COMPARATIVE *IN VIVO IN VITRO* SENSITIVITY OF *PLASMODIUM FALCIPARUM* TO SULPHADOXINE/ PYRIMETHAMINE IN ZARIA URBAN AREA

BY

SULLAYMAN IPEMIDA ADAOU

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DEPARTMENT OF PHARMACOLOGY AND CLINICAL PHARMACY
FACULTY OF PHARMACEUTICAL SCIENCES, AHMADU BELLO UNVERSITY, ZARIA

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DECLARATION

This is to certify that I carried out the work reported in this Thesis at the Ahmadu Bello University Teaching Hospital and the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University under the joint supervision of Dr. Lotte Lege-Oguntoye and Dr. B. Werblinska and Dr. W. N. Ogala. I Solemnly declare that no part of this Thesis has been submitted else where for a degree.



Sulayman. I. Adagu
Dept of Pharmacology & Clin. Pharmacy
Ahmadu Bello University, Zaria.

CERTIFICATION

This Thesis entitled "Comparative *in vivo/in vitro* sensitivity of *Plasmodium falciparum* to sulphadoxine/pyrimethamine in Zaria urban area", by Sullayman Ipemida Adagu meets the regulations governing the award of the degree of MASTER OF SCIENCE (Pharmacology) of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

Internal Examiners

1 ---------- Date 23/9/89

Dr. L. Lege-Oguntoye,

M.B., B.S., M.D., D.T.M. & H.

Reader, Dept Of Pharmacology & Clinical Pharmacy

Ahmadu Bello University, Zaria.

2 ---------- Date 23 Sept 1989.

Dr. W. N. Ogala,

M.B.B.S, F.M.C.Paed. F.W.A.C.P., D.C.H.,

Senior Lecturer/Consultant,

Department Of Paediatrics,

Ahmadu Bello University, Zaria.

3 ---------- Date--------

Dr. (Mrs) B. Werblinska,

M.D., Specialist Paed., F.M.C.Paed. F.W.A.C.P.

Reader, Department of Paediatrics

Ahmadu Bello University, Zaria.

External Examiner

---------- Date------ 23rd Sept 1989

Professor A. Olatunde,

M.B.,B.S., M.D., Dip. Clin. Pharm. F.W.A.C.P.,

Department of Clinical Pharmacology,

Faculty of Health Sciences,

University of Ilorin, Ilorin, Nigeria.

Head of Department

---------- Date------ 23rd Sept 89

Dr. I. Abdu-Aguye

M.B.,B.S, M.Sc., F.M.C.P.

Dept of Pharmacology & Clinical Pharmacy,

Ahmadu Bello University, Zaria

Dean, Postgraduate School

---------- Date------

Professor D. Saror, B.Sc., D.V.M., Ph.D

Postgraduate School,

Ahmadu Bello University, Zaria.

DEDICATION

To my

PRECIOUS WIFE, FATIMAT

and

OUR SON, LUQMAN AJIMASI

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ABSTRACT

44 children with *P. falciparum* malaria were treated with sulphadoxine/pyrimethamine combination corresponding to 35mg sulphadoxine plus 1.75mg pyrimethamine per kg body weight in a single dose using the WHO Extended Field Test. In 36 (81.8%) of the 44 patients, parasitaemia disappeared within 7 days after initiating therapy and did not reappear within the subsequent 21 day test period. The Mean Parasite clearance time for the 36 patients was 3.65±1.24 days. The remaining 8 (18.2%) were resistant at the RI level. Low level parasitaemia persisted in these 8 cases *in vivo* and all patients became asymptomatic within 7 days indicating that clinical and parasitological responses were well correlated. Because none of the children had evidence of clinical malaria, no second course of treatment was given. 42 isolates of the patients were cultured using the WHO standard mark II micro-test system. 8 (19%) of these successfully grew and they were all resistant. A comparison of the *in vivo* and *in vitro* studies showed that 6 (75%) of the *in vivo* resistant cases were resistant *in vitro*. The

remaining 2 (25%) *in vitro* resistant cases had delayed clearance *in vivo*. It is therefore concluded that in Zaria urban area there is high level resistance to sulphadoxine/pyrimethamine *in vitro* which corresponds to a low level (RI) resistance *in vivo* due to a high degree of immunity in children under 5 years of age.

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CHAPTER ONE

1.1 INTRODUCTION:

Malaria remains one of the most widespread infectious diseases in Africa. Over 90% of the 450 million persons in Africa south of the Sahara live in malarious areas. The epidemiology of malaria transmission and disease varies greatly and depends on topography, rainfall and human behaviour (Breman and Campbell, 1988). The prevalence of *Plasmodium falciparum* infection in endemic areas is frequently over 50%, with the highest rates occuring in children (Molineaux and Grammiccia, 1980). In most communities, the incidence of acute illness is difficult to measure because of the imprecision in clinical diagnosis and lack of microscopic confirmation. WHO considers that 80% of the 100 million cases of clinical malaria occurring in the world each year occur in Africa south of the Sahara (World Malaria Situation, 1985). The global situation can be described by using the following classification (World Health Statistics, 1988).

a. areas where malaria never existed or has disappeared spontaneously (1,327 million

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inhabitants).

b. areas where malaria has been eradicated by specific campaigns (768 million inhabitants)

c. areas where transmission is contained by constant control measures (2,318 million inhabitants)

d. areas where there is no organised large-scale control of transmission (405 million inhabitants).

Around the turn of the century there were about 2.5 million cases of malaria each year with 2.5 million deaths annually. The introduction of residual insecticides in about 1940 significantly improved prevention. About 1950 it was proven that DDT spraying inside houses interrupts transmission and this led to the assumption that eradication of malaria could be achieved within three years by exhausting the reservoir (World Health Statistics, 1988). Around the same time however, the discovery of the first cases of anopheline resistance to the residual insecticides introduced an element of urgency ((World Health Statistics, 1988). The principle of eradication had been adopted for the World as a whole by the World Health Assembly in 1955. The result of the first ten years (1957-1966) of the global

eradication programme were spectacular but uneven. There were reverses due to financial, administrative, operational problems, resistance, behaviour of the vector and inadequate development of basic health services. In Africa, the pilot projects generally failed to interrupt transmission. A new and more flexible strategy was adopted in 1969, and countries were invited to revise their programme to take local circumstances into account (World Health Statistics, 1988). Thus in the past 40 years, malaria control has been marked by a spectacular drop in morbidity and mortality, mared by a partial recrudescence a few years ago (World Health Statistics, 1988)

Recently, many countries have faced significant difficulties in ensuring that their health services are directed at priority problems and that they function effectively. there is no doubt that these problems are serious and have been compounded by current economic constraints. This situation further reinforces the need for a fundamental review and restructuring of the health system, as part of continued development in order to use the existing resources both in the general services and in specialized programmes within the health system in more productive ways to further

the goal of better health (WHO, 1984 (b). Many countries have made adjustments in their antimalarial programmes during the course of their transition from malaria eradication to malaria control. Blanket spraying has been replaced by selective and focal spraying in many countries, especially in South-East Asia and Latin America (WHO, 1984 (b)).

According to Breman and Campbell (1988) a strategy to ensure prompt recognition of probable malaria infections followed by effective treatment is the first step towards the control of malaria. Drug therapy may decrease the risk of severe infection, but will not, by itself, alter transmission in the community. The health care system, linked to effective community mobilization, is essential for these immediate drug distribution initiatives to succeed. This strategy is envisaged to provide stimulus for the development of a more comprehensive health care delivery system.

1.1.2 HISTORICAL BACKGROUND.

The word malaria is derived from the Italian "*mala*" and "*aria*" meaning bad air. The nature of malaria as prehistoric disease of animals and man

can only be guessed at. The aboreal hominids followed by the early man lived in close contact with various simian species in Africa and Asia. Anopheles which fed on them were able to transmit malaria parasites easily from one animal group to another (Bruce-Chwatt and Zulueta, 1980). The close relationship between simian and human malaria (Bray, 1963, Coatney, *et al*, 1971) suggests a common origin which Garnham (1963) placed in the early Tertiary age, some 60 million years ago when as he postulated, quartan-like parasites emerged in lemures and monkeys. He considered these to be benign tertian parasites originating from the Oligocene (30 million years ago) among lower monkeys and malignant tertian parasites during the Pleistocene, which began 2.5 million years ago and during which primate evolution emerged in higher apes and man.

The centre of *Plasmodial* evolution has been placed in tropical Africa (Bruce-Chwatt, 1965; Mattingly, 1973, 1976) as well as South-East Asia (Coatney *et al*, 1971) but there can be little doubt about the long association of man and malaria in the tropical region of the old World. It is not known, however, when the natural selection of the four human plasmodial species took place, although it is likely that it happened in the mid-Pleistocene (Garnham, 1966, Mattingly, 1976).

1.2 MALARIA WITH PARASITES

1.2.1 Parasitology

The *microorganisms causing malaria* are commonly referred to as malaria parasites; this term is usually restricted to the family *Plasmodiidae* within the order *coccidiidae*, sub-order *Heamosporidiidae*, which comprises various parasites found in the blood of reptiles, birds and mammals. (Bruce-Chwatt, 1980). According to Bruce-Chwatt, the zoological family *Plasmodiidae* includes the parasites which undergo two types of multiplication by asexual division (Schizogony) in the vertebrate host and a single sexual multiplication (Sporogony) in the mosquito host. The genus plasmodium has been defined on the basis of one of the asexual multiplication by division occuring in the parenchymal cells of the liver of the vertebrate host (exo-erythrocytic schizogony): the other characteristic of this genus is that the mosquito host belongs to the anopheles species.

Of the malaria parasites, four are infectious to man. These are *Plasmodium malariae, Plasmodium vivax, Plasmodium falciparum and Plasmodium ovale*; causing respectively, benign quartan malaria,

benign tertian malaria, malignant subtertian malaria and a mild form of benign tertian malaria. (Bowman and Rand, 1980).

1.2.2. Life cycle

Basically the life cycle of malaria parasites involves two types of hosts, one vertebrate and one invertebrate and it is within the latter that sexual reproduction occurs. (Peters, 1970).

Schizogony: Parasites of the human species undergo a sexual development (sporogony) in the mosquito and ultimately produce infective sporozoites which are injected into the vertebrate host when the female anopheline mosquito takes a blood meal. The sporozoites rapidly disappeares from the systemic circulation and enter the parenchymal cells of the liver, probably assisted by secretion of a proteolytic enzyme, where they commence cycles of asexual division called "schizogony" (Pinder, 1973). This process which constitutes the pre-erythrocytic or primary tissue phase, takes from 8 to 12 days and is essential to allow the parasites to undergo the necessary metabolic adaptation for the change from life in a poikilothermic insect to that in a warm-blooded vertebrate. The tissue

stage is regarded as the most fundamental phase in the life cycle from phylogenetic point of view, since it is common to all malaria parasites. (Garnham, 1966).

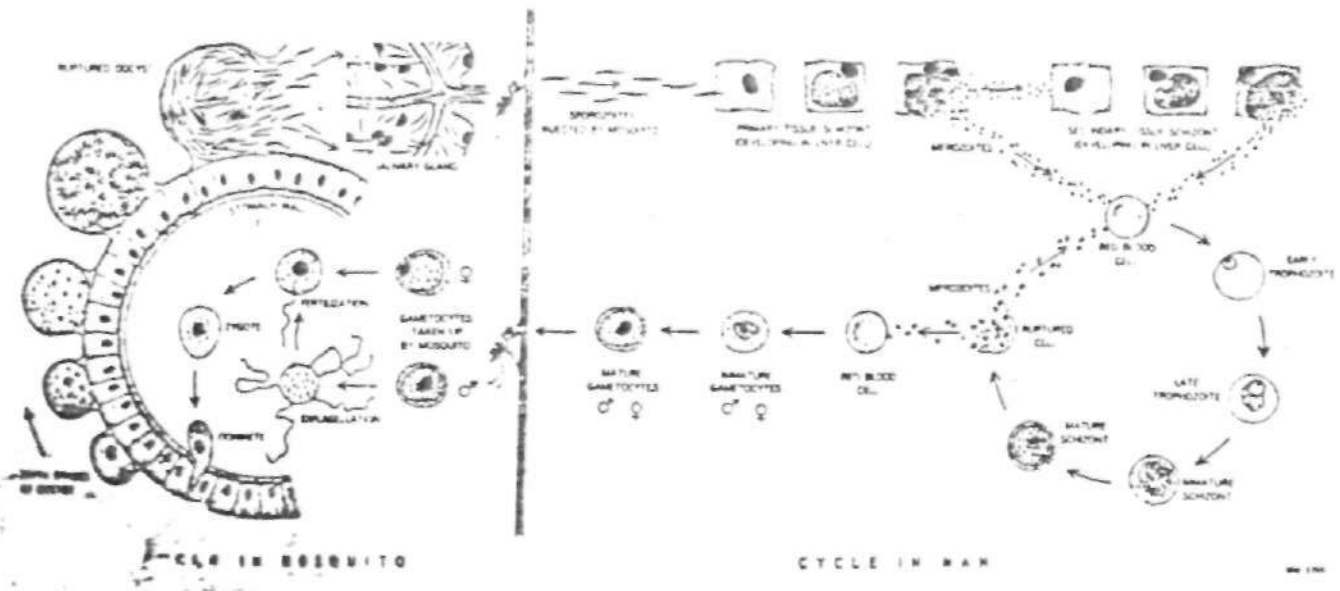
Primary exo-erythrocytic schizogony ends with the bursting of the parasitized liver cells and the release of merozoites, which invade red blood cells and initiate cycles of erythrocytic schizogony. Other merozoites reinvade fresh liver cells and undergo further cycles of asexual division. These "exo-erythrocytic" or "secondary" tissue forms (trophozoites) are believed to be responsible for the relapses that occur even after the blood cycle has been suppressed by drugs or immune processes of the body. *P. falciparum* does not have a secondary tissue phase in its life cycle. Tissue forms cannot be derived directly from the erythrocytic forms and relapses do not therefore occur in falciparum malaria, but renewed activity of the primary asexual phase can give recrudescences (Pinder, 1973).

The merozoites in the blood stream develop to trophozoites which in turn give rise to schizonts. A mature schizont is a solid body with usually between 8 and 24 nuclei. Cytoplasmic division gives rise to merozoites. The much damaged

erythrocyte now ruptures, liberating merozoites and unwanted material, including the haemozoin, into the blood stream. This release is responsible for the clinical symptoms of malaria. The released merozoites that evade destruction by the immune mechanism quickly invade fresh erythrocytes to perpetuate cycles of asexual multiplication, but several additional days elapse after first release of merozoites before levels of parasitaemia are such as to be detectable in blood smears and before symptoms are apparent.

Sporogony: At certain times, determined by the parasites and not the host, merozoites fail to reproduce asexually and differentiate into male and female forms called "gametocytes" (Pinder, 1973). Gametocytes take about 4 days to reach maturity, after which they circulate indefinitely until taken up by an invertebrate host, though they do not mature completely in an absolute sense until they are in the mosquito. Blood containing male and female gametocytes enters the alimentary tract of the mosquito after the act of biting an infected vertebrate. Any accompanying asexual forms are destroyed by digestion, and the gametocytes rapidly shed their erythrocytic envelopes and undergo maturation into male and female gametes.

When a female (macrogamete) is penetrated by a male (microgamete) a motile zygote is formed, the ookinete. This penetrates through the epithelial lining of the midgut wall and the lining of the body cavity of the mosquito. There the parasites form an oocyst within which develop several thousand sporozoites which are distributed passively wherever the body cavity extends. They are attracted towards the salivary glands probably by chemotaxis, penetrate the salivary gland and ultimately come to lie within the salivary gland duct (Peters, 1970). The female mosquito injects salivary fluid containing sporozoites into the subcutaneous tissues of another vertebrate during the process of feeding; the cycle of events starts all over again.



Life cycle of malaria parasite in the mosquito and in the human hosts. (WHO. 1963)

1.2.3. Parasite Morphology

P. falciparum Of all the species of human plasmodia, *P. falciparum* is the most highly pathogenic (Bruce-Chwatt, 1980). In a non-immune subject, it usually runs an acute course, and unless promptly treated with specific drugs, frequently terminates fatally. It is the chief infection in areas of endemic malaria in Africa. The infected red blood cells retain their normal size throughout all stages of development of the parasites. Cells habouring the older trophozoites and schizonts are frequently stippled with a few coarse reddish dots (Maurers'dots) scattered over about two-thirds of the erythrocyte (Bruce-Chwatt, 1980).

P. vivax is much less common in tropical Africa; it is absent in West Africa. It causes benign tertian malaria with frequent relapses. *P. vivax* has effect on the invaded red blood cells which gradually enlarge and become discoloured. A characteristic stippling in the form of small reddish points appear in the infected erythrocytes and is known as schüffner's dots.

P. malariae is the causal organism of quartan malaria. The course of the disease is not unduly severe but its long persistence is notorious. It

produces no evident changes in the host cells, although they may often appear some-what smaller than uninfected erythrocytes. A special staining may show the presence of discrete stippling, often called Ziemann's stippling, dark pigmentation, invaded red blood cells and schizonts which are rather small.

P. ovale infection produces tertian fever with prolonged latency, lesser trend to relapse and generally milder clinical symptoms. The young trophozoites measure about one-third the diameter of the red blood cells. Schüffner's stippling appears quite early and is more pronouced then in the case of *P. vivax* (Bruce-Chwatt, 1980).

1.2.4. Epidemiology of Malaria.

The epidemiology of malaria is concerned with the disease as it invades, inhabits and leaves people. (Russell, 1963). The authors considered that the essential epidemiological characteristics of any infective process, malaria included, is the number of infections distributed by any type of disease, a quantity dependent on the nature of the disease itself and also on the facilities locally available for its transmission from man to man.

The Parasites:

The natural life span of a malaria infection varies widely according to the involved *Plasmodium* species and the strain. *Plasmodium falciparum* averages 9-12 months, but a period of up to 3 years has been reported. (Verdrager, 1964). Infections with *P. vivax* and *P. ovale* usually last not more than 4 years but *P. malaria* infections can last for several decades (Wernsdorfer, 1980). In naturally-acquired falciparum malaria, an epidemiologically important feature is the relatively late appearance of gametocytes which in no case is earlier than 15 days after patency. Studies on *P. falciparum* indicate a periodicity and strictly limited duration of the infectivity of gametocytes which coincides with the period of highest biting activity of the vectors (Hawking, 1972).

The Host:

Ethnic genetic differences in the susceptibility to particular parasite species play an important role in the distribution of *P. vivax* which is virtually absent from West and Central Africa and shows very distinct difference of incidence in the Hamito-Semitic and Nilotic

populations of Ethiopia. (Armstrong, 1978). Innate and physiological factors may have a marked epidemiological impact. Carriers of the sickle cell gene (haemoglobin AS) are less susceptible to falciparum than non-sicklers. (Livingstone, 1971; 1976). The specific advantage of the heterozygous state has apparently offset the losses caused by the lethal homozygous state and perpetuated the gene in the highly malarious areas of tropical Africa. Also, glucose-6-phosphate dehydrogenase deficiency was believed to afford an attenuation of *falciparum* malaria and thus a relative protection of the afflicted person (Wernsdorfer, 1980).

The longevity of the vector is largely dependent on environmental conditions, especially relative humidity, and on species-specific inherent factors. The duration of sporogony, which correlates with the vector's longevity, in turn depends on the environmental temperature. Several variation in relative humidity may shorten the life expectancy of usually very potent vector species to the extent that malaria transmission ceases at temperatures which will favour rapid sporogony. This is seen for example with *A. gambiae* in the dry zones of the central Nile River

basin (Wernsdorfer, 1967) Mosquito density largely depends on the longevity of the adult mosquitoes and on the availability of suitable breeding places. The different anophelines species have very distinct ecological requirements for breeding - with wide variations regarding water flow, exposure to the sun, water temperature and aquatic vegetation. (Wernsdorfer, 1980). Malaria transmission requires that infected man and susceptible man be accessible to the vector which is normally confined to a flight range of up to 2 kilometers from its breeding place. The closer the breeding place is to human habitations, the higher the vector-man contact will be. Sporogony ceases below 16°C for *P. vivax* and below 20°C for *P. falciparum* (Wernsdorfer, 1980).

Endemicity: Malaria is endemic in a paticular area, when there is a constant measurable incidence of natural transmisson over a succession of years. Based on the spleen rate of children and adults, endemicity is classified into the following degrees: -

Holoendemic malaria, with childhood spleen rate constantly over 75% and a low adult spleen rate.

Hyperendemic malaria, with a childhood spleen rate

constantly over 50% and high adult spleen rate.
Mesoendemic malaria in which the spleen rate is between 11 and 50% in children.
Hypoendemic malaria in which the spleen rate is 10% or less in children.

1.2.5. Clinical features.

Malaria is a protozoal infection in which the causative agent, a member of the genus *Plasmodium*, undergoes a cyclical development both in the mammalian host and in the vector, the anopheles mosquito. (Pinder, 1973). The primary result of malaria is the rupture of infected erythrocytes and the escape of a "toxic" substance into the blood stream. The toxin gives rise to a sequence of events, which in man are a feeling of chilliness, shivering (rigor), headache and a rise in body temperature up to 39°C to 41°C. After 6 hours, the paroxysm ends with a profuse sweating, the headache disappears and the sufferer awaits for the next bout after the characteristic quotidian, tertian, or quartan interval that is inevitable in the absence of treatment. (Garnham, 1980). Other common charateristics of malaria are nausea, vomiting, diarrhoea and general muscle pain.

The risk of severe and potentially fatal *P. falciparum* infection is greater in persons with little or no acquired malaria immunity, such as young children and immigrants from malaria-free areas. Immunological changes during pregnancy appear to be associated with a higher frequency of infection during pregnancy and with low birth-weight new-borns in women with parasites in the placenta. (McGregor, 1984; Breman and Campbell, 1988).

1.2.6. Immunity

Malaria immunity may be defined as the capacity of the host to resist the infection brought about by all processes involved in destroying the *Plasmodia* or in limiting their multiplication. It also comprises the factors that modify the effects of the invasion of the organism by malaria parasites and aid in repair of damaged tissue (Bruce-Chwatt, 1986)

There are two types of immunity, natural and acquired immunity, respectively. Natural immunity is displayed by the host with previous experience of malarial infection, and it includes the effect of natural antibodies, the normal scavenging,

function of phagocytes or the unsuitability of the host for *Plasmodial* growth (Pinder, 1973). Acquired immunity is a state of partial or complete resistance existing in a previously susceptible host. The mechanism can only be set in motion by the presence of an erythrocytic infection. Such immunity may be non-specific, as when the host is infected with another organism that includes hyperactivity of reticulo-endothelial system, or specific, which derives from the recognition of and response to plasmodial antigen. The parasite is most susceptible to the effects of immunity when it is in contact with the body fluids, as with the sporozoite in the mosquito or the merozoite in the human circulation.

Natural immunity:

There at least two types of natural immunity, both based upon genetically inherited haemoglibinopathies. Sickle-cell anaemia provides a partial barrier to the extreme proliferation of the parasites in the red cells, and, while the likelihood of infection is as great among individuals with this trait as among normals, they do not show the very high parasitamia associated

with mortality in infections with *P. falciparum* (Pinder, 1973). Motulsky, (1964) gives a figure of one-twentieth of the expected mortality for an African group with the sickle-cell trait, the effect of which are particularly noticeable in young children who lack acquired immunity to malaria infection and who show much lower parasitaemia counts than their genetically normal peers. The degree of protection seems to be related to the amount of abnormal haemoglobin present, in that maximum parasitaemias in sickle-cell homozygotes are lower than in heterozygotes (Raper, 1959), but the exact mechanism is still unknown. It is unlikely that the mechanical effect of sickling is involved in view of the protection afforded by other abnormal haemoglobin types, and responsibility may lie with factors such as selective removal of sickled parasitized cells from the circulation or suboptimal utilization of sickle haemoglobin by parasites subsequently starved of essential amino-acids. (Pinder, 1973).

It appears that genetic deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD) also exerts a protective effect against severe infection with *P. falciparum*. The red cell enzyme defect appears to be harmless unless the red cells are challenged in some way, usually by exposure to oxidizing drugs (including the sulphonamides and primaquine). G6PD deficiency is inherited as a sex-linked trait with full expression in males. There are many variants of this enzymopathy. The evidence for the protective effect of G6PD deficiency against malaria is not as strong as for haemoglobin S.

Acquired immunity

The protection acquired by the host against subsequent reinfection with homologous strain of the relevant species of the malaria parasite is maintained for a variable period of time, depending on the degree and duration of the immune response to the antigen. There is little if any protective effect of such immunity against a species of plasmodium different from that which caused the initial infection. (Bruce-Chwatt, 1986). In highly endemic areas where transmission

continues throughout the greater part of the year, the population develops and maintains a high degree of immunity, while at the same time there is a nearly permanent presence of a very small number of malaria parasites in many subjects, mainly adults. The state of resistence in a previously infected host coupled with asymptomatic parasitaemia (premunition) is acquired slowly, and infants and young children may suffer severely from malaria and die. (Bruce-Chwatt, 1986).

Malaria immunity develops following invasion of the organism by the erythrocytic forms of malaria parasites. There is no convincing evidence that the exoerythrocytic stages have any significant effect on the immune response. (Bruce-Chwatt, 1986). There is no cellular response to these stages of the parasites in the liver. The physical basis of malaria immunity depends on the activity of both humoral and cellular factors, though the physiological conditions of the host also play some role. The immune response is an integrated phenomenon involving the T and B lymphocytes. T lymphocytes, which comprise the bulk of circulating lymphocytes, give rise to lymphoblasts, which can be cytotoxic to *Plasmodia* that they recognize. These lymphoblasts do not

synthesize antibody but stimulate B lymphocytes and the derived plasma cells to form antibody.

The working of protective immunity to malaria is seen best in holoendemic areas of tropical Africa. Although infection of the placenta is frequent, congenital malaria is rare because of the prenatal transfer of specific IgG antibodies from the maternal blood across the placenta. (Bruce-Chwatt, 1986). Such passively acquired immunity is transient, and after the first 4-6 months of life many young children have a high parasitaemia and severe clinical illness leading to a high fatality rate. In older children, clinical illness is less common, but an enlarged spleen and parasitaemia are common; in adults, parasitaemia becomes infrequent and of low density, clinical illness is rare, and palpable splenic enlargement is uncommon. (Bruce-Chwatt, 1986). Because of the protective effect of immunity to malaria acquired in the course of previous infections, it is commonly found that antimalarial drugs in individuals previously exposed to malaria have a more pronounced preventive and curative action than in non-immune subjects (Bruce-Chwatt, 1986).

1.2.7. Malaria Control

Malaria control can be achieved by vector control, adequate treatment of acute cases, chemoprophylaxis (Wernsdorfer, 1980) and malaria vaccine.

Vector control:

New insecticides and larvicides are tested as they become available from industry (WHO, 1984 (b). Those recently examined and found to be potentially valuable are bendiocarb, pirimiphos-methyl, and several synthetic pyrethroids. New methods of application such as selective spraying of houses and cattle shelters, based on the resting prefrences of the vectors, and spraying of bed-nets with synthetic pyrethroids are being tested in an effort to reduce both costs and the selection pressure for anopheline resistence. (WHO, 1985). These methods may be employed by the community with little associated hazards. Among the new products tested against mosquito larvae are some synthetic pyrethroids, insect development inhibitors, bacterial pathogens, fungi and nematodes. Useful larvivorous fish are being identified, cultivated and released.

Treatment of malaria:

Malaria transmission may be reduced or even interrupted through adequate and prompt treatment of malaria cases because this reduces or eliminates the parasite reservoir and thereby transmission (Black *et al*, 1986).

Chemprophylaxis: Widespread chemprophylaxis has been discouraged due to resistance and depression of immunity. (Molineaux and Grammiccia, 1980). Chemoprophylaxis is targeted (WHO, 1984 (a)). The target groups are:-

i Non-immune travellers to holoendemic malarious area.

ii Particularly sensitive or vulnerable groups of the holoendemic areas e.g pregnant woman and sickle cell children.

In the first group chemoprophylaxis and reduction in man-mosquito contact are equally important, whereas, chemoprophylaxis in pregnant women has currently been reviewed because there are no sufficient data uptill now that will back up recommendation of chemoprophylaxis in pregnancy. (Najera, WHO, personal communication).

Malaria Vaccine:

At present, there is no malaria vaccine in use, it is still being developed and tested. Most of the relevant research concerns *P. falciparum*.

The strategy for malaria vaccine development involves identification and characterization of "protective" parasite antigens, cloning of the corresponding genes and their expression in bacteria, analysis of their nucleotide sequence, and deduction of the amino acid sequence of the encoded molecule. The "protective" epitope (the immunogenic portion of the antigen molecule) may then be produced by genetic engineering methods or by chemical synthesis (WHO, 1986).

In attempting to identify "protective" *Plasmodial* antigens, attention has been focused on those antigens that are exposed to the immune system, either on the surface of the parasite or on the membrane of the infected erythrocyte. Vaccine targets currently envisaged are antisporozoite, antimerozoite and antigametocyte. Protective immunity in malaria is species-specific and largely stage-specific, so that each vaccine will protect only against the life-cycle stage or stage on which it is based.

Antisporozoite vaccine: This should prevent the establishment of plasmodia in the host and thus induce sterile immunity. In such a case, parasitaemia, asexual or sexual, would not occur, clinical illness will not supervene, and the subject will remain incapable of transferring malaria parasites. A vaccine of this type if applied to human populations sufficiently widely would interrupt natural transmission of malaria irrespective of the prevalent endemic level.

Antimerozoite vaccine: This is expected to induce immunity that operates by restricting the replication of asexual blood-stage parasites without necessarily inducing sterile immnuity. Consequently, its function will be to reduce the morbidity and mortality due to malaria. Such vaccines will be used in highly suceptible groups in endemic areas to induce a level of immunity that would prevent serious illness following infection. The objective will be to confer a degree of protection equivalent to that which develops only after several infections in endemic area. Infection might serve to boost the vaccine-induced immune response.

Antigametocyte vaccine: This will operates by inducing serum antibodies in the human host that block the fertilization of the female gametes by male gamates within the mosquito gut, or inactivate the fertilized zygote or ookinete. Such vaccines seems capable of interrupting malaria transmission at the mosquito level, but will neither protect the human host against sporozoites, hepatic forms, and asexual blood stages, nor prevent the development of gametocytaemia. Their use may reduce the overall rate of transmission in endemic areas and thereby play a role in malaria control. These vaccines would probably be used in combination with protective vaccines against sporozoites and/or asexual erythrocytic stages.

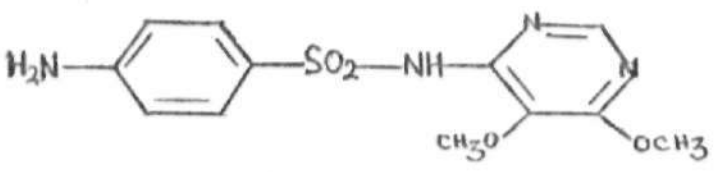
These three type of vaccines are currently being developed. The development of sporozoite vaccines, a natural precursor of which was first successfully tested in man more than ten years ago (Clyde, 1963) is at a more advanced stage than that of the other vaccines.

1.3 Pharmacology of antimalaria Compounds:

1.3.1 Sulphonamides

Shortly after introduction into therapeutics, the sulphonamides were shown to possess antimalarial activity. Much of the important work on sulphonamides was carried out during the intensive antimalarial programme during World War II. This and later work focussed attention on sulphadiazine. It was found, however, to be active only against the asexual blood forms of human malaria parasites and to act slowly. Subsequently, two long-acting sulphonamides, sulphadoxine and sulphalene have been used for treatment and suppression of malaria mainly in semi-immune patients infected with fully sensitive strains of *P. falciparum* (Goodman and Gilman, (1985)

Sulphadoxine



Sulphadoxine is of particular importance as it is rapidly absorbed from the gastro-intestinal tract. It has a remarkably long half-life of 100-200 hours in human plasma as compared to the 17 hours of sulphadiazine (Shepherd, 1970). It reaches peak plasma levels within 4 hours and its slow excretion of only 8% in the first 24 hours enables therapeutically effective levels of the drug to be maintained by weekly oral administration (Pinder, 1973). The real value of sulphadoxine lies in the synergistic combination with drugs like dihydrofolate reductase inhibitors such as pyrimethamine (Richards 1970). Sulphadoxine is active against *P. berghei* in the mouse, and oral doses of 0.8-1.0mg/kg effectively reduce parasitaemia by 50% in animals infected with either chloroquine sensitive or resistant strains (Richards, 1966; Aviado, 1967, 1969). Sulphadoxine is insecticidal to mosquitos when ingested in the diet. It is systemically effective in this respect when it is absorbed during the act of biting infected and sulphadoxine treated mice (Beesly and Peters, 1968; 1971). Thus sulphadoxine seems to have a triple mode of action by acting against both the oocysts and blood forms of

plasmodia and by being toxic when ingested by the insect (Ramkaran and Peters, 1969).

1.3.1.2 Sulphadoxine/pyrimethamine:

Combinations of antimalarial drugs have been used for many years. Many long-acting sulphonamides and a number of sulphones have a schizonticidal effect upon the blood forms of human malaria parasites (Pinder, 1973). Response to these is, however, charaterized by a relatively slow action, greater efficacy against *P. falciparum* than *P. vivax*, suppressive action against multiresistant *P. falciparum* but little action against sensitive strains and a reasonably good tolerance (Pinder, 1973). Synergism of antimalarial action was first noted between sulphonamide and pyrimethamine or proguanil against *P gallinaceum* (Rollo, 1955) and subsequently against human malaria (Hurly, 1959). Subsequent work has established the value of synergistic combinations of (i) drugs which inhibit dihydrofolate reductase, the enzyme that catalyses the reduction of dihydrofolate to tetrahydrofolate, and (ii) drugs which inhibit folate synthetase enzyme. (Richards, 1970). The synergism arises from the sequential blockade by

the two types of drug of steps in plasmodial synthesis of purines and pyrimidines.

The combination of sulphadoxine with pyrimethamine in a ratio of 20:1 is marketed as "Fansidar". The injectable forms of the drug contains 400mg of sulphadoxine to 20mg of pyrimethamine, while the tablet contains 500mg Sulphadoxine and 25mg of Pyrimethamine. The combination has mainly been used in South Eastern Asia for the treatment of naturally acquired infections with chloroquine resistant strains of *P. falciparum* In Laos, the weekly administration of 500mg of sulphadoxine and 25mg of pyrimethamine over 20 months proved to be an effective prophylactic regimen against falciparum infections (Ebisawa *et al*, 1979). Bartelloni *et al*, (1967). Successfully treated non-immune service men infected with chloroquine resistant *P. falciparum* in Viet-Nam and West Malaysia, respectively, with a single dose of 1g of Sulphadoxine combined with 50mg of Pyrimethamine.

Sulphadoxine/Pyrimethamine Toxicity

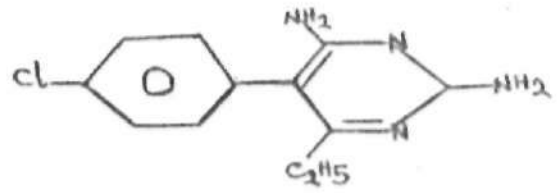
Kaufman (1963) reported that large doses of pyrimethamine, 25-50mg given daily for about 5 weeks have usually been employed in the treatment

of toxoplasmosis in man. The main side effects of such high doses of pyrimethamine with sulphonamide is a depression of the bone marrow with a reduction in the number of erythrocytes, leucocytes and thrombocytes. Others are gastrointestinal disturbance, buccal ulceration, alopecia and skin pigmentation (Weissbach, 1965; McGowan, 1964). An apparent Jarish-Herxheimer reaction during the treatment of falciparum malaria with sulphadoxine/pyrimethamine combination was reported by Kofoed (1984). Adverse reactions to the same drug combination were reported by United States Centers for Disease Control (1984) in the form of erythema multiforma, Stevens-Johnson syndrome and toxic epidermal necrolysis. The ten cases resulted in four deaths. A case of fatal multisystemic toxicity associated with Fansidar prophylaxis has been reported by Selby (1985).

1.3.1.3 Pyrimethamine:

Of the many 2,4-diaminopyrimidines synthesized and tested for antimalarial activity, two are outstanding - pyrimethamine and trimethoprim (Goodman and Gilman, 1985). Pyrimethamine was developed and used almost solely

as antimalarial agent. It is a 2.4-diamino-5-P-chlorophenyl-6-ethylpyrimidine of the following structure:



The 2.4-diaminopyrimidine is structurally similar to the biguanides e.g cycloguanil which is the active metabolite of proguanil. This structural similarity prompted the synthesis and introduction of pyrimethamine which bears a formal structural relationship to cyclognanil (Falco *et al*, 1951). The 2,4-diaminopyrimidine and the biguanides competitively inhibit plasmodial dihyrofolate reductase, and thus block the production of activated tetrahydrofolate needed for DNA synthesis.

Certain bacteria protozoa do not have the ability to transport preformed folate, and they therefore require a supply of paraaminobenzoic acid (PABA) to synthesize the compound (Ferone and Hitchings, 1966). The dihydrofolate reductase in intact cells is not exposed to these inhibitors. The "Small molecule" antifolates, such as

pyrimethamine and trimethoprim, seem to penetrate by non ionic diffusion, and their entrance is unrelated to the ability of the cell to assimilate folic acid (Hitchings and Burchall, 1965).

Pharmacokinetics:

Pyrimethamine is absorbed relatively rapidly from the intestines so that after an oral dose, the peak plasma level is reached between 2-6 hours. The absorption is almost complete. The drug is distributed throughout the body fluids and is moderately bound to tissues with an apparent volume of distribution of about 3 litres/kg (WHO, 1984a). It is about 80% bound to plasma proteins. Salivary concentration is about 20% that of plasma and reflects the unbound drug concentration (Ahmed and Roger, 1980). The drug persists in the plasma and continues to be excreted in the urine for as long as two weeks after the first administration of a single oral dose of 25mg. The elimination half-life is of the order 80-100 hours (Jones and Overall, 1979).

Uses:

Pyrimethamine is mainly used to suppress malaria. It has little value when given alone in

the treatment of an acute primary attack of malaria. It is slow in clearing parasitaemia, but it prevents the development of fertilized gamete and has causal prophylactic activity. It is not a radically curative agent in *P. vivax* malaria, although continuance of suppressive therapy for ten weeks after leaving a malarious area will provide "suppressive cure" with certain strains of *P. vivax* (Goodman and Gilman, 1985).

Toxicity

The use of pyrimethamine alone or in combination with a sulphonamide is not without toxic or side effects. The toxicity of pyrimethamine in normal human subjects has been determined relatively well. Adults tolorate oral doses of 50mg twice weekly for periods of 10-12 weeks without evidence of toxicity (Goodwin, 1952). Myatt *et al*, (1953) showed that daily doses of 25mg given for 49 days caused megalpblastic anaemia in 6 out of 12 subjects and rapid remission occured when the drug was stopped. Death was reported in two children who ingested 40-75mg/kg of pyrimethamine and the main sign of toxicity were convulsions and vomiting (Voorhoeve, 1963; Guignard, 1965). Extensive clinical

experience has shown that the standard weekly oral dose of 25mg for the suppression of malaria is usually well tolerated, but long term administration may result in megaloblastic anaemia which clears quickly when the drug is stopped (Covell *et al*, 1955). Morley *et al* (1964) observed no teratogenic effect in women given 50mg doses of pyrimethamine once a month during pregnancy. Harpey (1983), however, cited a possible case of pyrimethamine teratogenecity in a woman who had taken prophylactic dapsone and pyrimethamine three times after conception as well as chloroquine and gave birth to a male stillborn child with a severe defect of the thoracic and abdominal wall and a missing left arm. Gilbreath *et al* (1983) implicated pyrimethamine in the suppression of mitogenic lecithin-induced blast transformation of human peripheral blood mononucleated cells. Like the 4-aminoquinolines, pyrimethamine depresses cardiac function at dosas in excess of those usually used for therapeutic purpose (Aviado *et al*, 1968)

1.3.1.4 Chloroquine

Chloroquine is a synthetic 4-aminoquinoline derivative that was first synthesized and studied by the Germans under the name Resochin in 1934 (Goodman and Gilman, 1985). It is a 7- chloro-4-(4-diethylamine -1- methylbutylamine) quinoline. It has the same alkyl side chain as quinacrine but differs from quinacrine in having a quinoline instead of the acridine nucleus and lacking the methoxy radical.

Pharmacological action:

Chloroquine exerts no significant activity against sporozoites and exoerythrocytic tissue stages of plasmodia. It is not a casual prophlactic agent and does not prevent the establishment of infection. It is highly effective against the asexual erythrocytic forms of all four species of plasmodia infecting man, except in areas where drug resistant strains occur. The action is rapid, fever usually being absent after 24 hours and patent parasitaemia being eliminated in 48-72 hours following the standard therapeutic regimen (Bruce-Chwatt, 1986). Chloroquine and other 4-aminoquiolines act as gametocidal agents against *P. vivax*, *P. Ovale* and are effective

against the immature but ineffective against the mature gametocytes of *P. falciparum*. Chloroquine does not prevent relapse in vivax malaria, but it lengthens the interval between relapses.

Apart from its antimalarial action, chloroquine is effective in treating extraintestinal amoebiasis. It has anti-inflammatory properties and has been used occasionally in treating rheumatoid arthritis and discoid lupus erythematodes.

Pharmacokinetics:

Absorption

Recent studies in healthy adult volunteers and in children with malaria have shown that chloroquine is rapidly and almost completely absorbed from the intestinal tract. Adelusi *et al*, (1982) orally administered 10mg/kg chloroquine (tablets) to children with malaria. The peak plasma concentration of about 250µ.g/litre was reached in two hours with an absorption half-life of 0.56 hours. The concentration reached in the plasma in the first 30minutes after the oral administration of a 10mg/kg dose of chloroquine is

usually more than the therapeutic level for sensitive *P. falciparum* (i.e, 30ng/litre). In the study by Gustafsson *et al*, (1983). 300mg of chloroquine was given by slow intravenous infusion over a period of about 25 minutes. The peak plasma concentration was reached 5-15 minutes (Mean 12 minutes) after starting the infusion and was 10 times the peak concentration attained after an oral dose. Therefore, there is a very real danger of toxicity if chloroquine is given intravenously. There is no recent data on the rate of absorption of chloroquine after intramuscular administration in man (WHO, 1984a). Since chloroquine is rapidly and almost completely absorbed after oral administration even in the presence of pyrexia, there is no need to administer the drug parenterally.

Distribution

After absorption, chloroquine is widely distribution throuthout the body. Early studies showed that the drug accumulated in the tissues such as the spleen, kidney, lung, heart and liver where concentrations were 300 to 500 times higher than in the plasma (Berliner *et al*, 1948; McChesney and McAuliff, 1961; McCesney *et al*, 1966). The drug has particularly high affinity for melanin-containing tissues of the skin and eye.

These observations have been confirmed by more recent studies of Adelusi and Salako (1982a & 1982b). Because of its tissue distribution and binding, chloroquine has an exceptionally large volume of distribution which is about 200 times the total body fluid volume (WHO, 1986). Adelusi *et al*, (1982) showed that during the first 7 days of patent parasitaemia, the erythrocyte concentration of chloroquine is higher than the plasma concentration. The ratio was 21 at the height of parasitaemia and this fell to 5.3 after 4 days when parasitaemia had completely disappeared. This confirms that in man, there is pref rential concentration of chloroquine in red blood cells and that this is further increased by the presence of malaria parasites in the red cells.

Metabolism:

Chloroquine is metabolized by side-chain de-ethylation first to desethyl- and then bisdesethylchloroquine, which is a primary amine. This compound can be deaminated to form alcohol which is oxidized to the 4'-carboxylic acid derivative. Successive dealkylation of the side-

chain ultimately produces the compound 7-chloro-4-aminoquinoline (Kuroda, 1962 and McChesney *et al* 1966). The quinoline nucleus is resistant to degradation. Metabolism of chloroquine occurs slowly and the main metabolite varies in different species; in man it is desethylchloroquine (McChesney *et al*, 1967; Fletcher *et al*, 1975; Gustaffsson *et al*, 1983). Individual variations in the pattern of distribution of chloroquine and desethylchloroquine may have important bearing on the pattern of adverse reactions to the drug. Olatunde (1971), who studied the chloroquine and desethylchloroquine concentration in the plasma and skin of two groups of African subjects (i.e; those who itched after chloroquine and those who did not) found the plasma concentrations of both the parent drug and the metabolite to be similar in the two groups. However, skin from subjects prone to chloroquine-induced pruritus contained higher concentrations of chloroquine and lower concentrations of the metabolite than skin from other subjects. Recent studies have also shown that desethylchloroquine has about the same activity as the parent drug against chloroquine-sensitive plasmodium species (Aderounmu and Fleckenstein, 1983).

Elimination:

Chloroquine is eliminated from the body so slowly that after a single dose of 300mg the drug and its metabolite can be detected in plasma for up to 56 days, depending on the sensitivity of the assay method used. The decline of the erythrocyte chloroquine concentration after the peak level has been reached parallels that of plasma concentration, showing that equilibrium is reached quickly and is subsequently steadily maintained between these two media. They can therefore be regarded as behaving pharmacokinetically as a single compartment with regard to chloroquine (Adelusi *et al*, 1982; Gustaffsson *et al*, 1983). In contrast, the decline phase of the log-concentration time curves for the various tissues diverge from that of plasma curve with time. The tissues therefore behave as separate compartments with slower elimination rate constants than plasma (Adelusi and Salako, 1982a; 1982b). Estimates of the plasma half-life of chloroquine have varied, between 2.5 days obtained by Alving *et al*, (1948) and nine days obtained by Gustaffsson *et al* (1983), the range possibly reflecting the multicompartment distribution of the drug and differences in the duration of the sampling

period. The total plasma clearance of chloroquine varies between 750 and 1050ml/min of which renal clearance is between 400 and 450ml/min (Gustaffsson *et al*, 1983). Since the value for renal clearance is more than the glomerular filteration rate, the drug is probably excreted by both glomerular filtration and tubular secretion. The kidney is the main route of elimination of chloroquine from the body and the drug is detectable in the urine over a period of 120 days after a dose of 300mg. Maximum excretion occurs in the first 24 hours, about 10% of the administered dose being excreted in that interval. Chloroquine is excreted predominantly in the unchanged form, the excreted desethylchloroquine being only about 25% of the excreted parent drug.

Toxicity:

The earlier workers on chloroquine recorded blurring of vision, dizziness, mild pruritus, bleaching of hair and mild gastro-intestinal symptoms as side effects in individuals receiving chloroquine (Wiselogle, 1946). Itching in Africans, cardiovascular abnormalities, ocular damage, neuromuscular disorders, extrapyramidal dysfunction, photoallergic dermatitis and

pigmentation of the skin have also been reported. (Bruce-Chwatt, 1986). Cardiovacular effects of chloroquine in man and experimental animals were also observed by the earlier workers. Alving *et al* (1948) recorded dimunition in the height of the T - wave in the elecrocardiographic recording of 12 out of 20 volunteers taking 0.3mg/kg of chloroquine daily for eleven weeks. Olatunde (1970) demonstrated the hypotensive effect of chloroquine in anaesthetized cats and dogs. He observed that in children there is a slight but consistent fall in blood pressure after intramuscular chloroquine therapy for malaria fever. Cardiotoxic effects are most commonly observed after parenteral administration or in toxic overdosage and may take the form of hypotension or cardiac arrest and death (Ratliff *et al*, 1987). Ocular damage usually follows long term treatment of connective tissue disease with high dosage of chloroquine and long term suppressive therapy. It has also been described in workers exposed to chloroquine phosphate dust in the manufacture of chloroquine tablets (Salako, 1984).

Neuropathy results from the effects of the drug on the muscle, nerve or neuromuscular junction. The muscle fibres undergo autophagocytosis followed by segmental necrosis (Salako, 1984; Estes *et al*, 1987). Extrapyramidal effects similar to those occurring in parkinsonism have been decribed in patients treated with chloroquine. In Nigeria, Eronini *et al* (1977) reported an incidence of 1 in 5000 persons. The symptoms usually start within 24 hours of taking the drug.

1.3.1.5 Primaquine: It is a derivative of 8-aminoquinoline. Primaquine inhibits parasite mitochondrial respiration and this is probably the basis of its action against the primary and secondary liver stages as well as gametocytes (Bruce-Chwatt, 1986). It is highly active against the gametocytes of all human malaria species. The gametocidal action of a single dose of primaquine base against *P. falciparum* lasts for several days, rendering the gametocytes incapable of development in mosquitos that subsequently feed on the treated patients. Clinically, it is used together with blood schizontocides to achieve radical cure of relapsing malaria.

After oral administration, primaquine is rapidly absorbed from the intestine. A peak plasma level of up to 250µg/litre is reached within 1-3 hours after a single oral dose of 45mg. The drug is distributed throughout the body fluids with an apparent volume of distribution of about 3.5 litre/kg. (WHO, 1984a). Primaquine is eliminated rapidly from the body with a half-life of about 6 hours. At the recommended dosage, no symptoms of toxicity are likely. When the dosage is increased, toxic manifestations may include anorexia, nausea, epigastric distress, abdominal pain and cramps, cyanosis, the passage of dark urine and occasionally vomting. There could be leukopenia, methaemoglobinaemia, anaemia, and haemolytic anaemia in patients with glucose-6-phosphate dehydrogenase deficiency. These manifestations disappear when the drug is withdrawn (Goodman and Gilman, 1985).

1.3.1.6 Proguanil:

Proguanil is a biguanide derivative highly active against the primary exoerythrocytic forms of *P. falciparum*. It acts mainly by inhibiting plasmodial dihydrofolate reductase enzyme. Proguanil and its analogue chlorproguanil are

valuable drugs for causal prophylaxis in falciparum malaria (Bruce-Chwatt, 1986). Proguanil is not sufficiently rapid in action for the treatment of the acute attack in non-immune subjects. Its greatest drawback is a tendency to provoke resistance. Proguanil is rapidly absorbed from the intestine, the peak concentration after a single oral dose is reached in about 4 hours (WHO, 1984a). It is concentrated in the red blood cells where the concentration is 4-8 times greater than that of plasma. It is converted in the body to a triazine metabolite, cycloguanil, which is thought to be the active compound. Excretion is slow and takes place via the kidney. About 40% of a given dose can be recovered from the urine and faeces in the unchanged form, the rest as metabolite. The plasma elimination half-life is about 24 hours (Bruce-Chwatt, 1986).

1.3.1.7 Quinine:

Quinine is a cinchona alkaloid. It acts primarily as a schizontocide. It is inactive against the sporozoite and exoerythrocytic stages of malaria parasites. It is gametocidal against *P. vivax, P. ovale and P. malariae* (Bowman and Rand, 1980). Quinine is especially valuable for

the treatment of chloroquine resistant and multidrug resistant strains of *P. falciparum* where it is used alone or in combination with other drugs (Goodman and Gilman, 1985). Quinine has weak antipyretic action and weak analgesic action. It also stabilizes nerve terminal and skeletal muscle fibre membranes and inhibits responses to acetylcholine. It therefore relieves muscle cramps and other manifestations of hyperexcitability of the skeletal muscle system (Bowman and Rand, 1980).

Quinine is rapidly and almost completely absorbed from the intestine. Peak plasma concentrations are reached 1-3 hours after a single oral dose. After absorption, quinine is distributed throughout most of the body fluid. The concentration in the red blood cells is approximately one fifth of that in plasma and, in cerebral malaria, the concentration in the cerebrospinal fluid is approximately 7% of that in plasma. The apparent volume of distribution is about 2-5 litres/kg (WHO, 1984a). Quinine is metabolized in the liver and is excreted partly unchanged but mainly as the hydroxylated metabolite. Elimination from the body is rapid. The drug and its metabolite appear in the urine within 1 hour of its administration and little remains in the body after 48 hours (Hall *et al*, 1973 and White *et al*, 1982).

1.3.1.8 Other Antimalaria Drugs:

a. Mefloquine:

Mefloquine is a member of the 4-quinolinemethanols. It was developed for the treatment and prevention of chloroquine-resistant strains of falciparum malaria. Mefloquine in single well tolerated doses, has been shown to eliminate fever and parasitaemia rapidly in non-immune volunteers or patients in endemic areas infected with either chloroquine sensitive or highly chloroquine resistant strains of *P. falciparum* and to effect suppressive cure against all strains of *P. falciparum* and suppression of *P. vivax*. *In p. vivax* infections, however, malaria attacks recur sometimes after the end of the treatment (Rieckman *et al*, 1974; Trenholme *et al*, 1975; Clyde *et al*, 1976).

Mefloquine is well absorbed after oral administration and is extensively bound to plasma proteins. Peak plasma concentrations are attained in a few hours and decline slowly over a period of several days. After a single dose of 1g, the half-life in blood averages 17 days (Desjardins *et al*, 1979). Nausea, vomiting and dizziness may occur. These effects are dose related, self-

limiting and uncommon with single doses of 1g or less. There could be hallucinations, depression and disorientation during second week after drug administration. phototoxicity has not been associated with mefloquine.

b. Tetracyclines:

Tetracycilines given in doses of 250mg every 6 hours for 7 days produce a slow but complete clearance of parasitaemia and symptoms (Pinder, 1973). Their relative slowness of action makes concurrent treatment with quinine mandatory. It has no effect on the gametocytes, and patients on shorter less intensive regimens recrudesced, and it is not recommended for the treatment of acute attacks of malaria (Pinder, 1973). In a field trial in Thailand, a combination of tetracycline and quinine produced a radical cure in 97% of the patients, whereas treatment with quinine and chloroquine was successful in only 42% of cases (Bowman and Rand, 1980). Tetracycline in the oral dosage used for the treatment of malaria may cause nausea, vomiting and diarrhoea. There could be more serious effects on the skin, mucous membranes and gastrointestinal tract. Tetracycline chelates

heavy metals especially calcium and may be deposited in growing teeth and bones.

c. Halofantrine:

This is a member of phenanthremethanol. It is a new antimalarial with activity against highly resistant *P. falciparum*. Animal studies have shown evidence of activity against erythrocytic stages of parasites in all species of malaria tested (Canfield, 1980). Oduola *et al* (1987) showed full sensitivity of *P. falciparum* isolates to halofantrine and reduced susceptibility to mefloquine.

d. Qinghaosu (Artemisinine):

Qinghaosu is the active antimalarial component of the chinese herb, *Qinghao*. It represents a new chemical series of antimalarial compounds with a high level of blood schizonticidal activity against chloroquine resistant malaria parasites in laboratory model. Animal studies have shown quinghaosu to be widely distributed in the tissues and eliminated fairly rapidly. It may exert toxic effect on the erythroid haemopoietic cells of the bone marrow. High doses could cause liver damage (WHO, 1984a).

1.3.2 Clinical Treatment of falciparum malaria:

a. Infections sensitive to 4-aminoquinolines:

Chloroquine is still the drug of choice in infections caused by sensitive strains of *P. falciparum*. A course of treatment totalling 25mg/kg body weight of chloroquine base over 3 days in doses of 10mg/kg, 10mg/kg and 5mg/kg on the first, second and third day, respectively, is recommended for adults. Dosage administered to infants or children orally or intramuscularly should not exceed 10mg of chloroquine base per kg body weight per day. The normal dose is 5mg/kg of the base (WHO, 1984a).

b. Infections resistant at low to moderate level (RI and II) to 4-aminoquinolines:

Chloroquine is still the recommended drug for the treatment of infections resistant at low to moderate levels (WHO, 1984a). The dosage is the same as in (a). It is, however, advisable to have sulphadoxine/pyrimethamine combination at hand, in case the infection fails to respond to chloroquine treatment. A single therapeutic dose of the drug combination corresponding to 35mg/kg body weight of sulphadoxine plus 1.75mg/kg body weight of pyrimethamine is usually recommended.

c. Resistant cases of *P. falciparum* to chloroquine is treated with alternative drugs as outlined in 1.4.3.

1.4 Resistance of P. falciparum to antimalarial drugs:

1.4.1 Definition and underlying mechanism:

Drug resistance has been defined as the ability of a parasite strain to multiply or survive in the presence of a drug that normally destroys parasites of same species or prevents their multiplication. Such resistance may be relative (yielding to increased doses of the drug tolerated by the host) or complete (withstanding miximum doses tolerated by the host) (WHO, 1963).

In man the problem of resistance to the widely used antimalaria drugs such as chloroquine, pyrimethamine and sulphadoxine/pyrimthamine and the decreased effectiveness of quinine has so far been associated with *P. falciparum* infection alone (WHO, 1987a). There are several different mechanisms that can account for changes in drug sensitivity of various malaria parasitess (Beale, 1980). Examples are physiological adaptations due

to non-genetic changes, selection of previously existing drug resistant strains from mixed populations under drug pressure, spontaneous mutation or mutagenesis (due to antimalaria treatment or other drugs), mutation of extranuclear genes (e.g mitochondrial genes) and the existence of plasmids or plasmid-like factors. *Spontaneous gene mutation and selection of mutants* by the drug itself appear to be important in this phenomenon (Beale, 1980).

Resistance of *P. falciparum* to antimalarial drugs always seems to have occurred in areas that are under considerable drug pressure (WHO, 1987a). The selection of decreasingly sensitive *P. falciparum* may be promoted by the presence of subcurative concentrations of antimalarial drugs in the blood that only eliminate the most sensitive parasites allowing those that are less sensitive to survive. Spontaneous mutations will tend to reduce the sensitivity of some parasites even more and further drug-induced selection would reduce the response to an even lower level. (Wernsdorfer and Kouznetsov, 1980; Wernsdorfer, 1984). Subcurative drug concentrations in the blood frequently occur during mass suppressive treatment (prophylaxis), especially if drug intake

is irregular and insufficient; they also occur during the final phase of the elimination of long acting drugs (WHO, 1987 (a)). Thus subcurative drug concentrations may play an important role in the selection for drug resistance when such medicaments are used in areas of intensive malaria transmission. In addition, the use of dihydrofolate reductase and dihydropterate synthetase inhibitors against bacterial infections may induce the resistance of malaria parasites to pyrimethamine and sulphadoxine and compromise the efficacy of this combination for malaria control.

1.4.2. World Distribution

Historically, resistance of *P. falciparum* to quinine was noted in Brazil as far back as 1908 - 1910 (Peters, 1970). Sporadic cases of quinine resistance, although rarely substantiated, continued to be reported from various countries until widespread use of this drug was superceded by modern synthetic antimalarials (Wernsdorfer and Kouznestov, 1980). However, several strains encountered in south east Asia in recent years appear to have an RI level of resistance to the 14-day course of quinine (2g daily) and RIII level resistance has occurred. Studies in eastern

Thailand have shown true quinine resistance in this area (Reacher, 1981).

Chloroquine resistance of *P. falciparum* was suspected in south America in the late 1950 (Peters, 1970) and confirmed in 1959 in Thailand (Harinasuta *et al*, 1962). Around this period, resistance was discovered in other areas like Columbia. Chloroquine resistance *P. falciparum* has been discovered in almost every malarious area. Decreasing sensitivity of *P. falciparum* to an increasing number of antimalarial drugs is being reported from areas in Africa and Asia. The frequency and the degree of chloroquine resistant cases tend to increase with the length of time drug resistance has been present in the area concerned and are also related to the intensity of drug pressure and malaria transmission after the initial selection/introduction of resistant parasites. Thus a high frequency and a high degree of parasite resistance are observed in Kampuchea, Thailand and Vietnam, areas in which chloroquine resistance was first noted in the 1960s (WHO, 1984 (a)). In Nigeria in 1981, Olatunde *et al*, did not detect any level of resistance to both chloroquine and 'fansidar'. In 1985, Midala *et al*, (1988) observed a decreased chloroquine sensitivity of *P.*

falciparum in Northern Nigeria. Salako and Aderoumu, (1987) reported the presence of *in vitro* chloroquine resistance *P. falciparum* in Nigeria. In 1988 Breman *et al*, reported *P. falciparum* sensitivity to chloroquine in Southern Nigeria children andLege-Oguntoye *et al* (1989) found an incidence of 10% chloroquine resistance *in vivo* in Zaria urban area. Clinical trials in Thailand and eastern Africa, have not yet shown any evidence of a major problem of primary mefloquine resistance in *P. falciparum*. Cure rates in the dose range of 12.5-20mg of base/kg body weight (single dose) were constantly higher than 90% (WHO, 1984 (a)). The trials have included to date more than 2000 patients in Asia, Africa and south America. Most of the failures (only RI type up to now) were associated to drug loss (early vomiting) with resulting drug concentrations below MIC (WHO, 1984 (a)). However two cases of primary drug resistance have been observed, one in a patient in Thailand, Boudreau (1982) and the second in a patient who contracted the infection in East Africa (Bygbjerg 1983).

Resistance to sulphadoxine/pyrimethamine combination was first reported from Thailand (Hurwitz, 1981) and Tanzania (Vleugels, 1982).

Resistance to sulphadoxine/pyrimethamine was most pronounced on the eastern (Kampuchean and Laos) and the western Burmese borders (WHO, 1984(a)) of Thailand. In clinical trials conducted in patients suffering from paragonimiasis in Brazil (1980/81), some 25% of resistant cases were recorded, most of them at RI level. Increasing failure rates are now being reported from the Amazon basin and Columbia (WHO, 1984(a)). Resistance to this drug combination has later been reported from Burma, indonesia, Malaysia and Papua New Guinea (WHO, 1986). From America, the World Health Organisation reported that the efficacy of the combination had diminished by 30-40% (WHO, 1982). Resistance to this drug combination seems to be directly related to pyrimethamine resistance, the higher the degree of the latter, the more likely there is to be resistance to the combination (WHO, 1986). Resistance of *P. falciparum* to sulphadoxine/pyrimethamine seems to be linked to the presence of parasites highly resistant to pyrimethamine as the presence of a low medium level of pyrimethamine per se does not seem to preclude the curative effect of the combination. It may also be a necessary condition for the

development of resistance to the combination that *P. falciparum* should be resistant to both pyrimethamine and sulphadoxine, a likely event since sulphanamides have been widely used in Africa for other bacterial diseases (Onori, 1984). The sensitivity of most *P. falciparum* infections in Kenya to the drug combination may be a function of a low frequency of sulphadoxine resistance (Watkins *et al*, 1988). Resistance by *P. falciparum* to pyrimethamine or proguanil alone is present in certain localities of endemic regions, including Africa (WHO, 1973).

1.4.3 Management:

Resistance of *P. falciparum* to most of the current antimalarial drugs has emerged as the main technical problem in malaria control. A knowledge of the extent of the problem in terms of geographical distribution and degree of resistance is important for the selection of appropriate control measures and for the development of a policy for the rational use of antimalaria drugs. The presence of resistant parasites may necessitate the development of other drugs of which there is a limited range (WHO, 1986).

Curative treatment with 1,500mg chloroquine base is the least expensive regimen. The cost of the first and second line alternative drugs is considerably higher and their use puts a financial strain on individuals and the public economy in general. The alternative drug include amodiaquine, sulphadoxine/pyrimethamine, quinine alone, quinine plus tetracycline and mefloquine alone or in combination with sulphadoxine/pyrimethamine. Some of these drugs produce side effects and involve lenghty treatment regimens that often result in poor patient compliance as for example, the combination of quinine and tetracycline

Chloroquine is the drug of choice for the treatment of suspected or confirmed malaria in places where there is no known resistance or even in areas with RI-type resistance (WHO, 1986). In order to slow down the appearance of resistance, and also to ensure adequate treatment for those patients in whom the parasitological response cannot be monitored a full course of 25mg/kg of chloroquine base, given over 3 days is recommended. Amodiaquine has been shown to be somewhat more active than chloroquine against sensitive strains of *P. falciparum* and it also retains activity against some parasites possessing

RI-or RII-type resistance to chloroquine (Watkins, *et al*, 1984). The dose of amodiaquine usually employed is 25mg/kg body weight of amodiaquine base given over 3 days.

sulphadoxine/pyrimethamine.

In areas where there is a high level of resistance to chloroquine, sulphadoxine/ pyrimethamine is the drug combination of choice for the second line treatment of suspected or confirmed malaria if there is either no established resistance to it or the resistance is low grade or infrequent. A single dose of 75mg pyrimethamine (1.5mg/kg) given together with 1,500mg sulphadoxine (25mg/kg) is quite effective in treating chloroquine resistant *P. falciparum* (WHO, 1984b).

Mefloquine.

Mefloquine is highly effective in the treatment of multidrug resistant malaria. It can be given as a single dose of 25mg/kg body weight or in combination with other drugs. A combination of quinine and a single dose of 1.5g mefloquine achieves a 100% cure rate in the treatment of chloroquine resistant malaria (Hall, *et al*, 1977).

In order to delay emergence of resistance to mefloquine, the combination with sulphonamide/ pyrimethamine has been found to be most effective (WHO, 1984b). The combination of mefloquine/ sulphadoxine/pyrimethamine is in the ratio of 10:20:1 and in areas where resistance to sulphadoxine/pyrimethamine is rare, two tablets of the fixed combination each containing 250mg of mefloquine, 500mg sulphadoxine and 25mg pyrimethamine is given as a single dose. In places where resistance is high three tablets should be given as a single dose (WHO, 1984b).

Quinine.

Quinine is used alone or in conbination with other antimalaria drugs. For uncomplicated infections, 30mg/kg body weight is given orally, in 3 divided doses (10mg, 10mg, 10mg) daily for 7 days (Bruce-Chwatt, 1986). Alternatively, quinine may be given for 3 days in association with tetracycline (250mg, 4 times daily); the latter to be given for at least seven consecutive Days; Alternatives are doxicycline (0.2g daily) or minocycline (0.1-0.4g daily) (WHO, 1984b).

AIMS AND OBJECTIVES OF THE STUDY.

Malaria remains one of the most wide spread infectious diseases in Africa. Over 90% of the 450 million persons in Africa South of the Sahara live in malarious area (Breman and Campbell, 1988). The prevalence of *Plasmodium falciparum* infection in endemic areas is frequently over 50%, with the highest rate occurring in children (Molineaux and Grammiccia, 1980).

Resistance to most of the current antimalarial drugs has emerged as the main technical problem in malaria control. Resistance to pyrimethamine has been described in Northern Nigeria by Archibald (1960) and Dodge (1966). More recently, decreased sensitivity (Midala *et al*, 1988) and resistance (Lege-Oguntoye *et al*, 1989) of *P. falciparum* to chloroquine have been reported in Zaria, Northern Nigeria.

It is therefore the objective of this work to determine the sensitivity of *P. falciparum* to sulphadoxine/pyrimethamine which is an antimalarial drug combination used in the treatment of chloroquine -resistant strains of *P. falciparum*. *P. falciparum* resistance to this drug combination has been reported from Thailand

(Hurwitz, 1981) and Tanzania (Vleugels, 1982). Resistance of *P. falciparum* to sulphadoxine/pyrimethamine combination seems to be linked to the presence of parasites highly resistant to pyrimethamine (Onori, 1984). Watkins *et al*, (1988) reported that resistance of most *P. falciparum* infection to this drug combination in Kenya may be a function of a low sulphadoxine resistance.

No reports are available yet on the *in-vivo/in vitro* sensitivity of *P. falciparum* to sulphadoxine/pyrimethamine combination from the Guinea Savannah belt of Northern Nigeria. This study is therefore aimed at investigating the status of *P. falciparum* sensitivity to this drug combination in Zaria urban area using the WHO Extended Field Test and WHO standard mark II micro-test system. It is hoped that this study will present data on the level of response of *P. falciparum* to sulphadoxine/pyrimethamine combination. Such data will contribute to the present knowledge of the geographical distribution and degree of resistance which is important in the selection of appropriate control measures and for the development of a policy for the rational use of antimalarial drugs.

CHAPTER TWO

2. MATERIALS AND METHODS

1. MATERIALS

1. Pyrimethamine/Sulphadoxine Fansidar[12], each tablet containing 500mg sulphadoxine and 25mg pyrimethamine base. They were supplied by Roche (Nigeria) Limited. All were from the same batch number 880622A.

2. KH_2PO_4-potassium dihydrogen orthophosphate, BDH. Chemical Limited, Poole, England. Batch number 1884380

3. Na_2HPO_4-disodium hydrogen orthophosphate, BDH Chemical Limited, Poole, England. Batch No 6539490.

4. Giemsa stain was obtained from National Malaria and Vector Control Division, Federal Ministry of Health, Lagos.

5. Methanol, R-grade. May and Baker Limited, Degenham, England. Batch number MS, 1920.

6. Ethanol, absolute, May and Baker, Limited, Degenham, England . Batch number 71222.

7. Xylene (sulphur-free), May and Baker, Limited, Degenham, England. Batch number MS 2163.

8. Microslides, 75x25x1.0mm. Frosted one end both sides. Becton Dickinson and Company, Batch number 3058.

9. Microscope: Reichert Jung microscope. Reichert Scientific Instrument, Division of Werner-Lambert Technologies, Inc. Buffalo, New York. Made in USA. No. BG 341341.

10. Cotton wool

2.2 METHODS

2.2.1 Patients:

Patients of both sexes between the age of 6 months and 6 years residing in Zaria urban area were screened at the Paediatric Outpatients Department of Ahmadu Bello University Teaching Hospital where this study was conducted between August 1988 and January 1989. Rainfall during 1988 were recorded between May and October.

Informed consent was obtained from the parents of all enrolled children. Fever was defined as a body temperature of 37.5°C or above. Children were asked for any history of diarrhoea, pruritus and vomiting. The axilla temperature of each child was taken and recorded. The impression, 1, was used for fever or history of fever within the past 48 hours and ,0, for no measured fever or history of fever for past 48 hours. A child with decreased activity and mental alertness was also assigned ,1, while ,0, was assigned to any child who was well. A child was considered to have diarrhoea if the number of stools per day within the past 48 hours was 3 or more, (see appendix B)

History of antimalaria drug ingestion within the past 2-4 weeks was taken. If any, the name and

quantity of the drug were recorded. Such patients however were excluded from the study. Only children with negative urine sample to 4-aminoquinolines and sulphonamide were enrolled. The primary interest was that of the patients well being. Patients with complicating disease such as cerebral malaria, anaemia, jaundice and bronchopneumonia were excluded. Enrolled patients were registered and given serial numbers. The diagnosis for all the patients was confirmed by presence of asexual forms (trophozoites) of *P. falciparum* in the peripheral blood smear (thick film). Those who had mixed infections (*P. falciparum, P. malaria or P. ovale*) were excluded from the study. Patients whose parasite count was not less than 1,000 asexual parasites per mm^3 were selected for inclusion in the study and subjected to the 28-day W.H.O. Extended Field Test (WHO, 1973).

Shortly before drug administration the patients were weighed and their weights were recorded. The patients' name and residence were recorded. This enhanced smooth follow-up where the patients failed to turn up on any day. All patients were seen at the malaria clinic specially set up for the study. On day zero, a single oral

dose of sulphadoxine/pyrimethamine combination equivalent to 35mg sulphadoxine per kg body weight plus 1.75mg pyrimethamine per kg body weight was administered to each child by the investigator. To rule out vomiting all patients were observed for 30 minutes after drug administration. Any child that vomited two or more times after drug intake was withdrawn from the study. Patients were seen daily for the first one week and thereafter at weekly intervals for an additional period of 3 weeks. Thick and thin films were prepared immediately before drug administration and on each of the above days of follow-up. A thick film was considered negative only when the examination of 100 fields revealed no asexual parasites.

2.2.2 *In vivo*:

2.2.2.1 Urine Tests:

a. Dill-Glazko test:

The Dill-Glazko reagent was prepared by mixing 50mg eosin (Yellowish) with 100ml chloroform to which 1ml IN hydrochloric acid is added (Lelijveld. and Lortmann 1970). The mixture

was shaken until the chloroform assumed a light-yellow colour, after which the chloroform solution was separated in a separating funnel from the watery phase and stored in a glass-stoppered brown bottle. About 10 drops of the reagent were added to each 2ml of urine in a test tube. The presence of chloroquine in the urine is indicated by a change in colour from yellowish to violet-red in the chloroform layer which precipitates after shaking.

b. Lignin Test (Bratton-Marshall, 1939)

A drop of urine was placed on a blank strip of clean sterile newspaper and a small drop of 25% hydrochloric acid was added in the centre of the moistened area. Presence of sulphonamide was indicated by the immediate appearance of a yellow to orange spot.

2.2.2.2 Preparation of Phosphate Buffer Solution pH 7.2:

0.7g of potassium dihydrogen orthophosphate (KH_2PO_4) and 1.0g of disodium hydrogen orthophosphate ($Na_2 HPO_4$) were carefully weighed. The two salts were put together and dissolved in a small quantity of distilled water. The solution

was then made up to 1 litre and tested for PH. If neccessary, a little of either salt stock solution was added to correct the pH. The solution was stored in a refridgerator and only removed when needed.

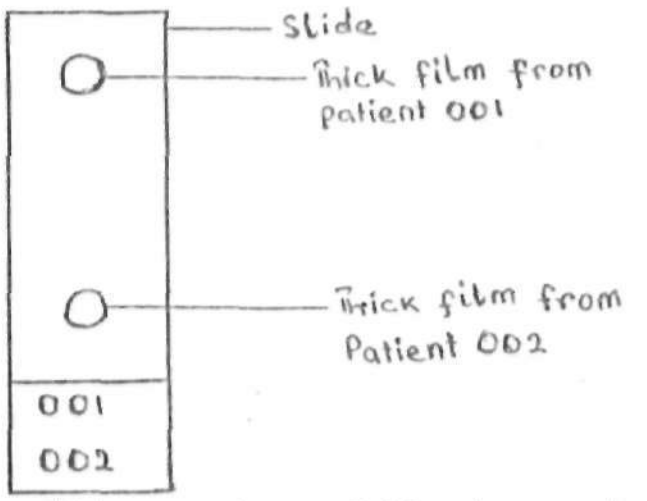
2.2.2.3. Preparation of Microscopic slides:

All slides were initially soaked for 24 hours in water containing detergent. At the end of this period, the slides were thoroughly washed using the smallest size test tube brush. The slides were then soaked in alcohol for another 24 hours to remove any impurities. Before use, the slides were thoroughly rinsed under running tap water. The water was then drained off and the individual slides were blotted and dried with a non-fibrous lint. All cleaned slides were kept in clean slide boxes and were only removed when needed.

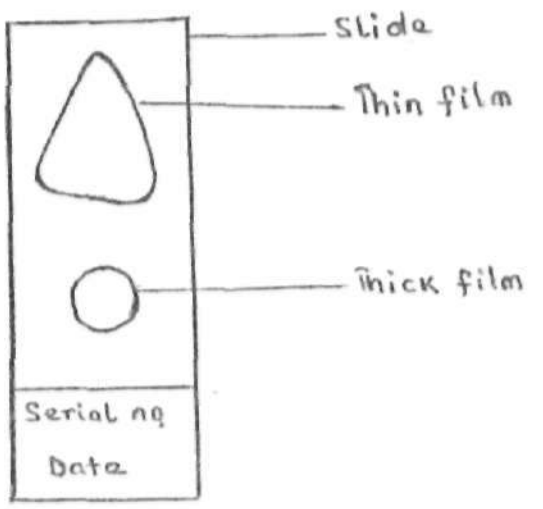
2.2.2.4 Preparation of Blood Smears:

The patient's finger was thoroughly cleaned with an alcohol swab, allowed to dry and then pricked with a disposable sterile 26G 3/8 injection needle. The first drop of blood was removed with a dry cotton swab. For screening, 3 drops of blood were then placed at the free end of

a slide and a thick round shape film was gently formed using an edge of a second clean slide. Usually, screening slides hold two thick blood films from different patients-with their serial number written on the frosted end of the slide. (Fig a).



For definitive slides, a drop of blood was placed in the centre of a clean slide. A thin film was made by holding a second clean slide at an angle of about 45° and spreading the drop along the first slide. Toward the frosted end of the same slide, a thick round shaped film was gently formed from 3 drops of blood. Each slide was labelled indicating the patient's serial number and the date (Fig b).



2.2.2.5 Preparation of Giemsa Stock Solution:

Care was taken in weighing 3.8g of Giemsa powder and in measuring 250ml of methanol and 250ml of glycerine. Both methanol and glycerine were thoroughly mixed and little was added at a time to the Giemsa powder contained in a mortar. The contents of the mortar were gradually ground until a solution of Giemsa was formed. The solution was filtered using filter paper and then kept for use.

2.2.2.6 Staining of Blood Films:

Giemsa aqueous stain was used. The stock solution was diluted with phosphate buffer PH 7.2. Different concentrations of Giemsa were used to stain slides for screening or for definitive counting.

Screening slides: 4ml of the Giemsa stock solution were added to 96ml of phosphate buffer PH 7.2 to obtain a 4% Giemsa solution. The slides were stained for a duration of 10 minutes.

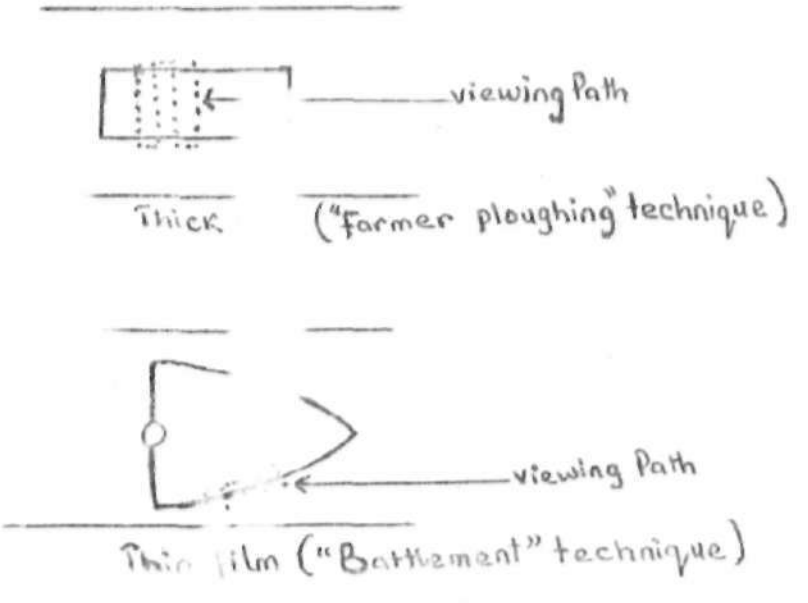
Definitive slides: 2ml of the Giemsa stock solution were added to 98ml of the phosphate buffer PH 7.2 to obtain a 2% Giemsa solution which

was used to stain slides for 30 minutes. Before staining, the thin blood smears were fixed for 3 minutes in methanol and air dried.

At the end of the staining period, the stained slides were gently rinsed in phosphate buffer PH 7.2 and carefully dried with a hair drier.

2.2.2.7 *Counting of Parasites:*

The stained slides were viewed in an oil immersion under a standard microscope with total magnification of x1000. The microscope was initially cleaned to remove all dust before use. After examination, the oil was carefully cleaned off the slides with sulphur-free xylene and kept in clean slide boxes for repeated examination. For screening and definitive counting, the technique described by Payne (1982) were adopted. The viewing path of these techniques are shown below.



In each case, the first field was focused, malaria parasites and leucocytes in this field were counted and recorded with two tally counters; one for malaria parasites and the other for white blood cells. Counting continued following the viewing path. For screening procedures the counting ended when the leucocytes number reached was 100. The corresponding number of malaria parasites was recorded. For definitive parasite counting, the procedure ended when either the parasite or leucocytes number reached 500 or 1000, respectively. In most cases, when these figures are reached some parasites and leucocytes still remain uncounted in the last field. In this case, counting continues until all the parasites and leucocytes in the last field have been counted.

2.2.2.8 Determination of Parasite Density (PD) per mm^3 blood:

The parasite density was determined by dividing the product of the parasite count and reference leucocyte count per mm^3 blood by the leucocyte count as described by the Payne's equation. That is:

$$PD/mm^3 = \frac{\textit{Parasites counted x standard leucocyte count (per } mm^3\textit{)}}{\text{leucocytes counted,}}$$

For example, if on day zero, the leucocyte count of a patient was 300 and parasite count was 520, then parasite density per mm^3 blood =

$$\frac{520 \times 6000}{300} = 10,400$$

2.2.2.9 Determination of Mean Parasite Density (MPD):

The mean parasite density for each day was determined by dividing the sum of the parasite densities of all the patients on that day by the total number of patients studied. (See tables 1 and 3) From these tables, the sum of the parasite densities for the 44 patients on day zero is 2,085,060. therefore, The MPD per mm3

$$= \frac{2,085,060}{44} = 47,388$$

2.2.2.10 Determination of Mean Parasite Clearance Time (MPCT) (days)

The MPCT can be determined only for patients with (i) regular follow-up over the first week and (ii) parasite clearance before or on day 7. The MPCT for each day was determined as follows: (Payne, 1982). The number of patients whose parasitaemia had cleared on each day was multiplied by the total number of days needed for parasite clearance, e.g if the MPCT (days) is calculated for 26 patients whose follow-up during the first one week was regular, the calculation will be done as follows: The symbols x represent days, f, frequency and x, mean.

x	f	fx	$f(x-\bar{x})^2$
1	0	0	0
2	6	12	16.335
3	6	18	2.535
4	7	28	0.8575
5	5	25	9.1125
6	2	12	11.045
7	0	0	0
	$\sum f=26$	$\sum fx=95$	$\sum f(x-\bar{x})^2= 39.885$

$$x = \frac{fx}{f} = \frac{95}{26} = 3.65 \text{ days.}$$

∴ MPCT = 3.65 days.

Standard deviation

$$= \sqrt{\frac{\sum f(x-\bar{x})^2}{\sum f}}$$

$$= \sqrt{\frac{39.885}{26}}$$

$$= 1.238$$

$$\simeq 1.24 \text{ days}$$

Thus the MPCT for the 26 patients is 3.65 ± 1.24 days.

2.2.2.11 Determination of Parasite Density Index (PDI):

The parasite density for each patient on day zero may be expressed as the Parasite Density Index according to Bruce-Chwatt criteria of classification (Payne, 1982). The frequency of occurence (f) of parasite density in each class (c) is recorded as shown below. The porduct (fxc) is then determined for each individual class and the sum of these products will be divided by the total number of patients studied. The table below illustrates this procedure.

Parasite Density/mm³	Class (c)	Frequency (f)	f x c
100 or less	1	0	0
101 - 200	2	0	0
201 - 400	3	0	0
401 - 800	4	0	0
801 - 1,600	5	0	0
1,601 - 3,200	6	1	6
3,201 - 6,400	7	4	28
6,401 - 12,800	8	6	48
12,801 - 25,600	9	7	63
25,601 - and above	10	26	260
	Total	44	405

$$\frac{f \times c}{\sum f} = \frac{405}{44} = 9.2$$

∴ PDI for day zero = 9.2

2.2.3 *IN VITRO*

2.2.3.1 Patients:

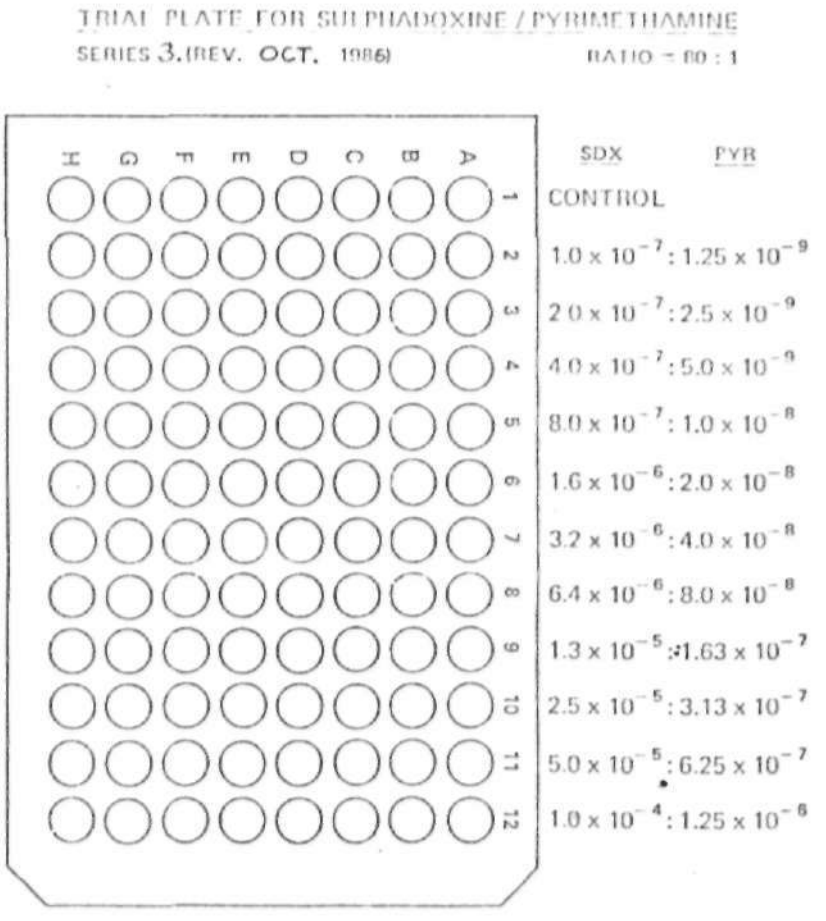
The WHO standard mark II micro-test system was adopted. For technical reasons, not all patients who qualified for the *in vivo* test were enroled for the *in vitro* test. Patient with parasitaemia lower than 1,000 or in excess of 90,000 per mm^3 had to be excluded (WHO, 1987 (b)).

2.2.3.2 Layout of Micro-culture Plates:

The standard WHO microtest plate contains wells labelled A to H. There are 12 vertical rows and 8 horizontal rows. Each vertical row is used for growing parasites from one patient. The patient's serial number is written against the row. The first well in a row (well A) contains no drug and therefore serves as control well. The other wells (B to H) are predosed with sulphadoxine/pyrimethamine combination in the ratio 80 : 1. Well B has the lowest concentration (Pmol) of sulphadoxine/pyrimethamine combination (10:0.125) while well H has the highest (10,000:125). The concentration of the drug is based on a geometrical progression with factors 3 and 3.33 used in alternation and the base 10 for sulphadoxine and 0.125 for pyrimethamine (see

Plate 1). Each plate has a plastic seal. In addition, the plate and its lid are kept in a sterile plastic bag. Continued cold storage of the plate at + 4°C has to be maintained until all wells of a plate are exhausted.

Plate 1



N.B.

1. Drug concentration is expressed per litre of blood/medium.
2. Standard well inoculum is 50 µl.

WHO 85473

2.2.3.3 Preparation of the Growth Medium:

The following procedures were all carried out under sterile conditions using an improvised wooden cabinet with a glass top and a hole on the right side. Shortly before the preparation of the growth medium, the inner compartment of the cabinet was sterilized using a large alcohol swab. It was then left to dry. The investigator in addition to wearing a face mask used a fresh alcohol swab to sterilize his hands up to the elbows.

The rubber seal of the LPLF liquid medium stock bottle containing 100ml of solution was wiped clean with an alcohol swab. It was allowed to dry and broken with a sterile needle. A second sterile needle was mounted on a sterile 20ml syringe and used to withdraw 10ml of LPLF liquid medium from the 100ml stock bottle. The syringe with the needle was then removed. The needle was recapped and the syringe was kept in a safe place. The seal-breaking needle was also removed wiped clean, allowed to dry and recapped. The seal of the L-glutamine vial was wiped clean with an alcohol swab and allowed to dry. The seal breaking needle was used to break the seal of the L-glutamine Vial. The needle of the 20ml syringe was

uncapped and the 10ml LPLF liquid medium was introduced into the L-glutamine vial. The vial was gently shaken to dissolve the L-glutamine. The seal breaking needle was removed and recapped. The 20ml syringe was also removed and its needle recapped for possible later use.

As many 6ml falcon presscap tubes as there were patients to be tested were labelled with the patients' serial number and kept upright in a tube rack. The alumimium cap and the rubber seal of the L-glutamine vial were carefully removed. A 1ml sterile syringe and needle was used to withdraw 0.9ml of the L-glutamine/LPLF liquid medium mixture into each 6ml falcon presscap tube. The partially used LPLF liquid medium stock bottle was returned to cold storage at + 4°C. The syringes and needles were discarded after use.

IN VITRO SUSCEPTIBILITY TESTS

Fig. I. PREPARATION OF THE BLOOD MEDIUM MIXTURE

(*a*) Draw 0.9 ml of growth medium into a 1 ml sterile disposable tuberculin syringe.

(*b*) Inject growth medium into sterile holding vial

(*c*) Clean and firmly prick the finger of the patient selected

(*d*) Draw 100 μl of blood into a sterile ant coagulant-treated micropipette

(*e*) Expel the blood from the micropipette into the vial

(*f*) Close the vial with a sterile cap and swirl the vial to provide a homogenized suspension of blood

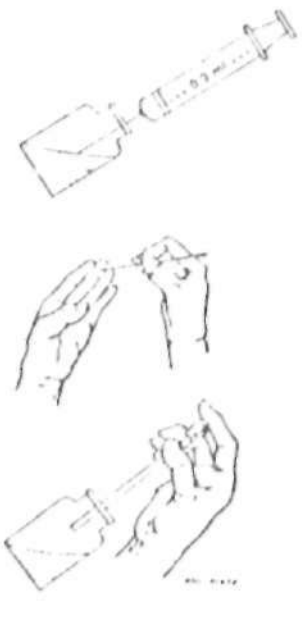


Fig. II. DOSING OF MICROPLATES

(*a*) Remove the predosed culture plate from the plastic bag using a surgical scalpel.

(*b*) Remove the plastic seal from the columns according to the number of tests to be performed.

(*c*) Remove the plate lid from the plastic bag and cover the plate.

(*d*) Shake the blood/medium mixture gently to resuspend the blood cells in the vial.

(*e*) Lift the lid and, with an Eppendorf pipette, add 50 μl of blood medium suspension to each of the wells of the appropriate column, starting with well A and continuing to well H

(*f*) Replace the lid and shake the plate gently to reconstitute the drugs.

(*g*) Write the patents' reference number on the lid over the appropriate columns.

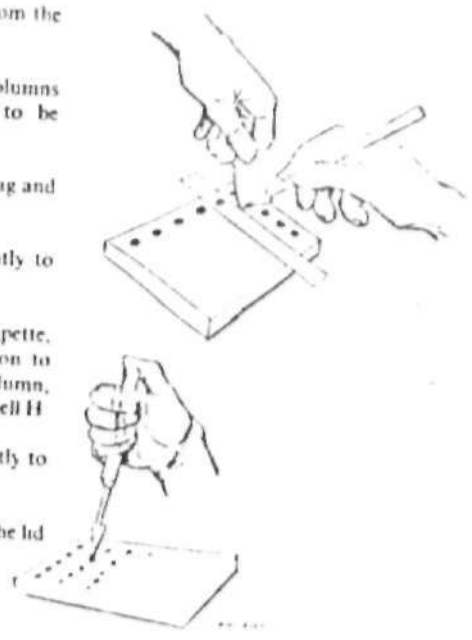
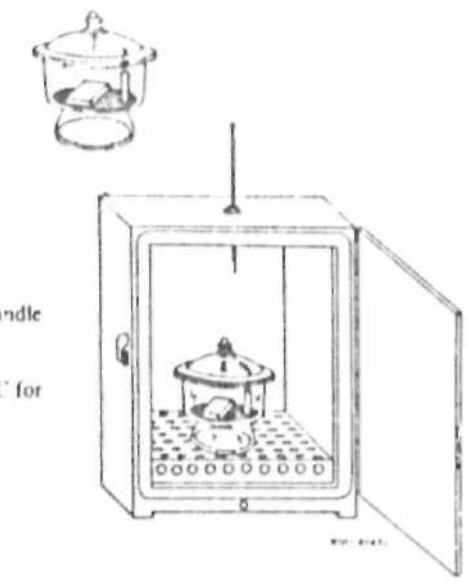


Fig III INCUBATION



(*a*) Place the plate in a candle jar.

(*b*) Light the candle.

(*c*) Replace the cover almost completely.

(*d*) Replace the cover completely when the candle is almost extinguished.

(*e*) Place the jar in an incubator at 37 38 C for 24 26 hours.

Fig IV. PREPARATION OF BLOOD FILMS



(*a*) Remove the plate from the incubator.

(*b*) Take the plate out of the candle jar.

(*c*) Using 50 μl (non-heparinized) micropipettes with a bulb, remove the growth medium.

(*d*) Draw blood from the well into a micropipette.

(*e*) Prepare a thick blood film.

NOTE: The micropipette tube must be changed for each well

2.2.3.4 Performance of the Mark II Micro-test:

Before drug administration on day zero, each patient had the tip of one finger cleaned with methylated spirit and after drying pricked with a sterile lancet. Dry sterile cotton wool was then used to wipe off the first drop of blood from the finger. A 100µl blood specimen was withdrawn into a sterile heparinized capillary tube and swiftly transferred into the L-glutamine/LPLF liquid medium mixture. The transfer was made possible by means of a small black 1ml rubber bulb which had been attached to the distal end of 100ul capillary tube before the tube was used to collect the blood. By sealing the hole in the end of the bulb with a finger tip and applying slight pressure on the bulb, the blood in the capillary tube was allowed to drop freely into the medium. The cap of the falcon tube was pressed firmly into place and the tube was gently agitated to mix blood and medium.

2.2.3.5 Aliquoting of Micro-Plates:

Using a sterile surgical blade the plastic bag containing the predosed micro-plate was opened and the plate removed. The lid of the plate was removed to one side and the plastic sealing strips

covering the required number were removed. This was done by cutting along the appropraite row with a sterile surgical blade and lifting up the required area of plastic lid with a sterile forceps, taking care not to contaminate the top of the plate.

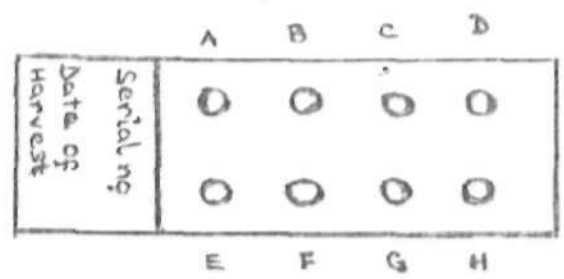
All the wells of the appropriate row (one vertical test row for each patient) were filled with 50µl aliquots of the blood/medium mixture (ratio 1.9) using a 50µl Eppendorf pipette with a disposable sterile tip. Aliquoting was always done starting with the control well (A) and following an increasing order of concentration, ending at well H (Plate 2). The falcon tube containting the blood/medium mixture was gently agitated from time to time to ensure that the blood was kept in suspension and thus evenly distributed into the wells. The sterile disposable tip was changed for each patient and a new sterile disposable tip was fitted to the Eppendorf pipette for each row of wells. Finally the lid of the microplate was carefully replaced. The serial number of each patient was indicated on the outside lid. The plate with its contents was carefully placed on a shaker and gently shaken for two minutes to enable any drug deposit to completely dissolve.

2.2.3.6 Incubation:

The incubator was set to give an internal temperature in the candle jar of 37.5°C. It was always prewarmed for at least one hour before being loaded with the plates. The candle jar was removed from the incubator and loaded with the plates. A thermometer was placed inside the jar to indicate the temperature in the jar as incubation progressed. Two pure paraffin candles were lit and placed on each side of the stacked plates. The candle jar lid was replaced and a good seal was ensured by use of Dow Corning® high vacuum grease. The exhaust cock was left in an open position and closed only when the second candle was at the point of extinction. The candle jar with its contents was then placed in the incubator and the starting time of incubation was recorded. An average incubation period of 27 hours was used to allow adequate growth of the parasites. (Plate 2, Figure III)

2.2.3.7 Harvesting:

After incubation, the contents of the wells were harvested. After removing the candle jar from the incubator all the plates were taken out. The contents of the wells were harvested row after row as follows. For each row, harvesting was started with well A and continued down to well H. Using a fresh 50ul non-heparinized capillary tube with a black rubber suction bulb the supernatant in well A was removed. The same capillary tube was used to remove the red blood cells deposit from the flat bottom of well A. Subsequently, the cell deposit from well A was transferred to a clean microscope slide to form a thick film. The same procedure was used to harvest wells B-H. A fresh capillary tube was used for each thick film. (see Plate 1, Figure IV). The 8 thick films obtained for each patient were arranged on the same slide as shown below:



The resulting thick films were carefully air dried for 24 hours before staining and thereafter stained for 30 minutes in a 1.5% giemsa solution PH 7.2. The stained slides were gently rinsed in phosphate buffer PH 7.2 and carefully dried with a hair drier.

2.2.3.8 Counting of Parasites:

Counting of thick films was based on the presence of trophozoites and normal schizonts with 8 or more nuclei. The farmer ploughing technique was applied. Counting was always done starting with well A and following an increasing order of drug concentration ending at well H. Counting was completed when the trophozoite number reached 200. Usually when this figure is reached some trophozoites still remain uncounted. Counting continues until all the trophozoites in the last field have been counted. The corresponding number of normal schizonts with 8 or more nuclei was recorded. A minimum of 20% schizont maturation in well A is required.

2.2.3.9 Probit analysis and preparatio of regression line:

A copy of the graph on which the *in vitro* micro-test results can be plotted was made from that included in the test kits(WHO, 1987 (b)). The *in vitro* test results obtained from schizont maturation or growth inhibition were plotted on this graph. The regression obtained through probit analysis of log dose/response tests can be used to make an estimate of the EC50, EC90 or EC99, ie the drug concentrations producing 50%, 90% or 99% inhibition of schizont maturation, respectively. It can also be used to estimate any intermediate value or for comparative purposes over space and time (Grab and Wernsdorfer, 1983). Sensitive isolates normally produce steep regression lines. The flatter the regression line and the higher the EC99, the more resistant the isolate (WHO, 1984 (a)).

2.2.4 Statistical Methods:

(a) Calculation of standard deviation (S.D) for the ages of the 44 patients:

x	f	mid x	f,mid x	$f(x-\bar{x})^2$
less than 1	7	0,5	3,5	57,2572
1 - 2	7	1,5	10,5	24,2172
2 - 3	7	2,5	17,5	5,1772
3 - 4	5	3,5	17,5	0,099
4 - 5	6	4,5	27,0	7,7976
5 - 6	6	5,5	33,0	27,4776
6 - 7	6	6,5	39,0	59,1576
	$\sum f=44$		$\sum f,midx=148$	$\sum(x-\bar{x})^2 = 181,1824$

$$\bar{x} = \frac{\sum f}{f} = \frac{148}{44} = 3.36$$

$$S.D = \sqrt{\frac{\sum f(x-\bar{x})^2}{\sum fx}}$$

$$= \sqrt{\frac{181.1824}{148}}$$

$$= 1.22$$

$$= 1.2$$

b. Calculation of standard deviation (S.D) for the weight of the 44 patients.

x	mid x	f	f.midx	$f(x-\bar{x})^2$
6 - 7	7	6	42	93.615
8 -10	9	8	72	30.42
10 -12	11	22	242	0.055
12 -14	13	1	13	4.2025
14 -16	15	4	60	65.61
16 -18	17	2	34	73.3205
18 -20	19	1	19	64.8025
	$\sum f=44$	$\sum fmidx=482$		$\sum f(x-\bar{x})^2=332.0255$

$$\bar{x} = \frac{\sum f}{f} = \frac{482}{44} = 10.95$$

$$S.D = \sqrt{\frac{\sum f\ (x-\bar{x})^2}{\sum fx}}$$

$$= \frac{332.0255}{482}$$

$$= 0.829 \qquad = 0.83$$

2.2.5 Frequency of Use of Sulphonamide Containing drugs:

A preliminary survey was carried out in Zaria urban area to investigate the extent of exposure of the population to any sulphonamide containing drug. Informations were collected to indicate how often sulphonamide alone or in combination with other drugs was prescribed, sold or dispensed on any day of a 30 day period between November and December 1989. This period corresponds to the cold season. The number of patients to whom such drugs were prescribed, sold or dispensed per day was recorded. In this survey, data were obtained from three sources:

a Pharmacy Department of the University Teaching Hospital, Tudun-Wada, Zaria.

b. University Health Services Clinic, Main campus, Samaru, Zaria.

c. Dangi Pharmacy, Samaru, Zaria

2.2.6 Determination of Percentage Content of Sulphadoxine/Pyrimethamine:

Quality control assays of sulphadoxine and pyrimethamine were carried out at the Department of Industrial Pharmacy and Quality Control, A. B. U. Teaching Hospital, to determine the percentage content of these drugs in sulphadoxine/ pyrimethamine combination.

CHAPTER THREE

3. RESULTS

3.1 In-vivo

575 children were screened in all and only 57(9.9%) were parasite positive. 4 (7%) of the 57 patients took a dose of chloroquine by day 2 or day 3 without prescription after a single dose of sulphadoxine/pyrimethamine combination. This was detected in their urine. They were therefore withdrawn from the study. 8 (14%) absconded while 1 (1.8%) had a mixed infection of *P. falciprum* and *P. malaria*. These 9 patients were also excluded from the study. One patient (1.8%) died on day 20 of severe pneumonia that was not treated on time because of social problems, but the results were included in the study. Of the 44 children admitted into the study, 2 were hospitalized for 2 days each because they were very ill; the remaining 42 patients were followed up as outpatients only.

Of the 44 patients, 22(50%)were male and 22(50%) female. The age of the children varied from 6 months to 6 years with an average age of3.6±1.2 years (see 2.2.4(a)). The range of body weight was between 6.2 and 16kg, with an average weight of 10.52±0.83kg (see 2.2.4 (b)). On first examination, all patients except 6 (Nos 98, 146, 172, 405, 532, 566) had fever with a body temperature of 37.5°C or more. The temperature of each of the patients with the above numbers was 37°C. Two patients (no. 234 and 401) had a body temperature of 40°C and 40.5°C respectively. 20 (45.5%) of the 44 children had diarrhoea on first examination and vomiting was recorded in 21 (47.7%), while 3 (6.8%) had pruritus.

Parasitological Response:

In the 44 children, parasite density on day 0 ranged from 1,635 to 222,857 per mm^3 with a mean parasite density of 47,388 per mm^3. 36 (81.8%) of the 44 patients had complete parasite clearance before day 7-and there was no reappearance of parasitaemia between day 7 and day 28. These were therefore considered fully sensitive to sulphadoxine/pyrimethamine. The parasite density of the 36 children over 28 days are shown in Table

1. It can be seen that none of the children became parasite negative on day one, 6(13.6%) became parasite negative on day two and 16(36.4%) were cleared of parasitaemia by day 3 and day 4. By day 7, all patients were parasite negative. Table 2 shows the mean parasite densities for the 36 patients for the 28-day test period. It can be seen that the decrease was gradual and that only by day 7 all patients had become parasite negative indicating a delay in parasite clearance. Figure 1 graphically shows the decline in mean parasite density over the 28-day test period. It can also be seen that by day 2, mean parasite density had fallen to 1.13% of the pretreatment density on day zero.

For 26 of the 36 children, parasite densities were recorded daily for the first 7 days. For these, the mean parasite clearance time (MPCT) was calculated to be 3.65±1.24 days. Table 3 presents the individual parasite densities of the remaining 8 (18.2%) patients. It would appear that 6(75%) of the 8 patients had persistent parasitaemia up to day 7. Parasitaemia was cleared on day 4 and reappeared on day 6 in one patient (No 145). In patient no. 143, parasitaemia was cleared on day 7 and reappeared on day 14. Though parasitaemia

persisted, a fall below 25% of the pretreatment level was observed in all the 8 patients except one (no 488) whose parasitaemia decreased by 30% on day 1 and by 67.8% on day 2. However by day 4, parasitaemia of this patient had decreased below 25% of the pretreatment level. By day 28, all 8 patients were cleared of parasitaemia. The mean parasite densities of the 8 patients is presented in Table 4. It can be seen that there was a sharp decline in parasite counts between day zero and day 3 and that thereafter a low level parasitaemia was maintained up to day 21, while on day 28, all the 8 patients were aparasitaemic.

The changes in Parasite Density Index (PDI) over the 28-day test period after a single dose of sulphadoxine/pyrimethamine in the 44 patients for both sulphadoxine/pyrimethamine sensitive patients and those with persistent parasitaemia are illustrated in Table 5. The PDI on day zero was similar for both groups. For the sensitive cases, the PDI was 9.2 on day zero and had dropped to 1 on day 6. Though the pretreatment PDI was high, by day 3, it had already fallen to 26.88% of the pretreatment level. This value is lower than the corresponding value (35.16%) for the remaining 8 patients. Figure 2 graphically illustrates the described changes of PDI in the 36 children.

Table 1

Parasite Density (per mm^3) of 36 children sensitive to a single standard dose of sulphadoxine/pyrimethamine in Zaria urban area, 1988.

S/No	0	1	2	3	4	5	6	7	14	21	28
1	080	7433	10625	109	0	0	0	0	0	0	0
2	098	30420	998	0	0	0	0	0	0	0	0
3	128	1635	6387	12	0	0	0	0	0	0	0
4	153	9741	484	167	18	36	-	12	0	0	0	0
5	160	41040	3102	54	18	0	-	0	0	-	0	0
6	172	13148	-	-	0	12	-	0	0	-	0	0
7	196	31408	27398	12	6	-	0	0	0	-	0	0
8	199	54915	9854	470	12	-	0	0	-	0	0	-
9	234	54102	103935	364	-	0	0	0	0	0	0	-
10	319	80341	35	0	0	0	0	0	0	0	0	0
11	369	7598	3692	23	0	0	0	0	0	0	0	0
12	378	23815	7194	2083	519	468	105	0	0	0	0	0
13	382	3756	2871	42	18	12	0	0	0	0	0	0
14	386	19200	11364	920	384	107	0	0	0	0	0	0
15	398	100400	-	599	24	24	0	0	0	0	0	0
16	400	158308	59172	1179	120	30	109	0	0	0	0	0

17	401	81405	50215	1552	546	370	36	30	0	0	0	0
18	404	45391	1800	607	0	0	0	0	0	0	0	0
19	406	222857	12850	-	179	0	0	0	0	0	0	0
20	414	43652	2285	1822	4962	1918	84	24	0	0	0	0
21	426	72679	60600	13000	1200	832	0	0	0	0	0	0
22	436	147500	110323	-	-	-	0	0	-	0	0	0
23	441	88390	42206	0	0	0	0	0	0	0	0	0
24	476	92000	67344	0	0	0	0	0	0	0	0	0
25	491	39853	33857	0	0	0	0	0	0	0	0	0
26	516	5680	2409	4713	60	0	0	0	0	0	0	0
27	517	50459	10936	1380	137	90	0	0	0	0	0	0
28	518	34532	2714	296	210	0	0	0	0	0	0	0
29	523	22699	9508	524	-	0	-	-	0	-	0	0
30	524	78255	12925	936	125	0	0	0	0	0	0	0
31	535	3633	6466	107	0	0	0	0	0	0	0	0
32	542	27655	7157	1302	647	0	0	0	0	0	0	0
33	544	10707	928	-	0	0	0	0	0	0	Died on day 20	
34	547	31842	998	48	0	0	0	0	0	0	0	0
35	566	171989	14874	1192	149	0	0	0	0	0	0	0
36	569	4990	2368	0	0	0	0	0	0	0	0	0

Table 2

Mean Parasite Density in 36 patients sensitive to a single dose of sulphadoxine/pyrimethamine in Zaria urban area.

DAY	MPD/mm³
0	92,720
1	47,938
2	1,047
3	280
4	118
5	10
6	2
7	0
14	0
21	0
28	0

Fig-1

Mean parasite density/mm³ in children with P.falciparum infection in response to a single therapeutic dose of sulphadoxine/pyrimethamine, Accra, 1986.

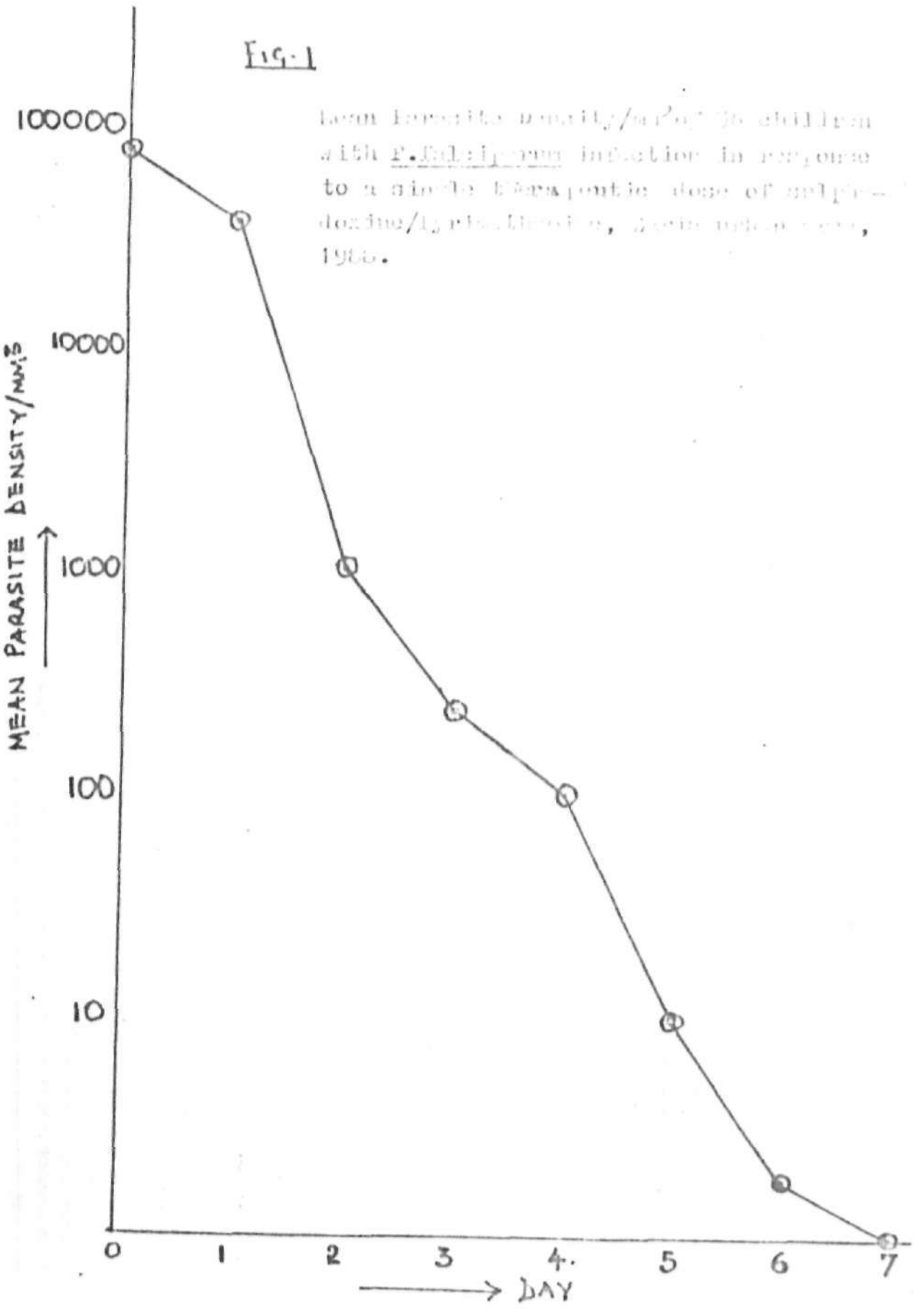


Table 3

Parasite Density of 8 children with persistent *P. falciparum* parasitaemia after a single therapeutic dose of sulphadoxine/ pyrimethamine in Zaria urban area.

S/No	0	1	2	3	4	5	6	7	14	21	28
1	135	21347	68591	542	183	108	18	29	6	12	0	0
2	143	9403	2778	6	12	6	-	6	0	96	12	0
3	145	14540	59	6	-	0	-	6	12	0	0	0
4	146	11333	437	122	36	12	12	18	30	18	18	0
5	191	33698	3828	2166	126	-	-	78	36	18	0	0
6	405	48545	9108	1008	222	165	161	138	68	108	24	0
7	440	90973	7260	2520	-	-	438	1253	959	576	0	0
8	488	21559	6483	14611	25814	850	1450	564	125	0	0	0

Table 4

Mean Parasite Density (per mm^3) of 8 children with persistent *P. falciparum* parasitaemia after a single therapeutic dose of sulphadoxine/pyrimethamine in Zaria urban area.

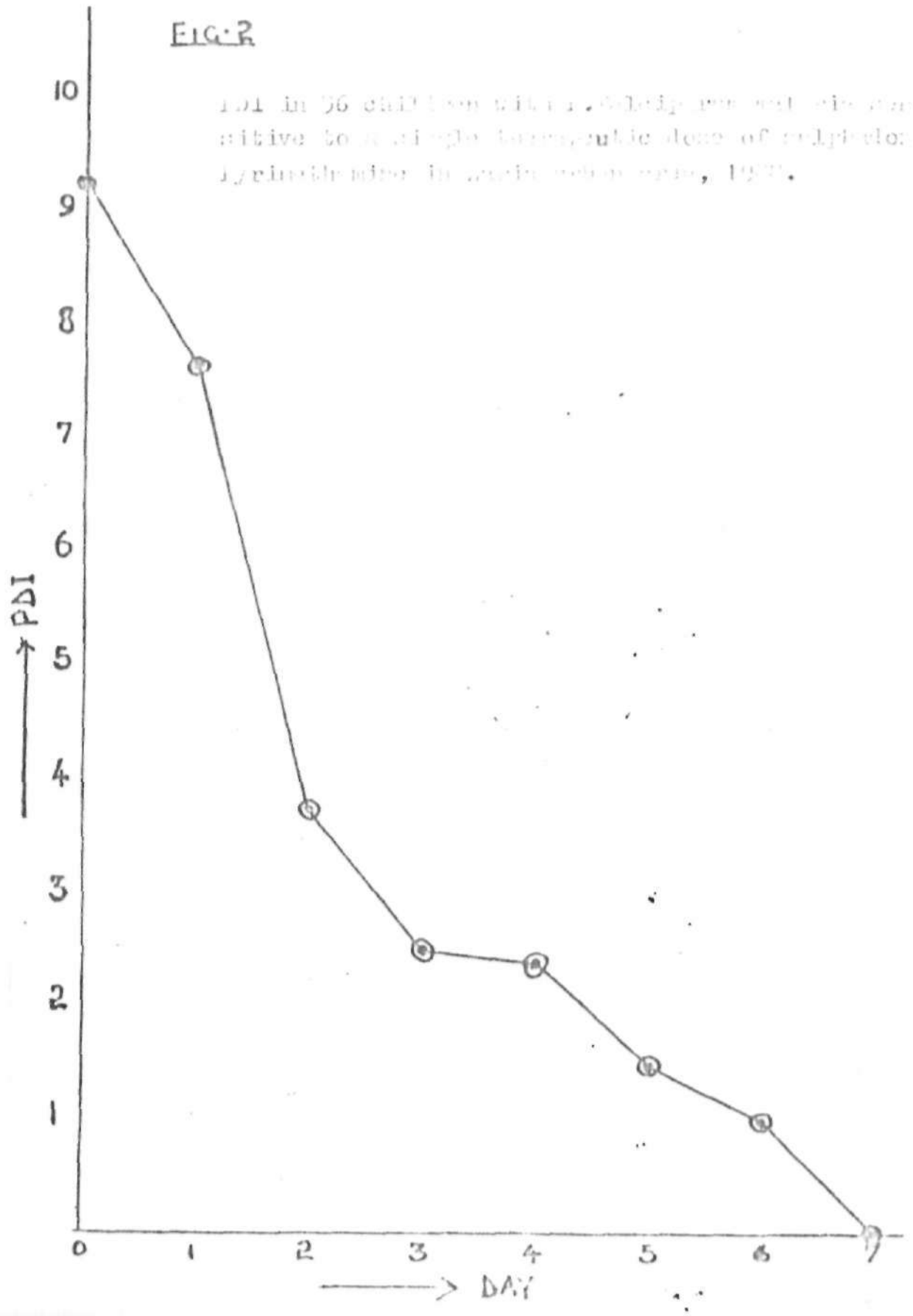
DAY	MPD/mm^3
0	29,022
1	12,318
2	2,623
3	3,299
4	143
5	260
6	262
7	157
14	104
21	6
28	0

Table 5

Parasite Density Index (PDI) over 28 days in sulphadoxine/pyrimethamine-sensitive and -resistant patients with *P. falciparum* malaria after a single therapeutic dose of sulphadoxine/pyrimethamine in Zaria urban area, 1988.

Day	36 *in-vivo* sensitive patients	8 *in-vivo* with persistent parasitaemia
0	9.2	9.1
1	7.6	6.5
2	3.7	4.3
3	2.5	3.2
4	2.4	2.2
5	1.5	2.6
6	1.0	2.0
7	0	1.7
14	0	1.7
21	0	1.0
28	0	0

FIG:2



rat in 96 children with *P. falciparum* per cent sensitive to a single intramuscular dose of sulphadoxine/pyrimethamine in Accra, 1973.

Clinical Response:

Table 6 presents the mean body temperature of the 44 patients over the first 7 days. It varies between 38.0°C on day zero and 35.3°C on day 5. Appendix C shows the body temperature of each of the 44 patient recorded over the 28-day test period. By day one patient no. 234 and no. 401 showed a decrease in body temperature from 40°C to 37.8°C and 40.5°C to 37°C, respectively. By this day only 7 patients, (nos. 080, 145, 404, 414, 440, 476, 544) still maintained a temperature of 37.5°C or more. By day 2, fever with a body temperature of 37.5°C and above was recorded in 4 patients (nos. 171, 191, 401, 426) and by day 3, the temperature of all patients had dropped below 37.5°C. No fever was subsequently recorded in any of the 44 patients over the remaining 25 day period except patient no. 488 who had developed broncho-pneumonia on day 19. She was admitted the same day and discharged on day 21 after significant clinical improvement.

Similar to the prompt decrease in body temperature, the gastrointestinal symptoms had resolved by day 5 in the 44 patients. There was thus a satisfactory correlation between parasitological and clinical responses for the 36

children who had been found sensitive to sulphadoxine/pyrimethamine *in-vivo*. However, there was no such correlation found for the remaining 8 patients with persistent *P. falciparum* parasitaemia. While they all became asymptomatic clinically within 3 days, parasitaemia had persisted for 21 days and did not clear until day 28. However, because of lack of clinical evidence of malaria in these 8 children, a second antimalaria course was not administered to any of them.

3.2 In-vitro

In-vitro sensitivity testing was carried out using the W.H.O. standard mark II micro-test system. In all, 42 isolates of *P. falciparum* were cultured. For the 42 patients, parasite density on day zero varied between 11,333 and 90,973/mm^3. There was satisfactory trophozoite maturation recorded for all of them. Schizont maturation, however, was recorded in only 13(30.95%) of the 42 isolates.

Table 6

Mean body temperature (°C) of 44 children with *P. falciparum* malaria over 7 days after a single therapeutic dose of sulphdoxine/pyimethamine in Zaria urban area, 1988.

Day	Mean body temp. (°C)
0	38.04
1	37.03
2	36.84
3	36.50
4	36.57
5	35.34
6	36.46
7	35.58

Table 7

In-vitro sensitivity of 8 *P. falciparum* isolates to sulphadoxine/pyrimethamine at Zaria urban area, 1988

Serial No	Schizont counts per test well of:							
	A 0pmol	B 10pmol	C 30pmol	D 100pmol	E 300pmol	F 1000pmol	G 3000pmol	H 10000#
145	53	55	49	43	42	33	31	26
146	84	90	90	71	101	74	69	74
191	47	42	54	37	63	58	51	41
378	34	35	37	37	61	51	53	29
386	36	40	62	66	53	48	59	31
405	44	47	27	29	35	37	37	33
440	52	46	43	47	25	31	21	5
488	108	113	93	91	112	100	54	40
Total schizont count	470	468	454	421	492	432	355	279
Mean of schizont	58,8	58,5	56,6	52,6	61,5	54,0	44,4	34,9
Percentage of control	100	99,6	96,6	89,6	104,7	91,9	75,5	59,4
Percentage maturation		99,6	96,6	89,6	104,7	91,9	75,5	59,4
Percentage inhibition		0,4	3,4	10,4	-4,7	8,1	24,5	40,6

= pmol

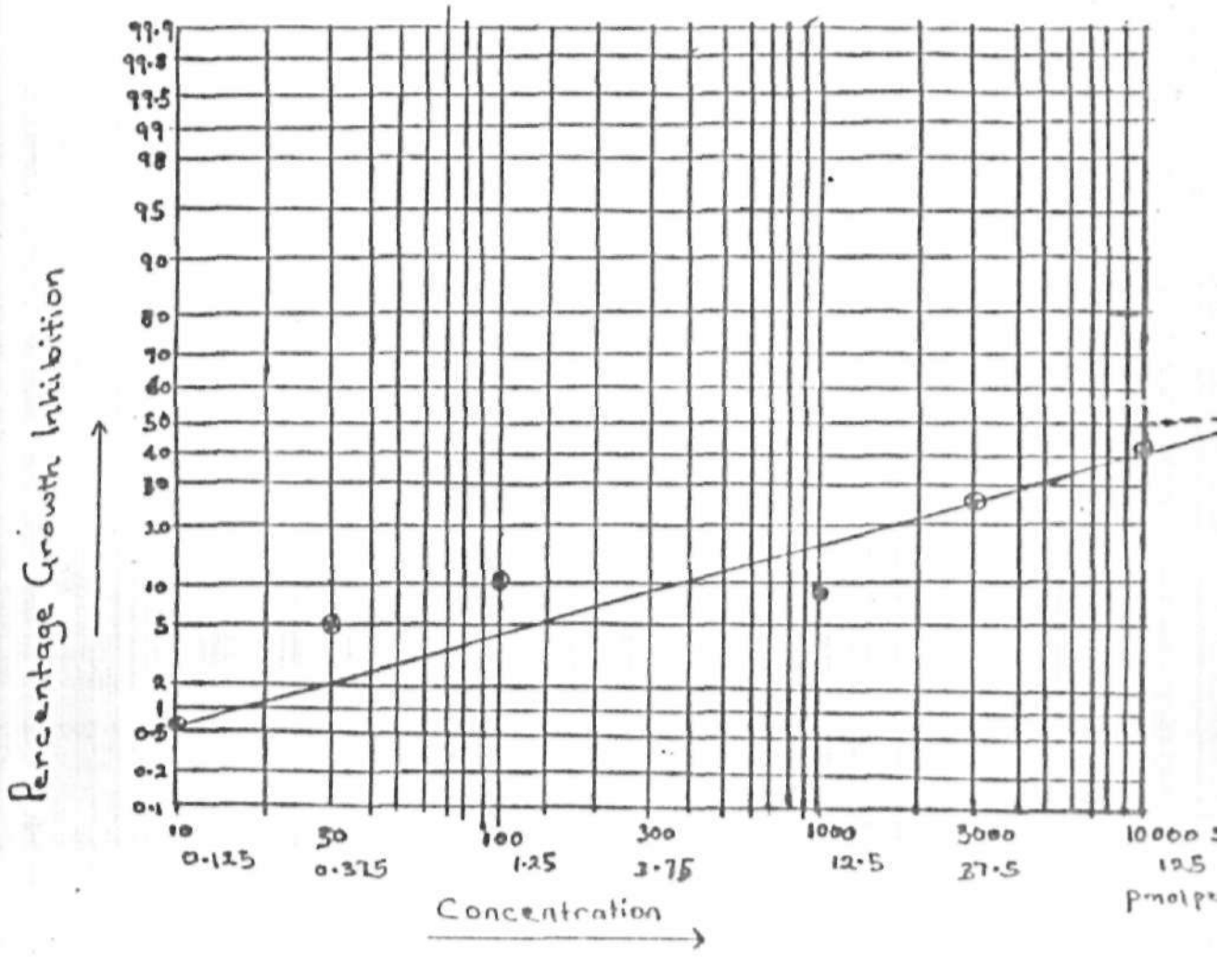
In 8 (61.54%) of the 13 isolates, schizont maturation was sufficient in the control well to permit interpretation of the test result, while schizont growth in 4 (30.77%) was not satisfactory, that is, percentage growth in the control well was below 20. The remaining isolate (No 192) was found to be a mixed infection with *P. malariae*, and this was therefore excluded. One isolate (12.5%) of the 8 isolates with sufficient schizont maturation was contaminated with yeast.

In all, no isolate was found sensitive to sulphadoxine/pyrimethamine combination. *Table 7* presents schizont counts in wells A to H for the 8 successful *P. falciparum* isolates. The table shows that for all isolates, schizont growth was present even at the highest sulphadoxine/ pyrimethamine concentrations in well H, indicating *in-vitro* resistance. It is also evident that inhibition of all 8 isolates was below 50% even in well H. Figure 3 shows the regression line as obtained in application of probit analysis to the result presented in table 7. On projection, ED50 is greater than the highest sulphadoxine/ pyrimethamine concentration of 10.000 pmol/125 pmol in well H. It was therefore not possible to precisely determine the ED50 for the 3 *P.*

falciparum isolates. There was correlation between *in-vivo* and *in-vitro* tests as 6 (75%) of the *in-vivo* resistant cases were resistant *in-vitro*, while the remaining 2 (25%) *in-vitro* resistant cases had delayed clearance *in-vivo*.

Figure 3

Percentage inhibition against concentration of 42 P.falciparum isolates cultured at A.B.U. Teaching Hospital, Zaria, 1988.



3.3 RESULT OF THE SURVEY:

The use of sulphonamide containing drugs [co-trimoxazole, sulphadoxine/pyrimethamine, phthalyl-sulphathiazole and sulphadimidine] was quantified over a period of 30 days. The survey reveals that of these drugs in Zaria, cotrimoxazole is the most frequently prescribed and used compound. This is followed by phthalylsulphathiazole. *Figure 4* presents the histogram showing the number of prescriptions of sulphonamide-containing drugs. It is seen that sulphadoxine/pyrimethamine was not as frequently used as cotrimoxazole and phthalyl-sulphathiazole and in 2 of the 3 investigation centres it was not even prescribed and dispensed. Due to some technical problems, it was not possible to record the overall use of antibiotics dispensed in each area. It was therefore difficult to express the figures obtained as the percentage of the total antibiotics issued. Table 8 presents these figures.

Table 8

Number of prescriptions of sulphonamide-containing drugs over a 30 day period in Zaria urban area, November - December, 1988

Sources	Drugs			
	1	2	3	4
A	353	84	60	7
B	67	26	1	8
C	352	0	0	0
Total	772	110	61	15

Key: -

A = A. B. U (Main Campus) Health Clinic

B = Dangi Pharmacy, Samaru

C = A. B. U. Teaching Hospital Zaria.

1 = Co-trimoxazole

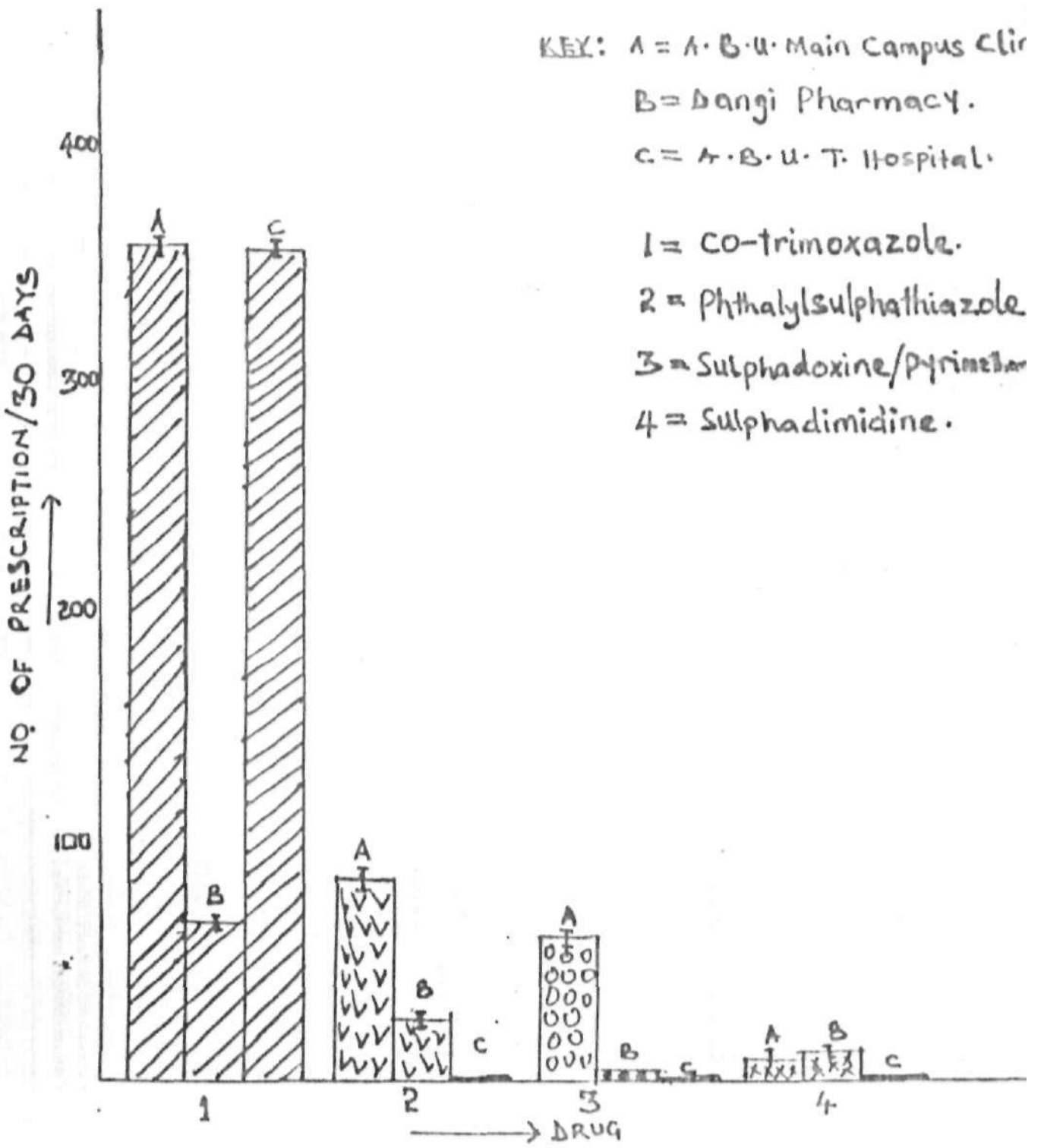
2 = Phthalylsulphathiazole

3 = Sulphadoxine/Pyrimethamine

4 = Sulphadimidine

FIG. 4

Number of prescription of cotrimoxazole - containing drugs over a 30 day period in Zaria urban area, November - December, 1988.



KEY: A = A.B.U. Main Campus Clinic
B = Dangi Pharmacy.
C = A.B.U.T. Hospital.

1 = Co-trimoxazole.
2 = Phthalylsulphathiazole
3 = Sulphadoxine/Pyrimethamine
4 = Sulphadimidine.

3.4 RESULTS OF THE QUALITY CONTROL TEST:

The results showed that the sulphadoxine/ pyrimethamine combination used in this study contains 108.54% (21.7mg) of pyrimethamine and 90.91% (454.45mg) of sulphadoxine.

CHAPTER FOUR

4 DISCUSSION

In this study a single dose of sulphadoxine/pyrimethamine combination given as 35mg sulphadoxine plus 1.75mg pyrimethamine per kg body weight cleared parasitaemia within 7 days of initiating therapy in 36(81.8%) of the 44 children treated. The parasites did not reappear within the remaining 21 day test period. *P. falciparum* was therefore fully sensitive to sulphadoxine/ pyrimethamine *in-vivo* in 81.8% of our children. These results are in agreement with previous reports of Olatunde *et al*, (1981) and Breman *et al* (1988) from Nigeria. Up to date, *P. falciparum* resistance to sulphadoxine/pyrimethamine has been sporadic in West Africa (Pierce *et al*, 1988; Gubler, 1988). Similarly, *P. falciparum* is still largely sensitive to sulphadoxine/pyrimethamine in East Africa (Watkin *et al*, 1988; Deloron *et al*, 1988). For the sensitive cases the mean parasite clearance time was 3.65±1.24 days indicating a delayed parasite clearance when compared to the results obtained by Olatunde *et al*, (1981) and

Nguyen-Dinh *et al*, (1984). The mean parasite clearance time of this study is similar to the ones obtained for chloroquine in the same area by Midala *et al*, (1988) (3.45±1.23 days in 1985) and Lege-Oguntoye *et al*, (1989) (3.65±1,23 days in 1987) respectively.

Our results for the 36 sensitive cases also indicate a good correlation between parasitological and clinical responses. The decline in fever and gastointestinal symptoms correlated well with the parasitological response. Though parasitaemia persisted beyond day 7 in the remaining 8 (18.2%) cases, parasite density sharply decreased below 25% of the pretreatment level on day 2 except in one case (No 488) where parasitaemia was markedly reduced on day 4. Similarly, the 8 patients became asymptomatic within 5 days, this is an indication of an initially good correlation of clinical and parasitological responses. There was however no such relationship found after day 5 in that there was a persistent low level parasitaemia up to day 21 without clinical evidence of malaria in the 8 children. As none of the 8 children had evidence of clinical malaria, a second course of treatment was not given. Surprisingly, all patients became

aparasitaemic by day 28. According to Wernsdorfer and Kouznestov (1980) these findings may be interpreted as low level (RI) resistance, in semiimmune populations. This interpretation seems to be supported by the fact that the 6 parasite isolates obtained from the 8 children were all found resistant to the drug combination *in-vitro*. The obvious disparity of *in-vivo* and *in-vitro* sensitivities in the 8 resistant cases may be explained by the high level of immunity that children of the study area were found to exhibit (Lege-Oguntoye *et al*, 1989)

A comparison of the *in-vivo* and *in-vitro* results of this study shows that all cases of *in-vivo* resistance except patients 135 and 143 were also resistant *in-vitro*. The isolates of these two patients could not be cultured due to technical problems. The remaining two *in-vitro* resistant cases (nos. 378 and 386) were sensitive *in-vivo* but showed a delayed parasite clearance on days 5 and 6, respectively. This further explains the influence of immune status of the patients. Similar to results obtained by Spencer *et al*, (1984) sulphadoxine/pyrimethamine combination failed to inhibit growth in 8 (19%) of the 42 *in-vitro* tests. This is therefore a clear indication

of resistance of *P. falciparum* to this drug combination *in-vitro*.

The reason for the appearance of *P. falciparum* resistance to sulphadoxine/ pyrimethamine in Northern Nigeria is not quite clear. Though pyrimethamine resistance of the parasite has been described in the area as early as 1960 (Archibald, 1960; Dodge, 1966), a moderate level of pyrimethamine resistance alone is probably not responsible (Onori, 1984). Resistance of *P. falciparum* to the sulphadoxine/pyrimethamine combination seems to be linked to pyrimethamine resistance(Lamont and Darlow, 1982; Spencer, 1985). However, Nuyen-Dinh *et al*, (1982) have reported that the presence of a medium level of pyrimethamine resistance per se does not preclude the curative effects of the drug combination. Onori, (1984) suggested that it may be a neccessary condition for the development of resistance to sulphadoxine/pyrimethamine combination that *P. falciparum* should be resistant to both pyrimethamine and sulphadoxine. Investigations in East Africa have shown that the parasites retain sensitivity to sulphadoxine/ pyrimethamine combination in an area of pyrimethamine resistance as long as they remain

sensitive to the sulphadoxine component (Watkins *et al*, 1988). It is unlikely therefore that parasitaemia in children persisted *in-vivo* because they were 'natural non-responders', a phenomenon that was reported from Thailand (WHO, 1984 (a)) but not comfirmed in investigations conducted in Nigeria (Olatunde *et al*, 1981) and East Africa (Spencer, 1985).

Our small drug survey indicates that sulphonamide-containing drugs are very often precribed in the study area, but further investigations are needed to assess the susceptibility of *P. falciparum* to the sulphonamides in Zaria urban area.

Subcurative doses were not used in this study as: sulphadoxine/pyrimethamine was administered under supervision. In addition patients were monitored for 30 minutes to rule out vomiting; and the drug combination was subjected to a quality control test where it was found to contain the standard doses of sulphadoxine and pyrimethamine.

It is apparent from this study that only those isolates that were resistant *in-vivo* grew *in-vitro*. No *in-vivo* sensitive isolates grew *in-vitro*. Why this is so, is not known, but it is an interesting revelation that requires further investigation.

CHAPTER 5

5. CONCLUSION

It can be concluded from this study that there is an incidence of 18.2% *in-vivo* *P. falciparum* resistance at RI level to sulphadoxine/pyrimethamine in Zaria urban area. The *in-vitro* results do not however permit definite conclusion regarding the degree of resistance, but schizont maturation at 10,000 pmol sulphadoxine/125 pmol pyrimethamine is a sign of high level of *P. falciparum* resistance to the drug combination.

In view of these findings therefore sulphadoxine/pyrimethamine combination should be strictly reserved for the treatment of *P. falciparum* malaria where parasitaemia and clinical evidence of malaria fail to respond to therapeutic doses of chloroquine. A policy for the rational use of antimalarials and antibiotics should be promulgated to reduce drug pressure, population of resistant mutants and to prevent cross-resistance among antimalaria drugs.

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APPENDICES

APPENDIX A

Absolute parasite and leucocyte counts and parasite densities per mm^3 of 44 patients with *P. falciparum* malaria followed up *in vivo* for 28 days after a single therapeutic dose of sulpadoxine/pyrimethamine in Zaria urban area, 1988.

L.C = leucocyte count; P.C = Parasite count; P.D = Parasite density/mm^3

S/No		0	1	2	3	4	5	6	7	14	21	28
	L.C	406	288	1002	1040	1010	1002	1010	1034	1020	1006	1000
080	P.C	503	510	18	0	0	0	0	0	0	0	0
	P.D	7433	10625	108	0	0	0	0	0	0	0	0
	L.C	100	1010	1020	1007	1000	1002	1000	1006	1040	98	-
098	P.C	507	168	0	0	0	0	0	0	0	0	0
	P.D	30420	998	0	0	0	0	0	0	0	0	0
	L.C	1020	481	1008	1015	1009	1000	1003	1007	1004	1000	1000
128	P.C	278	512	2	0	0	0	0	0	0	0	0
	P.D	1635	6,387	12	0	0	0	0	0	0	0	0

S/No		0	1	2	3	4	5	6	7	14	21	28
	L,C	147	44	1007	1016	1003	1026	1019	1020	1009	61	1016
135	P,C	523	503	91	31	18	3	5	1	2	0	0
	P,D	21347	68591	542	183	109	18	29	6	12	0	0
	L,C	335	1013	1011	1006	1000	-	1007	1009	1001	1007	1003
143	P,C	525	469	1	2	1	-	1	0	15	2	0
	P,D	9403	2778	6	12	6	-	6	0	95	12	0
	L,C	215	1020	1013	-	1004	-	1002	1006	1009	1005	1000
145	P,C	521	10	1	-	0	-	1	2	0	0	0
	P,D	14540	59	6	-	0	-	6	12	0	0	0
	L,C	270	1003	1036	1005	1001	1009	1003	1007	1010	1001	1004
146	P,C	510	73	21	6	2	2	3	5	3	3	0
	P,D	11333	437	122	36	12	12	18	30	18	18	0
	L,C	316	1009	1009	1000	1002	-	1000	1006	1001	1003	1000
153	P,C	513	81	28	3	5	-	2	0	0	0	0
	P,D	9741	484	167	18	36	-	12	0	0	0	0
	L,C	75	971	1006	1009	1003	-	1000	1001	-	1007	1001
160	P,C	513	502	9	3	0	-	0	0	-	0	0
	P,D	41040	3102	54	18	0	-	0	0	-	0	0

S/No		0	1	2	3	4	5	6	7	14	21	28
	L.C	230	-	-	1006	1003	-	1009	1000	-	1009	1003
172	P.C	504	-	-	0	2	0	0	0	-	0	0
	P.D	13148	-	-	0	12	0	0	0	-	0	0
	L.C	96	793	1000	1001	-	-	1006	1003	1001	1001	1004
191	P.C	539	506	361	21	-	-	13	6	3	0	0
	P.D	33668	3828	2166	126	-	-	78	36	18	0	0
	L.C	98	113	1016	1001	-	1003	1001	1000	-	1004	1002
196	P.C	513	516	2	1	-	0	0	0	-	0	0
	P.D	31408	27398	12	6	-	0	0	0	-	0	0
	L.C	59	316	1007	1001	-	1000	1007	-	1003	1000	-
199	P.C	540	519	79	2	-	0	0	0	0	0	-
	P.D	54316	9854	470	12	-	0	0	0	0	0	-
	L.C	59	31	1006	-	1015	1007	1001	1003	1004	1002	-
234	P.C	532	539	61	-	0	0	0	0	0	0	-
	P.D	54102	103935	364	-	0	0	0	0	0	0	-
	L.C	41	1002	1036	1021	1047	1051	1030	1039	1008	1008	1000
319	P.C	549	6	0	0	0	0	0	0	0	0	0
	P.D	80341	36	0	0	0	0	0	0	0	0	0

S/No		0	1	2	3	4	5	6	7	14	21	28
	L,C	413	819	1026	1031	1007	1007	1006	1011	1001	1020	1007
369	P,C	523	504	4	0	0	0	0	0	0	0	0
	P,D	7598	3692	23	0	0	0	0	0	0	0	0
	L,C	130	427	1008	1110	1000	1016	1000	1000	1002	1007	1001
378	P,C	516	512	350	96	78	18	0	0	0	0	0
	P,C	23815	7194	2083	519	468	106	0	0	0	0	0
	L,C	813	1001	1009	1014	1016	1021	1009	1013	1023	1003	1005
382	P,C	509	479	7	3	2	0	0	0	0	0	0
	P,D	3756	2871	42	18	12	0	0	0	0	0	0
	L,C	160	264	1017	1062	1013	1014	1000	1038	1014	1010	1000
386	P,C	512	500	156	68	18	0	0	0	0	0	0
	P,D	19200	11364	920	384	107	0	0	0	0	0	0
	L,C	30	-	1001	1000	1010	1000	1001	1008	1000	1000	1004
398	P,C	502	-	100	4	4	0	0	0	0	0	0
	P,D	100400	-	599	24	24	0	0	0	0	0	0
	L,C	26	59	1018	1000	1001	1050	1000	1002	1000	1000	1006
400	P,C	686	572	200	20	5	19	0	0	0	0	0
	P,D	158308	59172	1179	120	30	109	0	0	0	0	0

S/No		0	1	2	3	4	5	6	7	14	21	28
	L.C	37	65	1001	1000	1006	1000	1002	1000	1000	-	1009
401	P.C	502	544	259	91	52	6	5	0	0	-	0
	P.D	81405	50215	1552	546	370	36	30	0	0	-	0
	L.C	69	1000	1008	1000	1002	1005	1000	1010	1002	1000	1009
404	P.C	522	300	102	0	0	0	0	0	0	0	0
	P.D	45391	1800	507	0	0	0	0	0	0	0	0
	L.C	66	332	1000	1002	1018	1004	1000	1026	1001	1000	1003
405	P.C	534	504	168	37	28	27	23	15	18	8	0
	P.D	48545	9108	1008	222	165	161	138	88	108	24	0
	L.C	14	247	-	1004	1001	1000	1026	1002	1000	1007	1010
406	P.C	520	529	-	30	9	0	0	0	0	0	0
	P.D	222857	12850	-	179	0	0	0	0	0	0	0
	L.C	69	1024	1100	622	1001	100	1003	1007	1000	1000	1003
414	P.C	502	390	334	504	320	14	4	0	0	0	0
	P.D	43652	2285	1822	4862	1918	84	24	0	0	0	0
	L.C	53	50	240	1000	1010	1004	1002	1021	1012	1000	1021
426	P.C	642	505	520	200	140	0	0	0	0	0	0
	P.D	72679	60600	13000	1200	832	0	0	0	0	0	0

S/No		0	1	2	3	4	5	6	7	14	21	28
	L.C	24	31	-	-	-	1002	1010	-	1004	1007	1001
436	P.C	590	570	-	-	-	0	0	-	0	0	0
	P.D	147500	110323	-	-	-	0	0	-	0	0	0
	L.C	37	443	1000	-	-	1096	1020	1032	1000	1000	1000
440	P.C	561	536	420	-	-	80	213	165	95	0	0
	P.D	90973	7260	2520	-	-	438	1253	959	576	0	0
	L.C	41	87	1092	1011	1007	1000	1009	1002	1001	1007	1000
441	P.C	604	612	0	0	0	0	0	0	0	0	0
	P.D	88390	42206	0	0	0	0	0	0	0	0	0
	L.C	36	58	1020	1002	1001	1011	1004	1007	1005	1001	1000
476	P.C	552	651	0	0	0	0	0	0	0	0	0
	P.D	92009	67344	0	0	0	0	0	0	0	0	0
	L.C	145	404	216	129	1009	1004	1011	1004	1007	1010	1000
488	P.C	521	523	526	555	143	244	95	21	0	0	0
	P.D	21559	6483	14611	25814	850	1458	564	125	0	0	0
	L.C	95	98	1022	1012	1008	1020	1002	1007	1010	1004	1000
491	P.C	631	553	0	0	0	0	0	0	0	0	0
	P.D	39853	33857	0	0	0	0	0	0	0	0	0

S/No		0	1	2	3	4	5	6	7	14	21	28
	L.C	543	1051	639	1008	1003	1000	1007	1010	1000	1010	1004
516	P.C	514	422	502	10	0	0	0	0	0	0	0
	P.D	5680	2409	4713	60	0	0	0	0	0	0	0
	L.C	61	282	1000	1004	1000	1000	1004	1015	1000	1020	1001
517	P.C	513	514	230	23	15	0	0	0	0	0	0
	P.D	50459	10936	1380	137	90	0	0	0	0	0	0
	L.C	87	1008	1012	1002	1004	1000	1000	1006	1000	1011	1008
518	P.C	501	56	50	35	0	0	0	0	0	0	0
	P.D	34532	2714	256	210	0	0	0	0	0	0	0
	L.C	143	325	1007	-	1011	-	-	1000	-	-	1002
523	P.C	541	515	88	-	0	-	-	0	-	-	0
	P.D	22699	9508	524	-	0	-	-	0	-	-	0
	L.C	47	240	1000	1007	1009	1000	1000	1004	1000	1005	1010
524	P.C	613	517	156	21	0	0	0	0	0	0	0
	P.D	78255	12925	936	125	0	0	0	0	0	0	0
	L.C	834	476	1005	1000	1007	1000	1003	1000	1009	1008	1004
536	P.C	505	513	18	0	0	0	0	0	0	0	0
	P.D	3633	6466	107	0	0	0	0	0	0	0	0

S/No		0	1	2	3	4	5	6	7	14	21	28
	L,C	110	420	1009	1020	1000	1009	1000	1007	1005	1000	1008
542	P,C	507	501	219	110	0	0	0	0	0	0	0
	P,D	27655	7157	1302	647	0	0	0	0	0	0	0
	L,C	283	1002	-	1006	1002	1004	1000	1000	1010	-	-
544	P,C	505	155	-	0	0	0	0	0	0	-	-
	P,D	10707	928	-	0	0	0	0	0	0	-	-
	L,C	101	1022	1010	1000	1000	1008	1000	1005	1000	1000	1004
547	P,C	536	170	8	0	0	0	0	0	0	0	0
	P,D	31842	938	48	0	0	0	0	0	0	0	0
	L,C	19	215	1007	1005	1010	1000	1000	1005	1000	1010	1002
566	P,C	544	533	200	25	0	0	0	0	0	0	0
	P,D	171789	14874	1192	149	0	0	0	0	0	0	0
	L,C	612	1001	1000	1003	1000	1009	1007	1000	1008	1002	1000
569	P,C	509	395	0	0	0	0	0	0	0	0	0
	P,D	4990	2368	0	0	0	0	0	0	0	0	0

IN-VIVO RECORD FORM

SERIAL NO. _____ (DO) DATE _____

State: _____ Age _____

Local Govt: _____ Sex: /M/ or /F/

Name: _____ Weight: _____

Mother's Name: _____ Father's Name: _____

Address: _____ Address: _____

History of Antimalaria Drug Ingestion past 7 days* Yes or No

*If yes, circle Chloroquine, Pyrimethamine/Sulphonamide, Quinine, other (name) Quantity Given:

Activity	D0	D1	D2	D3	D4	D5	D6	D7	D14	D21	D28
Date											
Urine tract											
Temperature											
CQ base (mg)											
Tablet (No.)											
Syrup(ml)											
Time dose given											
Clinician's impression[b]											
Diarrhoea[b]											
Pruritus[b]											
Vomiting											
PARASITOLOTY											
Leucocytes											
Asexual parasite											
Parasites/mm^3											
Parasites/mm^3											

a. Fever (37.5°C) or history of fever in preceding 48 hrs = 1; no measured fever & no history of fever = 0; child not well (decreased activity & mental alertness) = 1; child well = 0. (Examples - 0+0 = 1+0 = 1; 1 + 1 = 2); - diarrhoea = 3 stools/day in past 48 hours

b. in past 48 hours.

Appendix C

Body temperature of the 44 patients with *P. falciparum* malaria after a single therapeutic dose of sulphadoxine/pyrimethamine in Zaria urban area, 1988

S/No	D0	D1	D2	D3	D4	D5	D6	D7	D14	D21	D28
080	38,5	37,5	36	36	36,3	36	36	-	-	-	-
098	37	36,5	36	37	36	36	37,8	36	-	-	-
128	37,5	37	36	36	37	36,8	36,5	36	36,8	36	36
135	37,5	36	36	36,5	36,6	36,3	36,0	36	36	36,5	36,5
143	36,5	36	36,4	36,5	36,5	-	37	36	36	36,5	36,5
145	39	37,5	36	36,5	36,3	-	37	37	37	36,5	36,8
146	37	36	36	36,5	-	-	36	36	36	36,5	36,8
153	37,5	36	37	36	37	-	36	36	36	36,5	36,5
160	37,5	36	37	36,5	36	-	36,8	36	-	36	36,6
172	37	-	37,6	36	-	-	37	37	-	36	36,6
191	38,7	37	37,8	37	-	-	36,5	36	36,5	36	36,5
196	38,9	37,3	36,8	36,3	-	36	36	36,5	-	-	36
199	39,5	37,8	37	37	-	36	36,3	-	36,5	36,6	-
234	40,0	37,8	37,3	-	36,6	36,5	36,6	36,6	36,6	36,6	36,8
319	36,3	37,5	36,5	36,5	36	36	36	36,7	36,8	-	-
369	38	37	-	36	36	36	36	36,5	36,8	36,9	36,9
378	38	37	36,8	36,6	36,5	36,5	36,5	36,5	36,3	36,5	36
382	38	37	37	36,8	37	36,8	36,5	36	37	36,8	36
386	37,8	37	36,5	36	36,5	36	37	36	36,8	37	36
398	38	-	37	36	36	36,5	36,5	36	36	37	37
400	39	37	37	36,5	36,5	36,5	36,8	37	36,8	-	37
401	40,5	37	38,5	37	36	36	36,3	36,8	36,8	-	36

S/No	D0	D1	D2	D3	D4	D5	D6	D7	D14	D21	D28
404	37,5	38	37	36	36	36	36,5	36,8	37	37	36
405	37	37	37,5	36	36,5	36,5	36,5	36,5	37	37	36
406	38,1	37	-	36	36,5	36,5	37	37	36,8	36,8	36
414	38,5	37,8	37,3	36,8	36,6	36,5	36,8	36	36	-	36
426	38,6	37	37,8	37	36,8	36,5	36,0	36	36	37	37
436	39,8	36,5	-	-	-	-	-	-	37	37	37
440	37,8	38,6	36,5	-	-	36,5	36,8	36	-	-	-
441	38,5	37	36	36	36,8	36	36	36	36	36	36,5
476	39,2	37,5	36,8	38,5	38	37	37	37	36	36	36,5
488	38,6	37	37	37	37	36	36	36	36	-	36,5
491	38	37	37,5	36,5	36	36,5	36	36	36	36,5	36,4
516	37,3	37	-	36,8	36,8	36,5	37	37	36,7	36,5	36
517	37,2	37,2	-	37,2	36,5	-	36	37	-	36	36
518	39	37	-	36,2	37	36,5	36,5	37	36	36	36
523	37	37	37,3	-	-	-	-	-	37	-	37
524	38,2	37	-	36	36,5	36,5	36	36,5	36,5	37	37
536	37,2	37	36,5	36,8	36,5	37	36,7	36,3	36,8	36	36
542	37,5	36									
544	39,1	38	37	37	37	36	-	36,5	-	-	-
547	38	37	-	36,8	36,5	36,8	36,9	36,6	36,4	36	-
566	37	37,2	36,5	36,8	36,5	37	37	37	36,8	36,8	37
569	37,8	36,7	36,8	36,5	36,5	37	37	36,6	36,5	36	37

APPENDIX D.

Schizont maturation for the 8 successful *P. falciparum* isolates.

Serial No. 145 Date 13.8.88

SDX/PYR	Trophozoites	Schizonts
A	205	53
B	210	55
C	204	48
D	203	43
E	207	42
F	213	33
G	201	31
H	205	26

Serial No. 146 Date 13.8.88

SDX/PYR	Trophozoites	Schizonts
A	204	84
B	204	90
C	202	90
D	203	71
E	204	101
F	201	74
G	205	69
H	206	74

REMARKS:

Appendix D.

Serial No. 191 Date: 24.8.88

SDX/PYR	Trophozoites	Schizonts
A	202	47
B	205	42
C	205	54
D	204	37
E	201	63
F	204	58
G	203	51
H	207	41

Serial No. 378 Date: 21.10.88

SDX/PYR	Trophozoites	Schizonts
A	202	34
B	205	35
C	203	37
D	203	37
E	204	61
F	204	51
G	206	33
H	207	29

REMARKS: 378 is sensitive *in-vivo* with delayed clearance

Serial No. 440 Date: 21.10.88

Appendix D_4

SDX/PYR	Trophozoites	Schizonts
A	201	52
B	207	46
C	208	43
D	207	47
E	201	25
F	204	31
G	202	21
H	205	5

Serial No. 488 Date: 8.12.88

SDX/PYR	Trophozoites	Schizonts
A	207	108
B	204	113
C	204	93
D	208	91
E	202	112
F	207	100
G	214	54
H	206	40

REMARKS:

Appendix E

In-vitro materials

	WHO standard *in-vitro* micro-test kits, containing:	Quantity
1.	Tissue culture plates, each plate has 12x8 wells predosed with sulphadoxine/pyrimethamine (SDX/PYS): 0,10,30,100,300,1000,3000,10,000 pmol SDX; 0,0,125, 0,375,1,25,3,75,12.5,37.5 pmol PYR per well (80:1)	6
2.	Eppendorf pipette, 50µl	1
3.	Eppendorf pipette tips, sterile , 0-0,2ml	100
4.	RPmi 1640 LPLF, sterile, 100ml, Batch III (1987) with gentamycin, Amimed A6 Austrasse 35, Switzerland,	1
5.	L-glutamine Vials, sterile for 10ml 1640 LPLF, Batch III (1987), Amimed AG, Basel, Switzerland,	10
6.	Presscap falcon tubes, sterile, 6ml	100
7.	Sterile disposable syringes, 20ml	50
8.	Sterile 18"x20 guage needle	50
9.	Microscope slides, frosted edge (box of 72 or 50) 75 x 25 x 1,0mm	10
10.	Scalpel holde and blade	1
11.	Forceps	1
12.	Aluminium foil (roll of 30m x 30cm)	1
13.	Heparinized sterile capillary tubes, 100µl (tubes of 20)	50
14.	Test tube rack	1

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WHO standard *in-vitro* micro-test kits, containing:	Quantity
15, Labelling ball pen	3
16, Cotton wool, 3 rolls /½g	15
17, Sterile 1ml syringes and needle	50
18, Untreated, non-sterile capillary tubes, 50ul	50
19, Bulb for capillary tubes	3
20, 266 3/8" disposable sterile needle or Autolet Fricker	300
21, Pure paraffin candle	4
22, Matches	1pkb
23, Incubator, Gallenkamp, size one	1
24, Candle jar	1
25, 1ml non-sterile pipette	1
26, Staining plates	2
27, Timer (stop - clock)	1
28, Ditilled water	
29, Heparin, 5ml (25,000 i,u perml) LEO pharmaceutical products, Denmark, Expiary date : 89 12	1
30, Buffer solution, pit 7.2	
31, Hydrochloric acid (25%), Sigma USA, Density, 1,18, purity 36%,	
32, Dowcorning® high vacum grease; N,V, Dow corning S,A; Snaffe Belgium,	1