (Males)

G6PD	Itchers	Non-Itchers	Total	% Itchers
A	2	5	7	28.6
B	18	27	45	40.0
A⁻	5	4	9	55.6
Total	25	36	61	41.0
% A⁻	20.0	11.1	14.8	

CAP and ABO Blood Groups

Results on Table 2.3.11 shows that there were 47.4% itchers among non-haemoglobinopathic patients with blood group A, 49.1% among group B, 42.9% among AB and 39.8% among group O. There was no difference in the prevalence of itching between the groups ($p > 0.05$). Also there was no difference in the frequency of the different blood groups among itchers and non-itchers ($p > 0.05$). The results therefore indicate that there was no association between itching to chloroquine and ABO blood groups.

Table 2.3.11

Distribution of CAP and ABO Groups among Non-Haemoglobinopathic patients

Blood Group	Itchers	Non-Itchers	Total	% Itchers
A	18(20.7%)	20(18.0%)	38	47.4
B	27(31.0%)	28(25.2%)	55	49.1
AB	3(3.5%)	4(3.6%)	7	42.9
O	39(44.8%)	59(53.2%)	98	39.8
Total	87(100%)	111(100%)	198	73.7

Results on Table 2.3.12 shows that there were 42.9% itchers among haemoglobinopathic patients with group A, 36.7% in group B, 50% in group AB and 42.6% in group O. There was no significant difference between the groups. Also there was no significant difference when the proportion of various blood groups among itchers and non-itchers were compared.

Table 2.3.12.

CAP and Distribution of ABO Blood Group Among Sicklers

Blood Group	Itchers	Non-Itchers	Total	% Itchers
A	9(21.9%)	12(31.1%)	21	42.9
B	11(26.8%)	19(33.3%)	30	36.7
AB	1(2.4%)	1(1.7%)	2	50.0
O	20(48.8%)	27(47.4%)	47	42.6
Total	41	59	100	41.0

Figures in parenthesis are the percentages of the blood groups among itchers and non-itchers

2.3.2. Elderly Patients

Thirty-one elderly patients (> 60 yr) 30 males and 1 female were recruited into the study. They had a mean age of (62±2 yr). Nine (30%) itched after taking oral chloroquine. With respect to haemoglobin types and CAP, Table 2.3.13 shows that there were 16.6% itchers among Hb AS individuals which was significantly lower than 30.4% among Hb AA and 50% in the Hb AC individuals ($p < 0.05$). Also the percentage of Hb AS among itchers (11.1%) was significantly lower than 22.7% among non-itchers ($p < 0.05$).

Table 2.3.14 shows that there was 50% itchers among G6PD deficient individuals as compared with 25% in non-deficient ones ($p < 0.05$). Also the percentage of G6PD deficient individuals among itchers and non-itchers was 33.3% and 14.3% respectively. This was also statistically significant ($p < 0.05$).

Eighteen of the patients reported not taking chloroquine in the last one year, 4 took the drug only once and 3 took it at least 3 times within the same period. The remaining 6 patients could not ascertain whether they took chloroquine or not.

Table 2.3.13

CAP and Haemoglobin Type Distribution Among Elderly Patients

Hb	Itcher	Non-Itcher	Total	% Itcher
A	7	16	23	30.4
AS	1	5	6	16.6
AC	1	1	2	50.0
Total	9	22	31	29.0
% AS	11.1	22.7	19.3	

Table 2.3.1-4

CAP and G6PD Type Distribution Among Elderly Patients

G6PD	Itcher	Non-itcher	Total	% Itcher
N	6	18	24	25.0
D	3	3	6	50.0
Total	9	21	30	30.0
%D	33.3	14.3	20.0	

N = Normal D = Deficient

2.3.3 PREGNANCY

Fifty-five pregnant women were recruited into the study. They were aged 21 to 36 years with a mean age of 28.5±4.8 years. Twenty-three (42%) of the pregnant women itched after taking the drug while 29(57%) out of 51 of the pregnant women reported itching to chloroquine outside pregnancy. There were 10 patients who did not itch during pregnancy but reported to be habitual itchers when chloroquine was taken outside pregnancy. This shows a reduced incidence of itching during pregnancy as compared to preconceptional period. Also 12 of the 23 (52.2%) itchers reported less intensity of itching during pregnancy as compared with the preconceptional period.

Tables 2.3.15a and b show the distribution of CAP and haemoglobin types during preconceptional period and pregnancy. During preconceptional period, the frequency of Hb AS among itchers was 20.1% and 36.4% in non-itchers. Although the frequency was lower among itchers, it was however not statistically significant ($p > 0.05$). Also there was no difference in the proportion of itchers among the different Hb types ($p > 0.05$). Similarly during pregnancy there was no difference in the proportion of Hb AS among itchers and non-itchers ($p > 0.05$).

Tables 2.3.16a and b show the distribution of CAP versus blood groups before conception and during pregnancy. It shows that there was no significant difference in the proportion of itchers within the different blood groups ($p > 0.05$). The result in Table 2.3.17 show the distribution of CAP with parity among the pregnant women. The percentage of itchers among those with parity 0 was 36.8, parity 1, 43.0%, parity 2, 50.0% and parity 3, 60.0%. This showed a progressive increase in itching with increasing parity ($r = 0.99$). It indicated that multiparous women itched more than nulliparous women.

Table 2.3.15

Distribution of CAP and Haemoglobin Type Among Pregnant Women

A. *Preconception*

Hb	Itcher	Non-Itcher	Total	% Itcher
AA	20	11	31	64.5
AS	6	8	14	42.9
AC	3	3	6	50.0
Total	29	22	51	56.9
% AS	20.1	36.4	27.4	

B. During Pregnancy

Hb	Itcher	Non-Itcher	Total	% Itcher
AA	16	17	33	48.5
AS	6	9	15	40.0
AC	1	5	6	16.7
Total	23	31	54	42.3
% AS	26.1	29.0	27.8	

Table 2.3.16

Distribution of CAP and Blood Group Among Pregnant Women

A. *Preconception*

Blood Group	Itcher	Non-Itcher	Total	% Itcher
A	5	5	10	50.0
B	6	9	15	40.0
AB	1	1	2	50.0
O	15	6	21	71.4
Total	27	21	48	56.3

B. During Pregnancy

Blood Group	Itcher	Non-Itcher	Total	% Itcher
A	5	6	11	45.5
B	4	11	15	26.7
AB	1	1	2	50.0
O	12	11	23	52.2
Total	22	29	51	43.1

Table 2.3.17

Distribution of CAP and Parity Among Pregnant Women

Parity	Itcher	Non-Itcher	Total	% Itcher
0	7	12	19	36.8
1	7	9	16	43.0
2	6	2	12	50.0
3	3	2	5	60.0
Total	23	29	52	44.2

2.3.4. Urban and Rural

Tables 2.3.18a, b and c show the results of the distribution of CAP with haemoglobin types, G6PD status and blood groups respectively among students in an urban area. One hundred and fifty subjects were recruited into the study from the urban school. Their age ranged from 14 to 18 years with a mean of 16.5±1.2 years. Eighty-five (56.7%) responded positively to CAP.

Table 2.3.18a shows that out of the 85 itchers, there were 18.8% Hb AS as compared with 30.6% among non-itchers. This showed a statistically significantly lower proportion of Hb AS among itchers when compared with non-itchers ($p <$

0.05). Also the percentage of itchers was lower ($p < 0.05$) among individuals with Ho AS (44.4%) when compared with those with Hb AA (60.2%).

Table 2.3.18b shows that the frequency of G6PD deficiency were 17.6% among itchers and 12.5% among non-itchers. Although the frequency of G6PD deficiency was higher in itchers than non-itchers, the difference was not significant ($p > 0.05$). Also there was no difference in the proportion of itchers among normal and deficient individuals ($p > 0.05$).

Table 2.3.18c shows that there was no difference in the percentage of itchers among the different blood groups ($p > 0.05$).

Tables 2.3.19a, b and c show the result of the distribution of CAP with haemoglobin types, G6PD status and blood groups, respectively among students in the rural community. Two hundred and nine students were interviewed accordingly but only 121 (58%) admitted to having taken chloroquine recently (within the preceding 2 months). They had an age range of 14 to 19 years with a mean of 15.2±6.0 years. Sixty-one (50.4%) responded positively to CAP.

Table 2.3.19a shows the percentage of Hb AS among itchers was 19.7% as compared with 31.7% among non-itchers ($p < 0.05$). Also there was a significantly lower proportion of itchers among Hb AS individuals when compared with Hb AA and other Hb types ($p < 0.05$).

Table 2.3.19b shows that the percentage of G6PD deficiency among itchers was 22.2% and 13.3% among non-itchers. This showed a higher proportion of G6PD deficiency among itchers than non-itchers ($p < 0.05$). However, there was no difference in the proportion of itchers among G6PD normal and deficient subjects ($p > 0.05$).

Table 2.3.19c shows that there was no statistically significant difference in proportion of itchers between the blood groups ($p > 0.05$).

0.05). Also the percentage of itchers was lower ($p < 0.05$) among individuals with Ho AS (44.4%) when compared with those with Hb AA (60.2%).

Table 2.3.18b shows that the frequency of G6PD deficiency were 17.6% among itchers and 12.5% among non-itchers. Although the frequency of G6PD deficiency was higher in itchers than non-itchers, the difference was not significant ($p > 0.05$). Also there was no difference in the proportion of itchers among normal and deficient individuals ($p > 0.05$).

Table 2.3.18c shows that there was no difference in the percentage of itchers among the different blood groups ($p > 0.05$).

Tables 2.3.19a, b and c show the result of the distribution of CAP with haemoglobin types, G6PD status and blood groups, respectively among students in the rural community. Two hundred and nine students were interviewed accordingly but only 121 (58%) admitted to having taken chloroquine recently (within the preceding 2 months). They had an age range of 14 to 19 years with a mean of 15.2±6.0 years. Sixty-one (50.4%) responded positively to CAP.

Table 2.3.19a shows the percentage of Hb AS among itchers was 19.7% as compared with 31.7% among non-itchers ($p < 0.05$). Also there was a significantly lower proportion of itchers among Hb AS individuals when compared with Hb AA and other Hb types ($p < 0.05$).

Table 2.3.19b shows that the percentage of G6PD deficiency among itchers was 22.2% and 13.3% among non-itchers. This showed a higher proportion of G6PD deficiency among itchers than non-itchers ($p < 0.05$). However, there was no difference in the proportion of itchers among G6PD normal and deficient subjects ($p > 0.05$).

Table 2.3.19c shows that there was no statistically significant difference in proportion of itchers between the blood groups ($p > 0.05$).

Tables 2.3.20a and b show the results of the distribution of CAP with haemoglobin types and G6PD status respectively among pre-school (under five years old) children in a rural community. One hundred and fifty-one were recruited into the study. They had an age range of 1 to 4.8 years with a mean of 3.1±1.3 years. Thirty-three (21.8%) itched after taking the drug.

Table 2.3.20a shows that the percentage of Hb AS among itchers was 3.0% and 16.9% among non-itchers. This shows a significantly lower proportion of Hb AS among itchers when compared with non-itchers ($p < 0.05$). Also it shows a statistically significantly lower proportion of itchers among Hb AS when compared with Hb AA and other Hb types ($p < 0.05$).

Table 2.3.20b shows that the percentage of G6PD deficiency among itchers was 36.4% and 15.3% among non-itchers. This shows a statistically significantly higher proportion of G6PD deficiency among itchers than non-itchers ($p < 0.05$). Also there was a higher proportion of itchers among G6PD deficient than normal subjects.

Table 2.3.18

Distribution of CAP Among Students in an Urban School

(A) CAP and Haemoglobin Type

Hb	Itcher	Non-Itcher	Total	% Itchers
AA	65	43	108	60.2
AS	16	20	36	44.4
AC	3	1	4	75.0
SS	1	1	2	50.0
Total	85	65	150	56.7
% AS	18.8	30.6	24.0	

(B) CAP and G6PD Status in Males

G6PD	Itcher	Non-Itcher	Total	% Itcher
N	28	14	42	66.7
D	6	2	8	75.0
Total	34	16	50	68.0
% D	17.6	12.5	14.6	

(C) CAP and Blood Groups

Blood Group	Itcher	Non-Itcher	Total	% Itchers
A	8	7	15	53.3
B	17	11	28	60.7
AB	1	3	4	25.0
O	32	16	48	66.7
Total	58	37	95	61.1

Table 2.3.19

Distribution of CAP Among Students in a Rural Community

(A) *CAP and Haemoglobin Type*

Hb	Itcher	Non-Itcher	Total	% Itcher
AA	43	35	78	55.1
AS	12	19	31	38.7
AC	4	4	8	50.0
SS	2	1	3	66.7
CC	0	1	1	0
Total	61	60	121	50.4
%AS	19.7	31.7	25.6	

Table 2.3.19

Distribution of CAP Among Students in a Rural Community

(A) *CAP and Haemoglobin Type*

Hb	Itcher	Non-Itcher	Total	% Itcher
AA	43	35	78	55.1
AS	12	19	31	38.7
AC	4	4	8	50.0
SS	2	1	3	66.7
CC	0	1	1	0
Total	61	60	121	50.4
%AS	19.7	31.7	25.6	

(B) CAP and G6PD Status in Males

G6PD	Itcher	Non Itcher	Total	% Itcher
N	28	26	54	51.9
D	8	4	12	66.7
Total	36	30	66	54.5
% D	22.2	13.3	18.2	

(C) CAP and Blood Group

Blood Group	Itcher	Non-Itcher	Total	% Itcher
A	13	13	26	50.0
AB	10	12	22	45.5
AB	1	3	4	25.0
O	29	29	58	50.0
Total	53	57	110	48.2

Table 2.3.20

Distribution of CAP Among Under Five Years Old Children in a Rural Community

(A) CAP and Haemoglobin Type

Hb	Itcher	Non-Itcher	Total	% Itcher
AA	25	91	116	21.6
AC	6	1	7	85.7
AS	1	20	21	4.8
SS	1	6	7	14.3
Total	33	118	151	21.8
% AS	3.0	16.9	13.9	

(B) CAP and G6PD Status in Males

Hb	Itcher	Non-Itcher	Total	% Itcher
N	7	61	68	10.3
D	4	11	15	26.7
Total	11	72	83	13.3
% D	36.4	15.3	18.1	

2.3.5 DISTRIBUTION OF CAP IN DIFFERENT GEOGRAPHICAL AREAS

2.3.5.1 Kano

Tables 2.3.21a, b, and c show the result of the distribution of CAP with haemoglobin types, G6PD status and blood group respectively among patients in Kano.

One hundred and twenty-one patients (82 children and 39 adults) were recruited into the study. They had an age range of 1 to 50 years with a mean age of 13.9±15.5 years. Sixteen 16(13.2%) patients (5 children and 11 adults) responded positively to CAP.

Table 2.3.21a shows that there was no individual with Hb AS among the 16 itchers whereas there were 14(13.3%) among the 105 non-itchers. There were 15.5% itchers among Hb AA and none among Hb AS individuals.

Table 2.3.21b shows that the percentage individuals with G6PD deficiency among itchers was 33.3% as compared with 11.4% in non-itchers. This shows a statistically significantly higher proportion of G6PD deficiency in itchers than in non-itchers ($p < 0.05$). Also the proportion of itchers among G6PD deficient patients was significantly higher than in normal patients ($P<0.05$).

Table 2.3.21c shows that there was no significant difference in the proportion of itchers among the different blood groups ($p > 0.05$).

2.3.5.2. Maiduguri

Tables 2.3.22a, b and c show the result of the distribution of CAP with haemoglobin type, G6PD status and ABO blood group respectively among patients in Maiduguri.

One hundred and sixty-five patients (24 children and 141 adults) were recruited into the study. They had an age range of 1 to 42 year with a mean age of 20.8±18.2 years. Forty (24.2%) patients (3 children and 37 adults) responded positively to CAP.

Table 2.3.22a shows that the percentage of Hb AS among itchers was 12.5% as compared with 20.8% in non-itchers. This indicates a statistically significantly lower proportion of Hb AS among itchers than non-itchers ($p < 0.05$). The percentage of itchers among patients with Hb AS was also significantly lower than among Hb AA patients ($p < 0.05$).

Table 2.3.22b shows that the percentage G6PD deficiency among itchers was 31.8% and 13.4% in non-itchers. This shows a statistically significantly higher proportion of G6PD deficiency among itchers when compared with non-itchers ($p < 0.05$). The percentage itchers among G6PD normal patients was 20.5% but 43.7% in G6PD deficient patients ($p < 0.05$).

Table 2.3.22c shows that there was no significant difference in the percentage of itchers among patients with the different blood groups.

2.3.5.3. Poliskum

Tables 2.3.23a, and b show the result of the distribution of CAP with haemoglobin types and G6PD status respectively among patients in Poliskum.

One hundred and thirteen patients (46 children and 67 adults) were recruited into the study. They had age range of 1 to 45 years with a mean of 14.7±10.1 years. Fifty-six (49.6%) patients (19 children and 37 adults) responded positively to CAP.

The percentage of Hb AS among itchers was 8.9% as compared with 26.3% in non-itchers (Table 2.3.23a). This also indicates a statistically significantly lower proportion of Hb AS among itchers than non-itchers ($p < 0.05$). The percentage of

itchers among Hb AA was 54.47 and 25.0% in Hb AS patients. This also showed significantly lower proportion of itchers among Hb AS than Hb AA ($p < 0.05$).

Table 2.3.23b showed that the percentage of G6PD deficiency among itchers was 28.6% and 13.3% among non-itchers. This indicated a significantly higher proportion of G6PD deficiency among itchers than non-itchers ($p < 0.05$). Also the percentage of itchers among G6PD normal patients (27.8%) was significantly lower ($p < 0.05$) than among G6PD deficient patients (50.0%).

Blood group was not done for the samples from Potiskum.

2.3.5.4 Port-Harcourt

One hundred and eight patients (all children) were recruited into the study. They had an age range of 1 to 14 years with a mean of 5.5±4.4 years. Nine (8.3%) responded positively to CAP. Table 2.3.24 showed a statistically significantly lower proportion ($p < 0.05$) of Hb AS among itchers (11.1%) relative to non-itchers (21.3%). Also the percentage of itchers among Hb AS (5.6%) was significantly lower ($p < 0.05$) than among Hb AA patients (11.3%). G6PD status and ABO blood group were not determined for these samples.

Table 2.3.21

Distribution of CAP in Patients from Kano

(A) *CAP and Haemoglobin Type*

Hb	Itcher	Non-Itcher	Total	% Itcher
AA	16	89	105	15.2
AS	0	14	14	0
SS	0	2	2	0
Total	16	105	121	13.2
% AS	0	13.3	11.6	

(B) *CAP and G6PD Status in Males*

G6PD	Itchers	Non-itchers	Total	% Itcher
N	6	39	45	13.3
D	3	5	8	37.5
Total	9	44	53	17.0
% D	33.3	11.4	15.1	

(C) CAP and ABO Blood Groups

Blood Group	Itcher	Non-itcher	Total	%Itcher
A	3	18	21	14.3
B	7	32	39	17.9
AB	1	4	5	20.0
O	4	35	39	10.2
Total	15	89	104	14

Table 2.3.22
Distribution of CAP in Patients from Maiduguri
(A) CAP and Haemoglobin Type

Hb	Itcher	Non-itcher	Total	% Itcher
AA	35	97	132	26.5
AS	5	26	81	16.1
SS	0	2	2	0
Total	40	125	165	24.2
% AS	12.5	20.8	18.8	

(B) CAP and G6PD Status in Males

G6PD	Itcher	Non-Itcher	Total	% Itcher
N	15	58	73	20.5
D	7	9	16	43.7
Total	22	67	89	24.7
% D	31.8	13.4	18.0	

(C) CAP and G6PD Status in Males

Blood Group	Itcher	Non-Itcher	Total	% Itcher
A	4	13	17	23.5
B	4	19	23	17.4
AB	1	3	4	25.0
O	22	55	77	28.6
Total	31	90	121	25.6

Table 2.3.23
Distribution of CAP in Patients from Potiskum
(A) *CAP and Haemoglobin Type*

Hb	Itcher	Non-Itcher	Total	% Itcher
AA	49	41	90	54.4
AS	5	15	20	25.0
SS	2	1	3	66.7
Total	56	57	113	49.6
%AS	8.9	26.3	17.7	

(B) CAP and G6PD in Males

G6PD	Itcher	Non-Itcher	Total	% Itcher
N	10	26	36	27.8
D	4	4	8	50.0
Total	14	30	44	31.8
% D	28.6	13.3	18.2	

Table 2.3.24
Distribution of CAP in Patients from Port-Harcourt

CAP and Haemoglobin Type

Hb	Itcher	Non-Itcher	Total	% Itcher
AA	8	63	71	11.3
AS	1	17	18	5.6
SS	0	1	0	0
Total	9	80	89	10.1
% AS	11.1	21.3	20.2	

DISCUSSION

Despite the emergence of chloroquine resistant parasites, chloroquine remains the first line drug for the treatment of malaria in the country. It is also the most widely used antimalarial drug in Nigeria (Ekanem *et al.* 1990). Chloroquine when used at therapeutic doses is safe, relatively non-toxic and is easily administered. Despite these obvious advantages, chloroquine associated pruritus is a very common phenomenon among those who frequently use the drug for therapeutic purposes. This is a big drawback on the antimalarial programme of those endemic areas where this occurs. The itching produced by the drug is so disturbing that those who have experienced it are often reluctant to take the drug for subsequent attacks of malaria and indeed do not often complete a single course (Osifo, 1984). The therapeutic consequences of such practices are enormous. Firstly, this may provide the ideal situation for the development of severe and complicated malaria because of inadequate treatment. Secondly, the contribution of this type of phenomenon to the development of resistance in the environment is hard to quantify because it may provide the right drug pressure for selecting out resistant parasites.

The way out of this therapeutic quagmire is either to develop drugs that will completely block the development of the pruritus associated with chloroquine or attenuate the response to chloroquine in people who are so predisposed. The other alternative is to use other drugs. In response to the first alternative, several regimes have been suggested for the control of CAP when it occurs (Ajayi *et al.* 1991). However none of them have been shown to be reliable. In response to the second alternative, several of the newer compounds for the treatment of malaria are definitely too expensive in relation to the gross national product, and therefore out of the reach of most people in endemic areas.

2.4.1. University College Hospital

The prevalence of CAP was 41.3% among UCH patients. This indicates that this is a very important and prevalent problem in our environment. The

contribution that this has made to the development of drug resistance in our environment is hard to ascertain as many of such patients are unable to complete a full course of chloroquine for malaria. This is because it has been shown that low drug pressure is able to select out resistant strains of *P. falciparum* (Bruce-Chwatt, 1986).

In our environment, patients have a preference for intramuscular administration of drugs as opposed to oral administration. In particular patients prefer intramuscular administration of chloroquine to oral administration (Walker, Unpublished data). One obvious reason is the unpleasant taste of chloroquine. In this study, it was demonstrated that there was less pruritus in the patients who had parenteral chloroquine compared to those who had it orally. It would therefore be expected that one easy way of circumventing the problem of CAP is to give the drug by the intramuscular route. This would in practice be fraught with a lot of dangers as it had been shown in the past that intramuscular administration of chloroquine had been associated with sudden deaths even when therapeutic concentrations of the drug had been used (Williams, 1966; Tuboku-Metzger. 1966; Walker *et al*., 1992). Salako *et al* 1987 had attributed these sudden deaths after the intramuscular administration of chloroquine to transient and very high plasma levels after intramuscular injections. Secondly, it is well recognized that one of the ways in which the AIDS virus is transmitted in developing countries is by the use of poorly sterilized needles in rural health centres. Therefore it is suggested that the WHO recommendation that parenteral chloroquine be used only in severe malaria when the patients cannot tolerate the oral dose, be adhered to.

2.4.1.1 *Genetic Influence:*

That CAP was more prevalent in some families was demonstrated in this study as it was more common amongst first degree relations (siblings and parents) of itchers than in non-itchers. Nevertheless it tended to appear at a certain age range. The reason for this is not clear. This lends credence to the hypothesis that there might be some heredo-familial basis to CAP. Thus supports the concept of a pharmacogenetic basis for the disorder as suggested by Haines and Chugh (1986)

The association between chloroquine intake and CAP observed in this study agrees with the reports of Spencer *et al.* (1987) and Salako (1984). Previously, Lindquist & Ullberg (1972) had shown that chloroquine cumulates in melanin containing organs like the skin and the retina. This affinity of chloroquine for melanin has been postulated to be the basis for the development of bulls eye retinopathy which is indeed the result of the cummulation of chloroquine in the retina (Voipio 1966). In the same way chloroquine cumulates in the skin. This indeed may in part explain chloroquine induced pruritus, and may provide a part answer for why it does not appear before a particular age range, and is seen only after several intakes of the drug. This may partly explain the reason why CAP is not experienced with the first ever dose of chloroquine as reported by Salako (1984). That CAP is more prevalent in adults compared to children supports the view that accumulation of the drug in the skin may have a significant role to play in the aetiogenesis of this phenomenon. It is also in agreement with the previous finding of Spencer *et al* (1987) and Osifo *et al* (1984) who observed a higher incidence of itching in adults compared to children.

24.1.2 *Parasitaemia:*

Previous workers had demonstrated that there was no relationship between CAP and parasitaemia (Olatunde 1969, Osifo, 1984; Burnham *et al*., 1989; Mnyika and Kihamia, 1991). This was confirmed in this study as there was no association between CAP and malaria parasitaemia in this study. Nevertheless, it had been shown that CAP is not common among those using the drug for prophylaxis (Salako 1984). This may mean that CAP is not due to parasite-drug interaction but an acute malaria febrile illness may create pathophysiological conditions that are conducive to the development of CAP (Osifo 1984).

2.4.1.3. *CAP and Haemoglobin Types*

Result in Table 3.8 showed that there was a significantly reduced frequency of sickle cell trait (Hb AS) among itchers relative to non-itchers as well as reduced frequency of itchers among Hb AS when compared with non-itchers. This observation suggests that the sickle cell trait in some way(s) may confer some protection against CAP on its bearers. One explanation to consider is that Hb AS individuals suffer far less intense malaria attacks per unit time as compared with individuals with other Hb types. That is the Hb AS is protective against severe malaria (Edington 1967, Hendrickse *et al* 1971). Therefore sickle cell trait bearers may be presumed to be less exposed cumulatively to chloroquine as compared with patients with other Hb types and hence develop CAP less often.

Other haematological indices did not show any differences between itchers and non-itchers. This is surprising as it might be expected that people who are more prone to CAP should have at least higher eosinophil counts. This finding may be an indication of the fact that histamines may not play a significant role in the genesis of CAP (Osifo 1989).

2.4.1.4. *CAP and G6PD*

Tables 2.3.9 and 2.3.11 showed that there were more itchers with G6PD deficiency than non-itchers. This indicates that G6PD deficiency may predispose to CAP. The validity of these relationships and their independence of haemoglobin types was demonstrated by the fact that these relationships were confirmed among sickle cell disease (haemoglobinopathic) patients as well as non-haemoglobinopathic patients.

The mechanism for this observation is not clear. However, G6PD deficient red cells have been reported to accumulate higher concentration of chloroquine than normal cells (Orjih, 1987; Jamney, 1984).

2.4.1.5. *CAP and ABO Blood Group*

As ABO blood groups had been used as internal controls, it was interesting to show that there was no difference in the frequency of CAP among the various ABO blood groups in both normal and sickle cell disease patients. As expected, there was no difference in the distribution of the ABO blood groups among itchers and non-itchers. This finding shows that in contrast to G6PD and Hb types, there was no association between CAP and ABO blood groups, as the ABO blood groups are not known to be associated with malaria.

2.4.2. CAP and Pregnancy

As chloroquine is used as a prophylactic in pregnancy even in areas with chloroquine resistance, it was important to find out the prevalence of CAP amongst pregnant women. This is because itching may reduce the enthusiasm of such patients to comply with a prophylactic regimen.

In this study, there was a reduced prevalence of itching of chloroquine in pregnancy (42%) as compared with non-pregnant women (57%). The mechanism for the lower frequency of CAP in pregnancy is not known. However, the physiological hormonal state in pregnancy may be involved, especially as it is known that certain steroids e.g. prednisolone suppresses itching (Abila and Ikueze, 1989, Ajayi *et al.*, 1991).

Nevertheless, there was an association between CAP in pregnancy and parity. It showed a higher frequency of itching in multipara than nullipara. This may reconfirm that CAP may be related to cumulative exposure to chloroquine.

2.4.3. Urban and Rural Population

The prevalence of CAP in the urban and rural school children (who were of similar age groups) was not significantly different. However, it was considerably lower among the rural pre-school children who were under five years of age. In all

the groups, there was an indication of an apparent protection against CAP by the sickle cell trait as there was a consistently lower proportion of Hb AS among itchers as compared with non-itchers. There was also a consistently higher frequency of G6PD deficiency among itchers than non-itchers, indicating susceptibility to CAP by the enzyme deficiency. Also the result of CAP and blood group showed that as previously observed, there was no difference in the frequency of CAP among the various blood groups.

2.4.4. Geographical Distribution of CAP

The population distribution of CAP in different geographical areas was investigated so as to be able to evaluate genetic as well as environmental factors on the prevalence of CAP. The prevalence of CAP was therefore evaluated in Kano, Maiduguri and Potiskum in the North and Port-Harcourt in addition to Ibadan in the South of Nigeria. The results from other areas were compared with results from U.C.H., Ibadan as reference standard.

2.4.4.1. *Prevalence of CAP*

The results show that the prevalence of CAP was 13.2% in Kano. This was low compared with U.C.H., Ibadan. This may partly be due to the fact that most of the recruited patients were children, 82 children and 39 adults.

The prevalence in Maiduguri was 24.2%. This was also considerably low despite that the patients studied were mostly adults; 141 adults and 24 children. It was 49.6% in Potiskum, and 8.3% among Port-Harcourt patients. The low prevalence in Port-Harcourt compared with U.C.H., Ibadan may be because all the patients studied in this area were children and the drug was administered to the patients via the parenteral route (subcutaneous or intramuscular).

It seems generally that the prevalence of CAP is particularly lower in the Northern population studied when compared with Ibadan. This may be due to the

fact that the malaria situation in the South is more endemic than in the North (Federal Ministry of Health, 1989).

2.4.4.2. *Haemoglobin Genotype and CAP*

The result from all the other 4 towns were in concordance with the finding in U.C.H. There was a consistently lower proportion of Hb AS among itchers as compared with non-itchers. Also the percentage itchers among Hb AS was lower than among Hb AA individuals. This suggests that the Hb AS just like in malaria confers some protection against CAP on its bearers

2.4.4.3 *G6PD Deficiency and CAP*

The result from the populations studied showed consistently higher frequency of G6PD deficiency in itchers as compared with non-itchers. Also there was a higher frequency of itchers in G6PD deficient than in non- deficient patients. This supports the hypothesis that G6PD deficiency may increase susceptibility to CAP

2.4.4.4. *ABO Blood Group and CAP*

Except in Port-Harcourt and Potiskum patients in whom ABO blood groups were not determined, the results like in Ibadan showed that there was no association between CAP and blood group in any of the populations

CHAPTER 3

3.0. THE DISPOSITION OF CHLOROQUINE IN SUBJECTS WITH AND WITHOUT CHLOROQUINE-ASSOCIATED PRURITUS AND THOSE WITH DIFFERENT HAEMOGLOBIN TYPES

3.1 INTRODUCTION

Interindividual differences in response to standard doses of drugs are well known in clinical practice. The major factor responsible for these differences is the marked variation in the capacity between individuals to metabolise drugs (Eichelbaum 1981). Genetic factors have been identified as being the main underlying principle involved in variations that exist in the phenotypic expression of drug metabolism.

Pharmacogenetics deals with clinically significant hereditary variations in response to drugs and has been found responsible for altered drug disposition and unusual reactions (Rawlins, 1988, Eichelbaum, 1981). Inter-individual variability in drug disposition and response is a therapeutic premise, thus evaluation and management of such variability is the basis for individualised pharmacotherapy.

Two broad types of pharmacogenetic phenomena are recognisable, those that are caused by pharmacokinetic differences and those that are due to altered tissue responses (Rawlins 1988). The most important pharmacokinetic differences are those concerned with drug metabolism. Genetic factors may change the response of target tissues to therapeutic drug concentrations that they lead occasionally to therapeutic failure or more often to toxicity. For example, hereditary anaemia may be precipitated by drugs in patients with hereditary peculiarities of red cell structure or function. An example is G6PD deficiency (Rawlins 1988). However, reports on chloroquine induced haemolysis is rare (Choudry et al 1977). Orjih (1987)

reported a lower plasma chloroquine concentration in Hb SS as compared with Hb AA subjects

Epidemiological studies from first part of this work have provided evidence that CAP is probably being influenced by hereditary factors. It was observed that the sickle cell trait protects against CAP whilst G6PD deficiency may increase susceptibility to CAP.

From the foregoing, it was thought necessary to investigate in healthy volunteers the pharmacokinetic and urinary excretion of chloroquine and its major metabolite in subjects with and without CAP and those with different haemoglobin types

3.1.1. *Review of Analytical Techniques*

The ideal method for the determination of drug concentrations should be specific, sensitive, accurate and simple. Various methods for the identification and determination of chloroquine in biological fluids have been used before the advent of the modern and more sensitive techniques (Bruce-Chwatt, 1959. Wilson and Edeson, 1954. Brodie *et al*., 1945). The methods were not specific and were also procedurally cumbersome. Paper and thin layer chromatographic techniques (Kuroda, 1962) allowed characterisation of chloroquine but are essentially qualitative methods. Spectrophotometric methods have also been described (Prouty and Kuroda, 1958. Robinson *et al*., 1970. Fairan *et al*., 1970). These methods gave quick quantitative and qualitative results but the sensitivity was about 1,000 ng/ml and the precision was very low.

Brodie *et al* (1947) first described spectrofluorimetric method for the determination of chloroquine in blood and urine. This method was based on double extraction and required UV-irradiation for two hours to develop fluorescence of the 4-aminoquinoline nucleus. Several workers (Alving *et al* 1948. Berliner *et al* 1948. Fellman *et al*, 1948. Coatney *et al*, 1949) used the method to determine chloroquine concentration. The method was later modified by McChesney *et al* (1956). A

further modification which required no irradiation was used by Gandette and Coatney (1961). The method was further modified by other workers in a stepwise progressive manner (McChesney *et al* 1962, 1966, 1967; Schulman and Young 1974; Bergqvist *et al*. 1976; Adelusi and Salako, 1980). However, some of these methods were not sensitive enough for pharmacokinetic studies and were also non-selective since the extraction step does not completely separate chloroquine from its metabolites which have the same fluorescence characteristics as the parent compound. Gas chromatographic techniques have also been described for the determination of chloroquine concentrations (Holtzman, 1965; Robinson *et al* 1970; Bergqvist and Ekerbom, 1981; Price-Evans *et al* 1979; Kuye *et al*., 1983) But costs and the cumbersome nature of these methods made them unpopular.

In recent years, immunoanalytical methods like the enzyme-linked immunosorbent assay have been applied in antimalarial assays (Escande *et al*., 1990). These methods may have a limited specificity and may cross react with metabolites. They are often only semi-quantitative (Churchill 1989).

The first reported specific High Performance Liquid Chromatographic (HPLC) method for determination of chloroquine was described in 1980 by Bergqvist and Frisk-Holmberg. HPLC applications for whole blood samples dried on filter paper have also been described (Patchen *et al* 1983; Lindstrom *et al*., 1985).

High Performance Liquid Chromatography

The principle of the technique of this separation method depends on the partitioning of the solute between two immiscible solvents, one of which is stationary and the other is the mobile phase or ion exchange (adsorption) between the mobile phase and the ionic sites of the packing of the stationary phase. The stationary phase which can be either polar or non-polar is coated onto an inert support and packed into a column. Resins, especially the sulphonic acid and quaternary amine types have been used as stationary phase. Separation of

components of a sample can be achieved by varying the amounts of the polar and non-polar compounds in the mobile phase.

Several authors have used different HPLC techniques for chloroquine analysis (Bergqvist and Frisk-Holmberg, 1980; Staiger *et al*, 1981; Alvan *et al*., 1982; Brown *et al*., 1982; Patchen *et al*., 1983; Essien *et al*., 1988; Ogumbona *et al*., 1986). Bergqvist and Frisk-Holmberg (1980) used ion-pair HPLC to separate chloroquine and its metabolite, desethylchloroquine in their protonated forms. They were able to detect chloroquine and desethylchloroquine in urine and plasma in the range of $10nMl^{-1}$ using UV detector and $0.5nMl^{-1}$ using the fluorescence detector. These are low enough concentrations for routine monitoring of therapeutic plasma level of chloroquine and desethylchloroquine. Patchen *et al* (1983) reported the determination of chloroquine and desethylchloroquine in finger prick blood samples absorbed on filter papers. A detection limit for chloroquine and desethylchloroquine of 5ng/ml was found using fluorescence detector.

A major advantage of the HPLC is its high sensitivity which permits the use of smaller volumes of blood which is possible to obtain on the field. Also the derivatisation procedure as in the gas chromatographic method for separating chloroquine and its metabolites is not necessary. A major disadvantage of the system is that the solvents used are too expensive and the technique is complex.

3.2. SUBJECTS, MATERIALS AND METHODS

3.2.1. *Subjects*

Fifteen healthy male volunteers who were medical students of the College of Medicine, University College Hospital, Ibadan participated in the study. They were aged 19-30 years (mean 23.1±4.70 years) and their weights ranged from 45.0-65.5 (mean 58.7±6.2 kg). All the subjects were non-smokers. Some took alcohol occasionally. None took any medication regularly. None of the volunteers had taken chloroquine in the 2 months preceding the investigation and none during the study period took any dose of the drug other than those administered as part of the

study. Measurements of haematological indices, liver enzymes, serum electrolyte and urea which were done at the beginning of the study were within normal limits in all the subjects. The urine were also tested for specific gravity, albumin and sugar. The tests were all normal. Haemoglobin type was determined for all the volunteers.

The subjects were divided into 2 groups; 7 itchers and 8 non-itchers. They were also grouped according to their haemoglobin genotype, 8 Hb AA and 7 Hb AS. It was not ethically possible to do kinetic study of this type in sicklers because they are always at the risk of anaemia.

3.2.2 *Materials*

Chloroquine base	May and Baker
Papaverine	BDH
Desethyl-chloroquine	May and Baker
Diethyl-ether	BDH Chemicals England
Sodium hydroxide	" " "
Hydrochloric acid (0.1M HCL)	" " "
Acetonitrile (HPLC grade)	Riedel-de Haen (Chromasolv)
Methanol (HPLC grade)	" " "
Sodium dihydrogen phosphate	BDH Chemicals England
Water (HPLC grade)	Riedel-de Haen (Chromasolv)
Perchloric acid	BDH Chemicals England
Lithium Heparin tubes	
Extraction tubes	Pyrex
Precision micropipettes	
Pasteur pipettes	
Centrifuge	MSE England.
HPLC System	Waters USA
pH Meter	Philips Zurich
Vortex Mixer	Gallenkamp England
Ultrasonicator (Brp,spm 220)	Branson 220 USA

3.2.3 *Study Design*

Each subject was given a single oral dose of (600mg) chloroquine base (Nivaquine, May and Baker) after an overnight fast with about 200ml of tap water. Food intake was only permitted 3 hours later.

Venous blood samples (5ml in heparinised tubes) were obtained before and at 0.5, 1, 2, 4, 8, 12, 24, 48, 96, 168 and 336 hours after drug administration.

The blood samples were centrifuged at 1200 g within 10 minutes of collection and plasma separated. This was done to minimise leakage of chloroquine from the cellular elements of blood into plasma while standing. The plasma was stored frozen at -20°C until analysed. Pooled 24 hours urine samples were also collected separately from each subject on days 1, 7 and 14 into plastic containers. The volumes were recorded and 10ml aliquots stored at -20°C until analysed.

3.2.4 *Analytical Techniques*

Plasma and urinary chloroquine and desethylchloroquine concentrations were analysed in duplicate using high performance liquid chromatography (HPLC). A simple sensitive and reproducible method based on the modification of the method of Ogunbona *et al* (1986) was developed. The new method was used for the assays

3.2.4.1 *Chromatographic Conditions*

A Waters model 440 liquid detector chromatograph fitted with a fixed-wavelength (254nm) and pump was used. The detector output was connected to a Perkin-Elmer recorder. The analysis was carried out on a Waters reversed phase silica based column (Bondapak, 3.9 x 300mm). The mobile phase consisted of 0.2M Sodium dihydrogen phosphate - Methanol - Acetonitrile in the ratio 65:30:5 with 1ml perchloric acid per 100ml of mixture. The pH of the mobile phase was

30. The pump was set at a flow rate of 1ml/min. All experiments were carried out at room temperatures. The internal standard (I.S.) used was papaverine from BDH.

3.2.4.2 *Plasma Assay*

Calibration curves based on peak-height ratios were first prepared by spiking drug-free plasma with standard chloroquine and desethylchloroquine (10ug/ml) to give concentration range of 20-200ng/ml).

To 1ml of plasma placed in a 10ml screw capped extraction tube were added 1ml of 2M sodium hydroxide, 10ul of I.S. solution (5ug/ml) and 4ml diethyl-ether. Each tube and its contents were then whirl mixed with a vortex mixer for 1 min and then centrifuged for 10 min at 2,000g. The ether layer was transferred into another tube. A 100ul volume of 0.1 N HCl was added to each tube containing the separated ether layer. The tubes were then whirlmixed for 1 min and centrifuged for 10 min. The ether layer was then discarded. A 10ul aliquot of the aqueous phase was injected into the HPLC.

The peak-height ratio for both the standard and test samples were recorded. Concentration of drug in test samples were determined by extrapolation from the standard calibration curve of concentration versus peak-height ratio.

3.2.4.3 *Urine Assay*

Calibration curves based on peak-height ratio for the concentration range 100-3000ng/ml were prepared for chloroquine and desethylchloroquine

A 1 in 10 dilution of urine was made with distilled water by adding 1ml of urine to 9ml of water. A 1ml aliquot of the diluted solution was taken through the extraction procedure as described for plasma samples above but for urine 20ul of internal standard (5ug/ml) was used. Drug concentrations were determined by extrapolation from the standard calibration curve

3.2.4.4 *Precision and Percentage Recovery of the Analytical Method*

Blank samples of plasma and urine were spiked with standard chloroquine and desethylchloroquine to give known concentrations of the drugs. A 10 or 20ul aliquot of the internal standard was added to blood and urine samples respectively. The samples which were prepared in duplicates were then taken through the extraction procedures as described previously. Peak-height ratios were calculated and concentrations determined by extrapolation from the calibration curves. Intra-assay and inter-assay coefficients of variation (C.V.) of the estimated concentrations were determined by comparing the standard deviation with the mean of the concentrations. This was used for the assessment of the precision of the method. Limits of detection of the drugs were also determined.

The percentage recovery was determined by comparing the peak-height ratios of the extracted drugs with those obtained by direct injection of same concentrations of the drugs.

3.2.5 *Pharmacokinetic Calculations*

The pharmacokinetic calculations were done using extended least square modelling programme (Holford 1986). The peak concentration (Cmax) and time to reach the peak (Tmax) were determined from the concentration - time profile.

The plasma concentration curves was approximated to a two compartment open model with kinetics as follows:

$$C = A e^{-\alpha t} + B e^{-\beta t}$$

where C is the concentration of the drug in the plasma. A and B are the biexponential functions of distribution and elimination phases respectively. While α and β are the distribution and elimination rate constants respectively and t is the time. At the cessation of distribution, the function, $e^{-\alpha t} = 0$ and elimination becomes significant.

The equation then becomes:

$$C = B e^{-\beta t}$$

$$\therefore \quad \text{Log } C = \text{Log } B - \beta t / 2.303$$

B is equivalent to Co, while β is equivalent to K where Co is the concentration of the drug at time, t = 0 in the plasma and K, the elimination rate constant in the plasma.

A plot of Log C against t gave a curve with terminal portion as straight line representing the B elimination phase, the slope of which equals -K/2.303 and intercept equals Co.

K was calculated by linear regression of the log concentration-time profile from the monoexponential terminal portion, using the last three or four time points. Half-life ($t^1/_2$) was then calculated from the relation:

$$t^1/_2 = 0.693/K$$

The area under the plasma concentration-time curve (AUC) was estimated using the trapezoidal rule.

The area to infinite time was calculated by integrating C_{last}

$$\int_{t_{last}}^{\infty} C dt = C_{last} / \beta$$

where C_{last} is the last value of chloroquine concentration on the calculated β slope of the last squares linear regression line of the log concentration-time plots. The infinite area is defined as the percentage of the total area derived from the concentration-time plots after extrapolation of the last time point of infinity as

Extrapolated area/total area x 100.

Renal clearance (CL_R) was calculated from urinary and plasma data according to the relation

$$CL_R = \frac{\text{Amount of drug excreted unchanged per unit time}}{Cp}$$

Where Cp is the plasma concentration at the mid-point of the urine collection interval.

The urinary recovery of chloroquine and desethyl-chloroquine up to day 14 was approximately estimated by calculating from the area under the urinary concentration time curve using the trapezoidal rule and data from days 1, 7 and 14. Excretion to infinite time was estimated assuming the same half-life of chloroquine or desethylchloroquine in urine after day 14 as that calculated from the straight line connecting excreted amount on days 7 and 14. The sum of the total areas under the excretion curves of CQ and DCQ is an estimate of the total amount of CQ and DCQ recovered in urine.

Values are given in the text and tables as means ± S.D.

Differences between means were tested for significance using Students t-test and P-values less than 0.05 were taken as significant.

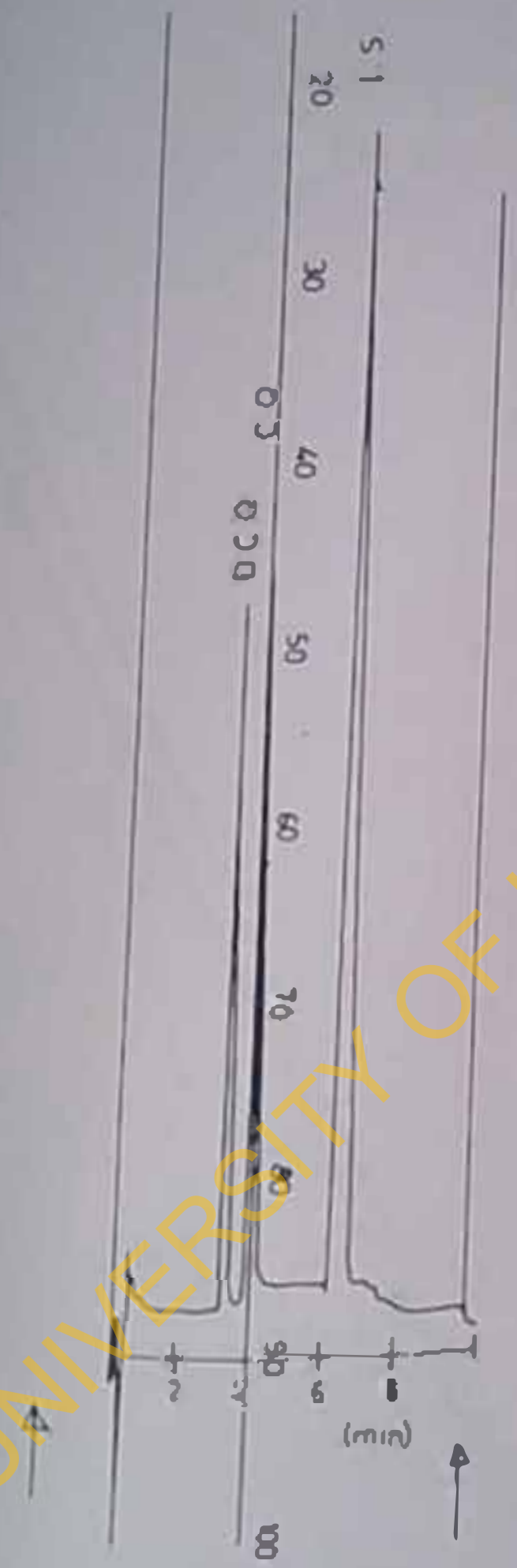
33 RESULTS

33.1. *Liquid Chromatographic Method for Assay of Chloroquine and Desethylchloroquine*

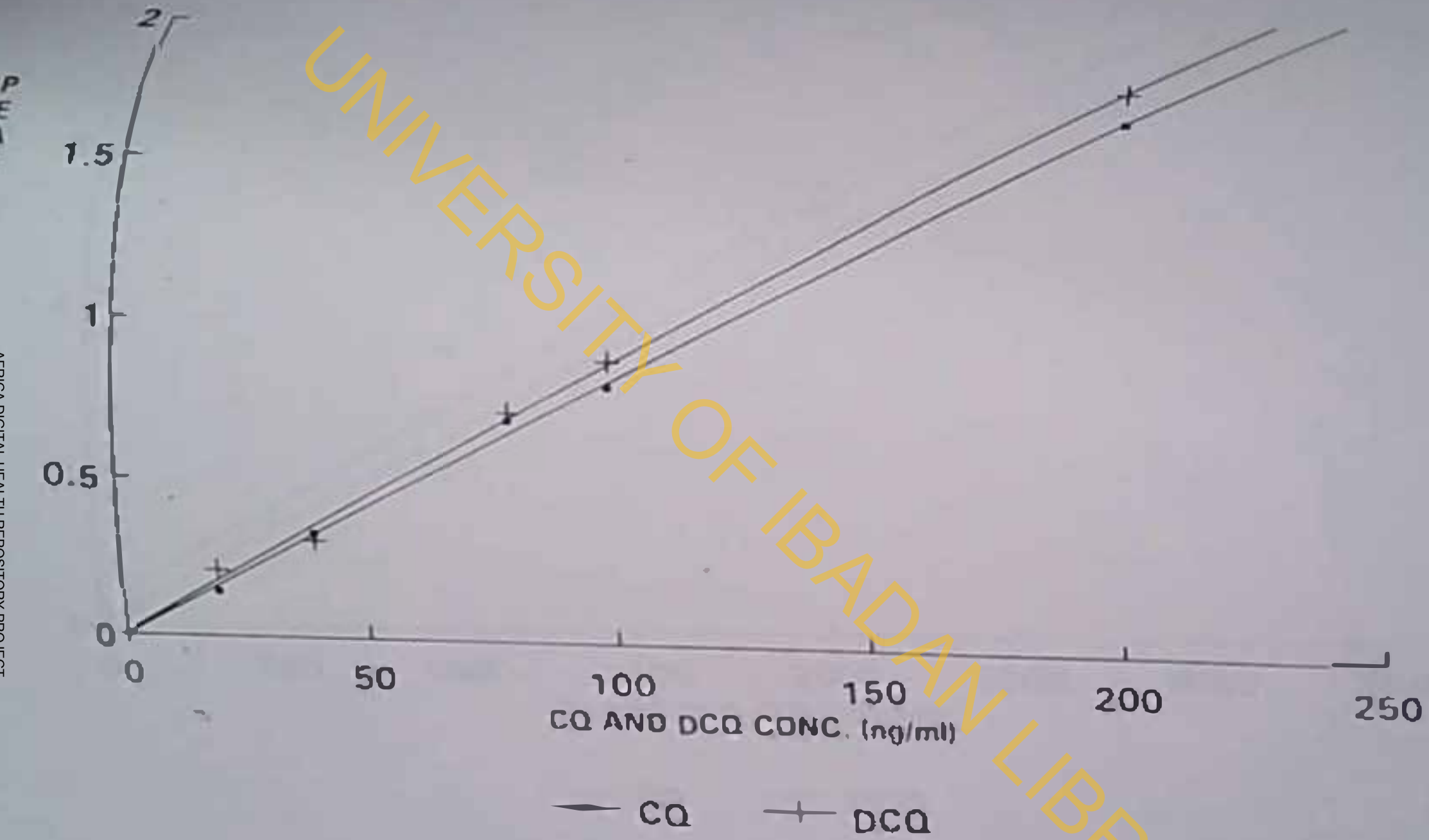
Chloroquine, Desethylchloroquine and I.S were all well separated on the chromatograms. They were eluted in the order, desethylchloroquine, chloroquine and I.S with retention times of 3, 4 and 6 minutes respectively (Fig 33.1). There was no interference from other drugs like pyrimethamine, proguanil, sulphadoxine, amodiaquine, paracetamol, promethazine or chlorpheniramine etc which may be present in the body. The limit of detection for chloroquine and desethylchloroquine were 1.0 and 0.5ng/ml respectively. Table 3.3.1 shows the precision of the analytical method. The intra-assay C.V. of chloroquine and desethylchloroquine in plasma were at 20ng/ml 3.0% and 4.7%, 100ng/ml 3.2% and 3.9%, and 400ng/ml 3.3% and 4.1% respectively. While in urine it was at 50ng/ml 4.2% and 5.0% for chloroquine and 100ng/ml 4.1%, and 4.0% and 4,000ng/ml 4.2% and 5.0% for chloroquine and desethylchloroquine respectively. The interassay C.V. of chloroquine and desethylchloroquine in plasma were at 20ng/ml 4.9% and 7.2%, 100ng/ml 4.8% and 6.8%, and 400ng/ml 4.8% and 4.6% respectively. While in urine it was at 50ng/ml 4.5% and 6.3%, 1000ng/ml 4.8% and 6.3% and 4,000ng/ml 5.1% and 6.6% for chloroquine and desethylchloroquine respectively.

Table 3.3.2 shows the absolute recovery of chloroquine and desethylchloroquine. In plasma the percentage recovery was at 100ng/ml 94.5±3.1 and 94.0±4.7 and at 200ng/ml 92.7±4.7 and 86.1±2.9 for chloroquine and desethylchloroquine respectively. In urine, the percentage recovery was at 1,000ng/ml 89.9±6.6 and 87.0±7.2, and 2,000ng/ml 91.0±5.0 and 92.2±2.7 for chloroquine and desethylchloroquine respectively. The concentration-time curves for chloroquine and desethylchloroquine in plasma and urine showed a linear response (Fig 3.3.2 and 3.3.3) with regression coefficients (r^2) > 0.99 in all cases (Tables 3.3.3 and 3.3.4).

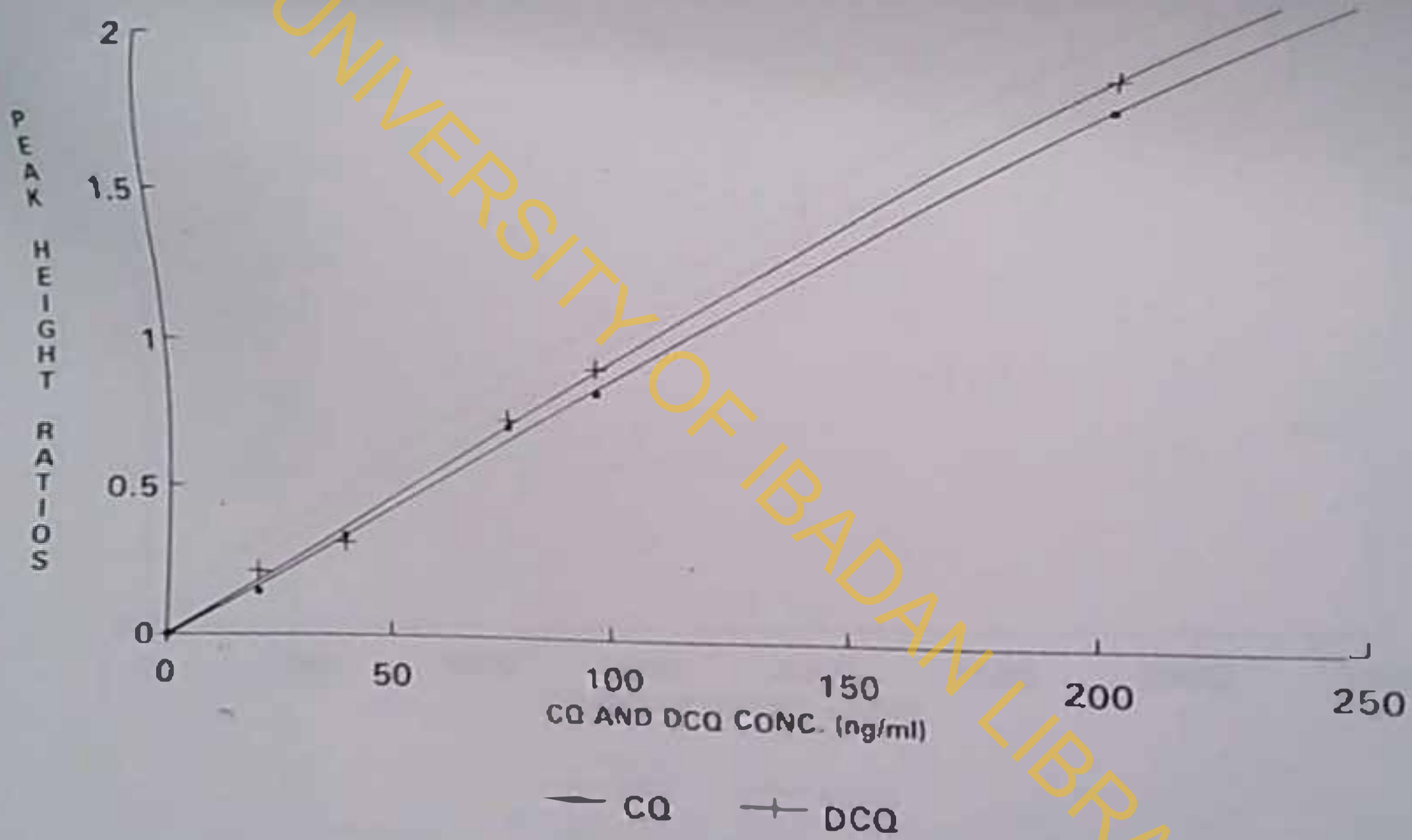
Fig 33 :



A TYPICAL CHROMATOGRAM OF CHLOROQUINE ANALYSIS.



CALIBRATION CURVES FOR CHLOROQUINE AND DESETHYL-CHLOROQUINE IN PLASMA



CALIBRATION CURVES FOR CHLOROQUINE AND DESETHYLCHLOROQUINE IN URINE

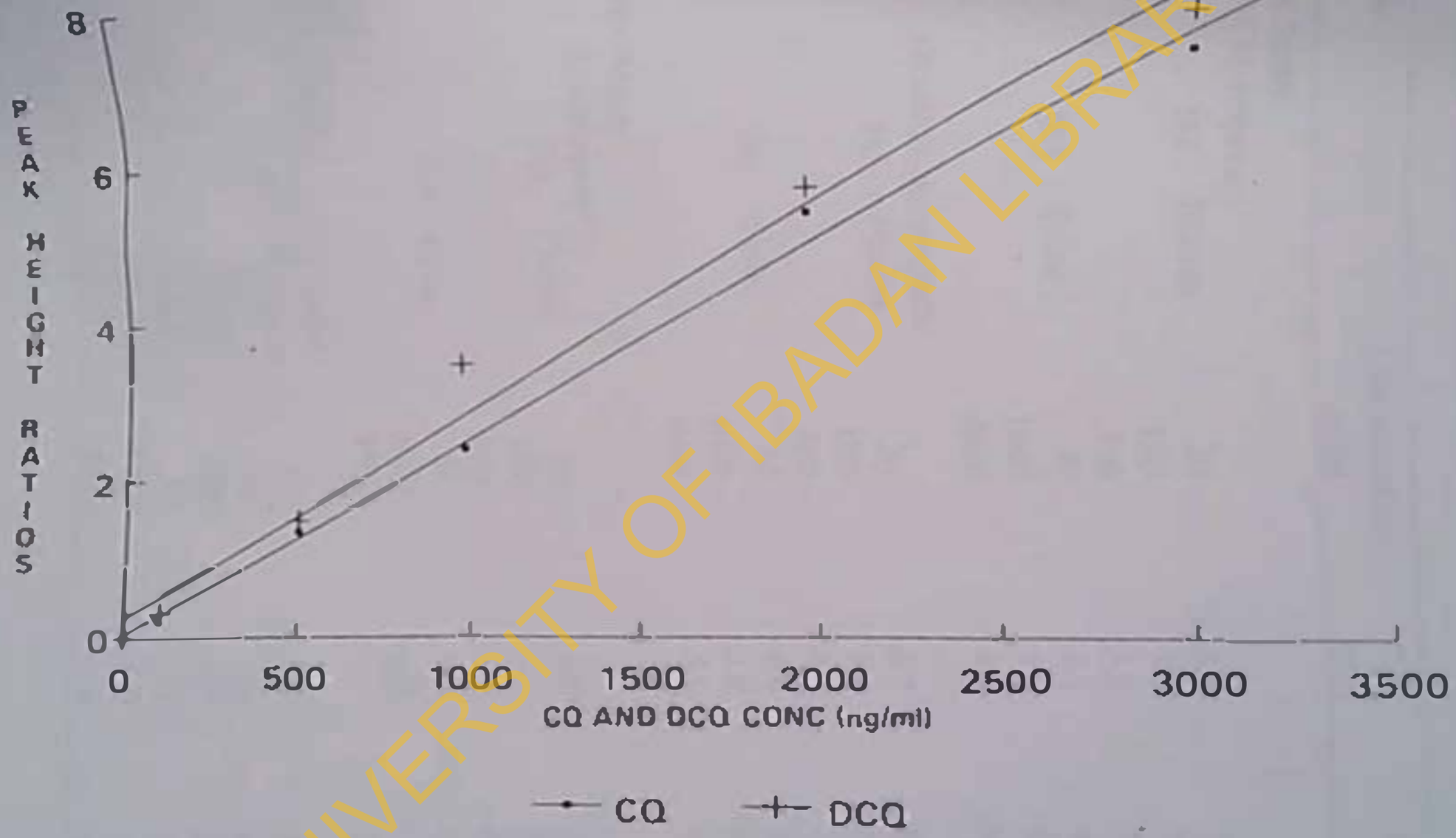


Table 3.3.1 Precision of Analytical Method

Sample	Concentration ng/ml	C.V (%)	n
Intra-Assay			
1. *Chloroquine:*			
(a) Plasma	20	3.0	7
	100	3.2	7
	400	3.3	7
(b) Urine	50	4.2	6
	1000	4.1	6
	4000	4.2	6
2. *Desethyl-chloroquine:*			
(a) Plasma	20	4.7	7
	100	3.9	7
	400	4.1	7
(b) Urine	50	6.9	6
	1000	4.0	6
	4000	5.0	6
Inter-Assay			
1. *Chloroquine:*			
(a) Plasma	20	4.8	5
	100	4.8	5
	400	4.8	6
(b) Urine	50	4.5	6
	1000	4.8	6
	4000	5.1	4
2. *Desethyl-chloroquine:*			
(a) Plasma	20	7.2	5
	100	6.8	5
	400	4.6	6
(b) Urine	50	6.3	6
	1000	6.3	6
	4000	6.9	4

Table 3.3.2.

Absolute Recovery of the Analytical Method

Sample		Concentration ng/ml	% Recovery ± S.D.	n
1. *Chloroquine:*				
(a)	Plasma	100	94.5±3.1	5
		200	92.7±4.7	5
(b)	Urine	1000	89.9±6.6	5
		2000	91.0±5.0	4
2. *Desethyl-chloroquine:*				
(a)	Plasma	100	94.0±4.7	5
		200	86.1±2.9	5
(b)	Urine	1000	87.0±7.2	5
		2000	92.2±2.7	4

Table 3.3.3

The Calibration of Chloroquine and Desethyl-chloroquine in Plasma

Conc (ng/ml)	Peak Height (mm)			Peak Height Ratio	
	CQ	DCQ	I.S	CQ / I.S.	DCQ / I.S.
0	0	0	38.5	0.00	0.0000
20	8.5	12.5	59.5	0.1428	0.2100
40	32.0	30.0	97.0	0.3298	0.3092
80	58.5	60.5	83.5	0.7005	0.7245
100	30.0	33.0	37.0	0.8108	0.8918
200	57.0	60.5	34.5	1.6521	1.7536

r_{CQ} = 0.9991

r_{DCQ} = 0.9988

Table 3.3.3

The Calibration of Chloroquine and Desethyl-chloroquine in Plasma

Conc (ng/ml)	Peak Height (mm)			Peak Height Ratio	
	CQ	DCQ	I.S	CQ / I.S.	DCQ / I.S.
0	0	0	38.5	0.00	0.0000
20	8.5	12.5	59.5	0.1428	0.2100
40	32.0	30.0	97.0	0.3298	0.3092
80	58.5	60.5	83.5	0.7005	0.7245
100	30.0	33.0	37.0	0.8108	0.8918
200	57.0	60.5	34.5	1.6521	1.7536

r_{CQ} = 0.9991

r_{lxCQ} = 0.9988

Table 3.3.4

The Calibration of Chloroquine and Desethyl-chloroquine in Urine

Conc (ng/ml) CQ	Peak Height (mm) DCQ	I.S.	I.S.	Peak Height Ratio CQ / I.S.	DCQ
0	0	0	62.0	0.0000	0.0000
100	12.0	18.0	60.0	0.2000	0.3000
500	81.5	90.5	61.0	1.3360	1.4836
1000	143.0	159.0	46.0	2.3833	3.4565
2000	189.0	200.0	36.0	5.2500	5.5556
3000	145.0	155.0	20.5	7.0732	7.5609

r_{CQ} = 0.9968

r_{DCQ} = 0.9914

3.3.2. *Haemoglobin AA Versus AS*

3.3.2.1 *Plasma*

The log concentration-time curves for chloroquine and desethylchloroquine after oral administration of 600mg chloroquine base are shown in Fig. 3.3.4 and 3.3.5. The pharmacokinetic parameters derived from the individual concentration-time data are shown in Tables 3.3.5 and 3.3.6. The peak plasma concentration (Cmax) of chloroquine was higher in Hb AA as compared with Hb AS volunteers

It was 395.4±102.5ng/ml for Hb AA and 283.7±91.0ng/ml for Hb AS. This shows a significantly lower Cmax in Hb AS than Hb AA subjects ($p < 0.05$). The time to reach peak concentration (Tmax) was 3.5±2.1 h in Hb AA and 3.7±2.1 h in Hb AS ($p > 0.05$). The plasma chloroquine concentrations declined slowly afterwards and the drug was still detectable 14 days after its administration. The apparent terminal half life ($t^1/_2$) was 111.4±52.9 in Hb AA and 121.0±50.0 h in Hb AS ($p > 0.05$). The coefficient of regression for the calculation of $t^1/_2$ was 0.98±0.04. The area under the plasma concentration-time curve (AUC) was 22665±9101 ng/ml h for Hb AA and 15164±8791 ng/ml h for Hb AS ($p > 0.05$). The extrapolated AUC as a percentage of the total AUC was less than 20% in both groups. Renal Clearance (Cl_R) was 243.2±106.5 ml/min-1 for Hb AA and 316.1±122.8 ml/min-1 for Hb AS. There was no statistically significant difference between the 2 groups ($p > 0.05$).

Desethylchloroquine due to the administered drug was detectable in plasma 30 min after drug ingestion in the 2 groups. The pharmacokinetic parameters of desethylchloroquine in the Hb AA and Hb AS are shown in Table 3.3.6. The Cmax was 107.6±39.1 ng/ml in Hb AA and 90.6±62.9 ng/ml in Hb AS. There was no statistically significant difference between the 2 groups ($p > 0.05$).

The Tmax for desethylchloroquine was 5.3±3.0 h in Hb AA and 5.1±3.6 h in Hb AS ($p > 0.05$). Desethylchloroquine was still detectable in plasma 2 weeks after drug administration. The $t^1/_2$ of the metabolite was similar in Hb AA and Hb AS volunteers and was 103.4±55.2 h and 146.9±165.9 h respectively ($p > 0.05$). The renal clearance was 242.8±163.2 ml/min for Hb AA and 269.8±163.2 ml/min for Hb AS ($p > 0.05$). The AUC was 7750±1765 ng/ml h and 5783±5305 ng/ml h for Hb AA and Hb AS respectively ($p > 0.05$). Also the ratio of the AUC of the metabolite to the AUC of the parent drug was similar in both groups. It was 0.37±0.19 for Hb AA and 0.31±0.11 for Hb AS ($p > 0.05$). This is indicative of a similar rate of metabolism in the 2 groups.

3.3.2.2 Urinary Excretion

The analysis of the excretion rate of chloroquine and desethylchloroquine in 24 h urine collections showed an exponential decline of both compounds with time

in both groups. Table 3.3.7a and b shows the urinary excretion data in Hb AA and Hb AS respectively. The total chloroquine and desethylchloroquine excreted in the first 24 h was 83.0±36.6 mg in Hb AA and 89.3±44.8 mg in Hb AS which is approximately 13.8% and 14.9% of the administered dose respectively. This was similar in both groups ($p > 0.05$). The estimated total chloroquine and desethylchloroquine recovery in individual subjects was 432.8±216.2 mg for Hb AA and 408.9±167.9 mg for Hb AS which is approximately 72.1% and 68.2% of the administered dose respectively ($p > 0.05$). Of the estimated total recovered quinoline, 77% and 81% were due to the parent compound and 23% and 19% due to the metabolite, giving a ratio of 3.3 and 4.3 for Hb AA and Hb AS respectively.

Table 3.3.5.

The Pharmacokinetic Parameters of Chloroquine

Subject Code	Cmax (ng/ml)	Tmax (h)	$t^1/_2$ (h)	Cl_R (ml/min)	AUC (ng/ml.h	Extrapolated AUC as % of total
(a) Hb AA Volunteers						
MA	403.5	2	69.2	131.1	24003	3.0
OO	400.3	4	146.1	159.4	36611	18.2
AO	431.4	2	113.0	234.0	21995	8.0
OD	399.0	2	83.3	283.0	14166	1.5
EH	259.8	8	221.2	237.0	31163	31.6
BH	589.3	4	107.8	455.3	22044	10.0
OK	411.0	4	91.3	ND	24048	8.7
AD	269.2	2	84.5	201.0	7292	4.5
Mean	395.4	3.5	111.4	243.2	22665	10.7
±S.D	102.5	2.1	52.9	106.5	9010	9.9
(b) Hb AS Volunteers						
IA	360.4	4	170.7	183.0	12635	14.2
IT	264.4	2	208.3	463.0	11368	17.0
BS	264.2	4	67.9	386.0	9098	7.3
EC	321.3	8	105.4	445.0	30704	11.2
OS	421.5	2	106.7	171.0	24477	8.7
AK	176.4	2	84.4	225.8	8290	5.9
AL	178.0	4	103.9	338.8	9776	8.4
Mean	283.7	3.7	121.0	316.1	15164	10.4
±S.D	91.0	2.1	50.0	122.8	8790.7	4.0

Table 3.3.6.

The Pharmacokinetic Parameters of Desethyl-chloroquine

Subject Code	Cmax (ng/ml)	Tmax (h)	$t^1/_2$ (h)	Clr (ml/min)	AUC ng/ml h	AUC DCQ / AUC CQ
(a) **IIb AA Volunteers**						
MA	142.0	2	72.6	158.3	18243	0.76
OO	37.2	8	209.7	197.1	5437	0.15
AO	101.4	8	157.6	234.2	4495	0.20
OD	106.8	2	71.1	331.9	5176	0.37
EH	89.8	8	39.9	342.9	7212	0.23
BH	121.6	4	92.3	272.5	8817	0.40
OK	168.4	8	110.5	ND	9524	0.40
AD	90.5	2	73.6	162.4	3096	0.42
Mean	107.2	5.3	103.4	242.8	7750	0.37
±S D	39.1	3.0	55.2	75.9	4765	0.19
(b) **Hb AS Volunteers**						
IA	50.0	4	518.2	347.2	2942	0.23
TT	37.5	2	106.7	514.5	3341	0.30
BS	118.0	4	92.3	65.3	3639	0.40
EC	212.4	12	95.6	382.6	16847	0.55
OS	113.4	2	53.2	72.9	8518	0.35
AK	43.5	4	44.8	260.7	2510	0.30
AL	59.7	8	117.8	245.6	2686	0.27
Mean	90.6	5.1	146.9	269.8	5783	0.34
±S D	62.9	3.6	165.9	163.2	5305	0.11

Table 3.3.7

Mean Urinary Recovery and Estimated Total Excretion of Chloroquine (CQ) and Desethyl-chloroquine (DCQ)

(a) Hb AA

Day	Amount Recovered (mg)			Estimated Total Recovery (mg)	Recovery as % of dose given
	1	7	14		
CQ	62.3±26.0	9.5±6.9	5.1±4.7	332.6±164.9	55.4
DCQ	20.7±10.6	3.1±2.7	0.9±0.9	100.2±51.3	16.7
Total	83.0±36.6	12.6±9.6	6.0±5.6	432.8±216.2	72.1
Total recovered as % of dose	13.8	2.1	1.0	72.1	

(b) Hb AS

Day	Amount Recovered (mg) 1	7	14	Estimated Total Recovery (mg)	Recovery as % of dose given
CQ	71.8±30.4	8.0±3.0	3.4±1.0	333.3±122.8	55.6
DCQ	17.5±14.4	2.1±1.2	1.1±1.0	75.6±45.1	12.6
Total	89.3±44.8	10.1±4.2	4.5±2.0	408.9±167.9	68.2
Total recovered as % of dose	14.9	1.7	0.8	68.2	

FIG. 3.3.4

THE DISPOSITION OF CHLOROQUINE IN HBAA AND HBAS VOLUNTEERS

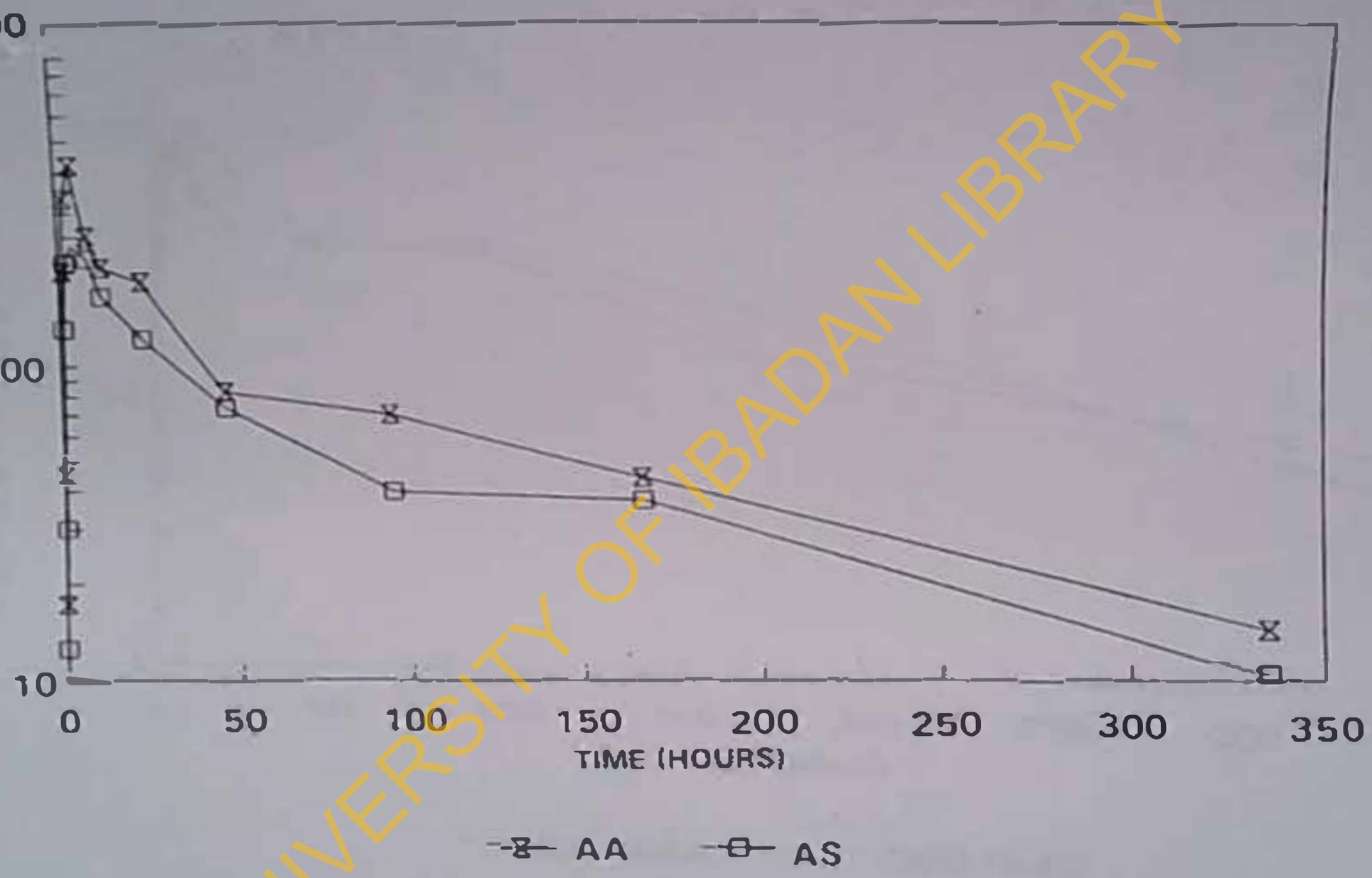
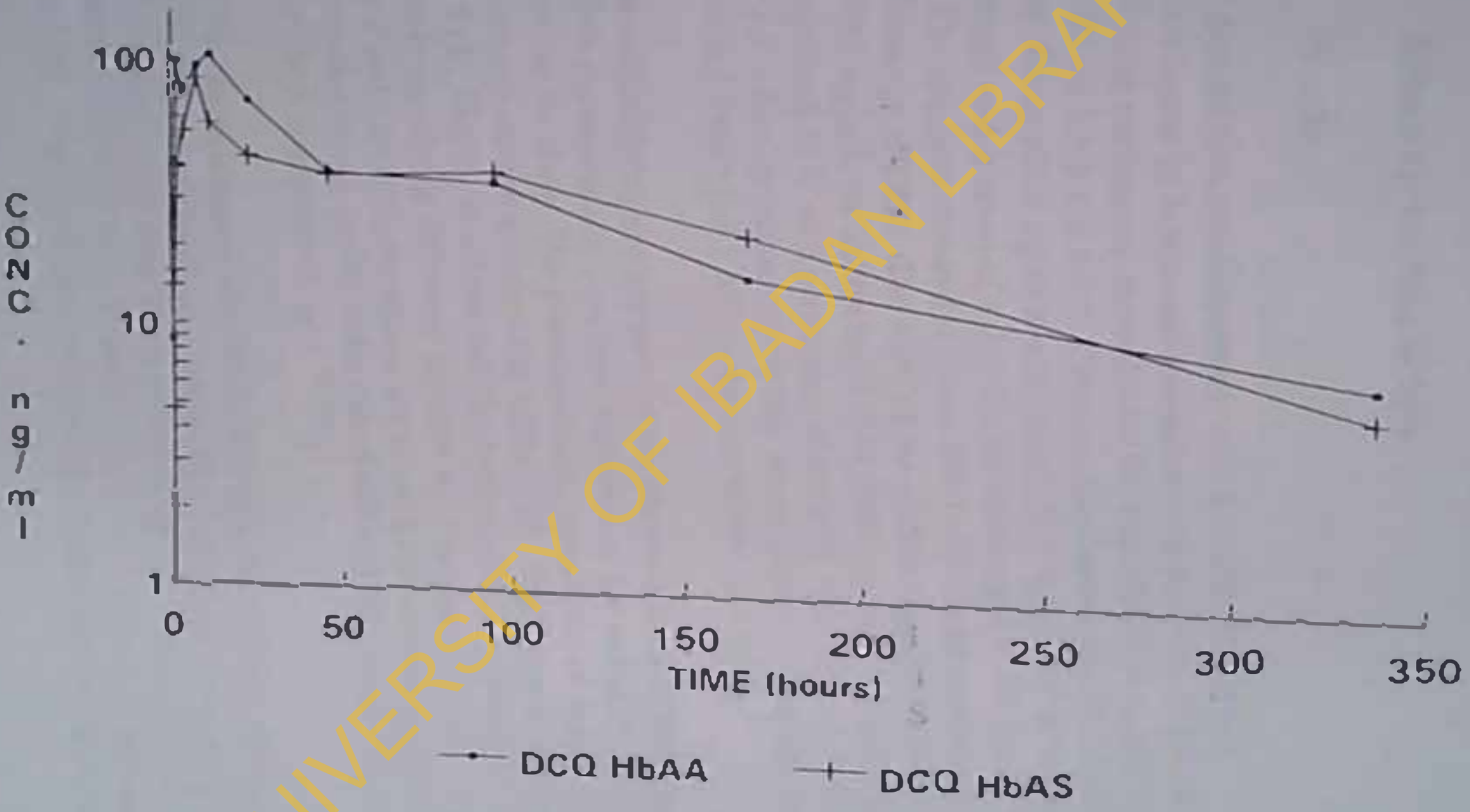


Fig 3.3.5

PLASMA CONCENTRATION TIME PROFILE OF DESETHYLCHLOROQUINE IN Hb AA AND Hb AS VOLUNTEERS



3.3.3 Itchers Versus Non-Itchers

3.3.3.1 Plasma

The plasma concentration-time profile for chloroquine and desethylchloroquine in itchers and non-itchers are as in Fig. 3.3.6 and 3.3.7. The pharmacokinetic parameters derived from the individual concentration-time data are shown in Tables 3.3.8 and 3.3.9. Cmax of chloroquine was 367.1±120.6ng/ml in itchers and 362.4±103.6 ng/ml in non-itchers ($p > 0.05$). Tmax ranged from 2 to 8 h in both groups with a mean of 4.0±2.0 h for itchers and 3.3±2.1 h for non-itchers ($p > 0.05$). The apparent terminal t1/2 was 148.1±55.8 h for itchers and 98.3±17.7 h for non-itchers ($p > 0.05$). The AUC of the plasma concentration-time profile was 22562±1185 ng/ml h for itchers and 17844±9000 ng/ml h for non-itchers ($p > 0.05$). The extrapolated AUC as a percentage of the total AUC was 15.2±8.4% for itchers and 6.5±3.3% for non-itchers ($p > 0.05$). Renal clearance was 302.8±129.8 ml/min for itchers and 2565±108.1 ml/min for non-itchers ($p > 0.05$).

Desethylchloroquine appeared in the plasma quickly in both groups such that measurable concentrations were seen in plasma by the end of 30 min of administering the drug. The pharmacokinetic parameters of desethylchloroquine in itchers and non-itchers are shown in Table 3.3.9. The Cmax of desethylchloroquine was 79.8±37.6 ng/ml in itchers and 113.2±61.0 ng/ml in non-itchers. There was no statistically significant difference between the 2 groups ($p > 0.05$). The Tmax was 6.6±3.4 h and 5.0±4.0 h for itchers and non-itchers respectively ($p > 0.05$). The $t^1/_2$ of the metabolite was similar in the itchers and non-itchers and was 173.8±161.2 h and 79.9±26.0 h respectively ($p > 0.05$).

The renal clearance was 280.4±76.2 ml/min and 280.7±97.0 ml/min in itchers and non-itchers respectively ($p > 0.05$). The ratio of the AUC of the metaboline to the AUC of the parent drug was significantly greater in the non-itchers compared to the itchers and were 0.25±0.08 and 0.43±0.15 ($p < 0.05$) in itchers and non-itchers respectively. This is indicative of a decreased metabolism in itchers relative to non-itchers.

3.3.3.2. *Residual Chloroquine in Plasma*

Table 3.3.11 shows that of the 9 habitual itchers 7 actually itched after taking the drug. None of the non-habitual itchers reacted. Six of the 7 current reactors had residual chloroquine in zero hour plasma. The concentration was 26.7±11.2 ng/ml whereas the remaining 1 itcher as well as the 2 who did not itch had no measurable quantity of chloroquine in zero hour plasma. Four of the 6 non-habitual itchers also had residual chloroquine with concentration of 16.2±9.7 ng/ml. This shows that residual chloroquine was significantly more in the plasma of itchers than non-itchers.

3.3.3.3. *Urinary Excretion*

The pattern of excretion of chloroquine and desethylchloroquine in 24 h urine collections in itchers and non-itchers are shown in Table 3.3.10. The total chloroquine and desethylchloroquine excreted in the first 24 h was 101.8±43.3 mg in itchers and 73.4±28.0 mg in non-itchers ($p > 0.05$) which is approximately 17 and 12% of the administered dose respectively.

The estimated total chloroquine and desethylchloroquine recovery in individual subjects was 496.3±211.2 mg in itchers and 345.3±122.6 mg in non-itchers. This corresponds to approximately 83% in itchers and 58% in non-itchers. There was no significant difference between the two groups ($p > 0.05$). From the estimated total urinary excretion, 81% and 75% of the recovered quinoline in itchers and non-itchers respectively were due to the parent compound and 19% and 25% due to desethylchloroquine giving a ratio of approximately 4.3 and 3.0 for itchers and non-itchers respectively.

Table 3.3.8.

The Pharmacokinetic Parameters of Chloroquine

Subject Code	Cmax (ng/ml)	Tmax (h)	$t^1/_2$ (h)	Cl_R (ml/min)	AUC (ng/ml.h	Extrapolated AUC as % of total
(a) Itchers						
OO	400.3	4	148.1	159.4	36611	18.2
AO	431.4	2	113.0	234.2	21995	8.0
EH	259.8	8	221.2	237.0	31163	31.6
BH	589.3	4	107.8	455.0	35269	10.0
IA	360.4	4	170.7	183.0	12635	14.2
TT	264.4	2	208.3	463.0	11168	17.0
BS	264.2	4	67.9	386.0	9098	7.3
Mean	367.1	4.0	148.1	302.0	22562	15.2
±S.D	120.5	2.0	55.8	129.8	1185	8.4
(b) Non-Itchers						
MA	403.5	2	69.2	131.1	24003	3.0
OD	399.0	2	53.3	283.0	14166	1.5
OK	411.0	4	94.3	ND	24048	8.7
EC	321.3	8	105.4	445.0	30704	11.2
OS	421.5	2	106.7	171.0	24477	8.7
AD	269.2	2	84.5	201.0	7292	4.5
AK	176.4	2	124.2	225.8	8290	5.9
AL	178.0	4	103.9	338.8	9776	8.6
Mean	322.4	3.3	98.3	256.5	17844	6.5
±S.D	103.6	2.1	17.7	108.1	9000	3.3

Table 3.3.9

The Pharmacokinetic Parameters of Desethyl-chloroquine

Subject Code	Cmax (ng/ml)	Tmax (h)	$t^1/_2$ (h)	Cl_R (ml/min)	AUC ng/ml.h	AUC DCQ / AUC CQ
(a) Itchers						
OO	37.2	8	209.7	191.7	5437	0.15
AO	101.4	8	157.6	231.2	4495	0.20
EH	89.8	8	39.9	342.9	7212	0.23
BH	121.6	4	92.3	272.5	8817	0.25
IA	50.0	4	518.2	347.2	2942	0.23
TT	37.5	2	106.7	514.5	3341	0.30
BS	118.0	4	92.3	65.3	3639	0.40
Mean	79.4	5.4	173.8	282.1	5125	0.25
±S.D	37.1	2.5	161.2	140.6	2185	0.08
(b) Non-Itchers						
MA	142.0	2	72.6	158.3	182.43	0.76
OD	106.8	2	71.1	331.9	3176	0.37
OK	168.4	8	110.5	ND	9524	0.40
EC	212.4	12	95.6	382.6	16847	0.55
OS	113.4	2	53.2	72.9	8518	0.35
AD	90.5	2	73.6	162.4	3096	0.42
AK	43.5	4	44.8	260.7	2510	0.30
AL	59.7	8	117.8	245.6	2686	0.27
Mean	117.1	5.0	79.9	256.4	8325	0.43
±S.D	55.9	3.9	26.0	123.9	6272	0.15

Table 3.3.10

Mean Urinary Recovery and Estimated Total Excretion of Chloroquine (CQ) and Desethyl-chloroquine (DCQ)

(a) Itchers

Day	Amount Recovered (mg)			Estimated Total Recovery (mg)	Recovery as % of dose given
	1	7	14		
CQ	82.1±31.5	9.8±6.1	5.5±4.3	104.2±157.0	67.4
DCQ	19.7±11.8	2.4±2.0	0.9±1.0	92.1±53.2	15.3
Total	101.8±43.3	12.2±8.1	6.4±5.3	496.3±211.2	82.7
Total recovered as % of dose	17.0	2.0	1.1	82.7	

(b) Non-Itchers

Day	Amount Recovered (mg)			Estimated Total Recovery (mg)	Recovery as % of dose given
	1	7	14		
CQ	55.0±14.3	7.7±4.2	3.0±1.8	261.7±76.2	43.6
DCQ	18.4±13.7	2.8±2.4	1.1±0.9	83.6±46.4	13.9
Total	73.4±28.0	10.5±6.6	4.1±2.7	345.3±122.6	57.5
Total recovered as % of dose	12.2	1.8	0.7	87.5	

Table 3.3.11

The Relationship Between Residual Chloroquine and CAP in Itchers and Non-Itchers

	Subject	Previous History of CAP	Current Reaction to Chloroquine	Residual Chloroquine (ng/ml)
(a)	EH	Yes	Yes	16.57
	BH	"	"	40.82
	AO	"	"	40.55
	OO	"	"	17.00
	BS	"	"	26.82
	TT	"	"	18.76
	IA	"	"	0.00
	AK	"	No	0.00
	OD	"	No	0.00
(b)	MA	No	No	14.56
	EC	"	"	30.25
	OC	"	"	11.47
	AD	"	"	0.00
	OK	"	"	8.35
	AL	"	"	0.00

(a) Subjects with previous history of CAP
(b) Subjects without previous history of CAP

THE DISPOSITION OF CHLOROQUINE IN ITCHERS AND NON-ITCHERS

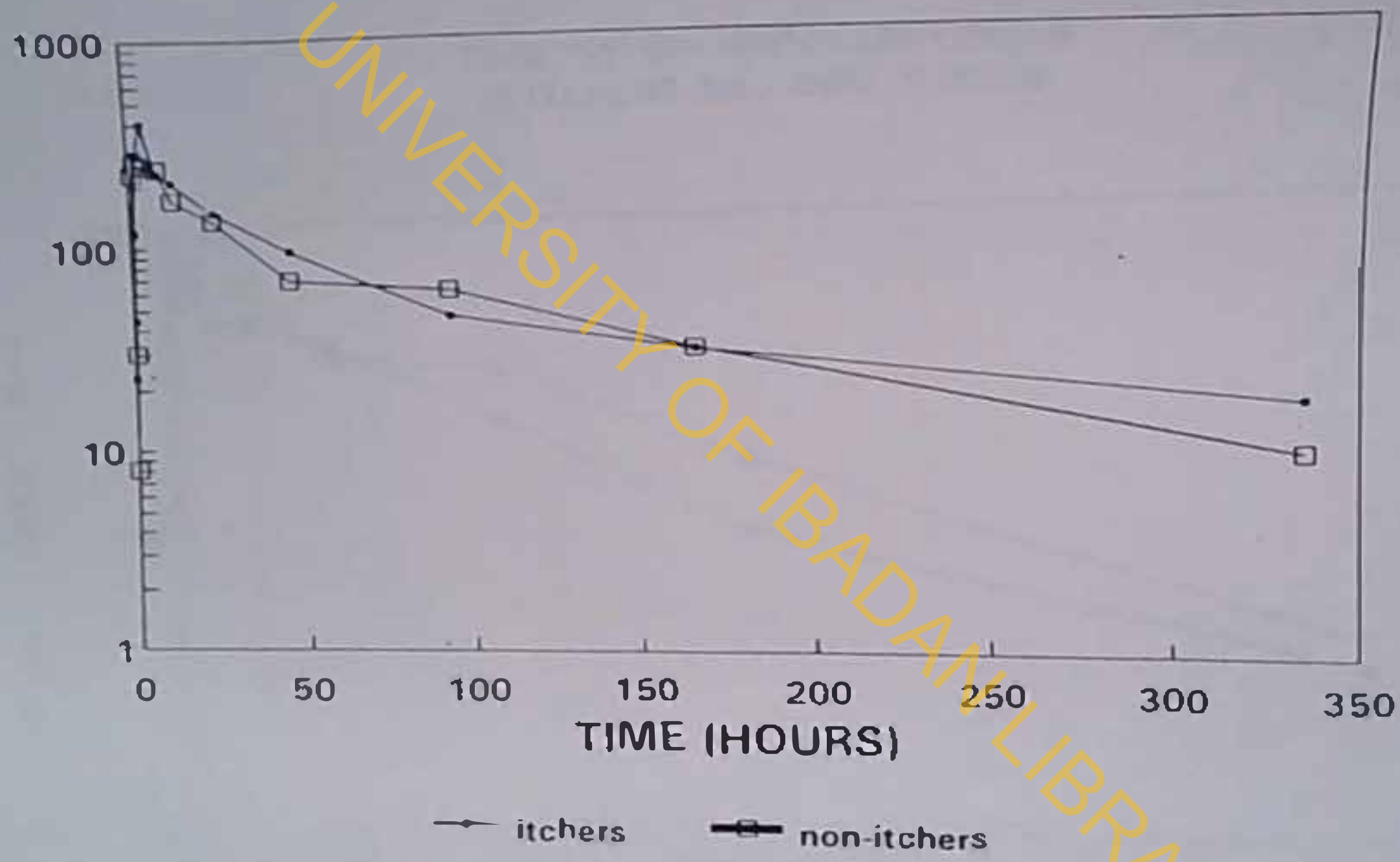
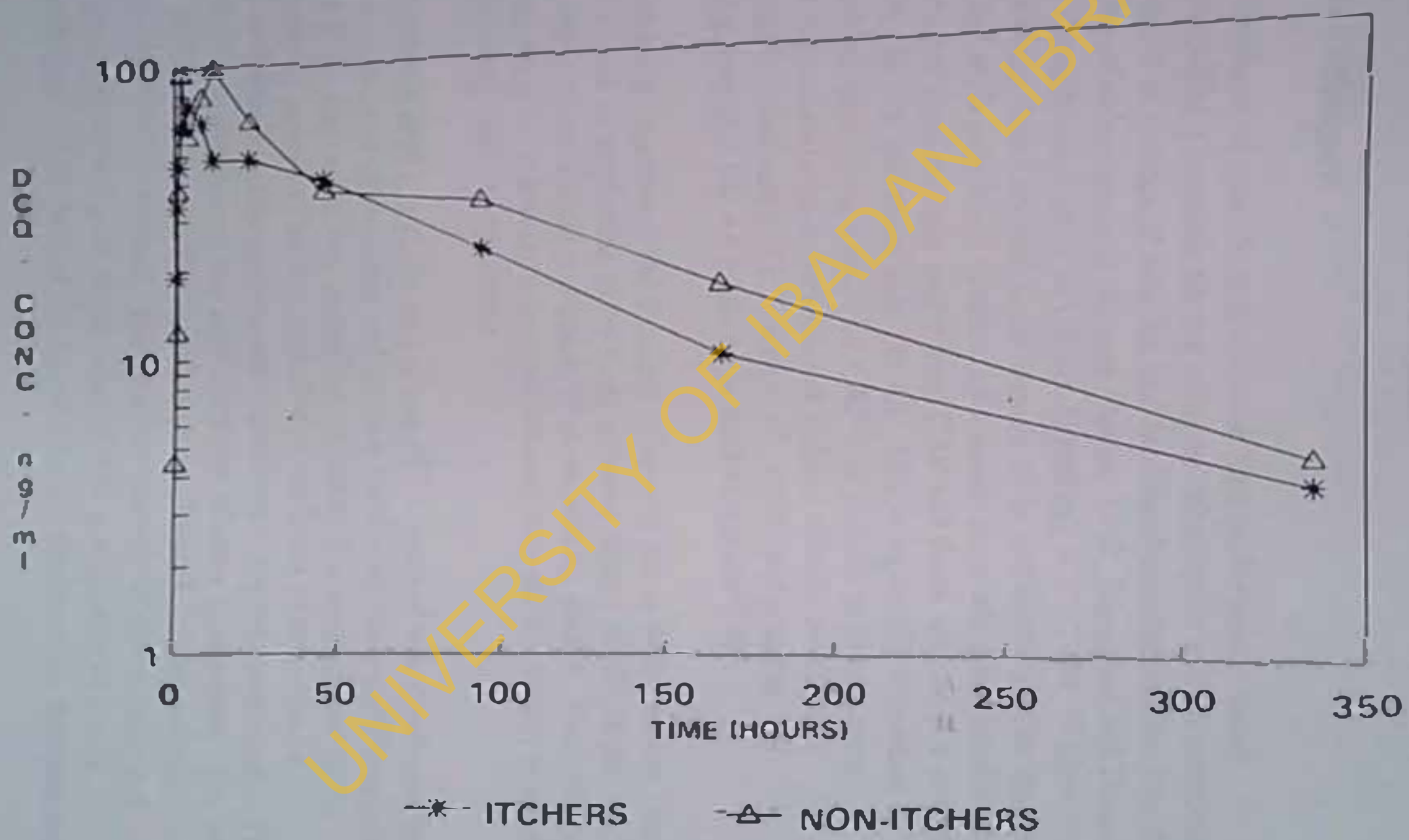


FIG 3.3.7

DESETHYLCHLOROQUINE LEVELS IN ITCHERS AND NON-ITCHERS



3.4 DISCUSSION

3.4.1 *Analytical Techniques*

The development of specific and sensitive high performance liquid chromatographic (HPLC) methods for the assay of chloroquine and its metabolite, desethylchloroquine in biological samples had facilitated detailed studies into the pharmacokinetics of chloroquine in the past (Alvan, 1982; Bergqvist and Domeij-Nyberg, 1983; Gustafsson *et al*., 1983; Frisk-Holmberg *et al*., 1984; Walker *et al*., 1987). Nevertheless, there are still some aspects of its pharmacokinetics that need be clarified. One such area is the problem of whether there are pharmacokinetic differences between patients who suffer from CAP and those who do not as well as in subjects with Hb AA and those with Hb AS. This is especially important as various attempts at identifying a basis for CAP with respect to chloroquine metabolism in itchers and non-itchers has not yielded reliably conclusive appreciable results (Olatunde 1971; Essien *et al*., 1989) and also that our observation suggests that Hb AS might be protective against CAP.

In developing countries if the frontiers of science are to be improved, then methods that would be applicable to the local situation should be developed e.g. in most developing countries there is a dearth of chemicals for assays. Even where they are found, they are relatively expensive because they are not locally sourced. This had hamstrung many investigators.

In light of the above, a cheap and simple HPLC method was developed for the pharmacokinetics of chloroquine and its main metabolite (desethylchloroquine). This method is a 2 step extraction procedure, therefore it is simpler than earlier ones. The method proved to be very sensitive as the limits of detection of chloroquine and desethylchloroquine were 1ng/ml and 0.5ng/ml respectively. Thus makes it possible to detect very low concentrations of these compounds. The low intra-assay and inter-assay coefficients of variation (Table 3.3.1) are indicative of high reproducibility of the method. The absolute recovery of chloroquine and desethylchloroquine which were in the order of 90% is indicative of a high extraction efficiency. The method can be used for the simultaneous determination of

chloroquine and its metabolites in biological samples. This method therefore is easily adaptable for therapeutic drug monitoring and research, since in these instances accuracy and sensitivity are two properties required of analytical techniques.

3.4.2 *Pharmacokinetics*

The results from the study showed that the pharmacokinetics of chloroquine and its main metabolite were similar in both itchers and non-itchers in this study. The production of the metabolite appears to be limited by the disposition of chloroquine as the $t^1/_2$ of both compounds were similar. However, the lower AUC generated by the metabolite in the itchers is an indication of possibly an inherent ability by the itchers to be less disposed to metabolizing chloroquine. This finding is contrary to the previous report (Essien *et al.*, 1989) that CAP is related to the production of higher quantities of metabolite in itchers. It however confirms the previous finding of Olatunde (1974) who demonstrated an equivalent concentration of chloroquine in itchers and non-itchers.

A comparison of the pharmacokinetics of chloroquine in subjects who have Hb AS and Hb AA like in itchers and non-itchers showed a similar disposition of chloroquine. Even though the Cmax of chloroquine was lower in Hb AS individuals when compared with Hb AA. However, absorption problems in the Hb AS individuals could not be ruled out. This needs to be further investigated.

Similarity of drug levels in all the groups might be due to the reported large apparent volume of distribution of chloroquine which has been attributed to its extensive tissue distribution and binding (Gustafsson *et al.*, 1983; Walker *et al.*, 1987; WHO, 1990).

The pharmacokinetic parameters of chloroquine found in this study were comparable to those previously reported in literature. The mean peak plasma concentration (Cmax) of chloroquine from one group to the other ranged from 283.6 to 374.8 ng/ml and time to peak (Tmax) ranged from 2 to 8 h. These are in

chloroquine and its metabolites in biological samples. This method therefore is easily adaptable for therapeutic drug monitoring and research, since in these instances accuracy and sensitivity are two properties required of analytical techniques.

3.4.2 *Pharmacokinetics*

The results from the study showed that the pharmacokinetics of chloroquine and its main metabolite were similar in both itchers and non-itchers in this study. The production of the metabolite appears to be limited by the disposition of chloroquine as the $t^1/_2$ of both compounds were similar. However, the lower AUC generated by the metabolite in the itchers is an indication of possibly an inherent ability by the itchers to be less disposed to metabolizing chloroquine. This finding is contrary to the previous report (Essien *et al*., 1989) that CAP is related to the production of higher quantities of metabolite in itchers. It however confirms the previous finding of Olatunde (1971) who demonstrated an equivalent concentration of chloroquine in itchers and non-itchers.

A comparison of the pharmacokinetics of chloroquine in subjects who have Hb AS and Hb AA like in itchers and non-itchers showed a similar disposition of chloroquine. Even though the Cmax of chloroquine was lower in Hb AS individuals when compared with Hb AA. However, absorption problems in the Hb AS individuals could not be ruled out. This needs to be further investigated.

Similarity of drug levels in all the groups might be due to the reported large apparent volume of distribution of chloroquine which has been attributed to its extensive tissue distribution and binding (Gustafsson *et al*., 1983; Walker *et al*., 1987; WHO, 1990).

The pharmacokinetic parameters of chloroquine found in this study were comparable to those previously reported in literature. The mean peak plasma concentration (Cmax) of chloroquine from one group to the other ranged from 283.6 to 374.8 ng/ml and time to peak (Tmax) ranged from 2 to 8 h. These are in

agreement with the report of Walker *et al* (1987) and Salako *et al* (1987). Desethylchloroquine which was detectable half an hour after drug administration had mean Cmax range of 81.3 to 108.8 ng/ml and Tmax range of 2 to 12 h. The values also agreed with those of Walker *et al* (1987) and Salako *et al* (1987). The mean terminal half life ($t^1/_2$) in the groups ranged from about 4 to 5 days. The $t^1/_2$ was lower than those reported by Gustafsson *et al* (1983), Salako *et al* (1987) and Walker *et al* (1987) because the sampling period was smaller in this study. It however agreed with the reports of McChesney *et al* (1967); Brohult *et al* (1979) and Adelusi *et al* (1982) who sampled for less than 10 days.

Despite the fact that none of the subjects took any dose of chloroquine at least in the last 2 months preceding the study, 9 of the 15 subjects had residual chloroquine confirming that chloroquine can still be found in the blood many months after its administration (Essien and Ifudu 1984; Tanenbaum and Tuffanelli, 1980). The result showed that residual chloroquine was more in the zero hour plasma of itchers as compared with non-itchers. This is suggestive that residual chloroquine may contribute to the pathogenesis of the itching process in those prone to CAP after taken further dose of the drug. However, this needs be clarified in further studies. This may explain the fact that it is unusual for CAP to be experienced on the first exposure to the drug, that the people in which it occurs are frequent users of chloroquine for the treatment of malaria (Olatunde 1981; Salako 1984; Spencer *et al*., 1987) and that the reaction usually do not occur in reactors after long time of not taking the drug but reappears in such individuals after a fairly frequent administration of the drug (Ekpechi and Okoro 1964; Salako 1984).

The urinary excretory pattern of chloroquine and desethylchloroquine were similar in itchers and non-itchers as well as in Hb AA and Hb AS subjects. This is despite the fact that Hb AS individuals produced relatively more urine volumes than Hb AA ones. Some Hb AS individuals have been demonstrated to have impaired ability to concentrate urine (Taylor *et al* 1978; Elebute, 1974; Statius van Eps *et al*., 1970). However, this does not constitute overt renal disease.

The mean renal clearance (Cl_R) of chloroquine in the 4 groups in the study ranged from 2.13 to 3.16 ml/min. This is substantially more than the normal range of

glomerular filtration rate and it is in agreement with the finding of Gustafsson *et al*., (1983); Walker *et al*., (1987) and Salako *et al*., (1987) who also reported that the renal excretion of chloroquine probably takes place by both glomerular filtration and tubular secretion.

A substantial amount of the drug and its metabolite were excreted within the first 24 h. About 14% and 15% of the oral dose of chloroquine were excreted in the ratio of 3.0 and 4.0 in Hb AA and Hb AS respectively. Also about 17% and 12% of the drug dose were excreted within the first 24 h in the ratio 4.0 to 6.0 in itchers and non-itchers respectively.

The total recovery of the drug and its metabolite after 14 days as a percentage of the administered dose in all the groups were similar to the findings of Walker *et al* (1987).

From the foregoing, it is suggested that pharmacogenetic studies be done to confirm the above findings as it has been demonstrated in the past that there is polymorphism in drug metabolism (Sjoqvist and von Bahr 1973)

5.5

GENERAL CONCLUSION

Although there was no difference in the pharmacokinetics of chloroquine in itchers and non-itchers or in Hb AA and Hb AS, however, non-itchers produced a significantly higher level of desethyl-chloroquine relative to itchers. Results from family studies which indicated influence of heredo-familial factors in the prevalence of CAP, the association of CAP with some genetic red-cell markers (Hb and G6PD types) as well as the report of racial predisposition of CAP are indicative of influence of genetic factors; hence a pharmacogenetic basis to CAP may be considerable. Although comparison of the concordance of this phenomenon in identical or non-identical twins could not be carried out, this may not be critical as concordance may merely be due to environmental factors.

Despite genetic predisposition to CAP, cumulative exposure to chloroquine was found to influence the incidence of CAP. This may be indicative of the importance of environmental factors in the aetiology of CAP.

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APPENDIX

QUESTIONNAIRE

CHLOROQUINE-ASSOCIATED PRURITUS (CAP) STUDY

1. Serial No. /____/ 2. Hospital No. /____/

3. Date /__/__/__/

4. Name .....

5. Sex 1. Male /__/ 6. Age Month/Year /____/

2. Female /__/ 6(a) Date of Birth /__/__/__/

7. Weight (kg) /__/

8. Occupation (Specify) /__/

1. Unemployed
2. Unskilled worker e.g. labourer, cleaner, petty-trading, vendor
3. Semi-skilled e.g. Typist, Clerk
4. Skilled worker e.g. teaching, technician, nurse, banker
5. Student
6. Others

9. If subject is a student/dependant

Occupation of Parent/Guardian check (8) /__/

10. Have you ever taken chloroquine 1. Yes /__/ 2 /__/

3. Dont know /__/

11. Last dose /__/__/__/

12. Have you any history of itching to chloroquine Yes/No /__/

13 - 22 Applicable to only those with history of itching.

13. CAP experienced /__/
1. Never
2. As far back as can remember
3. At a particular point

14. CAP onset on /__/ 14(a) At age /__/ years.

15. CAP disappeared /__/ 1. Yes 2. No.

16. CAP associated drugs /__/
1. Nivaquine
2. Resochin
3. Norolon
4. Dagaquine
5. Others
6. All

17. Itching to Camoquine (Amodiaquine) 1. Yes /__/ 2. No /__/
3. Never taken /__/

18. Pruritus usually starts /__/ hours after 1st dose of chloroquine course

19. Pruritus lasts /__/ hours

20. Which drug(s) suppress(es) the itching effect in you /__/ e g
(1) Piriton (2) Aspirin (3) Sedatives like valium (specify)
(4) Phenergan (5) None (6) Others (specify)

21. Pruritus peak intensify / / 1. Mild
2. Slightly incapacitating
3. Incapacitating

22. You itch only when you take the drug / / 1. Orally
2. By injection
3. Both

23. How often do you have fever in a year / / (Specify)

(1) Once (2) Twice (3) Occasionally (4) Often

24. How many tablets of chloroquine have you taken in the past one year.
Specify / /

25. How many times did you take chloroquine injection in the past one year.
Specify / /

26. Does any of your parents itch to chloroquine

(1) Father (2) Mother (3) Both (4) Not known / /

27. Does any of your relations itch to chloroquine / /

(1) Brother (2) Sister (3) Cousin (4) All (5) Not known

28. Blood group (ABO and Rh) / / / /

29. Genotype / /

30. G6PD screening (1) Normal (2) Delicient / /

31. G6PD electrophoresis / /

32. PCV / / 32(a) Retics / /%

33. WBC /____/ 33 (a) Eosinophil (count) /____/

Direct Observation of Chloroquine Administration

34. Malaria parasite (1) Positive (2) Negative /____/

35. Percentage parasitaemia /___/

36. (a) Parasite density (thick) /___/ x 10^{-3}/ml

37. Serum stored during CAP attack (1) Yes (2) No /____/

38. Urine stored during CAP attack (1) Yes (2) No /____/

39. Drug taken/Dose /___/ Time /___/ Route /____/

40. Pruritus /____/ (1) Yes (2) No

41. (a) Onset /___/ h after drug

QUESTIONNAIRE

CHLOROQUINE-ASSOCIATED PRURITUS IN PREGNANT WOMEN

1. Serial No. /___/
2. Hospital No. /___/
3. Date /_/_/_/
4. Name .....
5. Age: Month/Year /___/
6. Weight /___/
7. Occupation Specify /___/

i. Unemployed
ii Unskilled worker e.g Labourer, cleaner, petty trading etc.
iii Semi-skilled e.g Typist, Clerk etc
iv. Skilled worker e.g Teacher, technician, nurse, banker etc
v. Student
vi. Others

8. Have you ever taken Chloroquine 1. Yes /_/ 2. No /_/
3. Don't know /_/

9. Last dose when /___/
10. Parity at time of study /___/
11. Have you any history of itching to chloroquine?

(a) When pregnant Yes/No /___/

(b) When not pregnant Yes/No /____/

12. If yes, compare the itching during pregnancy and at preconception

13. Whether children itch to chloroquine /_____/
1. Yes
2. No
3. Don't know

14. Whether husband itch to chloroquine /_____/
1. Yes
2. No
3. Don't know

15. At what age does itching starts in the family /_____/

CHLOROQUINE CONCENTRATION (ng/ml) AMONG ITCHERS

Time(h)	OO	AO	EH	BH	IA
0	17.00	40.55	16.57	40.82	0.00
0.5	-	-	-	124.42	4.96
1	91.46	364.90	79.05	148.11	46.64
2	-	431.40	207.38	464.63	250.87
4	480.28	-	-	589.26	360.44
8	271.95	279.94	259.75	208.93	-
12	229.35	338.16	185.88	180.00	163.41
24	203.11	205.26	168.92	157.63	84.27
48	137.75	-	97.58	72.70	-
96	91.86	51.11	71.57	66.42	17.09
168	79.17	23.57	39.29	42.18	21.58
336	31.27	14.84	30.91	14.27	7.36

CHLOROQUINE CONCENTRATION (ng/ml) AMONG NON-ITCHERS

Time(h)	MA	QD	OK	EC	OS	AD
0	14.59	0.00	8.35	30.25	11.47	0.00
0.5	17.84	-	18.04	18.21	29.80	20.39
1	402.18	103.92	-	-	370.38	-
2	403.47	190.02	153.14	16.81	421.51	269.15
4	-	282.73	411.00	-	251.40	-
8	340.17	285.08	284.08	321.28	367.59	133.00
12	295.00	170.84	369.46	121.63	283.90	139.66
24	264.98	72.11	341.86	157.81	222.53	53.02
48	105.29	77.45	63.20	-	93.22	26.13
96	76.14	66.14	100.00	107.27	64.44	20.14
168	42.79	25.82	29.14	75.46	45.02	9.69
336	7.12	2.92	15.40	22.78	13.90	2.72

DESETHYLCHLOROQUINE CONCENTRATION (ng/ml) AMONG FCHERS

Time(h)	[illegible]	AO	EH	IH	IA
0		22 [illegible]	X 24	15 XX	0 [illegible]
0.5		-	-	43 21	0 [illegible]
1		[illegible]	11 XX	[illegible]	-
2		[illegible]	65 35	[illegible]	2X X4
4	20 20	-	-	121 [illegible]	50 [illegible]
8	17 23	[illegible]	X9 75	93 91	-
12	22 22	[illegible]	[illegible]	-	10 X0
24	[illegible]	[illegible]	51 [illegible]	[illegible]	13 57
48	11 [illegible]	-	45 [illegible]	5X 22	-
[illegible]	13 5X	[illegible]	47 13	57 13	2 41
[illegible]	[illegible]	[illegible]	[illegible]	31 42	2 67
[illegible]	5 33	2 92	10 67	[illegible]	1 X5

DESETHYL-CHLOROQUINE CONCENTRATIONS (ng/ml) AMONG NON-ITCHERS

Time (hr)	MA	OD	OK	EC	OS	AD	AK	AL
0	-	0.00	32.29	2.00	1.28	0.00	0.00	0.00
0.5	1.03	-	49.06	4.12	4.19	-	-	3.37
1	117.45	9.92	-	-	44.03	-	5.26	12.47
2	141.96	106.84	88.54	21.91	113.37	90.54	11.53	7.30
4	-	60.64	97.16	-	61.98	-	43.48	27.10
8	108.70	70.89	108.43	76.60	89.70	51.18	33.58	29.84
12	124.26	31.76	119.14	212.75	97.85	62.44	32.04	-
24	137.51	29.25	-	146.25	81.59	89.88	23.78	15.24
48	72.78	66.74	26.11	-	50.47	13.70	18.50	10.09
96	79.05	15.55	40.11	45.94	31.15	11.52	13.00	-
168	48.44	5.12	11.10	38.36	18.81	4.00	2.97	6.11
336	8.38	1.36	7.32	8.74	1.51	1.00	-	1.90

DATA OF URINARY DRUG RECOVERY

Subject	Urine Volume (ml)			Amount of Drug Recovered (mg)					
				D1		D7		D14	
	D1	D7	D14	CQ	DCQ	CQ	DCQ	CQ	DCQ
OO	1,550	1,140	950	52 58	6 3	6 50	0 30	2 10	0 15
AO	780	1,520	1,840	77 3	15 74	22 7	25 7	14 5	2 3
EH	880	650	720	63 52	31 9	5 69	2 10	4 27	0 1
BH	1,950	780	1,380	118 00	36 70	9 72	4 30	6 96	1 4
IA	1,230	1,030	900	43 17	9 9	4 8	0 69	2 67	0 10
TT	1,250	1,320	1,400	122 9	26 9	10 47	2 70	3 94	1 83
BS	2,300	2,110	1,940	97 2	11 10	8 34	1 14	3 86	0 10
MA	1,250	870	1,310	55 73	24 73	4 07	7 84	5 60	1 43
OD	1,690	530	1,950	49 23	15 18	4 69	0 22	1 16	0 11
OK	NC	1,120	2,200	ND	ND	ND	ND	ND	ND
EC	1,200	620	700	77 93	46 52	9 09	4 10	4 30	2 40
OS	1,430	1,400	640	69 90	10 27	8 48	1 20	2 34	0 20
AD	1,610	1,130	860	40 63	14 60	3 13	1 80	1 15	0 5
AK	2,000	1,150	820	51 7	11 33	11 6	2 79	4 45	2 03
AL	2 150	1,720	1,830	40 0	6 01	3 06	2 07	2 00	0 70

D = Day
NC = Not Collected
ND = Not done

DATA OF ESTIMATED TOTAL DRUG RECOVERY

Subject	Estimated Total Recovery (mg)		Total Drug Recovered (mg)	Percentage of 600 mg Recovered
	CQ	DCQ		
OO	237.43	25.17	262.15	43.69
AO	594.00	119.29	713.29	118.80
EH	298.21	123.40	421.61	70.26
BH	536.44	161.16	697.60	116.27
IA	206.70	39.90	246.60	41.11
TT	529.15	128.45	657.60	109.60
BS	427.70	47.45	475.15	79.19
MA	276.10	149.50	425.60	70.93
OD	213.26	55.35	268.62	44.77
OK	ND	ND	ND	ND
EC	370.99	149.80	520.71	86.66
OS	321.00	44.44	365.44	60.90
AD	72.40	67.30	239.70	39.95
AK	301.53	71.90	372.62	62.10
AL	76.30	46.47	222.77	37.12