BIOCHEMICAL STUDIES ON SOME PLANT EXTRACTS IN USE AS ANTI-MALARIALS IN WESTERN NIGERIA

BY

ADEBIMPE ADETOUN ODETOLA
B.Sc. (Hons), (Ibadan)

A thesis in the Department of Biochemistry

Submitted to the Faculty of Medicine
in partial fulfilment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

September 1975

ABSTRACTS

In order to investigate the antimalarial property of some local medicinal plants namely Azadirachta indica, Morinda lucida, Alstonia boonei, Enantia chlorantha and some mixtures of plants used especially in the Western State of Nigeria, experimental malaria, induced in mice and chicks with Plasmodium berghei and Plasmodium gallinaceum respectively, was treated with the plant extracts prepared as used traditionally using as criteria of cure their effects on

(a) the level of parasitemia in the treated animals compared with untreated controls,

(b) the body temperature

(c) serum total proteins

(d) serum protein fractions

(e) serum glucose level

(f) serum total bilirubin

(g) serum enzymes, glutamic pyruvate transaminase (GPT), leucine amino peptidase (LAP) and alkaline phosphatase.

The results of such experiments in mice showed that none of the six plant extracts tried had anti-malarial properties against rodent malaria. In chicks only the water extract of leaves of Morinda lucida at a high concentration had some

suppressive action against this avian malaria but the effect was insignificant compared with the effect of Chloroquine which was used as the standard drug. In both mice and chicks the extracts had no beneficial effect on the values of some serum biochemical constituents that were studied.

Results of an investigation of the extracts of the two most popular plants - Morinda lucida and Azadirachta indica on some hospitalised malaria patients showed no beneficial effect on the patients as evidenced by deteriorating clinical symptoms which improved after administration of chloroquine sulphate.

When tried on normal animals, some of the plant extracts had some adverse effects on the animals. Prolonged use of some of them might be toxic to the liver.

Since these drugs do not possess antimalarial properties comparable with any of the existing antimalarial drugs, and in view of their toxic effect on the liver, it is puzzling to note their wide spread use as curative agents in peasant communities in Nigeria.

This thesis is dedicated to my husband, Adebayọ and my children, Ibironkẹ, Olumide and Ọpẹyẹmi.

ACKNOWLEDGEMENTS.

I wish to express my profound gratitude to my supervisor, Professor Olumbe Bassir, for his valuable guidance, interest and encouragement during the period of this study. I am also very grateful to him as the Head of the Department, for placing every facility in his department at my disposal.

I would like to thank the University of Ibadan for giving me a scholarship for this course.

I am grateful to the academic and technical staff of Biochemistry Department and my fellow research students, for their academic advice and technical assistance.

My gratitude goes to the Deputy Director of the Health Centre, Dr. S. O. Ademle and his staff, for their cooperation in the clinical studies carried out in the health centre.

Finally, my profound gratitude goes to my husband, Bayo, for his ~~patience, understanding~~, support and encouragement throughout the period of study; my parents Rev. and Mrs. E. A. Adedayo and every member of my ~~family~~ for their constant encouragement and to my husband's relations who have been most understanding and kind. Lastly, I would like to thank Dr. and Mrs. Adetuyibi and family for their most generous hospitality while I lived with them.

CERTIFICATION.

I certify that this work was carried out by Mrs. Adebimpe Adetoun Odetola in the Department of Biochemistry University of Ibadan, Ibadan, Nigeria.

SUPERVISOR.

Professor O. Bassir Ph.D. D.Sc.
Head of Department of Biochemistry,
University of Ibadan, Ibadan,
NIGERIA.

September, 1975.

CERTIFICATION

I certify that this work was carried out by Mrs. Adebimpe Adetoun Odetola in the Department of Biochemistry University of Ibadan, Ibadan, Nigeria.

SUPERVISOR.

Professor O. Bassir Ph.D. D.Sc.

Head of Department of Biochemistry,

University of Ibadan, Ibadan,

NIGERIA.

September, 1975.

TABLE OF CONTENTS

CONTENTS

TABLE OF CONTENTS

CONTENTS

CHAPTER THREE: METHODS.

CHAPTER FOUR: EXPERIMENTS AND RESULTS.

CHAPTER ONE

INTRODUCTION

1 History of herbal medicine in Nigeria.

The use of locally prepared medicine has been long in existence in Nigeria. Herbalists, 'native doctors' and 'juju men' usually dispense these local medicines and their treatment often involves the use of entire plants, roots, stems, barks, fruits, woods, juices or exudates of local plants. The local medicines in use are by no means limited to plant materials. Sometimes whole animals, blood, saliva and even human parts are included in the preparation of some of these medicines. Usually these medicines are in the form of infusions, powders, soaps, ointments and fumigations which are far from the usual presentation of orthodox medication.

The knowledge of the properties of drug plants shown by local medicine men may either have been passed on to them by their elders or may be based on experience. Frequently neither the 'doctor' nor the patient attributes the healing power to the plant itself but to some occult power or spirit of the plant which is stronger than the disease spirit, (Lambo 1974) In such cases the 'doctor' claims to get his directions from his god, which in many parts of the Western State is the Ifa, and in such cases the preparation of the medicine may involve sacrifices to the gods and incantations over the plant materials to be used.

Thus the traditional medicine is still very much linked with local religions and cults.

Sometimes uses of medicinal plants are also empirical and are based on the belief that nature has provided a plant for every disease and has indicated by an obvious sign for which disease or for which part of the body each drug plant is to be used. This belief also existed in Europe in the Middle Ages, and a classical example given by Oliver (1960), was the walnut which, having the shape of the brain should therefore be used for diseases affecting this. Similarly in West Africa, plants with white latex are used to increase milk production; a typical example is the use of palm wine, an exudate of palm trees, by some Yorubas to improve lactation in nursing mothers.

In many cases, a serious ailment appears to the local medicine men to call for violent actions; for instance leprosy or snake-bite are often treated with strong vesicants while dysentery is treated with a violent purge.

It was the belief in the past that diseases were caused by evil spirits. Therefore purgatives, emetics and diuretics were amongst the favourable drugs used to chase the evil spirit from the body and are therefore used indiscriminately for fevers, leprosy, venereal diseases, dysentery or even rheumatism.

In some cases the treatment may be purely symptomatic. For example the drug may be able to relieve pains due to an infection but with no destructive action on the organism responsible for the infection.

These methods of selection and use of drug plants explain why in some cases, the recovery of the patient may be attributed to his faith or his natural resistance rather than to the herbalist's skill.

2 Some medicinal plants used for malaria fever in some parts of the Western State of Nigeria

A general survey of the plants commonly used against malaria was conducted in some local markets where medicinal plants are sold. During this survey it was necessary to differentiate between the different types of fevers. The dealers were able to differentiate malaria fever from other types of fevers by calling it 'rain fever', an indication of the fact that it is commonest during the rainy season. Others described it as '4 O'clock fever', an indication of its regular and intermittent nature. However, there was some overlap in the use of some of the plants, for while some were used exclusively for malaria others were used for both malaria and yellow fevers.

There were variations in the types of plants used from place to place and in the combination of the plants but the most frequently used and the most popular ones are those described below.

a Azadirachta indica.

Azadirachta indica which is also known as Neem, or Dogonyaro tree is known locally as 'Dogonyaro'. It is an evergreen tree which is about 80 foot tall. The leaves are usually bipinnate and it bears abundant pinacles of white flowers and small green yellow fruits. Hooking (1959).

It is a native of India but it has been introduced into other parts of the world (Hooking, 1959; Watt and Breyer-Brandwijk.1962). In Nigeria where it has naturalized, it is used to line avenues and fences. It is also planted in some hocrs because of its accredited medicinal value.

In India, its home of origin, the bark as well as that of its closely related Melia azadirach was used for the treatment of malaria before the introduction of quinine(Oliver, 1961). The seed oil, known as margosa oil was used in India in skin diseases like leprosy, but it is now used largely in the manufacture of cooking fats and soaps.

In Nigeria the plant is widely used. It's most important medicinal use is for fevers, especially malarial fever. For this, the leaves and bark are used in various ways. While some people boil only the leaves in water others boil the leaves, bark and other plant materials together in water or palm wine and not only drink the extract obtained but also bath with it and inhale the vapour.

The first reliable chemical investigations on the tree were carried out by Songupta et. al. 1960, who isolated nimbidin, nimbidol, nimbinin and nimbin from the tree. They also isolated a flavone called nimbicetin; a sterol called nimbosterol and a glycoside of nimbosterol called nimbosterin. Recently Songupta isolated sigiol and nimbiol from the trunk bark of the plant.

It has been reported that crude extracts of both Azadirachta indica and Melia azadirach show distinct anti-feeding activity against locusts and the plants themselves are unpalatable to and are not eaten by the desert locusts, (Nitra, 1963). Recently a locust phagorepellent called meliantriol was isolated by fractionation of the seed oil.

Fashina (1969) isolated nimbolide from the leaves and nimbin from the bark while Fabunle (1972) isolated some limonoids from the plant. It is interesting that so far no alkaloid has been isolated from the plant and that it is not possible to relate the bitterness of the extracts to the compounds so far isolated (Fashina, 1969). There is no doubt that this plant has some useful actions.

b. Morinda Lucida.

Morinda lucida is the common species of this plant in Nigeria. It is known locally as 'Oruwo' and appears to be the oldest known plant used against malaria.

It is a shrub with terminal clusters of white fragrant flowers and green fruits [illegible] locally the leaves

sometimes the bark and roots are all used in the treatment of malaria, yellow fever and jaundice, (Oliver 1960 and Singha, 1965) and sometimes in dysentery (Singha, 1965).

In Central Africa the leaf is used as a diuretic by inhaling the vapour from a boiling decoction or by bathing in the decoction. A decoction of the root and leaf is used as a purgative while scraping of the stem rubbed on the abdomen is used as an oxytocic (Watt and Breyer-Brandwijk, 1962). In the Ivory Coast the plant has been used as a leprosy remedy.

Chemically most morinda species contain tannins and methylanthraquinones as well as an alizarin derivative (Oliver, 1960). Morindin was isolated from Morinda citrifollia. A glycoside related to or identical with morindin was found in the root-bark of Morinda geminata Morinda umbellata and Morinda tinctora. On hydrolysis morindin yields a colouring matter, morindon, which is a trihydroxymethylanthraquinone (Hollroy, 1950).

c. Alstonia boonei

Alstonia boonei is known as Pattern wood. It is the common species of Alstonia in Nigeria. It is a tall forest tree with whorled leaves and small greenish white flowers.

Locally its wood is used for light carpentary; the leaves are used for malaria while its bark is sometimes used as a febrifuge and in external application for rheumatic pains. The latex is smeared on malabar swellings caused by a filarial parasite, Filaria malabarensis

In East Africa the various Alstonia barks are used in the localities where they occur as remedies for malaria (Watt and Breyer-Brandwijk, 1962).

The bark of Alstonia constricta is known to contain alkaloid echitamine, $C_{22}H_{28}O_4N_4$, (Henry, 1925). Goodson and Henry (1925) also showed the presence of echitamidine and a lactone in the bark. The root and leaf contain the alkaloid ditamine $C_{16}H_{19}O_2N$.

Alstonia constricta, another species of this plant is known to contain alstonine and it is reported to exert some antimalarial properties (Gollman, 1962). Hutchinson and Dalziel (1962) reported that the alkaloidal sulphates of Alstonia constricta are inactive against malaria but that some unspecified components of the leaves and twigs of Alstonia yunnanensis have slight anti-malarial activity.

It has been reported by Goodson and Henry (1925) that echitamine hydrochloride is toxic to mice in doses of 0.3 to 0.5 mg per 20gm and that it acts by paralysis of the medulla.

d. Enantia chlorantha

Enantia chlorantha is known as 'Afrio-yellow wood'. It is a tall forest tree with yellow wood, solitary flowers and black fruit carpels.

Both the alcoholic and water extracts of the bark of this tree are used against malaria and sometimes it is used as an antipyretic. It is said to be used as an ingredient in guéré arrow poison and as the basis of a yellow dye (Oliver, 1960).

Chemically, all Enantia species are known to contain the alkoloid berberine which is responsible for the bitter taste.

e. Various other drug plants.

In addition to the above named plants which are used singly in the preparation of malarial drugs, various other plants are used as components of drugs of more complex nature. These plants are not regarded as antimalarial drug plants on their own but they are used in combination with some of those mentioned earlier in various proportions. In particular the following are generally used:

(i) Cymbopogon citratus (lemon grass)

(ii) Psidium guajava (guava)

(iii) Mangifera indica (mango)

(iv) Carica papaya (pawpaw)

(i) Cymbopogon citratus

This plant is commonly known as lemon grass and it is a popular component of most drugs. It is used not only for malaria but also as a febrifuge. Its leaves contain a volatile oil, citronella, (Singha 1965).

(ii) Psidium guajava

The leaves of this plant are used most popularly for gastro-enteritis and diarrhoea; but they are also used in the preparation of malarial drugs. The plant is known to contain tannins, resin and essential oil, (Singha, 1965; Oliver 1960).

(iii) Mangifera indica

Mangifer- indica is commonly known as the mango tree. It's bark and leaves are used as astringent; lotion for mouthwash for relief of toothache, sore gums, sore throat and other mouth infections. An infusion of the root-bark is used for diarrhoea and dysentery while the juice of the trunk is used as an antisyphilitic. Its bark is used as a component of some malarial drugs. The plant contains tannins and rosins (Oliver 1960, Singha, 1965).

(iv) Carica papaya.

Carica papaya is known as the pawpaw tree. Medicinally the leaves and the latex of raw fruits are used for fevers, beriberi and as anthelmintic. Its infusion is used for stomach ache (Singha 1965). The plant contains papayotin.

3 MALARIA

a. Definition of malaria

The word 'malaria' in some dictionaries, is defined as an unwholesome or poisonous air (Webster, 1964). This definition is archaic and it reflects the belief in the 17th century that people who died of the disease succumbed to d'aria ("the air") or to mal'aria ("bad air") as an indication that the fatal illness had been caused by breathing noxious marsh air or 'miasma' (Wareshaw, 1949; and Russell et. al. 1963). Today malaria is recognized as a disease characterized by intermittent febrile paroxysms, anaemia and splenic enlargement. Caused by infection with parasites generally

(iii) Mangifera indica

Mangifera indica is commonly known as the mango tree. It's bark and leaves are used as astringent; lotion for mouthwash for relief of toothache, sore gums, sore throat and other mouth infections. An infusion of the root-bark is used for diarrhoea and dysentery while the juice of the trunk is used as an antisyphilitic. Its bark is used as a component of some malarial drugs. The plant contains tannins and resins (Oliver 1960, Singha, 1965).

(iv) Carica papaya.

Carica papaya is known as the pawpaw tree. Medicinally the leaves and the latex of raw fruits are used for fevers, boriberi and as anthelmintic. Its infusion is used for stomach ache (Singha 1965). The plant contains papayotin.

3 MALARIA

a. Definition of malaria

The word 'malaria' in some dictionaries, is defined as an unwholesome or poisonous air (Webster, 1964). This definition is archaic and it reflects the belief in the 17th century that people who died of the disease succumbed to d'aria ("the air") or to mal'aria ("bad air") as an indication that the fatal illness had been caused by breathing noxious marsh air or 'miasma' (Worsham, 1943; and Russell et. al. 1963). Today malaria is recognized as a disease characterized by intermittent febrile paroxysms, anaemia and splenic enlargement, caused by infection with parasites generally assigned to the genus Plasmodium (Russell et. al. 1963).

Chemically, all Znantic species are known to contain the alkoloid berberine which is responsible for the bitter teste.

e. Various other drug plants.

In addition to the above named plants which are used singly in the preperetion of malarial drugs, various other plants are used as components of drugs of more complex nature. These plants are not regarded as antimelarial drug plants on their own but they are used in combination with some of those mentioned earlier in various proportions. In particular the following are generally used:

(i) Cymbopogon citratus (lemon grass)

(ii) Psidium guajava (guava)

(iii) Mangifera indica (mango)

(iv) Carica papaya (pawpaw)

(i) Cymbopogon citratus

This plant is commonly known as lemon grass and it is a popular component of most drugs. It is used not only for malaria but also as a febrifuge. Its leaves contain a volatile oil, citronella, (Singha 1965).

(ii) Psidium guajava

The leaves of this plant are used most popularly for gastro-enteritis and diarrhoea; but they are also used in the preparation of malarial drugs. The plant is known to contain tannins, resin and essential oil, (Singha, 1965; Oliver 1960).

(iii) Mangifera indica

Mangifera indica is commonly known as the mango tree. It's bark and leaves are used as astringent; lotion for mouthwash for relief of toothache, sore gums, sore throat and other mouth infections. An infusion of the root-bark is used for diarrhoea and dysentery while the juice of the trunk is used as an antisyphilitic. Its bark is used as a component of some malarial drugs. The plant contains tannins and resins (Oliver 1960, Singha, 1965).

(iv) Carica papaya.

Carica papaya is known as the pawpaw tree. Medicinally the leaves and the latex of raw fruits are used for fevers, boriberi and as anthelmintic. Its infusion is used for stomach ache (Singha 1965). The plant contains papayotin.

MALARIA

a. Definition of malaria

The word 'malaria' in some dictionaries, is defined as an unwholesome or poisonous air (Webster, 1964). This definition is archaic and it reflects the belief in the 17th century that people who died of the disease succumbed to d'aria ("the air") or to cal'aria ("bad air") as an indication that the fatal illness had been caused by breathing noxious marsh air or 'miasma' (Wareham, 1949; and Russell et. al. 1963). Today malaria is recognized as a disease characterised by intermittent febrile paroxysms, anaemia and splenic enlargement, caused by infection with parasites generally assigned to the genus Plasmodium (Russell et. al. 1963).

Malaria has been, and still is, perhaps the most serious protozoan disease of man in many countries. In India alone, malaria was responsible for over a million deaths annually with nearly 100 million persons who suffered from it. In Southern Asia, Central parts of South America and Equitorial Africa, malaria appears to be still prevalent. Russell et. al. (1963) estimated the number of victims of malarial infection throughout the world at more than a quarter of a billion with about one per cent mortality rate. The World Health Organization is succeeding in bringing about practically a complete eradication of this disease in several countries. Today while about 1.926 billion suffer from the disease annually, about 1.346 are protected from the disease (WHO, Twenty-fifth World Assembly 1972).

In the United States, for instance, the disease had been prevalent in places in the southern states. In 1935 more than 150,000 malaria cases were recorded, but ten years later the number declined to 60,000 cases. In 1957 the total number of malaria cases diminished to 572 and in 1958, there were only 94 cases (Brody and Overman, 1959). Today in that country malaria has been virtually eradicated.

In malarious countries, the disease is a serious economic and social problem since it affects the majority of the population and brings about a large number of persist ni sickness, the loss of man power and retardation of both mental and physical development among children. In the words of Sir Ronald Ross quoted from

b. Economic importance of malaria.

Malaria has been, and still is, perhaps the most serious protozoan disease of man in many countries. In India alone, malaria was responsible for over a billion deaths annually in nearly 100 million persons who suffered from it. In South-East Asia, China, parts of South America and Equitorial Africa, malaria appears to be still prevalent. Russell et. al. (1953) estimated the number of victims of malarial infection throughout the world at more than a quarter of a billion with about one per cent mortality rate. The World Health Organization is succeeding in bringing about practically a complete eradication of this disease in several countries. Today while about 1.826 billion suffer from the disease annually, about 1.346 are protected from the disease (WHO, Twenth-fifth WHO Assembly 1972).

In the United States, for instance, the disease had been prevalent in places in the southern states. In 1935 more than 150,000 malaria cases were recorded, but ten years later the number declined to 56,000 cases. In 1955 the total number of malaria cases diminished to 522 and in 1958, there were only 94 cases (Brody and Dunn, 1959). Today in that country malaria has been virtually eradicated.

In malarious countries, the disease is a serious economic and social problem since it affects the majority of the population and brings about a large number of persistent sickness, the loss of man power and retardation of both mental and physical development among children. In the words of Sir Ronald Ross quoted from

Marshall (1949), "The malaria fever is important not only because of the misery which it inflicts upon mankind, but because of the serious opposition which it has always given to the march of civilisation in the tropics. Unlike many diseases, it is essentially endemic, a local malady, and one which unfortunately haunts more especially the fertile, well-watered and luxuriant tracts - precisely those which are of the greatest value to man. There it strikes down not only the indiginoous populations but, with still greater certainty, the pioneers of civilisation - the planter, the trader, the missionary and the soldier. It is therefore the principal and gigantic ally of barbarism. No wild deserts, no savage races, no geographical difficulties have proved so inimical to civilisation as this disease. We may also say that it has withheld an entire continent from humanity - the immense and fertile tracks of Africa. What we call the Dark Continent should be called the Malarious Continent and for centuries the successive waves of civilisation which have flooded and fertilised Europe and America have broken themselves in vain upon its deadly shores.

While it is true that malaria occurs mainly in tropical and semi-tropical regions, it is by no means confined to these localities. Malaria has been reported north of the Arctic Circle and as far south as the extreme tip of South America, and from an altitude of over 9,000 feet in Bolivia to 1,312 feet below sea level in the basin of the Dead sea (Cromlman, 1962).

c. Classification of malaria parasites.

The classification of malaria parasites according to those given by Garnham (1966) and Kudo (1966) is as shown below.

Phylum : Protozoa.

Subphylum : Plasmodroma.

Class : Sporozoa.

Order : Haemosporido.

Sub-order : Haemosporidiidea.

Families. : (a) Plasmodiidae Koehl.
(b) Haemoproteidae Doflein.
(c) Leucocytozoidea Wallis and Bennett.

Peters (1970) reported that all the species of plasmodium employed for chemotherapeutic studies fall within the family Plasmodiidae which by definition includes parasites which have a sexual phase in the mosquito and asexual cycles in tissues and blood cells of the vertebrate host. Gametocytes are produced and develop in mature erythrocytes.

d. Life history of malaria parasite

The pursuit of better drugs against the malaria parasite went hand in hand with research on the biology of the parasite. With the newer knowledge of the parasite the imperfections of the earlier drugs were understood, and the search for new ones encouraged.

Basically the life history consists of three phases and two types of hosts; one vertebrate and one invertebrate host. Male and female gametocytes within erythrocytes enter the stomach of a

female mosquito that feeds on the blood of an infected vertebrate. Within the mid-gut of the mosquito the gametocytes mature into male and female gametes, the former by a process known as ex-flagellation and the latter by emergence from the host cell. (Peters, 1970). Fertilisation of a megagamete by a microgamete results in the formation of a zygote called the ookinete or "wandering vermicille". These ookinetes transverse the main tissues of the stomach wall to just beneath the outermost membrane of the stomach. The parasites then encyst and grow rapidly as spherical forms called oocysts within which develop several thousand sporozoites which are liberated into the body cavity of the mosquito on maturation. The sporozoites find their way into the salivary gland ducts of the mosquito where they can initiate a new cycle of development.

Infected mosquitoes introduce sporozoites into man. They enter the circulation and are carried in the peripheral circulation until they reach the tissue in which they will commence the next phase of their life history. During this phase which is known as primary exo-erythrocytic schizogony(James and Tate, 1938) each sporozoite develops within a single host tissue cell into what have been variously called cryptozoites (Huff and Coulston, 1944); primary ex-erythrocytic forms (Davey, 1944); early exo-erythrocytic forms or pre-erythrocytic forms or schizonts inside which are formed numerous daughter cells or cryptozoites.

The type of mesodermal tissue cell in which this process takes place and the subsequent fate of the cryptozoites differ within the different subgenera. For example Jones and Tate (1938) showed that the pre-erythrocytic schizogony takes place in the endothelial cells of the spleen, heart, liver, lung and brain of the birds infected with Plasmodium gallinaceum, while Garnham et. al.(1955) showed that they develop in the parenchyma cells of the liver in man infected with Plasmodium falciparum and Plasmodium ovale. There is more than one exo-erythrocytic generation in certain subgenera (eg Haemamoeba). The ensuing metacryptozoites in their turn may give rise to further generations of metacryptozoic schizonts in the tissue or enter erythrocytes to commence the next phase of development.

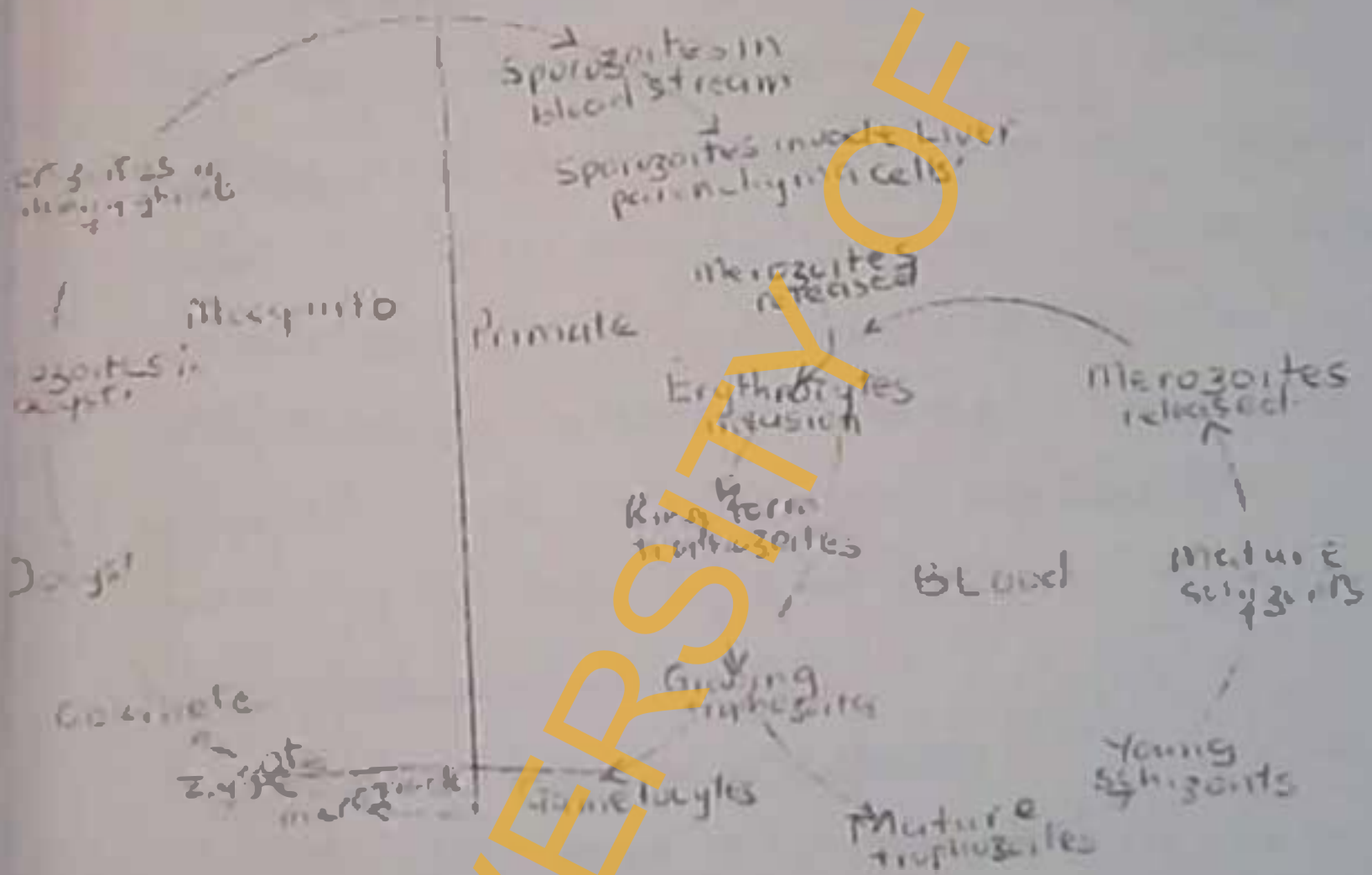
The entire process of pre-erythrocytic schizogony is essential to allow the parasites to undergo the necessary metabolic and other adaptations for a change from life in the poikilothermic insect to that in a warm blooded vertebrate.

As a rule large numbers of cryptozoites are produced in the schizonts by the time the erythrocytic phase commences, although the early generation of some avian species may contain relatively few (Peters, 1970).

Some of these parasites enter the red blood cells and again multiply by schizogony and the numbers build up and up. Unless a drug is administered or immunity against the infection is developed death usually occurs.

Some of the daughter merozoites of the red blood cell phase grow into male and female gametocytes, not schizonts. In these parasites the chromatin does not divide and no further development of them takes place unless they are sucked up by the appropriate mosquito within which the development is completed.

The life cycle can be represented diagrammatically as shown below:

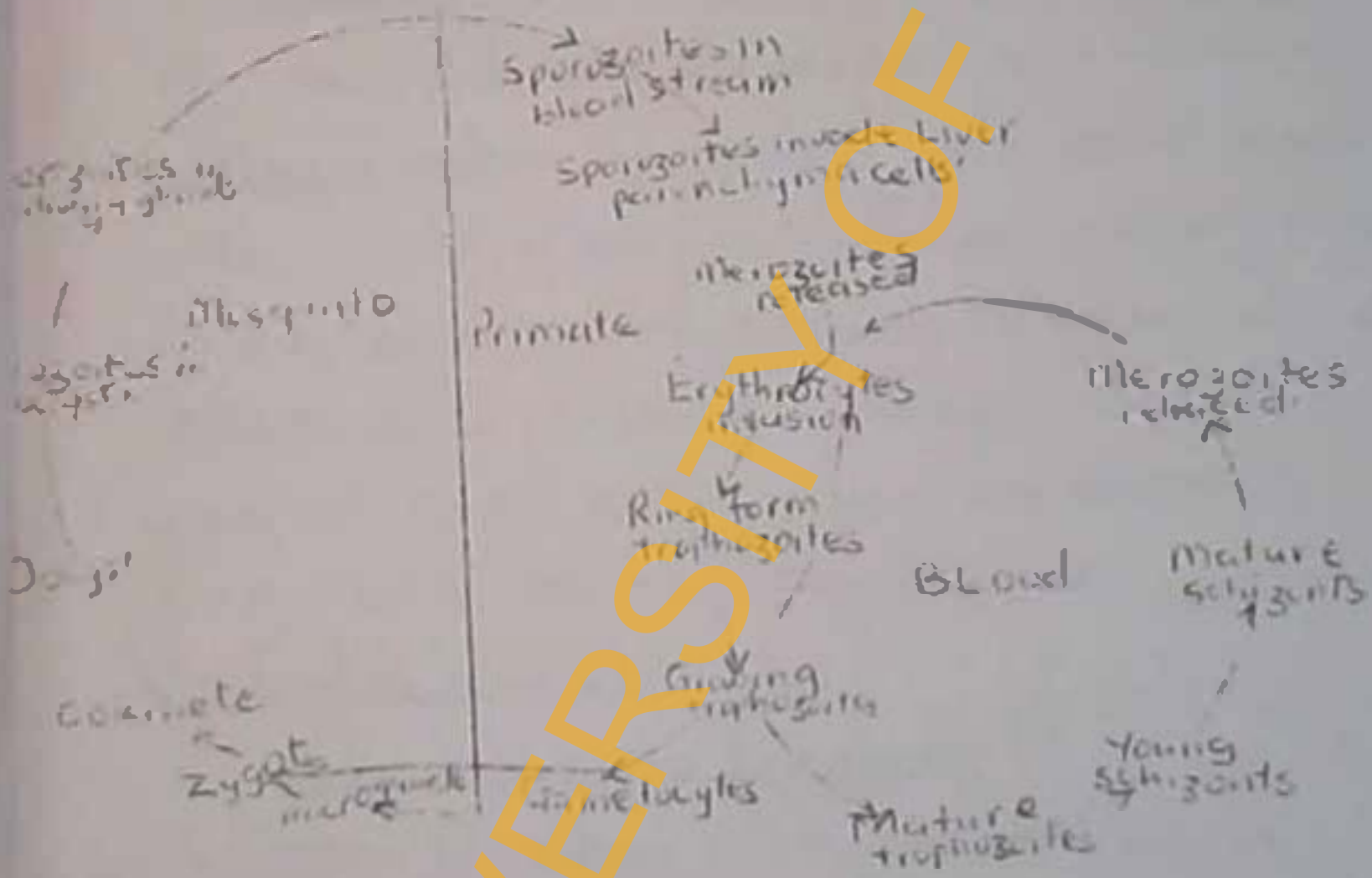


Schematic life cycle of plasmodia in lower primates and man Thompson and Gerbal (1972).

It is essential to bear in mind the

Some of the daughter merozoites of the red blood cell phase grow into male and female gametocytes, not schizonts. In these parasites the chromatic does not divide and no further development of them takes place unless they are sucked up by the appropriate mosquito within which the development is completed.

The life cycle can be represented diagrammatically as shown below:



Schematic life cycle of plasmodia in lower primates and man Thompson and Werbel (1972).

It is essential to bear in mind the

fundamental difference in the life histories of the individual parasite species when considering the basis of antimalarial chemotherapy. Plasmodium falciparum, for example, undergoes only one generation of pre-erythrocytic schizogony (Thompson and Werbel, 1972). Once this is completed only the erythrocytic cycle continues the infection in the human host and if the erythrocytic parasites are all destroyed there can be no relapse.

Plasmodium vivax, Plasmodium ovale and Plasmodium malarae on the other hand develop secondary exo-erythrocytic parasites. Plasmodium gallinaceum erythrocytic parasites may be killed by appropriate medication only for the hosts to die later from overwhelming secondary exo-erythrocytic schizogony in the reticulo endothelial cells lining the capillaries of the brain, (Davey 1948; Garnham, 1948; Huff, 1949). Failure to appreciate these fundamental points may lead to the blame for failure of a particular course of treatment.

4 Drug therapy in malaria.

a. Historical review of drug therapy

Before the outbreak of the first world war, the only specific antimalarial drugs known were the cinchona alkaloids, of which the most commonly used was quinine.

Cinchona is the name given by Linnaeus to a family of plants indigenous to the eastern slopes of the Andes in memory of the Countess of Cinchon, wife of an early Viceroy of Peru, to whom legend attributes the introduction of the bark to Europe (Covell et. al. 1955).

There is evidence (Jaramillo Arango, 1950) that the value of the bark of the cinchona tree in the treatment of malaria was known to the natives of the New World before the arrival of the Spaniards, but the evidence presented by Suppan (1931) does not support this view. Rather, the properties were probably first discovered by the Jesuit missionaries who made a habit of chewing the bark of trees in order to distinguish their different kinds, and in this way, they noticed the extremely bitter taste of the bark. Those of them who practiced medicine then tried an infusion of it on a certain ague which was common in that part of South America.

Powdered bark had reached England by the middle of the seventeenth century under the name Jesuits powder and was prescribed for the treatment of intermittent fevers for the next two centuries. It was not until 1712, that Torti suggested that it was of use only for agues and not for other types of fevers (Findlay, 1951). The active principles of this bark were isolated in 1820 by Caventon and Pelletier (Krueger, 1931; Russell, 1960). The first known active principles were quinine and cinchonine. Other alkaloids were seperated in ~~1846~~ and 1847, and about this time their use became widespread throughout the world.

The importance of cinchona trees as the only source of quinine resulted in their unbridled exploitation and near extinction since removal of the bark results in the death of the tree. It was in order to avoid this catastrophe that attempts were made to introduce

the tree into other tropical regions. Plantations of the tree were established in Java and India but were later abandoned by the British because the project was uneconomical. The Dutch East Indies came to supply almost the whole world's demand for quinine at this time.

During the period 1880-1890, reckless over production led to a fall in prices which proved ruinous to planters. The planters in Java managed to survive the slump and at the outbreak of the second world war, about 97% of the world's supply of quinine came from Java. This situation was important in the development of the synthetic antimalarials, for the fact that the Germans were cut off from all sources of quinine during the first world war was the stimulus which inspired the intensive search for synthetic substitutes. Also, during the second world war it was the fear that the cinchona plantations would fall into Japanese hands that stimulated the British and American scientists to find synthetic remedies. On both occasions, however, the war which provided the initial stimulus was over before the important discoveries were made.

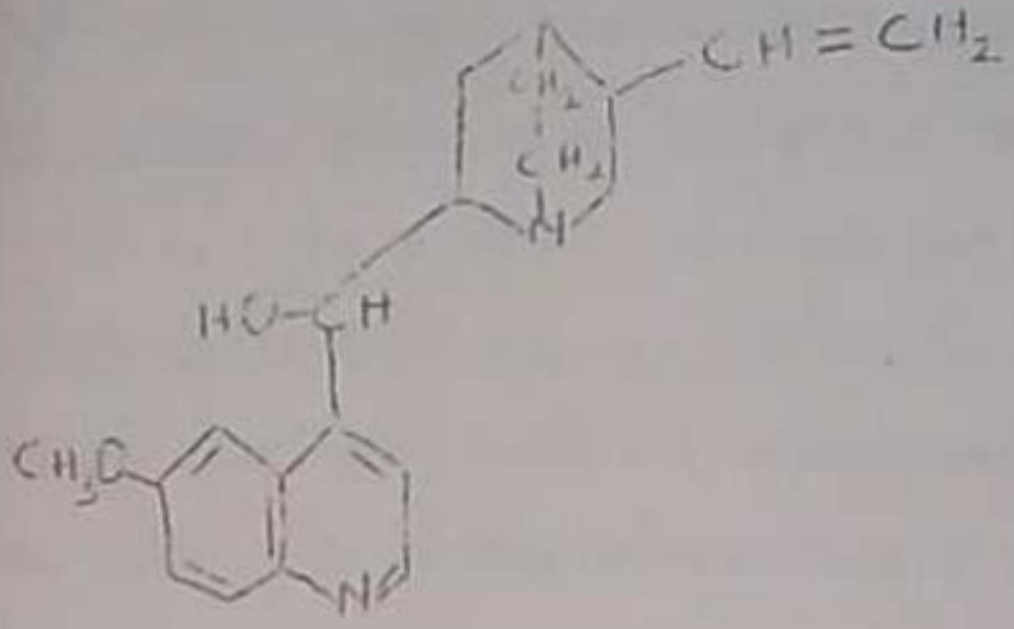
b. Active principles of the cinchona tree.

Quinine was the first alkaloid isolated from cinchona bark by two French Scientists, Caventon and Pelletier in 1820 (Russell et.al 1963). Other major alkaloids of the bark are cinchonine, quinidine and cinchonidine.

They all possess antimalarial properties although their relative potencies vary with the species of Plasmodium under consideration. For example, quinidine is more effective than quinine against Plasmodium gallinaceum (Marshall, 1945) and Plasmodium falciparum (Schmidt, 1956). Also quinine is about twice as active against Plasmodium relictum as cinchonidine (Buttle et.al., 1938).

Quinine remains the only drug to which most resistant strains of Plasmodium falciparum are susceptible. Its exact mode of action is not known but it has been shown to inhibit lactate formation from glucose in Plasmodium gallinaceum, (Silverman et.al., 1944). It also inhibits glycolytic enzymes of Plasmodium gallinaceum (Marshall, 1945). Quinine has also been shown to prevent P^{32} labelled phosphate incorporation into RNA and DNA by Plasmodium gallinaceum and Plasmodium berghei (Schellenberg and Coatney, 1960). Quinine has both toxic and side effects on man and other animals. Repeated full doses frequently cause cinchonism, which in its mildest form includes nausea, headache, and slight visual disturbances and in its more severe forms can cause gastrointestinal symptoms and disturbances in hearing and vision are increased (Hallman, 1962).

Chemical structure of Quinine.



c. Synthetic antimalarial drugs.

(1) Saminoquinolines

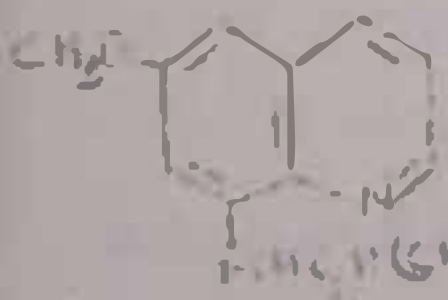
The search for synthetic antimalarials other than quinine initiated by the Germans during the first world war when they were out off from all sources of quinine resulted in the production of Pamaquine (Plasmoohin). The discovery of pamaquine is generally believed to be based on the observation of Guttman and Ehrlich in 1891 (Thompson and Werbel 1972) that the dye - methylene blue - had some chemotherapeutic effect on malaria in patients. Keeping this observation in mind various basic side chains were introduced into the formula of methylene blue and eventually resulted in the synthesis of 8-aminoquinolines of which pamaquine emerged as the first synthetic antimalarial agent.

Although it has remarkable antimalarial properties, it was unsuitable as a therapeutic agent in several respects, the most important being its relatively high toxicity and the fact that it has little action on the asexual erythrocytic forms of Plasmodium falciparum (Alving et.al. 1948; Schmidt and Coatney, 1955).

In order to find, less toxic and more effective analogues of pamaquine more work was done on the 8-aminoquinoline and by the end of the second world war, pentaquine, a less toxic analogue emerged. With further work primaquine was synthesized and it became the least toxic and most effective 8-aminoquinoline tested (Alving et.al. 1952;

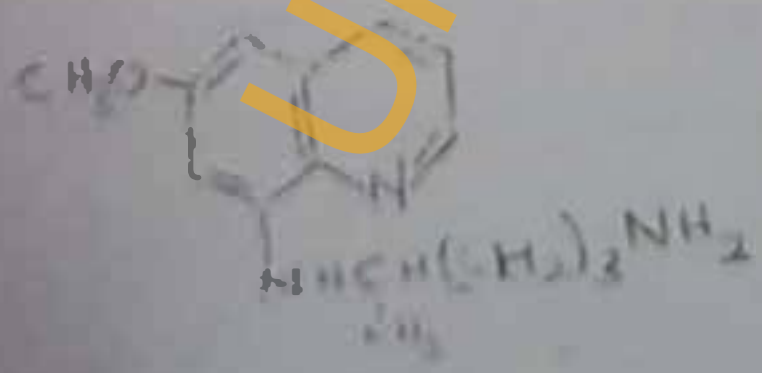
Chemical structure of 8aminoquinolines

Pamaquine



Pentaquine

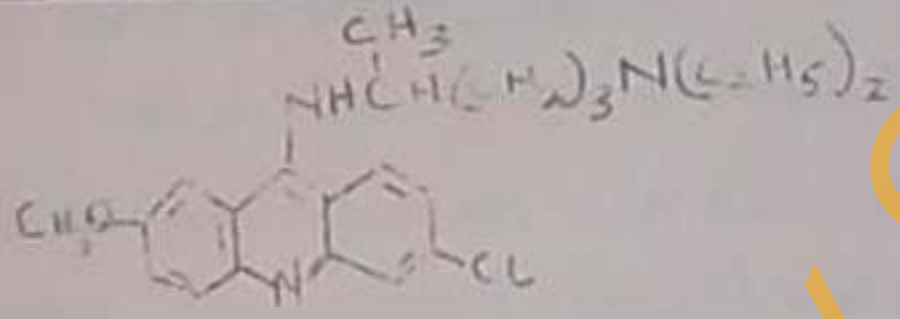
Primaquine



(ii) Acridines.

Introduction of the basic side chain which is considered essential for antimalerial efficacy into variety of heterocyclic systems was unsuccessful. Its introduction into the acridine nucleus led to the discovery of quinacrine which is also called atebrin Atabrine or mepacrine (Thompson and Werbel, 1972). Other acridine derivatives were synthesized and tried but quinacrin. remained the most important of this group.

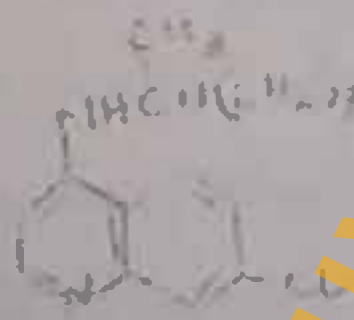
Chemical structure of quinacrine.



(iii) 4-aminoquinolines

The search for antimalarial agents superior to the existing ones led to the synthesis of 4-aminoquinolines of which chloroquine emerged as the most effective and important.

Chemical structure of chloroquine.

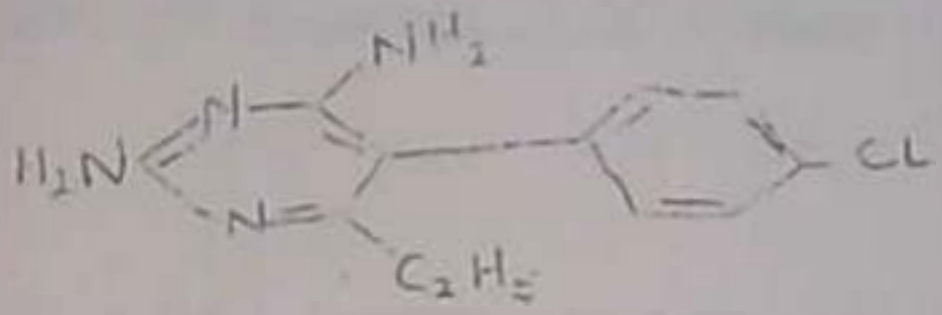


(iv) Pyrimethamine derivatives.

Further work in malarial chemotherapy led to the discovery of pyrimethamine (daraprim) which was found to be highly active against blood forms of Plasmodia generally.

It was reported to be several hundred times as active as quinine against Plasmodium gallinaceum in chicks and Plasmodium berghei in mice (Falco et, al. 1951; Singh et.al., 1953).

Chemical structure of daraprim.



(v) Other synthetic compounds.

Various other groups of compounds are known to possess antimalarial properties and these have been well documented by Wiselogle (1946). Some of these groups of compounds are the sulfonamides, sulfones, some antibiotics like aureomycin, terramycin, and chloramphenicol (Coatney and Greenberg 1952; Wiselogle, 1946).

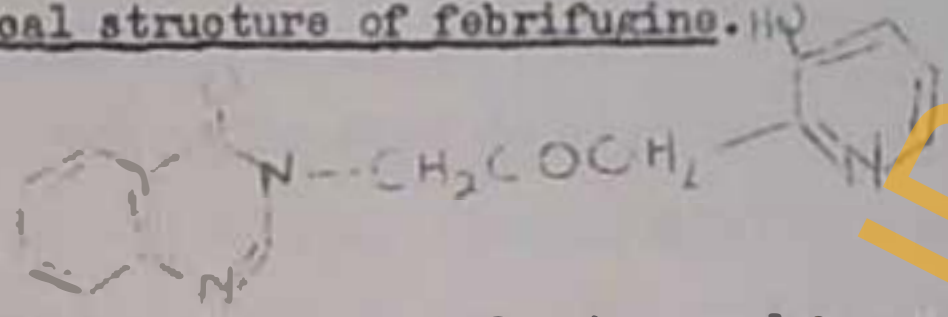
(vi) Other natural products.

Thompson and Wartel (1972) reported in their book that only one natural material other than quinine is known to possess potent antimalarial activity.

An extract, Ch'ang Shan, obtained from the powdered roots of Dichroa febrifuga, has been known for its antimalarial properties in China for many centuries (Russell, 1960). Febrifugine, the active principle in this plant, was isolated and shown to possess antimalarial activity (Jang et.al., 1948; Koepfli et.al., 1947).

Febrifugine has also been isolated from leaves of a variety of Hydrangea species. The drug is an extremely potent antimalarial agent against experimental infections. It has been estimated to be from 16-64 times as active as quinine against Plasmodium gallinaceum in chicks (Koepfli et. al., 1947).

Chemical structure of febrifugine.



Experimental malarial infection in laboratory animals.

a. Choice of experimental animals

Plasmodia parasitise an unusual range of animals which include in addition to man, lizards, birds, rodents and lower primates (Thompson and Werbel, 1972). Like most intracellular parasites the various species tend to be specie specific and cross infection is not usual except between closely related hosts. Bray (1957a,b; 1958 and 1959) showed that malaria parasites of man have only been transmitted to splenectomized chimpanzees and only two species of simian parasites have been transmitted to man. More recently some strains of human plasmodia have been adapted to small primates, - especially Plasmodium falciparum in the owl monkey (Aotus trivirgatus) (Porter and Young, 1967; Cieman et.al. 1969).

Lower down the scale, Plasmodium berghei, a natural parasite of the true rat in the Zaire Republic, can be transmitted to laboratory mice and rats (Vincke and Lips, 1948). Also P. vinckei and P. petteri, parasites of the common mongoose, can be transmitted to canaries and pigeons.

It follows from what has been said above that the experimental chemotherapist who is unable to work with malaria in man may not be able to work with the human parasites in animals and must therefore make a choice of both parasite species and host. If his aim is the discovery of a drug for use in man, and not fundamental research, he can be misled by the reaction of both parasites and host to his compounds. Infact the specificity of action of some antimalarial substances is most marked. The sulfonamides, for instance, have a most striking curative action against Plasmodium knowlesi in monkeys (Coggeshall, 1938); a marked action against Plasmodium lophurae in ducklings (Marshall et. al., 1942) and Plasmodium gallinaceum in chicks but no detectable action against Plasmodium inui in monkeys (Coggeshall, 1940) or Plasmodium relictum and Plasmodium cathemerium in canaries. If the compound is metabolised, the rate at which this occurs may vary according to the host, and if a metabolite is the active principle (as it is with proguanil) both the amount formed and the rate of its formation may vary and influence the overall result considerably.

The host may also influence the behaviour of the parasite. Plasmodium knowlesi, for example, is extremely virulent in the Indian rhesus monkey Macaca mulata, giving rise to a fulminating infection which readily kills, whereas in its natural host it is relatively benign.

The choice of the experimental animal is important. Monkeys are expensive and awkward to handle, they will consume a lot of drug because of their size. Canaries are not easy to get in large numbers, they are relatively expensive and they are delicate creatures. Mice are common, cheap, easily housed and handled. Chicks are also cheap and easy to handle but they require extra heating which may make their use inconvenient. Pigeons require much space, turkeys are expensive .

Considering the ease with which mice and chicks are housed and handled, we have chosen mice and chicks as the experimental models for primary testing of our drugs using Plasmodium berghei in mice and Plasmodium gallinaceum in chicks.

b. Plasmodium berghei - drug system.

The superiority of rodent malaria over avian malaria as models for screening and evaluating suppressive and curative effects of antimalarials is now recognized (Peters, 1967; Bruce-Chwatt, 1967). Of the several species of rodent plasmodia now available, Plasmodium berghei is the species most commonly used.

It is believed to have many similarities to human plasmodia in general and to Plasmodium falciparum in particular. Its sporogenic cycle under optical temperature conditions lasts 11 to 13 days, more or less the same length of time the sporogonic cycles of human plasmodia requires for completion in mosquitoes. Its tissue stages differ only in their rapid rythm of development from that of Plasmodium falciparum. (Yeoli and Most 1965). Plasmodium berghei resembles chloroquine resistant strains of Plasmodium falciparum in its susceptibility to the action of sulfonamides, sulfones and pyrimethamine, and like Plasmodium falciparum, it fails to produce late exo-erythrocytic schizonts (Yeoli et.al., 1966).

Plasmodium berghei is still considered one of the most economical and conveniently handled model for primary drug screening in spite of the criticism of Schneider (1954) that certain drugs such as paraquine and proguanil are relatively ineffective in this model and could be missed. Until recently, it was thought that results obtained in this species should be referred with care to the primate malaria since it was uncertain whether it belonged to the same genus (Findley, 1951). However, Yeoli and Most's report (1965) that the pre-erythrocytic cycle bears a strong resemblance to that of the primate malaria has helped to dispel this doubt.

In spite of its convenience, Plasmodium berghei is very sensitive to variations in the experimental conditions involved in chemotherapeutic studies.

Precautions to be observed in this system have been very well reviewed by Peters (1970). Factors which require standardisation are enumerated below.

(i) Mouse strain.

All strains of mouse are not equally susceptible to infection with blood forms of Plasmodium berghei and the course of infection may differ (Cadun et.al. 1965).

(ii) Sex and age.

The sex and age of the mice are very important factors which cannot be neglected in this study. Konopka et.al. (1966) have demonstrated clear differences in the response of male and female mice to Plasmodium berghei infections. They have found that the response of female animals to chemotherapy may vary at different times during the oestrus cycle. Welldo et.al. (1966) showed that mice tend to become less susceptible to Plasmodium berghei infections with advancing age while mice smaller than 18gm are more difficult to handle. Hence male mice weighing between 18 and 22gm are regarded as suitable for routine studies.

(iii) Concomittant infections.

Serial transfer of blood usually causes infection with Eperythrozoon coccoides or Haemobartonella muris. The type of infection caused with contaminated Plasmodium berghei inoculum may differ considerably from that produced by the experimental agent alone.

A competitive action of B.coccoides has been described with Plasmodium berghei by Kretschmar (1965) and by Peters (1965); and by Babesia rodhaini by Peters (1963).

Neoarsphenamine (NAB) has been used to cure mice of E.coccoides (Peters, 1970). More recently Thompson and Boyles, (1966) have successfully used trivalent arsenical oxophenarsine hydrochloride to clear Plasmodium berghei of side infections.

Peters (1970) has suggested that the presence of Erythrozoon coccoides should always be suspected if an undue scatter is observed if the parasitemia levels of untreated controls animals, with only some reaching the anticipated numbers.

(iv) Environment and diet.

Environmental conditions such as temperature and the stress of handling may influence the course of infection with Plasmodium berghei. Lack or insufficient level of para-amino-benzoic acid or folic acid in the diet may be responsible for a low level of parasitemia (Peters, 1970). This can be rectified by supplementing the diet with PABA or folic acid in their drinking water.

(v) Strain of parasite.

The strain of parasites used is also important and the choice depends on the requirements of the experimenter. But generally it is known that the most consistent results are obtained with very old strains that have been maintained only by blood passage and have lost the ability to produce sporozoites.

Younger strains are labile in their response to various drugs (Peters, 1968; Diggens and Gregory, 1969), and hence may give different results, depending on the time when they were employed. Old laboratory strains tend to increase in their sensitivity towards all active compounds and hence are more suitable for detecting low levels of antimalarial activity.

(vi) Other factors.

The success of the course of infection depends directly on the infecting dose (Wellde et.al., 1966) and the effective dose of a schizontrocidal drug depends on the intensity of infection at the time of administration and the level of immune response that the host has developed at that point. It is therefore important to work with standardised infective dose level. Other factors which may also affect the course of infection and therefore have to be standardised are

(i) the time required in preparing inocular.

(ii) route of drug administration

(iii) timing of the first drug dose

(iv) frequency and duration of administration.

(v) timing of termination of experiments.

(vi) techniques of reading blood films.

C Plasmodium gallinaceum - chicks system.

Plasmodium gallinaceum was discovered by Brumpt in 1935 (Garnham, 1966) and has since been widely used in chemotherapeutic studies in malaria. Other avian malaria parasites are known and no onetype is considered superior to the others. But Davey(1963) endorsed the use of Plasmodium gallinaceum in chicks because of its amenability to studies on both blood forms and tissue stages, relative ease of vector aspects and sporozoite supply and such host considerations as cost, availability and freedom from complicating intercurrent infections.

6 In-vivo techniques for testing drug activities.

a. Plasmodium berghei - mouse system.

The methods employing Plasmodium berghei in mice have been reviewed by Wiselogle (1946), Thurston (1953), Peters (1967a) and Peters (1970). One of the first people to establish a standard test for activity against blood forms was Thurston. In her test, albino mice weighing about 20gm were inoculated with about 5-15 million infected red blood cells on DO (that is the day on which the mice were infected). They were treated by oral administration of the test compounds once daily for four days starting three or four hours after infection. Parasite counts were made on blood films made from tail blood on D+4 and D+6 in both controls and treated mice. The minimum effective dose was attained when the mean of less than 1% of the red cells is parasitized. In her modification of this method, Thurston gave smaller inoculum of about one million

parasitized cells and expressed the minimum effective dose as that which reduced parasitemia to 2% of that of the control (Thurston, 1950).

Most of the methods employed later were based on this general principle with some variations. Schneider et.al. (1949) adopted a different criterion for expressing their results. They adopted the MED which they defined as the lowest dose that delayed the appearance of parasitemia in 75% of their animals for three days after the end of treatment. They treated the mice subcutaneously on DO, D+1 and D+2. Rollo (1952) and Siddons (1953) adopted the ED50 instead of MED as their measure of activity. In India, Krishnaswami et.al. (1954) classified their results as Class III (Clinical cure), Class II (no parasites seen for three days after the end of treatment) and Class I (no effect).

Other workers used the drug-diet method in which treatment was started the day before parasites were injected and continued for six consecutive days and blood smears were prepared on the fifth day after the animals were infected. Darrow et.al. (1952).

Thurston's test and those of other workers mentioned above have the disadvantage that they only demonstrate the action of a compound on the course of infection starting at a time when parasitemia is minimal. They gave no indication of its action in overcoming heavy parasitemia or its activity to eradicate parasites completely.

In order to overcome these drawbacks, Peters (1965b) differentiated between "suppressive test" and "therapeutic test". The suppressive test follows the general lines of Thurston's method. In the therapeutic test, treatment was started only when the parasitaemia in the control animals reached 10% from which day the animals received a single daily treatment for four consecutive days (T_1 to T_4 inclusive). Blood films were made daily from T_1 and activity was assessed by two parameters. These were the degree by which the anticipated increase in parasitaemia between T_1 and T_4 had been depressed by treatment (as compared with the rise in control levels during the same period) and the number of days (if any) that treated mice remained parasite free after therapy. The latter gave an indication of ED_{100} level.

One of the major disadvantages of this method according to Peters (1965b) is the amount of work entailed in making daily examinations of blood which when animals become parasite free, must be continued for either an indefinite or pre-selected but extended period of time. Also in certain drug-resistant strains parasitaemia increases very slowly and by the time the 10% level is reached, immunity is already having a marked influence on its progress.

Whichever method of screening is adopted, it is useful to apply a reference drug with which the test drug can be compared. Quinine, chloroquine, pyrimethamine and quinacrine have been used.

b. Plasmodium gallinaceum - chick system.

The procedures for testing drugs against Plasmodium gallinaceum in chicks have been described by Davey (1948) and adopted by other workers (Wiselogle, 1946). The procedure entails the use of groups of five or six 6-days old chicks in which infections are induced by the intravenous injection of about 50 million parasitized cells. The chicks are kept at 32°C on a standard diet free of antibiotics as these may interfere with the progress of infection. The drugs were given orally starting about 4 hours after infection. Two doses were given on each of the next three days, blood smears were prepared on the next day after the end of treatment (that is fifth day) and the percentage of parasitised cells was determined by examination of about 500 erythrocytes. Usually in the untreated controls the parasitaemia rose to about 70%. An irregularity in the parasitemia of treated chicks compared with controls may indicate marginal drug action, but a definite activity is reflected in a much lower count than that of the controls. Such testing permits assessment of short-term effects on asexual blood forms, but the assessment of long-term effects or eradication of blood forms may be complicated by the presence of secondary tissue stages.

c. Other criteria useful in drug screening.

Jacobi (1965) Schneider (1960) have adopted broader criteria of activity in drug screening. Jacobi found that the temperature fell in infected mice with the increase of parasitemia

and that successful therapy prevented this fall. He therefore recorded body temperature daily as well as the erythrocyte infection rate and survival time. Schneider (1968) also recorded both the body temperature and spleen weight.

Several workers have shown that malaria infections cause various biochemical changes in the host. For instance it has been shown that during the course of vivax or falciparum malaria, serum albumin decreases, (Boyd and Kreiko, 1941; Dolo and Emerson, 1945; Taylor et.al. 1943); alpha1 globulins increase (Taylor et.al. 1949); alpha 2 globulins occassionally decrease (Dolo and Emerson, 1945); and gamma globulins increase (Lunn et.al., 1966). The beta globulins showed no consistent changes (Lunn et.al. 1966). Both increases and decrease in glucose have been reported in human malaria depending on the stage of the disease at which the blood was examined and on the severity of the infection. In infections with Plasmodium vivax and Plasmodium falciparum a rise in serum glucose levels during fever was observed (Fulton and Kasparian, 1950).

More recently similar biochemical changes have been observed in other animals infected with malaria parasites. For instance in mice infected with Plasmodium berghei, Briggs et.al. (1960) observed marked alterations in level of serum proteins. They also reported that when treated with primaquine diphosphate early in the course of infection most of the mice survived as carrier animals and serum protein patterns returned to normal.

Sadun et. al. (1955 and 1966) also observed significant biochemical changes in the serum of mice infected with Plasmodium berghei. They reported marked increases in serum glutamic pyruvate transaminase and serum glutamic oxalate transaminase as early as two days after infection. Lower fasting glucose levels occurred in heavily infected mice while a moderate reduction in alkaline phosphatase and albumin values was observed. Other changes observed include increases in BSP retention and positive copalin flocculation reaction. Minimal or no changes were reported in total protein, non-protein nitrogen, phosphorus, globulins, bilirubin, calcium and some other metals.

Sadun et. al. (1966) have reported changes similar to those observed in man and mice in monkeys infected with Plasmodium falciparum.

In the process of screening some plant materials for their antimalarial properties, a few of the above biochemical changes were used. These criteria are serum total protein, serum protein fractions, serum glucose level, serum glutamic pyruvate transaminase (SGPT), Leucine amino peptidase (LAP), alkaline phosphatase and bilirubin.

7 In-vitro techniques

Efforts to devise conventional in-vitro test procedures for the large-scale evaluation of drugs against asexual blood forms have been limited by the complexity of the culture procedures, inability to achieve substantial growth for prolonged periods and

the lack of defined culture media. A few studies have however been done. The various parameters which have been employed to ascertain whether parasites have survived exposure to the drugs are infectivity of the parasites to clean vertebrate hosts, effects of drugs on morphology, growth and reproduction and also on metabolic reactions.

a. Infectivity

This involves incubation of the parasite with known quantities of antimalarial drugs and injecting the inoculum into fresh animals. The degrees of parasitemia produced by suspensions of erythrocytes with drugs and the controls are then compared. Warhust (1966) and Warhust and Powell(1968) recommended that the results can be based on the time required for parasitemia to reach 2% level. This method was used in studies by Greenberg et.al.,(1951), Taylor et.al. (1952) and Josephson et.al. (1953) with Plasmodium gallinaceum. They found that drugs which are effective in vivo against all stages of asexual development in the blood were active. Such drugs are chloroquine, quinine, quinacrine and naphthoquinones.

b. Morphological effects.

Morphological changes as a reflection of the mode of drug action is difficult to ascertain and could be erroneous as parasites treated with drugs might undergo morphological changes other than those directly brought about by the drugs (Peters, 1970).

c. Growth and Reproduction.

In this method the progress of cultures is judged not only by observation of morphological damage but also by laborious counts of the numbers of parasites at different stages of development. Thus Black (1946) found that proguanil arrested growth at the early schizont stages and sulfonamides, a little later during chromatin division at certain concentrations. Gioman et.al. (1966) found that growth of Plasmodium cynomolgi is arrested with high concentrations of pyrimethamine. In their own experiments, Rieckman etal. (1968) found that chloroquine or quinine inhibited maturation of the earliest stages of P. falciparum exposed to them while cycloguanril did not inhibit preschizont stages but led to abnormal appearing schizonts.

d. Biochemical studies.

All antimalarial drugs induce a number of metabolic changes in the parasites by interfering with one or more of their vital processes. Several studies have been carried out along this line. Automated techniques for measuring the effects of drugs on several metabolic systems concourrently are being developed (Canedella et.al. 1970). Drug effects are assessed by inhibition of glucose consumption, lactate production and the release of free amino acids during a one-hour incubation of Plasmodium berghei infected rat erythrocytes.

Effect of drugs on synthesis of RNA and DNA by Plasmodium gallinaceum in chick erythrocytes have been studied by Schellenberg and Coatney (1961) who found that chloroquine, quinacrine and quinine inhibited synthesis of both RNA and DNA.

8 Clinical trials in hospital patients with naturally acquired infections

a. Selection of patients

In selecting patients suitable for clinical trials, it is necessary to distinguish between those who are partially protected by previously acquired immunity and the non-immune patient suffering from his first attack of malaria. Besides it is also necessary to distinguish and identify the type of Plasmodium responsible for the disease. The age of the patients must be taken into consideration. Children between the ages of 5 and 15 years are considered suitable for trials as they show a high degree of uniformity and are often more readily accessible for clinical investigations. Bruce-Chwatt (1967) in clinical trial of some antimalarial drugs found school children most suitable.

b. Parameters useful in assessing antimalarial action.

Peters (1970) recommended that a suitable battery of physical and biochemical tests should be applied in each patient in order to observe possible toxic effects of new compounds being used.

Effect of drugs on synthesis of RNA and DNA by Plasmodium gallinaceum in chick erythrocytes have been studied by Schellenberg and Coatney (1961) who found that chloroquine, quinacrine and quinine inhibited synthesis of both RNA and DNA.

8 Clinical trials in hospital patients with naturally acquired infections

a. Selection of patients

In selecting patients suitable for clinical trials, it is necessary to distinguish between those who are partially protected by previously acquired immunity and the non-immune patient suffering from his first attack of malaria. Besides it is also necessary to distinguish and identify the type of Plasmodium responsible for the disease. The age of the patients must be taken into consideration. Children between the ages of 5 and 15 years are considered suitable for trials as they show a high degree of uniformity and are often more readily accessible for clinical investigations. Bruce-Chwatt (1967) in clinical trial of some antimalarial drugs found school children most suitable.

b. Parameters useful in assessing antimalarial action.

Peters (1970) recommended that a suitable battery of physical and biochemical tests should be applied in each patient in order to observe possible toxic effects of new compounds being used.

The progress of the malaria infection itself can be judged by the patients clinical picture like the body temperature and pulse. The degree of parasitaemia found in the peripheral blood should also be noted. Biochemical tests similar to those employed in the primary drug testing in mice and chicks can also be used in this test.

CHAPTER TWO
MATERIALS

1 The Medicinal Plants.

The antimalarial properties of the following commonly used medicinal plants were investigated.

Botanical Name	Common Name	Local Name
Morinda lucida	Brimstone Tree	Oruwo
Alstonia boonei	Pattern Wood	Ashfoyaje
Azadirachta indica A.Juss.	Neem or Margosa Tree	Dogonyaro
Buantia chlorantha	African Yellow Wood.	Awopa
Cymbopogori citratus	Lemon grass	Lacon grass
Carica papaya	Papaya, Pawpaw	Ibepo
Mangifera indica	Mango	Mango
Psidium guajava Linn	Guava	Guava

The plant materials used in this study were usually bought locally from either Bugbe or Oba's market in Ibadan.

2 Malarial Parasites

a Plasmodium berghei.

This was obtained from Professor A. Jones of the department of Pharmacology, University of Ibadan. It has been maintained in white mice by weekly transfer from infected mice to fresh mice.

b Plasmodium gallinaceum.

This was obtained from Mr. Sergeant of London School of Hygiene and Tropical Medicine. It was maintained in white leghorn chicks by weekly transfer from infected chicks to 6-days old chicks.

c Plasmodium falciparum.

This was obtained from the out-patients department of the University College Teaching Hospital. It was usually obtained from children under the age of ten.

3 Experimental Animals

a Mice

Albino mice, Musculus species, usually weighing between 18 and 25gms were used in this study. As much as possible, mice belonging to the same litter were selected. The mice were supplied by the Pre-clinical experimental animal house of the University of Ibadan. They were kept on the standard stock diet obtained from Livestock Feeds Company, Lagos.

b Chicks

6-days-old white leghorn chicks were used. They were obtained from the experimental and research farm of Ibadan University. They were kept in cages at a constant temperature of 32°C and fed on antibiotics free diet obtained also from the experimental and research farm.

4 Diet of Animals.

a Mice.

Mice were fed on laboratory diet (mice and rat) supplied by Livestock Feed Limited, Lagos. The diet contains about 21.0% protein, 4.0% fibre and 3.5% oil made from the following ingredients:

maize	molasses,
guinea corn	pellet binder,
middlings	bone meal,
wholemeal flour	Oyster shell,
fish meal	salt,
milk powder	Vitamin Premix,
groundnut cake	Mineral Premix,
brewers yeast.	Antioxidant.

b Chicks

The experimental chicks were fed on chick mash which contains the following ingredients:

grains,	bone meal
middlings,	Oyster shell,
fish meal,	salt,
groundnut cake,	Vitamin premix,
dried brewers grains,	Mineral premix

5 Reagents for the preparation of inoculum.

a Citrate anti-coagulant

Most workers used the sodium-citrate anti-coagulant in their studies (Cenedella, 1968). This solution was prepared by dissolving 3.69gm of trisodium citrate and 1.13gm of citric acid in 100ml solution.

b Isotonic saline.

Isotonic saline solution was always used in the dilution of blood to required red cell density or required level of parasitized rod blood cell. It was prepared by dissolving 0.89gm of sodium chloride in 100ml solution.

6 Reagents for staining blood films

a Leishman's stain.

It was used as bought from Hopkins and Williams Limited.

b Giemsa's stain.

This was also obtained from Hopkin and Williams Limited and diluted 1 in 10 in buffered water before use.

c Buffered water.

It was always better to wash stained slides in buffered water which was prepared by dissolving 2gms of a 7:4 mixture of disodium hydrogen phosphate (Na_2HPO_4) anhydrous and potassium dihydrogen phosphate (KH_2PO_4) anhydrous, in a litre of water. (Blacklock and Southwell, 1969)

7 Reagents for estimation of serum proteins in serum.

The reagents used in this method are those described by Varley (1962).

a(i) Stock biuret reagent: Solution A.

45gm of sodium potassium tartrate (Rochelle salt), $NaKC_4H_4O_8.4H_2O$, was dissolved in 400ml of 0.2N sodium hydroxide in a beaker. 15gm of copper sulphate ($CuSO_4.5H_2O$) was added and allowed to dissolve completely by stirring. Then 5gm of potassium iodide was added. The solution was transferred to a litre flask and made up to the mark with 0.2N sodium hydroxide solution.

(ii) Stock biuret reagent: Solution B.

This was a solution of 5% potassium iodide in 0.2N sodium hydroxide.

(iii) Working biuret solution.

50ml of solution A was diluted to 250ml with solution B.

b Versatol A standard.

Versatol A, was obtained from General Diagnostics Division of Warner-Chilcott Laboratories, Morris Plains, New Jersey. The solution was prepared as instructed by dissolving the content of each vial in 5ml water to give a solution containing 7.1gm serum proteins per 100ml.

8 Reagents for protein separation by electrophoresis.

The reagents used were those recommended by Bailey (1957) for paper electrophoresis.

a Veronal buffer, pH 8.6, 0.1/2

20.6gm of sodium diethylbarbiturate was added to a hot solution of 3.68gm of diethyl barbituric acid and the solution made up to 1 litre with more water. This reagent was prepared fresh when needed.

b Staining dye.

Amido black 10B (Naphthalene Black) was used. A saturated solution of amido black in 10% acetic acid was filtered and used. It could be used repeatedly.

c Washing solution.

Electrophoretic papers were washed free of excess dye in a solution ofmixture of methanol, acetic acid and water in the ratio 5:1:5. volume by volume.

d Eluting solution.

This could either be a 10% aqueous acetic acid or a 50% methanol or ethanol and 0.5N sodium hydroxide in the ratio 1:1 volume by volume.

e Electrophoretic Paper.

Whatman chromatographic paper 3mm in strips 3.5cm wide and about 40cm long were always used.

9 Reagents for the determination of serum glucose.

For this determination, the method of Nelson and Somogyi described by Annino (1964) was used. The reagents were those reported by Annino (1964).

a 0.3N Barium hydroxide.

28.4gm barium hydroxide monohydrate $[Ba(OH)_2.H_2O]$ or 47.2gm barium hydroxide octahydrate $[Ba(OH)_2.8H_2O]$ was dissolved in one litre of solution which was left to stand for several days in a covered container. The supernatant was decanted and stored in a polythylene bottle and protected from air.

b Zinc sulphate 5% w/v.

50gm of Zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) was dissolved in a litre of solution and stored in a brown bottle.

c Copper sulphate solution.

29gm of anhydrous disodium phosphate (Na_2HPO_4) or 70.6gm of the dodecahydrate ($Na_2HPO_4.12H_2O$) and 40gm of potassium sodium tartrate ($KNaC_4H_4O_6.4H_2O$) were dissolved in about 700ml of distilled water. 100ml of N.NaOH solution was added. Then 80ml of a 10% (w/v) solution of hydrated copper sulphate ($CuSO_4.5H_2O$) was then added with stirring. To this solution, 180gm of anhydrous sodium sulphate (Na_2SO_4) was added. When all the salts were dissolved, the solution was diluted to one litre with distilled water, and allowed to stand a day or two before it was filtered. The reagent keeps indefinitely.

d Colour reagent.

50gm of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, was dissolved in 900ml of water. To this was added 42ml of concentrated sulphuric acid and 6gm of sodium arsenate ($Na_2HAsO_4.7H_2O$) dissolved in 50ml water. The solution was mixed thoroughly and incubated for 48hours at 37°C. It was stored in a brown bottle and keeps well.

e(i) Stock glucose standard.

25.0gm reagent grade glucose was dissolved in approximately 300ml of 0.15% w/v benzoic acid. The solution was made up to 500ml with more benzoic acid. This solution keeps at -4°C.

(ii) Working glucose solution.

About 6ml of stock was warmed to room temperature. 5ml was then diluted to 100ml with water; 1ml of this solution was then diluted to 50ml with 0.15% benzoic acid. This working standard keeps well even at room temperature.

10 Reagents for the determination of glutamic pyruvate transaminase, GPT.

The method of Rietman and Frankel, (1957) was used. The reagents were obtained from BDH Chemicals Limited. The enzyme assay set contained the following reagents.

a SGPT buffered substrate.

The content of each tube labelled as substrate was dissolved in 60ml of solution to give a solution containing 2mM of 2-oxoglutaric acid, 200mM DL-d-alanine and 0.1M phosphate buffer. This solution is stable for six weeks if stored in a deep freeze and preserved with 2 drops of chloroform.

b 2:4-dinitrophenyl hydrazine (1mM).

One volume of the concentrated (5mM) solution was diluted with four volumes of N/1HCL before use. This solution keeps at room temperature.

c Pyruvate standard, 2.0mM solution.

The content of the pyruvate standard vial was dissolved in sufficient distilled water and made up to a total volume of 10ml in a volumetric flask.

d Sodium hydroxide solution, 0.4M.

This solution was prepared by diluting 4M. NaOH solution (free from carbonate) ten times with freshly deionised water.

11 Reagents for the determination of serum leucine-amino peptidase, LAP

The reagents for this determination were those contained in the Biochemical Test Combination Kit obtained from the Biochemical Department of Boehringer Mannheim Gebh. The set contained the following reagents.

a Phosphate buffer pH 7.2.

The content of the bottle labelled 1 was dissolved in 100ml redistilled water as directed. It contained 100mM phosphate buffer at pH 7.2.

b Leucine-p-nitranilide.

The content of bottle 2 was dissolved in 3.5ml methanol to give a solution containing 25mM leucine-p-nitranilide which keeps well for six months at about 4°C.

.2 Reagent for the determination of serum phosphatase.

The method of King, [illegible] was used in this determination and involved the use of the following reagents.

a Alkaline buffer m/10.

6.36gm of sodium carbonate anhydrous and 3.36gm of sodium bicarbonate were dissolved in water and made up to 1 litre.

b Acid buffer m/10

21.0gm of citric acid crystals and 188ml N/1 sodium hydroxide were mixed and made up to 500ml with distilled water. The pH was adjusted to 4.9 with N/1 HCl or N/1 NaOH if necessary. It was preserved with a little chloroform and stored at 4°C.

c Substrate m/100.

2.18gm disodium phenyl phosphate was dissolved in distilled water and made up to 1 litre. It was preserved with chloroform and stored at 4°C.

d Tartrate, m/1

15.0gm tartaric acid, anglar, was dissolved in about 70ml water. 18.5ml of 10N.NaOH was added and the pH. adjusted to 4.9. The solution was then made up to 100ml with more distilled water and preserved with chloroform.

e Sodium Hydroxide.

Both 0.5N and 2% NaOH were prepared.

f Sodium carbonate.

Both 0.5% and 2% Na_2CO_3 were prepared.

E 4-amino-phenazone - 6%

6gm of solid was dissolved in 100ml solution.

h Potassium ferricyanide, 2.4%.

2.4gm of solid was dissolved in 100ml of solution.

i Stock standard phenol.

0.1gm of phenol was dissolved in 100ml of solution in HCl to make a solution of 1.0mg/ml.

Low and High working standards containing 0.01mg and 0.05mg per ml were prepared and stored at +4°C.

13. Reagents for the determination of serum bilirubin.

The method of Powell, described by Varley (1962) was used and the reagents were those given by Varley.

a(i) Diazo reagent: 'A'

1.0gm sulphanilic acid was dissolved in 15ml of conc. HCl and made up to one litre with distilled water.

(ii) Diazo reagent 'B'

0.5gm of sodium nitrite was dissolved in 100ml solution to give a 0.5% solution.

(iii) Diazo reagent: working solution.

5.0ml of reagent 'A' was added to 0.15ml of reagent 'B'. This working solution was always prepared fresh.

(iv) Diazo blank.

This was 1.5% HCl (v/v) in water.

b Benzoate-urea solution.

10gm sodium benzoate and 10gm urea were dissolved in water and made up to 100ml. The solution was filtered before it was stored at $+4^{0}C$.

c Methyl-red standard solution.

(i) Stock standard.

0.290gm methyl red was dissolved in glacial acetic acid and made up to 100ml.

(ii) Working standard.

5.0ml glacial acetic acid was added to 1.00ml of stock standard. To this solution was added 11.4gm sodium acetate and the volume made up to one litre with water. The pH was adjusted to 4.63.

14. Reagents for estimation of free fatty acid produced 'in vitro'

The reagents used in this work were those used by Dole(1956) and Loreh and Ley. (1966).

a Krebs phosphate buffer pH 7.4 (Ca^{2+} omitted).

This phosphate buffer was prepared from the following solutions:

(I) Sodium chloride 0.9% (0.15M).

The solution contained 9gm of salt per litre of solution.

(II) Potassium chloride 1.19% (0.15M)

It contained 11.9gm of salt per litre of solution.

(III) Magnesium sulphate 3.38% (0.154M).

It contained 33.8gm of salt per litre of solution.

(IV) 0.1M phosphate buffer pH 7.4

17.8gm $Na_2HPO_4.2H_2O$ was dissolved in 20ml NaCl and diluted to 1 litre with distilled water.

Krebs phosphate buffer was obtained by mixing the above solutions in the given proportions.

100 parts of solution I

4 " " " II

1 part " " III

20 parts " " IV

b Kreb's phosphate buffer (pH7.4, Ca^{2+} omitted) containing 0.2% glucose.

This was prepared by dissolving 7.29gm glucose in 1 litre of Kreb's phosphate buffer.

c Extraction mixture.

This is a mixture of isopropyl alcohol, n-heptane and sulphuric acid in the proportions 40:10:1.

d Titration mixture.

(i) Stock thymol blue

0.1g thymol blue was dissolved in hot water and made up to 100ml.

(ii) Working thymol blue.

The stock thymol blue reagent was diluted 10 times with redistilled ethanol.

e Alkali. approximately 0.01N sodium hydroxide.

This was prepared by $\frac{1}{1000}$ dilution of a saturated sodium hydroxide solution with carbon dioxide free distilled water. The alkali was protected with a soda lime column stored in a small reservoir mounted above a 0.100cc. burette.

f Palmitic acid standard.

265.4mg palmitic acid was dissolved in 100ml n-heptane to give a solution containing 10meq./litre. This solution was stored at -15°C to minimize evaporation. Standard serial dilutions of it were usually used along with each experiment.

CHAPTER THREE

METHODS

Preparation of inoculum containing parasitized red blood cells.

The parasitised inoculum used in this work always contained a known amount of parasites. In the case of Plasmodium berghei, the inoculum was prepared to contain approximately 5 million parasitised red blood cells per ml of inoculum while the Plasmodium gallinaceum inoculum always contained about 50 million parasitised blood cells per ml. This was done by first finding the percentage parasitemia of the infected blood and then the red blood cell count. The blood was then diluted with isotonic saline in the proportions indicated by both determinations.

a Determination of % parasitemia.

The method used was that suggested by Marshall et.al. (1942) in which the number of parasitised red blood cells out of 200 red blood cells was found by examining a thin film of the blood prepared as described below.

i Preparation and staining a thin blood film.

The method used was that of Blacklock and Southwell (1969) in which a drop of blood was placed near one end of a clean slide which was laid flat on a table. Steadying the slide with the left forefinger and thumb at the end remote from the drop of blood, another slide, the spreader, was held at an angle, up against that edge of the drop which was nearest the center of the slide;

the blood will run along the back of the spreader's edge and the spreader was then pushed towards the other end of the slide with a smooth steady movement. The spreader had both corners broken off, so that the resultent film was narrower than the slide on which it was spread. The drop of blood to be spread was usually so small that the film would terminate, well before reaching the end of the slide, in drawn-out tails.

(ii) Staining blood film with Leishman's stain.

With the aid of a pipette, enough Leishman's stain just to cover the film was dropped on the slide; after about 15 seconds twice as many drops of buffered water as stain was added and mixed. After the slide had been left to stain for about ten minutes the stain was rapidly washed off by flooding it with neutral water. It was then allowed to dry in an upright position. The dry film was examined under a drop of immersion oil.

b Determination of the red blood cell count.

The haemocytometer described by Baker et. al. (1957) was used to determine the red blood cell count. In this method, a drop of blood from the pool of blood was sucked up to the 0.5 mark (or slightly beyond it) on the red blood cell pipettes. The mouth piece of the pipette was closed and excess blood was wiped away from the outside of the pipette. If the blood was beyond the 0.5 mark, the excess was removed by touching the tip gently against the back of the hand till the blood was exactly at the 0.5 mark.

A small bubble of air was drawn into the capillary and immediately the diluting fluid was sucked up to the 101 mark, rotating the pipette vigorously all the time to mix blood and solution thoroughly. After mixing for about a minute about a quarter of the content was blown out so as to remove the pure diluting fluid in the stem.

The glass bars on either side of the counting chamber were moistened with the tip of the finger and the coverrlip firmly pressed down on them so that a series of concentrically arranged rings, (Newton's rings) was seen. The tip of the pipette was quickly, but gently placed on to the surface of the counting platform where it projected beyond the coverglass. A small amount of the solution flowed under the coverglass. The platform should be covered but if the fluid flowed over the edge of the chamber, or if bubbles of air appeared in it, the slide was washed up, dried and the process repeated.

As soon as the cells had settled down (that is after about two minutes) the count was made as the rulings and the cells were then in the same plane. If the distribution of the cells was not uniform when observed under the low power objective, the counting chamber was cleaned and filled again. The microscope should be horizontal.

The total number of cells in eighty small squares using the high power objective, was counted. Cells on the upper line and left side of each square were included in the count for that square.

The number of cells per cubic millilitre of blood was obtained by multiplying the total count in eighty squares by a factor, which in this case was 10,000. The factor was obtained from the calculation below.

Calculations

The depth of each chamber $= \frac{1}{10}$ mm

Each square $= \frac{1}{400}$ sq.mm.

$\therefore$ Volume of one square $= \frac{1}{4000}$ cu.mm.

Total volume counted $= 80 \times \frac{1}{4000}$ cu.mm.

If n is the number of cells in 80 squares, number per cu.mm. in diluted blood $= n \times \frac{4000}{80}$

Blood was diluted 200 times.

$\therefore$ Total cell per cu.mm. $= n \times \frac{4000}{80} \times 200$

$= n \times 10{,}000$/cu mm.

$= n \times 10^4$/cu mm.

2. Inoculation of experimental animals

Mice weighing between 18 and 25gm were injected intraperitoneally with 0.2ml of infected blood containing about 5 million parasitized blood cells per ml. This was considered suitable for producing an infection in mice used for chemotherapeutic studies (Jacobs et.al., 1963).

7-days-old chicks were inoculated intravenously through the jugular vein with about 0.5ml of an inoculum containing 50 million parasitized red blood cells per ml. (Coatney et.al. 1953).

Methods of drug preparation.

a. Preparation of solutions of standard antimalarials.

Aqueous solutions of each of the antimalarial drugs employed were prepared fresh weekly in 0.01NHCl and stored at 2°C using the method of Jacobs. et. al. (1963).

b. Preparation of drugs from the local plant materials.

(i) Traditional method of preparation of drugs

Traditionally most herbal drugs are prepared by boiling the components in enough water to cover the materials. The cooking pot which traditionally is a clay pot with a narrow neck and a lid is never removed from the fire place. It is replaced on the fire place after each cooking so as to keep the content hot and perhaps prevent spoilage. More water is usually added to the drug as often as necessary to keep the components constantly submerged in water. This method makes it difficult to estimate what quantity of water is used to prepare a particular amount of drug plant.

In order to find an approximate idea of the weight of drug plants used, ten market samples were bought and weighed in each case and the average weight was found. From this weight recommended for an adult man the equivalent weight for mice weighing

about 20gm was calculated.

The approximate weight of plant materials used are given below:

1 Morinda lucida - leaves — 540gm per litre of water.

2 Azadirachta indica - leaves — 320gm " " " "

3 Alstonia boonei - leaves — 400gm " " " "

4 Enantia chlorants - bark — 250 gm per litre alcohol.

5 Mixture A

a, Asadirachta indica - leaves

b, " " - bark

c, Cymbopogori citratus - leaves

d, Carica papaya - leaves — 35gm

e, Mangifera indica - bark — 60gm.

All boiled in 2 litres of water.

6 Mixture B

a, Morinda lucida - leaves — 150gm

b, Azadrachta indica - leaves

c, " " - bark — 55gm.

d, Enantia chlorant - bark — 35gm

e, Carica papaya - leaves

f, Cymbopogori citratus - leaves — 40gm

g, Mangifera indica - bark — 50gm.

All the plant materials are boiled in 2 litres of water.

(ii) Method II

The method of Berg et.al. (1962) was used to prepare water extract of the various plant materials. In this case, about 1kg of freshly collected plant material was macerated and boiled in about 2.5 litres of water for 1hour. After filtration, the water extract was then concentrated under reduced pressure to a small volume, usually about 200ml. This concentrate is diluted to required strength before use.

Method III

Preparation and separation of water soluble components.

Water extract of drug plants prepared as described earlier was separated into different fractions using the method described below:

The concentrated water extract was made acidic with 2% HCl and immediately extracted by shaking with 2 portions of Pet ether (40^{0}-30^{0}) in a seperating funnel. This will remove oil, fat and waxy colouring matter. The pet ether is evaporated under reduced pressure and the residue obtained constitute extract 1.

The aqueous acid solution left after the Pet ether extraction was extracted with two portions of chloroform and then with chloroform-alcohol mixture (3:2 volume by volume). The chloroform and chloroform-alcohol extracts were combined and evaporated to dryness by distillation under reduced pressure.

The residue obtained constitutes fraction II and should contain glycosides and weakly basic alkaloids.

The aqueous acid solution left is basified by adding a 20% solution of ammonium hydroxide drop by drop until the solution is distinctly alkaline. The strong base is extracted with chloroform. The residue after distillation constitutes fraction III and should contain basic alkaloids.

The ammoniacal solution left is filtered through whatman No.1 filter paper using vacuum. The clear filtrate is acidified with dilute HCl and may contain some quarternary alkaloids.

4. Estimation of serum total protein.

The biuret method of King described by Varley (1962) was used.

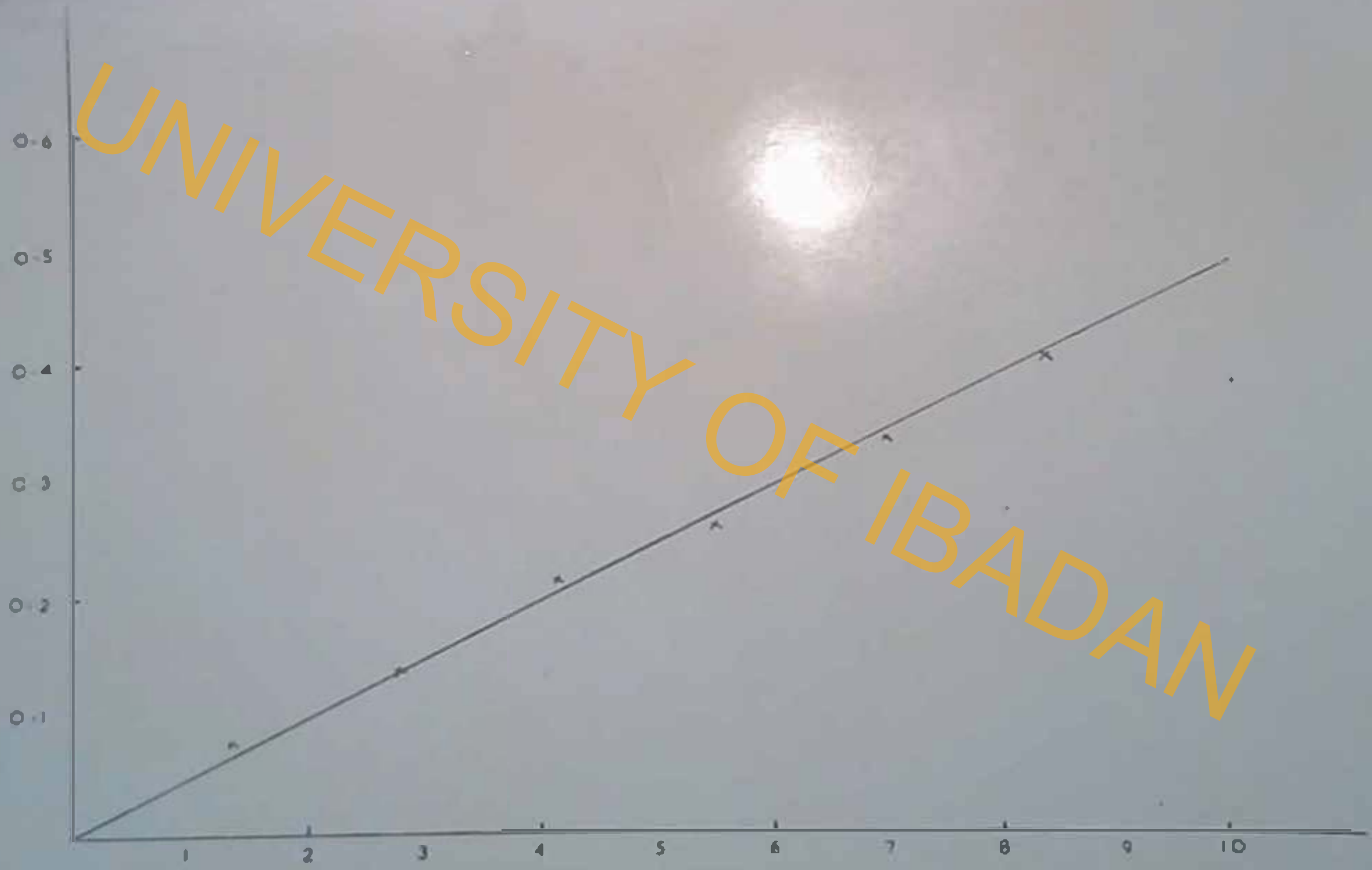
To 0.1ml of each serum sample in a test tube, 2.9ml water was added. Each test solution was prepared in triplicate. To each test tube and to a blank containing 3ml of distilled water, 3ml of working biuret reagent was added. All tubes were incubated in a water bath at 37°C for 10 minutes. The optical density of each solution was read in an SP600 Spectrophotometer at 540 m/u setting the instrument to zero with the blank.

The protein concentration in gm/100 ml was read from the calibration curve prepared as described below.

Preparation of a standard curve for protein.

A standard curve for protein was prepared using Versatol 'A', a reconstituted human serum protein. The content of the vial was dissolved in 2ml of distilled water to give a solution containing 7.1Gm serum proteins per 100ml solution. Several dilutions of this solution were made and each dilution was treated with biuret reagent as in the case with the serum samples. The optical densities were plotted against the concentrations of the proteins to obtain a standard protein curve, (Fig 1)

FIG. 1



5 Fractionation of serum proteins by paper electrophoresis.

A modification of the methods of Bailey (1962) and Briggs et.al. (1960) was used to fractionate the serum proteins. Chromatographic paper 3mm in strips of 3.5cm wide and 40cm long were first moistened with buffer solution and partially dried between two dry filter papers before the serum sample was applied. 0.02ml serum was applied to the paper with a smooth-tipped pipette, care being taken not to scratch the surface of the paper. The line of application was usually about mid-way of each strip and was always clear of both edges of the paper by 7 to 8cm.

The strips were then put into the electrophoretic tank which had previously been filled with buffer solution such that the levels of buffer at each end of the strips were equal in order to avoid syphoning of solution from one end to the other. Absorbent cotton wool was used to connect the buffer solution on either side of each buffer compartment.

When the papers were set, the tank was covered with a glass cover on which silicone grease has been applied to make the tank air-tight. This was necessary to prevent evaporation of the buffer as this will change the concentration of the buffer and consequently its pH.

A current of 3ma per centimeter and voltage of about 150 volts were applied. The sera were fractionated over 20 hours for good separation.

5 Fractionation of serum proteins by paper electrophoresis.

A modification of the methods of Bailey (1962) and Briggs et.al. (1960) was used to fractionate the serum proteins. Chromatographic paper 3mm in strips of 3.5cm wide and 40cm long were first moistened with buffer solution and partially dried between two dry filter papers before the serum sample was applied. 0.02ml serum was applied to the paper with a smooth-tipped pipette, care being taken not to scratch the surface of the paper. The line of application was usually about mid-way of each strip and was always clear of both edges of the paper by 7 to 8cm.

The strips were then put into the electrophoretic tank which had previously been filled with buffer solution such that the levels of buffer at each end of the strips were equal in order to avoid syphoning of solution from one end to the other. Absorbent cotton wool was used to connect the buffer solution on either side of each buffer compartment.

When the papers were set, the tank was covered with a glass cover on which some silicone grease has been applied to make the tank air-tight. This was necessary to prevent evaporation of the buffer as this will change the concentration of the buffer and consequently its pH.

A current of 4ma per centimeter and voltage of about 100 volts were applied. The sera were fractionated over 20 hours for good separation.

After the fractionation the strips were dried in an oven at about 110°C for 20 minutes to render the proteins insoluble, before they were stained in amido black stain for about 10 to 20 minutes. The excess dye was washed off with many changes of the washing mixture until the back-ground was as clean as possible.

The strips were dried in air. The coloured zones containing the protein fractions were cut in strips of 0.5cm and eluted with 5ml of the clutin solution over two hours. The optical densities of the eluent were read at 620 m/u on spectrophotometer SP600 and the readings were plotted against the distance of each strip to obtain a curve from which the relative values of the fractions can be obtained. A typical example of the curve obtained is shown in Fig 2. The absolute value can be obtained from this value and the value of the total protein in the serum.

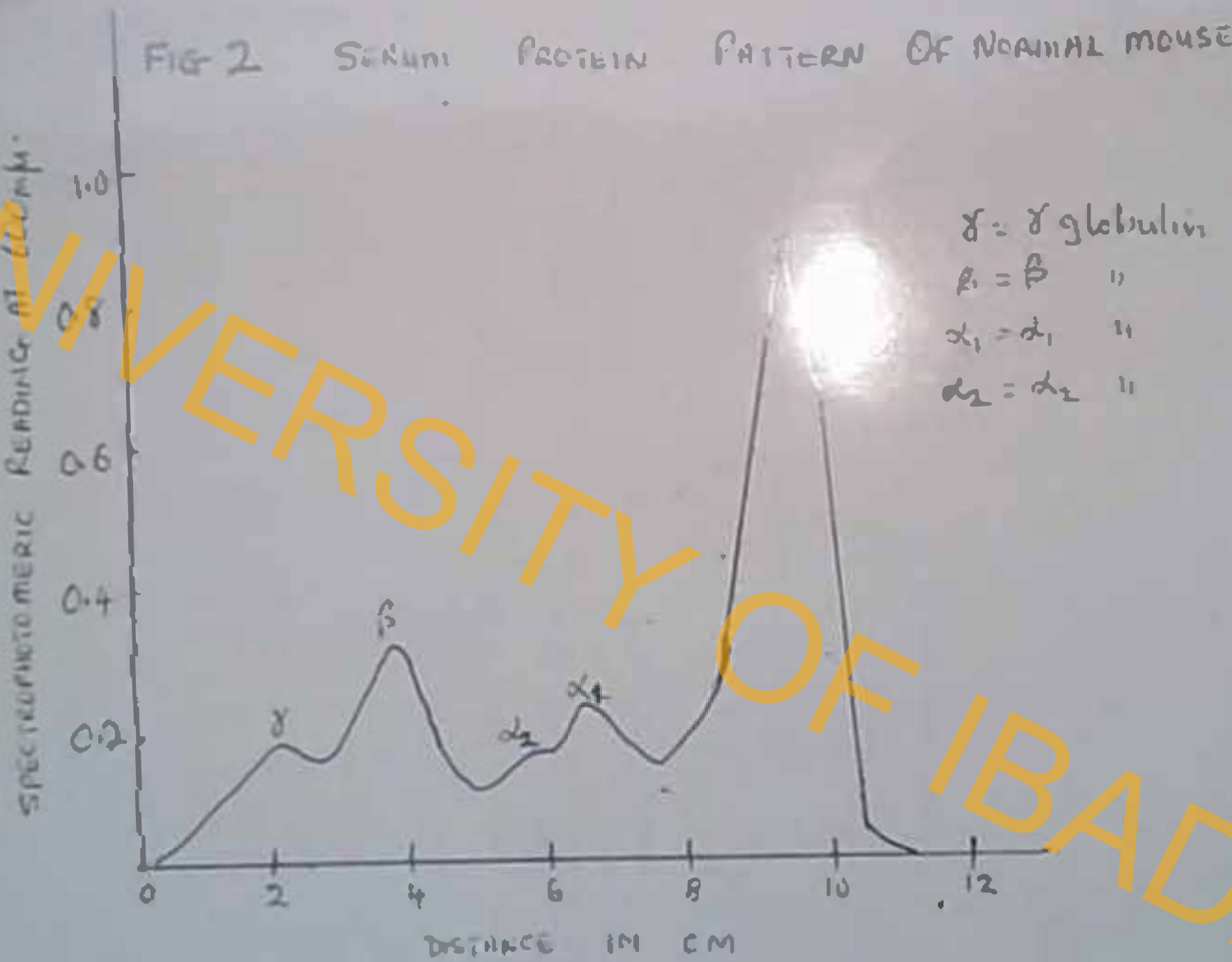
6 Estimation of serum glucose.

This was determined by using the method of Nelson (1944).

The barium hydroxide and zinc sulphate solutions prepared as described in chapter two were first tested as follows before they were used.

To a 50ml flask, 5ml zinc sulphate, 10ml water and one drop of 0.1% phenolphthalein were added. This mixture was titrated against barium hydroxide solution from a 1ml burette until a faint pink end-point was reached.

FIG 2 SERUM PROTEIN PATTERN OF NORMAL MOUSE



γ = γ globulin
β_1 = β "
α_1 = α_1 "
α_2 = α_2 "

Usually this required about 4.7 to 4.8ml. If the titration was outside these limits, the stronger solution was usually adjusted. For instance if the titre was less than 4.7ml, the barium hydroxide solution was stronger and the concentration factor was $\frac{4.75}{\text{litre}}$. This factor was multiplied by the total volume of barium solution to be adjusted and the difference between this calculated result and the actual volume of the barium solution represented the amount of water to be added.

And if the titre was greater than 4.8ml, the concentration factor was $\frac{\text{litre}}{4.75}$ and this was applied to the total volume of the zinc sulphate solution to calculate the amount of water to be added to it.

When this had been standardised then to 0.2ml serum in a 12ml heavy walled centrifuge tube, 3.2ml water and 0.4ml barium hydroxide reagent added, mixed thoroughly and allowed to stand for about one minute or until the solution turned brown. Then 0.4ml zinc sulphate solution was added, mixed well and allowed to stand for at least two minutes before it was centrifuged for 10 minutes at 2000 rpm.

2ml of the clear supernatant fluid was pipetted into a thick-walled test tube with cover. To this solution, 1ml copper sulphate solution was added and mixed. The tube was then placed in a boiling water bath for 10 minutes.

A blank of 2ml water and 2ml of working standard sugar solution were also treated like the supernatant.

FIG 3.

STANDARD GLUCOSE CURVE

The tubes were cooled thoroughly in ice. 1ml of colour reagent was added to each tube, mixed and left to stand for at least two minutes after which it was diluted to 25ml with water. The absorbance in the SP600 at 490m/u was read setting the instrument at zero with the blank.

Calculation.

$$\frac{\text{Concentration of standard}}{\text{Reading of standard}} \times \text{reading of unknown} = \text{glucose mg/100ml.}$$

Standard containing 100mg per 100ml was always used. A standard curve was also prepared using serial dilutions of the standard glucose as shown in the figure. (Fig. 3)

Estimation of serum glutamic pyruvate transaminase (GPT).

The method of Reitman and Frankel (1957) was used.

Principle.

The method is based on the principle that this enzyme catalyses the reaction.

L-Alanine + 2Oxoglutarate $\rightleftharpoons$ L-glutamate + pyruvate.

The pyruvate produced reacts with 2:4-dinitrophenyl hydrazine to produce an intensely coloured hydrazone on the addition of sodium hydroxide. Measurement of optical density at 505nm provides a measure of enzyme activity when compared with a standard graph.

Procedure

The procedure was that included in the [illegible] biochemical reagent kit for this enzyme determination. Blood specimen was separated immediately and for each assay the sets of tubes were prepared as follows.

	Test	Control
Buffered substrate	1.0ml	1.0ml.
Serum	0.2ml	-
Mixed, incubated in water bath at 37°C for 30 minutes		
2-4 dinitrophenyl hydrazine reagent	1ml	1ml.
Serum	-	0.2ml
Mixed and incubated for 20 minutes room.		
0.4N sodium hydroxide solution	10ml	10ml.
Mixed and allowed to stand for 10 minutes after which O.D. was taken at 540/um against a water blank.		

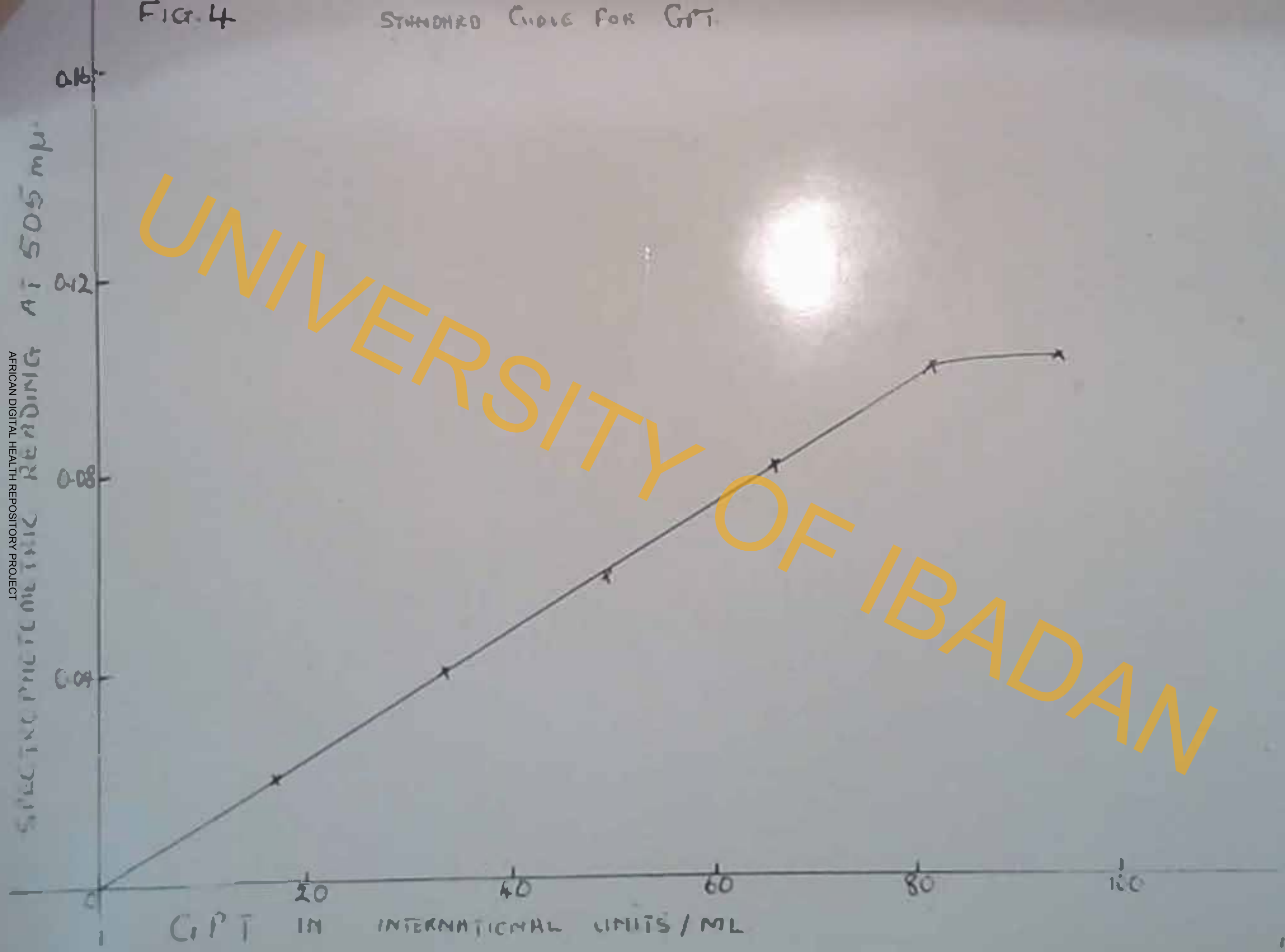
Preparation of a calibration curve for GPT.

Seven tubes were prepared as follows:

Tube No.	Test	2mM Standard	Substrate	/u mole pyruvate per mg. per litre	International unit per litre.
1	0.2ml	0.0ml	1.0ml	-	-
2	0.2"	0.05ml	0.95 "	17	6.5
3	0.2"	0.10 "	0.90 "	33	12.5
4	0.2"	0.15 "	0.85 "	50	12.0
5	0.2"	0.20 "	0.80 "	67	30.5
6	0.2"	0.25 "	0.75 "	83	32.5
7	0.2"	0.30 "	0.70 "	100	36.0

FIG. 4

STANDARD CURVE FOR GPT.



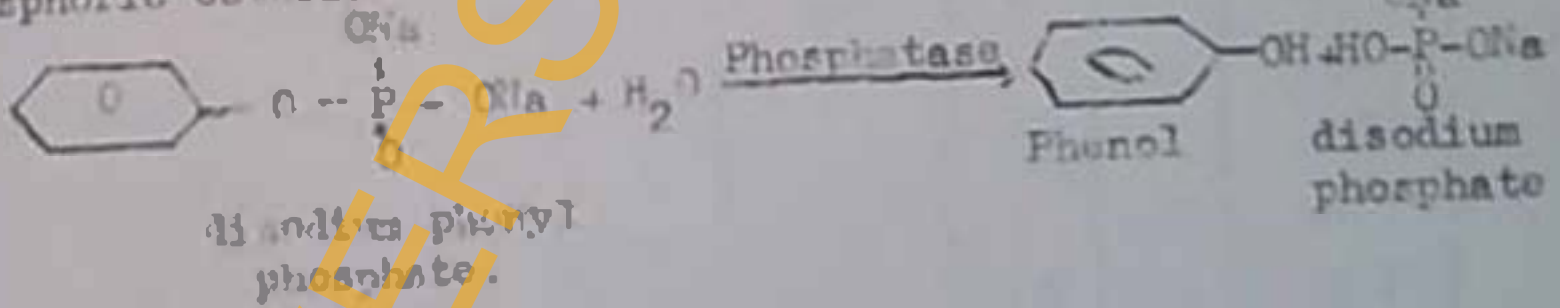
The solutions were mixed and incubated at 37°C for 30 minutes; and to each tube 1ml of 2.4-dinitrophenyl hydrazine reagent was added, mixed and incubated for a further 20 minutes. 10ml of 0.4N NaOH was added and mixed. After allowing the tubes to stand for 10 minutes the optical densities of each solution against a water blank at 505mm in a 1cm cell were read.

From the optical densities obtained a standard curve for reagents in the set was plotted. From this curve, the values of GPT in the test samples were read Fig 4.

8 Estimation of phosphatases.

Principle.

Phosphatases are enzymes which catalise the hydrolysis of monophosphoric esters:



disodium phenyl phosphate.

Two types of phosphatases are commonly estimated in serum, an alkaline phosphatase showing maximum activity at pH 10, and acid phosphatases with maximum activity about pH 5.

The method most commonly used is that of King and Armstrong, in which disodium phenyl phosphate is hydrolysed by phosphatase (acting as a catalyst only) under standard conditions.

The amount of phenol or phosphate so liberated may be taken as a measure of the amount of creatine present.

4 amino phenazone forms a red quinone with phenol in the presence of an alkaline oxidising agent at pH 10.4 and potassium ferricyanide. The optical density of the solution is then read at 510m/u. The reagents do not react with serum proteins and removal of the latter is therefore unnecessary.

Procedure for estimation of alkaline phosphatase

Test-tubes were set up and reagents were added and mixed thoroughly in the proportions indicated in the table.

	Test	Blank	Standards Low	Standards High	Standard blank
Alkaline buffer	1.0	1.0	1.0	1.0	1.0ml
Substrate	1.0	1.0	-	-	-ml
Distilled water	-	-	-	-	1.0ml
Low standard phenol	-	-	1.0	-	-ml
High " "	-	-	-	1.0	-ml.
Sodium hydroxide 0.5%	-	0.8	0.8	0.8	0.8ml
Incubate test and blank at 37°C for 5 minutes					
Serum	0.1	0.1	-	-	-ml
Incubate test and blank at 37°C for 15 minutes					
Sodium hydroxide 0.5%	0.8	-	-	-	-ml
Sodium carbonate	1.2	1.2	1.2	1.2	1.2ml
Amino-phenazone	1.0	1.0	1.0	1.0	1.0ml
Potassium ferricyanide	1.0	1.0	1.0	1.0	1.0ml

The pink colours which developed were compared at 510m/u using the appropriate blank to set to zero.

Calculation.

$$\frac{\text{test reading}}{\text{standard reading}} \times \frac{0.01(0.03) \times 100}{0.1}$$

$$= \frac{\text{test reading}}{\text{standard reading}} \times 10(\text{or } 30) \text{ King-Armstrong unit per 100ml.}$$

The King-Armstrong unit is the amount of enzyme which will set free 1mg of phenol in the given time under the conditions of the test. This time is 15 minutes at pH10.5 for Alkaline phosphatase while it is one hour for acid phosphatase at pH5.0.

9 Estimation of serum leucine amino peptidase (LAP).

Principle.

The determination of leucine amino peptidase activity (LAP) in human serum is considered superior to older methods, like the assay of alkaline phosphatase, for the differential diagnosis of jaundice.

Peptidases hydrolyse terminal peptide bonds. Unlike the proteinases they do not attack native proteins. Their substrates are di-and polypeptides. Peptidases can be subdivided according to their substrates into dipeptidases, aminopeptidases and carboxypeptidases. Aminopeptidases hydrolyse peptides containing free terminal amino groups.

In the method used, the enzyme is made to act on l-leucine-p-nitranilide in a buffer solution pH 7.2.

Procedure:

The method which accompanied the LAP reagent of Biochemica Test Combination obtained from Boehringer Mannein GMbh, was used. In this method test tubes were set up as shown below. Solution one is the phosphate buffer at pH 7.2 while solution two is the leucine-p-nitranilide solution, that is the substrate in this case. Into each test tube 3ml. of solution one (phosphate buffer) and 0.1ml of solution two (substrate) were added, mixed and incubated in a water bath at 25°C for 5 minutes. Then 0.1ml of the serum sample was added and mixed. The optical density of this mixture was read immediately at 405/4m. and recorded as E_1. After exactly 30 minutes incubation at 25°C, the optical density was again read and recorded as E_2. The difference between both is ΔE.

With optical density differences above 0.600, the serum was diluted ten times with physiological saline. The result should be multiplied by 10.

Calculation.

LAP activity of the enzyme is obtained from a table supplied with the reagents or calculated as below.

$E_2 - E_1 = \Delta E$

$108 \times \Delta E =$ mU/ml.

Estimation of serum total bilirubin.

Principle.

Bilirubin reacts with diazotized sulphanilic acid to form the red coloured azobilirubin. In aqueous solutions only conjugated bilirubin (direct bilirubin) reacts. When benzoate-urea is added, all the bilirubin (conjugated and free) react.

Methyl red (2.9mg per litre at pH 4.65 in acetate buffer) is used as a standard. The colour of this solution accurately matches the colour obtained when 0.01mg of bilirubin is treated with the diazo reagent in a final volume of 4ml.

Procedure.

The method used is that of Powell described by Varley (1962).

Into 4 sets of test tubes the following solutions prepared as described earlier were added.

	Total		Conjugated	
	Test	Blank	Test	Blank
Serum	0.4	0.4	0.4	0.4ml
diazo reagent	0.2	-	0.2	-ml
diazo blank.	-	0.2	-	0.2ml
benzoate-urea solution	3.4	3.4	-	-ml
distilled water	-	-	3.4	3.4ml

The solutions were mixed and allowed to stand at room temperature for 10 minutes and their optical densities were read at 520m/u using the appropriate blank to set the spectrophotometer at zero.

Standard.

The optical density of the standard methyl red solution was also read using distilled water to set the instrument to zero.

Calculation.

$$\frac{\text{test reading}}{\text{Standard reading}} \times 0.015 \times \frac{100}{0.4}$$

$$= \frac{\text{test reading}}{\text{Standard reading}} \times 4 \text{ mg per 100ml.}$$

For values of over 10mg per 100ml, smaller quantity of serum was used and the amount of benzoate-urea solution (or distilled water) was increased accordingly.

10. Estimation of total lipids and free-fatty acid produced by Plasmodia in 'in-vitro' systems.

The method used in this determination was a combination of the techniques of Dole(1956); Loroh and Cey,(1966); Canedella (1968) and Canedella et. al. (1969).

a Preparation of Plasmodium suspension.

Blood from 30 to 40 normal mice or parasitized by Plasmodium berghei was collected by cardiac puncture into 5ml syringe containing 0.1 ml sodium citrate anticoagulant and then pooled. All parasitized mice were used between the 5th and 8th days of infection.

Blood from normal or parasitized chicks were also collected by heart puncture.

Thin smears were prepared from the infected blood pools, stained and counted to determine the percentage parasitaemia. Total red cell counts were also established. Subsequently the parasitised and unparasitised blood were centrifuged at about 1000g ~~per minute~~ for 10 minutes at 5°C. The plasma was removed and discarded and the cells were resuspended to the original volume in Krebs phosphate buffer (pH 7.4, Ca^{2+} omitted) and centrifuged again. This washing procedure was repeated twice. The washed cells were then resuspended in the buffer to a specific parasite density or total red cell count. Equal volumes of parasitized or unparasitized cell suspensions and Krob's phosphate buffer (containing 0.6% glucose) were added and mixed. The incubated cell suspensions were thus 0.022m with respect to glucose.

Aliquots of the remaining pool cell suspensions were removed for determination of the pre-incubation levels of both total lipid and free fatty acid and the remaining suspensions were incubated for four hours at 37°C with shaking. Another aliquot was withdrawn for determination of total lipid and free fatty acid after four hours.

Extraction and estimation of total lipid content of the cell suspensions.

Aliquots of the whole cell suspension to be analysed for total lipid were extracted overnight with 20 volumes of 2:1 Chloroform - methanol. The Chloroform-methanol extracts were then warmed to about 50°C and filtered. The lipid extract was evaporated to dryness and the total phospholipid content of the residue was determined by the method of Bassir (1971) in which the lipid phosphorus was determined.

The residue was first dissolved in 0.4ml perchloric acid followed by 5ml distilled water. Then 0.4ml concentrated ammonium molybdate solution and 0.4ml of 0.2% ascorbic acid were added. The contents of the tubes were mixed by gentle shaking and allowed to stand at room temperature for 20 minutes. A standard phosphate solution of potassium dihydrogen phosphate (5ml =0.2mgP) was similarly treated. The colours of the solutions were read in a colorimeter using a red filter and setting a water blank at zero.

Calculation.

$$\text{mg. P per 100ml} = \frac{\text{Reading of Test}}{\text{Reading of Standard}} \times 0.2 \times \frac{100}{0.5}$$

Extraction and separation of FFA from cell suspension.

Free fatty acids were extracted from cell suspension using the Trout et.al. (1960) modification of Dole (1956) extraction procedure.

In this method, to 2ml of the cell suspension in a glass-stoppered test tube was added, with shaking, 10ml "extraction mixture (isopropanol, n-heptane and $1N_2SO_4$ in ratio 40:10:1 volume by volume); then 5ml of n-heptane and 4ml water were introduced and the mixture was shaken for at least 2 minutes. 4ml to 5ml aliquots of the upper heptane layer was removed into a glass stoppered centrifuge tube and then vigorously shaken for five minutes with an equal volume of 0.75 per cent aqueous sulphuric acid. The tube was then centrifuged at about 500g for 5 minutes. To the last tube which contained Lowry-Lobo's "titration" mixture, 1ml of the washed heptane layer was transferred and it was then titrated with 0.01N sodium hydroxide while being agitated with a stream of nitrogen gas delivered to the bottom of the tube with a fine glass capillary. The stream of nitrogen should expel carbon dioxide from the sample and keep the two phases mixed during titration. As the green-yellow end-point was approached the gas stream was interrupted from time to time for examination of the indicator colour in the alcoholic phase. Good lighting was found essential as the end-point was difficult to see under poor lighting.

The heptane layers from appropriate titration blanks and palmitic acid standards were similarly washed before titration. A series of standards freshlyprepared daily and ranging from 50 to 500/ug palmitic acid per litre were used for comparison.

Determination of growth and infectivity of Plasmodia after exposure to drugs 'in-vitro'.

Various methods are used for cultivation of Malarial parasites 'in-vitro'. Each species requires its own variation of the general medium.

a. Cultivation of Plasmodium berghei in vitro.

A combination and modification of the methods of Polet and Barr, (1968), Trager (1967) and Polet (1966) in which parasitized cells were suspended in a nutrient medium for 24 hours failed due to loss of viability and extensive haemolysis. The method of Rickman et. al. (1968) was used. This involves the incubation of citrated parasitised red blood cells with some glucose in screw cap bottles for about 6 hours.

b. Cultivation of Plasmodium gallinaceum in-vitro

The method of Taylor et. al. (1951) was used in the cultivation of Plasmodium gallinaceum.

Parasitized blood was obtained from chicks with approximately 60% of their erythrocytes parasitised. The blood was diluted with isotonic saline to contain about 10×10^8 parasitised cells per ml. Ideally rocker-dilution boats like those described by Gieman et. al. (1966) should

Determination of growth and infectivity of Plasmodia after exposure to drugs 'in-vitro'.

Various methods are used for cultivation of malarial parasites 'in-vitro'. Each species requires its own variation of the general medium.

a. Cultivation of Plasmodium berghei in vitro.

A combination and modification of the methods of Polet and Barr, (1968), Trager (1967) and Polet (1966) in which parasitized cells were suspended in a nutrient medium for 24 hours failed due to loss of viability and extensive haemolysis. The method of Hockman et. al. (1968) was used. This involves the incubation of citrated parasitized red blood cells with some glucose in screw cap bottles for about 6 hours.

b. Cultivation of Plasmodium gallinaceum in-vitro

The method of Taylor et. al. (1951) was used in the cultivation of Plasmodium gallinaceum.

Parasitized blood was obtained from chicks with approximately 60% of their erythrocytes parasitized. The blood was diluted with isotonic saline to contain about 10×10^8 parasitized cells per ml. Ideally rocker-dilution boats like those described by Cioman et. al. (1966) should

be used as containers. In their absence 25ml conical flasks were used. To each sterile flask were added 4.4ml chicken blood, 0.1ml of penicillin in isotonic saline to give 5000 units of sodium penicillin G, and 0.5ml of parasitised chick blood containing about 5×10^8 parasitised cells.

The flasks were then incubated at 37°C for 6 hours with gentle shaking.

Two methods were suggested for assaying survival of parasites. These were inoculation of an aliquot of the cultures into susceptible six-days old chicks and determining the period before detection of parasitemia and secondly by direct examination of films prepared from the cultures flasks at regular intervals.

In the former method, 0.1ml of the culture was inoculated into six-days old white leghorn chicks. Blood smears of the chicks were examined daily beginning the day after inoculation and continuing until parasites were detected on two consecutive days or if negative for 14 days.

CHAPTER FOUR

EXPERIMENTS AND RESULTS

Investigation 1

Clinical and biochemical effects of

(a) Plasmodium berghei in mice

(b) Plasmodium gallinaceum in chicks.

Experiment 1a

Clinical and biochemical effects of Plasmodium berghei in mice

It has been shown that all strains of mice are not equally susceptible to infections with the blood forms of Plasmodium berghei and that the course of infection varies greatly from one strain of mice to another (Box et. al. 1954). It is therefore considered essential to find out the course of infection of the available strain of Plasmodium berghei in the strain of white mouse (Musculus species) supplied by the Pre-clinical Animal House of the University of Ibadan.

Procedure

A strain of Plasmodium berghei which has been maintained for several years by weekly blood passage in mice was used in this experiment.

Twelve litters of mice consisting of at least 5 males and 5 females and weighing between 18 and 22gms were selected for this experiment. Two litters were infected with 1 million parasitized

CHAPTER FOUR

EXPERIMENTS AND RESULTS

Investigation 1

Clinical and biochemical effects of

(a) *Plasmodium berghei* in mice

(b) *Plasmodium gallinaceum* in chicks.

Experiment 1a

Clinical and biochemical effects of *Plasmodium berghei* in mice

It has been shown that all strains of mice are not equally susceptible to infections with the blood forms of *Plasmodium berghei* and that the course of infection varies greatly from one strain of mice to another (Box et. al. 1954). It is therefore considered essential to find out the course of infection of the available strain of *Plasmodium berghei* in the strain of white mouse (Musculus species) supplied by the Pre-clinical Animal House of the University of Ibadan.

Procedure

A strain of *Plasmodium berghei* which has been maintained for several years by weekly blood passage in mice was used in this experiment.

Twelve litters of mice consisting of at least 5 males and 5 females and weighing between 18 and 22gms were selected for this experiment. Two litters were infected with 1 million parasitised

erythrocytes contained in 0.2ml of an inoculum prepared from an infected donor mouse as described on page 66. These mice were used for daily determinations of body temperature, parasitemia and red blood cell counts using methods that have already been described in detail in chapter three.

The other 10 litters were divided into 8 groups of 5 mice. Four groups were infected with 0.2ml of the infected inoculum while the other four groups were inoculated with normal mice erythrocytes contained in 0.2ml inoculum. In grouping the mice, care was taken to ensure that the infected and uninfected groups had identical compositions. On the day of inoculation and at two days interval, one group of 5 mice from the infected lot and a corresponding group from the uninfected lot were selected. After their body temperature and percentage parasitemia had been determined, all the 5 mice in each group were killed and their sera were pooled for the determination of serum proteins; glutamic pyruvate transaminase GPT, leucine-amino peptidase, LAP and alkaline phosphatase; serum bilirubin and serum glucose using methods that have already been described in detail in chapter three.

erythrocytes contained in 0.2ml of an inoculum prepared from an infected donor mouse as described on page 65. These mice were used for daily determinations of body temperature, parasitemia and red blood cell counts using methods that have already been described in detail in chapter three.

The other 10 litters were divided into 8 groups of 5 mice. Four groups were infected with 0.2ml of the infected inoculum while the other four groups were inoculated with normal mice erythrocytes contained in 0.2ml inoculum. In grouping the mice, care was taken to ensure that the infected and uninfected groups had identical compositions. On the day of inoculation and at two days interval, one group of 5 mice from the infected lot and a corresponding group from the uninfected lot were selected. After their body temperature and percentage parasitemia had been determined, all the 5 mice in each group were killed and their sera were pooled for the determination of serum proteins; glutamic pyruvate transaminase GPT, leucine-amino peptidase, LAP and alkaline phosphatase; serum bilirubin and serum glucose using methods that have already been described in detail in chapter three.

erythrocytes contained in 0.2ml of an inoculum prepared from an infected donor mouse as described on page 66. These mice were used for daily determinations of body temperature, parasitemia and red blood cell counts using methods that have already been described in detail in chapter three.

The other 10 litters were divided into 8 groups of 5 mice. Four groups were infected with 0.2ml of the infected inoculum while the other four groups were inoculated with normal mice erythrocytes contained in 0.2ml inoculum. In grouping the mice, care was taken to ensure that the infected and uninfected groups had identical compositions. On the day of inoculation and at two days interval, one group of 5 mice from the infected lot and a corresponding group from the uninfected lot were selected. After their body temperature and percentage parasitemia had been determined, all the 5 mice in each group were killed and their sera were pooled for the determination of serum proteins; glutamic pyruvate transaminase GPT, leucine-amino peptidase, LAP and alkaline phosphatase; serum bilirubin and serum glucose using methods that have already been described in detail in chapter three.

Results.

The results of these experiments are shown in tables 1a, 1b, 2a and 2b. Tables 1a and 1b show the temperatures, percentage parasitamia and RBC counts in both sexes. The results are expressed as the mean with their standard deviations. Tables 1a and 1b show that in both sexes parasitemic progressed from the first day after inoculation to about the sixth day when there was a decrease in the rate of increase of parasitemia. Most of the mice infected had died by the eight day after inoculation and those that survived showed a drop in the level of parasitemia.

With progress of parasitemia there was a slight increase in the body temperatures above normal and a more or less continuous drop with increased parasitemia. This finding compares favourably with those reported by Jacob-et.al. (1963) and Welldo et. al (1966) who have also observed a drop in the body temperature of mice infected with Plasmodium berghei.

Also with increased parasitemia there was a continuous fall in the red blood cell count from about 8.27×10^6 red blood cell per cumm on the first day of infection in the male mice to about 2.85×10^6 red blood cell on the 8th day after inoculation. This observation is similar to that reported by Zuckerman (1957) who showed that in most rodents which succumb to Plasmodium berghei infections, marked anaemia usually proceeds and may precipitate death.

TABLE 1a

Percentage Parasitemia, Temperature and Red Blood Cell count in male mice infected with Plasmodium berghei

Day	No of mice	Percentage parasitemia	Temperature oF	Red blood cell x 10^6/cumm
0	10	-	98.8 ± 0.5	8.27 ± 0.63
1	10	2.5 ± 0.8	99.1 ± 0.8	
2	10	6.8 ± 1.5	98.6 ± 0.8	6.84 ± 0.58
3	10	20.3 ± 6.6	96.2 ± 1.3	
4	10	38.9 ± 4.3	95.8 ± 1.1	5.19 ± 0.81
5	8	55.3 ± 6.1	94.3 ± 1.6	
6	6	65.5 ± 7.3	< 94	3.55 ± 0.44
7	3	58.2 ± 3.7	< 94	
8	2	41.7 ± 1.4	< 94	2.85 ± 0.51

TABLE 1b

Percentage Parasitemia, Temperature and Red Blood Cell count in female mice infected with Plasmodium berghei

Day	No of mice	Percentage parasitemia	Temperature °F	Red blood cell X 10^6/cumm
0	10	-	98.8 ± 0.2	8.81 ± 0.72
1	10	2.8 ± 0.5	99.0 ± 1.4	
2	10	7.9 ± 2.2	97.9 ± 1.2	7.35 ± 0.61
3	10	24.4 ± 3.7	96.4 ± 2.6	
4	9	45.9 ± 6.2	94.9 ± 1.4	5.52 ± 0.65
5	9	58.4 ± 5.3	94.4 ± 0.9	
6	5	69.6 ± 6.4	<94	4.28 ± 0.79
7	2	56.2 ± 4.1	<94	
8	2	50.7 ± 3.5	<94	3.68 ± 0.55

Tables 2a and 2b show the results of the effect of this infection on some biochemical values in the serum. The values are expressed as the mean of 4 determinations with their standard deviations. It was observed that as the level of parasitemia increased, there was also an increase in the levels of serum enzymes glutamic pyruvate transaminase and leucine amino peptidase There was a drop in serum glucose levels. No marked changes were observed in the levels of serum alkaline phosphatase, bilirubin and total proteins but a marked decrease in the albumin and a corresponding increase in the B-globulin fractions were observed. These findings are similar to those reported by Sadun et.al. (1965) who have also observed increase in serum glutamic pyruvate transaminase, decrease in serum glucose and variations in serum protein patterns in mice infected with Plasmodium berghoi.

Conclusion

From the result discussed, it has been shown that the course of infection of the Plasmodium berghoi available in the white mice supplied is similar to that reported by other workers. It can therefore be considered suitable for this study.

Also there is no sex differences in the response of mice to this infection.

TABLE 2a

The effect of Plasmodium berghei infection on some biochemical values of serum constituents in male mice

Day	Sample	No of mice	% Parasitemia	Temperature oF	Glutamic pyruvate transaminase	Leucine amino peptidase mu/ml	Total bilirubin mg/100ml	Alkaline phosphatase King Armstrong unit/100ml	Glucose mg/100ml	Total protein gm/100ml	Albumin	Total globulin	β-globulin	γ-globulin
											Percentage composition			
0	Normal	5	-	98.86±0.21	25.63±0.58	4.95±0.12	0.58±0.01	6.14±0.09	109.5±1.6	6.42±0.14	52.4±1.3	18.8±2.5	22.4±2.6	6.2±0.6
	Infected	5	-	98.84±0.14	27.21±0.43	5.36±0.25	0.68±0.05	6.06±0.13	122.5±2.4	5.98±0.13	58.4±1.0	19.5±1.9	16.8±1.3	5.4±0.5
+2	Normal	5	-	98.83±0.16	24.92±0.15	5.17±0.19	0.64±0.03	6.02±0.07	116.7±0.9	6.09±0.08	56.5±0.8	16.7±0.5	21.2±1.1	5.6±0.2
	Infected	5	7.2±2.4	97.46±1.05	63.85±0.34	6.75±0.84	0.93±0.01	5.68±0.02	57.3±3.1	6.04±0.15	46.0±1.1	20.5±1.2	26.8±0.7	6.1±0.2
+4	Normal	5	-	98.90±0.43	25.39±0.20	4.88±0.43	0.66±0.02	6.31±0.11	112.6±1.1	6.15±0.03	53.2±2.5	17.4±2.0	22.6±1.8	6.6±0.4
	Infected	5	35.3±1.9	96.42±2.14	119.35±0.55	10.51±0.79	1.21±0.02	4.21±0.18	65.1±2.6	5.12±0.11	48.7±1.9	21.0±0.9	32.0±1.4	8.1±0.3
+6	Normal	5	-	98.85±0.33	26.79±0.14	5.03±0.26	0.59±0.03	6.22±0.02	118.6±1.4	6.20±0.04	55.6±0.8	17.5±1.6	22.0±0.3	5.9±0.5
	Infected	4	60.5±5.8	94.52±1.84	81.65±0.54	16.32±0.23	6.85±0.11	6.89±0.06	60.0±1.8	5.81±0.02	41.1±2.3	25.6±3.1	34.2±0.7	9.6±0.2

TABLE 2a

The effect of Plasmodium berghei infection on some biochemical values of serum constituents in male mice

Day	Sample	No of mice	% Parasitemia	Temperature oF	Glutamic pyruvate transaminase	Leucine amino peptidase mu/ml	Total bilirubin mg/100ml	Alkaline phosphatase King Armstrong unit/100ml	Glucose mg/100ml	Total protein gm/100ml	Albumin	Total globulin	β-Globulin	Globulin
											Percentage composition			
0	Normal	5	-	98.86±0.21	25.63±0.58	4.95±0.12	0.58±0.03	6.14±0.09	109.5±1.6	6.42±0.14	52.4±1.3	18.8±2.5	22.4±2.6	6.2±0.6
	Infected	5	-	98.84±0.14	27.21±0.43	5.36±0.25	0.68±0.05	6.06±0.13	122.5±2.4	5.98±0.13	58.4±1.0	19.5±1.9	16.8±1.3	5.4±0.5
+2	Normal	5	-	98.83±0.16	24.92±0.15	5.17±0.19	0.64±0.03	6.02±0.07	116.7±0.9	6.09±0.08	56.5±0.8	16.7±0.5	21.2±1.1	5.6±0.2
	Infected	5	7.2±2.4	97.46±1.05	63.85±0.34	6.75±0.84	0.93±0.01	5.68±0.02	57.3±3.1	6.04±0.15	46.0±1.1	20.5±1.2	26.8±0.7	6.1±0.2
+4	Normal	5	-	98.90±0.43	25.39±0.20	4.88±0.43	0.66±0.02	6.31±0.11	112.6±1.1	6.15±0.03	53.2±2.5	17.4±2.0	22.8±1.8	6.6±0.4
	Infected	5	35.3±1.9	96.12±2.14	119.35±0.55	10.51±0.79	1.21±0.02	4.21±0.18	65.1±2.6	5.12±0.11	48.7±1.9	21.0±0.9	32.0±1.4	8.1±0.3
+6	Normal	5	-	98.85±0.33	26.79±0.14	5.03±0.26	0.59±0.03	6.22±0.02	118.6±1.4	6.20±0.04	55.6±0.8	17.5±1.6	22.0±0.3	5.9±0.5
	Infected	4	60.5±5.8	94.52±1.84	81.65±0.54	16.32±0.23	6.85±0.11	6.89±0.06	60.0±1.8	5.81±0.02	41.1±2.3	25.6±3.1	24.2±0.7	9.6±0.2

TABLE 2b

The effect of Plasmodium berghei infection on some biochemical values of serum constituents in female mice

Day	Sample	No. of mice	% Parasitemia	Temperature oF	Glutamic pyruvate transaminase	Leucine amino peptidase mu/ml	Total bilirubin mg/100ml	Alkaline phosphatase King Armstrong unit/100ml	Glucose mg/100ml	Total protein gm/100ml	Albumin	Total globulin	β-Globulin	γ Globulin
											Percentage composition			
0	Normal	5	-	98.82±0.30	30.12±0.41	4.97±0.14	0.59±0.02	6.36±0.13	98.80±0.92	6.28±0.12	55.0±1.2	19.2±0.7	20.4±0.6	7.0±0.3
	Infected	5	-	98.80±0.26	28.54±0.36	5.13±0.22	0.66±0.04	5.94±0.27	112.45±1.03	6.40±0.08	59.1±0.8	16.6±1.1	21.8±1.5	6.5±0.3
+2	Normal	5	-	98.83±0.24	28.75±0.19	5.26±0.09	0.57±0.02	6.00±0.19	102.56±0.76	6.23±0.20	53.8±1.1	19.6±0.2	19.9±0.4	6.5±0.5
	Infected	5	10.16±1.4	98.45±0.98	79.33±0.25	6.62±0.51	0.91±0.12	5.73±0.06	45.03±1.41	6.25±0.14	43.2±0.9	23.4±1.3	27.5±0.8	6.1±0.4
+4	Normal	5	-	98.86±0.07	27.82±0.14	5.03±0.11	0.61±0.05	6.25±0.12	102.32±0.90	6.31±0.06	58.1±0.7	17.8±0.8	19.5±0.3	6.0±0.2
	Infected	5	38.35±3.9	96.78±1.5	136.36±0.76	9.80±0.14	1.47±0.16	5.45±0.15	48.66±0.57	5.50±0.16	32.4±1.6	23.1±0.4	39.3±0.7	5.2±0.6
+6	Normal	5	-	98.80±0.34	29.14±0.23	5.38±0.25	0.59±0.03	6.17±0.20	100.51±1.02	6.27±0.19	53.0±0.5	18.9±0.3	21.4±0.6	6.3±0.3
	Infected	3	65.52±4.1	94.82±1.56	109.55±0.81	13.91±0.37	1.96±0.07	6.09±0.14	52.74±1.13	5.36±0.07	35.5±0.9	22.9±0.7	35.2±1.3	8.4±0.4

detec

about

Experiment 14.

Clinical and biochemical effects of a strain of Plasmodium gallinaceum on white leghorn chicks.

Procedure

It was desirable to know the course of Plasmodium gallinaceum infection in the strain of white leghorn chicks obtainable from the Experimental and Research Farm of the University of Ibadan.

Forty six-day old white leghorn cockrels were infected with about 25 million parasitized red blood cells intravenously. Twenty others were inoculated with 25 million erythrocytes obtained from a normal chick. The chicks were kept warm at about 32°C and fed on chick mash which contained no anti-biotics. While 10 infected chicks were examined daily for their level of parasitemia, body temperature and red blood cell count, others were placed in groups of five and were sacrificed at three days interval to determine the effect of Plasmodium gallinaceum infection on some serum biochemical values. The same methods that were used in the mice were also used to determine serum total protein; enzymes LAP, GPT and alkaline phosphatase; serum bilirubin and serum glucose.

Results.

The results are shown in tables 3 and 4. There was a prepatent period of approximately three days when no parasites were detected in the blood of the infected chicks. Peak parasitemia occured about the 6th day after which a drop was observed.

Some of the infected chicks died before the 9th day by which time the level of parasitemia had fallen significantly.

There was an inconsistent change in the body temperature of the chicks, but usually a higher body temperature was recorded before the peak parasitemia and a drop in temperature just before death. Like in the case of Plasmodium berghei infection in mice, there was a marked decrease in the red blood cell count of the infected chicks.

Table 4 shows the effect of Plasmodium gallinaceum infection on the value of some biochemical constituents of the serum. Marked increases were observed in the serum level of Glutamic Pyruvate Transaminase, leucine amino peptidase and bilirubin. There was only a slight decrease in the serum glucose and total protein but there was a marked drop in the level of albumin and a significant increase in the level of γglobulin. The total alpha globulins and βglobulins showed only slight variations. These observations are in line with those reported by Stauber (1954) and Rao and Cohly (1953) who reported marked decreases in the albumin level with a marked increase in the gamma globulin fraction in chicks infected with Plasmodium gallinaceum.

Conclusion

From the results of these experiments it is observed that the strain of Plasmodium gallinaceum supplied follows the expected course in white leghorn chicks. It can therefore be used for this study.

Percentage parasitemia, temperature and red blood cell count in white leghorn chicks infected with Plasmodium gallinaceum

Days	Percentage parasitemia	Temperature °F	Red blood cell X 10^6/cumm.
0	-	105.6±0.7	1.85±0.62
1	-	105.1±1.2	
2	-	106.4±2.3	
3	-	106.1±3.7	1.67±0.38
4	8.4±1.5	105.1±1.6	
5	30.1±4.6	104.8±3.5	
6	67.5±5.3	104.5±2.7	1.52±0.41
7	52.6±4.4	105.6±3.2	
8	28.2±3.3	103.8±2.2	
9	10.1±5.2	104.3±4.6	1.15±0.35

TABLE 4

The effect of Plasmodium gallinaceum infection on the values of some biochemical constituents in the serum

Day	Sample	No of mice	% Parasitemia	Temperature °F	Glutamic pyruvate transaminase IntUnit/ml	Leucine amino peptidase mu/ml	Total bilirubin mg/100ml	Alkaline phosphatase-King Armstrong unit/100ml	Glucose mg/100ml	Total protein gm/100ml	Albumin	Total alpha globulin	β-globulin	γ-Globulin
											Percentage composition			
0	Normal	5	-	105.91±0.72	8.02±0.16	47.71±0.82	0.61±0.03	7.41±0.33	220.2±1.7	4.22±0.31	56.9±1.7	15.3±0.5	10.1±1.6	17.7±1.5
	Infected	5	-	105.84±0.36	8.42±0.51	45.63±0.26	0.56±0.05	7.80±0.24	214.6±0.9	4.45±0.24	51.3±2.0	15.0±1.6	11.5±0.5	21.2±2.9
+3	Normal	5	-	105.81±0.22	7.75±0.42	49.61±0.33	0.66±0.02	7.53±0.19	217.5±0.8	4.32±0.09	52.5±1.2	17.5±1.5	11.6±1.3	18.8±2.2
	Infected	5	-	106.60±0.88	17.56±0.38	56.85±0.41	0.91±0.12	7.66±0.28	201.4±2.2	4.06±0.11	33.6±1.7	23.1±2.4	10.6±1.9	25.7±1.4
+6	Normal	5	-	105.86±0.51	8.31±0.22	45.39±0.25	0.59±0.07	7.12±0.30	221.6±0.7	4.47±0.16	54.7±0.5	16.1±1.8	11.8±2.0	17.3±2.5
	Infected	5	66.2±3.4	104.36±2.61	20.24±0.15	80.46±0.92	1.72±0.15	6.41±0.29	204.3±1.6	3.82±0.07	32.1±1.4	28.9±2.7	10.3±1.1	28.7±2.6
+9	Normal	5	-	105.81±0.35	8.19±0.34	48.24±0.51	0.63±0.04	7.36±0.17	219.6±0.5	4.25±0.08	55.9±0.8	17.5±0.6	10.7±0.9	19.9±1.7
	Infected	3	24.8±6.9	105.9±3.14	33.35±0.26	113.50±0.39	2.61±0.23	6.84±0.20	198.5±0.8	3.14±0.12	36.8±1.2	23.1±1.2	9.8±0.5	32.0±1.1

INVESTIGATION 2

Effect of Chloroquine on

a Plasmodium berghei infection in mice.

b Plasmodium gallinaceum infection in chicks

Experiment 2a

Effect of Chloroquine on Plasmodium berghei infection in mice

In screening drugs for their anti malarial properties, it is always useful to use a reference drug. Chloroquine which has been reported to have a curative effect on both Plasmodium berghei and Plasmodium gallinaceum as well as human malarial parasites was chosen as the reference drug. Thompson and Herbol (1972) have suggested that the effects of drugs on the infection may be assessed by comparing the mean percentage of cells parasitised in treated and control groups. In addition to this, the effect of this drug on the values of some biochemical constituent in the serum which we have shown vary with infection in mice was also investigated.

Procedure

Twenty litters of mice comprising at least 4 males and 4 females were selected. Fifteen litters of these were divided into three sets of 5 litters. Each set was subdivided into 4 groups of identical composition each consisting of 5 mice from the 5 litters. In each set, one group of 5 mice was inoculated with normal mouse erythrocytes while the other 3 groups were inoculated with one million

parasitised erythrocytes. Of the infected mice one group was left as untreated control while the other two groups were treated with about 300µg of chloroquine daily starting 6 hours and 48 hours respectively after inoculation. The drug was administered orally through a catheter attached to a syringe. Treatment was continued for five days. At 2 days interval all the five mice in one group were killed and their sera pooled for analysis of glutamic pyruvate transaminase, leucine amino peptidase, alkaline phosphetase, bilirubin, glucose and protein fractions. Before the mice were killed, their body temperatures and percentage parasitemia were also recorded. The experiment was repeated three times.

Results.

The results of these experiments are shown in tables 5A and 5C. The temperatures and percentage parasitemia were expressed as the mean of the number of mice indicated in each group with their standard deviations while the serum biochemical values are expressed as the means of three determinations with their standard deviations. The tables of results indicate that when mice were treated with chloroquine they showed a significant drop in the level of parasitemia compared with the control. There was also a smaller decrease in temperature of the mice in which treatment was started after 48 hours than the controls. When treatment was started early the drop in temperature was insignificant (tables 5B and 5D).

When treatment was started early there were significantly lower levels of serum glutamic pyruvate transaminase and leucine amino peptidase and higher levels of glucose in the treated mice compared with the untreated control. Although no significant differences were observed in the serum total protein values, significant differences in the albumin and βglobulin fractions were observed. In the mice in which treatment was started early, increased value of albumin and a corresponding decreased value of βglobulins observed showed that the serum protein levels were becoming normal in those mice.

A similar reaction was observed in the serum biochemical values of mice in which treatment was delayed although less significant differences occured between their values and those of the controls.

Conclusion.

Chloroquine when administered early cures mice of *Plasmodium berghei* infection. Its effect on parasitemia, body temperature, serum glutamic pyruvate transaminase, leucine amino peptidase, glucose and protein fractions are statistically significant enough to use such values as a measure of the effectiveness of the drug.

TABLE 5A

Effect of Chloroquine on Plasmodium berghei infection in male mice

Days after inoculation	Sample	No. of mice.	Parasitemia %	Temperature °F	Glutamic pyruvate transaminase Int-Unit/ml.	Leucine amino peptidase mu/ml.	Total bilirubin mg/100ml	Alkaline phosphatase King Armstrong (Unit/100ml	Glucose mg/100ml	Total protein mg/100ml	Albumin	Total alpha globulin	β Globulin	γ Globulin
											Percentage composition			
0	Normal	15	-	98.6±0.4	26.04±0.83	4.78±0.62	0.68±0.21	5.84±0.51	105.0±6.45	5.56±0.30	57.1±2.75	21.6±1.9	20.0±2.1	6.3±1.6
2	Normal	15	-	98.5±0.2	22.46±0.38	4.96±0.73	0.68±0.43	5.34±0.77	112.0±4.8	5.40±0.42	54.0±2.4	17.1±2.2	20.2±1.9	6.6±0.5
	C	15	10.7±1.1	98.5±1.5	58.92±4.19	8.21±1.16	0.95±0.45	5.53±0.96	47.7±8.5	5.42±0.51	40.7±3.9	23.5±1.1	33.0±4.5	5.1±1.2
	T1	15	2.4±0.6	98.3±0.8	31.44±2.30	5.11±0.62	0.84±0.35	5.12±0.44	86.3±5.6	5.61±0.48	44.3±4.4	22.6±0.7	27.6±2.5	6.2±1.8
	T2	15	11.3±2.6	98.3±1.2	62.60±4.71	8.56±0.92	0.91±0.13	5.04±1.19	42.0±6.9	5.59±0.43	41.6±3.0	21.7±0.9	32.5±4.5	5.6±0.7
4	Normal	15	-	98.6±0.3	23.76±1.25	4.65±1.04	0.59±0.26	6.03±1.41	106.4±3.8	5.23±0.54	56.2±1.8	18.8±2.1	19.6±1.2	6.2±1.1
	C	15	47.4±6.3	97.0±1.3	106.50±7.21	14.31±3.2	1.16±0.42	5.10±0.84	64.0±7.3	5.08±0.11	36.6±5.8	22.9±1.7	32.0±4.6	8.6±1.4
	T1	15	1.5±0.7	98.1±0.5	28.81±2.26	6.80±1.80	0.89±0.24	5.00±0.35	94.3±5.1	5.56±0.32	42.5±6.2	21.4±1.8	27.7±5.8	8.4±0.5
	T2	15	10.2±3.5	98.0±1.1	43.54±5.80	8.39±2.65	1.09±0.32	5.82±0.76	48.0±8.5	5.35±0.66	41.7±5.2	24.3±1.0	26.9±4.2	6.9±1.3
6	Normal	15	-	98.8±0.4	25.59±0.81	4.90±0.91	0.66±0.13	5.59±1.03	110.7±6.0	5.44±0.35	53.8±2.7	21.5±0.9	19.4±2.3	6.2±0.9
	C	11	68.5±10.5	95.1±2.4	120.61±9.56	24.92±4.40	1.38±0.54	4.84±0.65	58.7±9.2	4.69±0.42	31.2±5.5	25.6±2.7	33.2±3.8	8.0±1.6
	T1	15	1.4±1.1	98.3±0.5	30.32±4.15	8.16±2.21	0.78±0.32	5.21±0.56	98.5±4.6	5.19±0.38	51.9±2.3	20.5±1.9	20.8±2.6	8.8±1.2
	T2	15	3.1±1.9	98.2±0.8	37.01±5.81	10.38±2.16	1.12±0.26	5.15±0.62	69.6±7.8	5.24±0.36	38.6±4.9	24.6±2.2	30.3±5.5	8.5±0.8

C = Control mice.
T1 = Treatment commenced 6 hours after infection.
T2 = Treatment commenced 48 hours after infection.

TABLE 5B

Statistical comparison of the effect of chloroquine on Plasmodium berghei infection in male mice 6 days after infection.

STUDENT'S 'T' VALUES

Days after inoculation	Sample	Parasitemia	Temperature	Glutamic pyruvate transaminase	Leucine amino peptidase	Alkaline phosphatase	Total bilirubin	Glucose	Total protein	Albumin	Alpha globulin	βGlobulin	γGlobu
6	C, T1	24.0^{xx}	7.7^{xx}	14.9^{xx}	5.8^{x}	0.8$^{.}$	1.6$^{.}$	6.6^{x}	1.5$^{.}$	5.9^{x}	0.09$^{.}$	3.9^{x}	0.5$^{.}$
6	C, T2	20.4^{xx}	7.0^{xx}	12.9^{xx}	5.1^{x}	0.4$^{.}$	1.0$^{.}$	1.5$^{.}$	1.7$^{.}$	1.7$^{.}$	0.1$^{.}$	1.6$^{.}$	0.4$^{.}$

C = Control
T1 = Treatment commenced 6 hours after infection
T2 = Treatment commenced 48 hours after infection

. Not significant
x Significant at 5% level
xx Significant at 1% level.

TABLE 5C

Effect of Chloroquine on Plasmodium berghei infection in female mice

Days after inoculation	Sample	No. of mice	Parasitemia %	Temperature °F	Glutamic pyruvate transaminase Int-Unit/ml	Leucine amino peptidase mu/ml	Alkaline phosphatase King Armstrong Unit/100ml	Total bilirubin mg/100ml	Glucose mg/100ml	Total protein mg/100m	Albubin	Total alpha	βGlobulin	γGlobulin
											Percentage composition			
0	Normal	15	-	98.8±0.3	27.91±3.47	5.34±1.51	5.22±1.76	0.81±0.15	114.6±4.4	5.81±0.52	55.7±3.4	19.4±2.2	17.3±2.8	5.6±2.0
2	Normal	15	-	98.7±0.3	29.33±2.58	5.28±0.94	5.42±0.53	0.75±0.10	112.5±7.8	5.64±0.47	57.6±2.2	20.4±1.6	19.2±1.7	5.9±1.8
	C	15	13.5±2.3	98.0±1.7	63.52±3.40	8.71±1.26	5.91±0.63	0.98±0.12	62.2±8.5	5.59±0.38	38.5±4.6	23.5±1.9	28.2±4.6	8.8±2.1
	T1	15	1.0±0.4	98.5±2.4	32.18±1.29	5.90±1.32	5.66±0.88	0.82±0.06	97.5±4.6	5.72±0.54	45.1±2.6	20.7±2.7	23.8±2.4	7.8±1.7
	T2	15	10.7±3.1	97.7±1.2	59.71±2.55	8.42±1.53	5.56±0.57	0.95±0.24	58.5±5.4	5.66±0.53	40.2±5.8	22.4±2.0	30.3±3.5	7.1±1.2
4	Normal	15	-	98.8±0.2	26.65±1.38	5.06±0.82	5.39±0.95	0.85±0.08	112.7±3.5	5.78±0.36	56.6±2.9	17.2±1.4	18.9±2.4	6.3±0.8
	C	15	43.6±4.5	97.5±1.6	98.42±4.86	12.83±2.51	5.02±1.29	1.35±0.38	49.6±10.1	5.25±0.45	38.5±6.8	23.3±4.1	29.3±5.2	6.8±1.2
	T1	15	4.2±1.5	98.2±0.7	33.69±2.46	5.77±1.43	5.40±0.63	0.94±0.25	89.2±4.9	5.40±0.29	49.5±5.2	20.5±2.7	22.9±1.8	7.1±0.9
	T2	15	7.9±2.1	98.0±1.1	42.97±3.61	9.26±1.19	5.17±0.71	1.16±0.32	63.5±6.7	5.18±0.63	42.8±4.7	24.9±2.8	24.0±3.4	8.3±1.5
6	Normal	15	-	98.8±0.2	28.16±2.34	5.11±2.24	5.52±0.86	0.67±0.13	108.3±5.4	5.64±0.27	51.2±4.5	21.7±1.1	18.7±1.8	6.4±1.1
	C	12	64.4±9.8	95.7±2.1	112.52±8.75	18.85±2.71	4.66±0.58	1.51±0.49	55.2±9.8	4.82±0.79	41.7±5.8	22.7±2.5	32.1±4.0	7.4±1.9
	T1	15	1.5±0.9	98.3±0.4	32.15±3.22	6.24±0.85	5.11±0.47	0.82±0.14	93.4±3.2	5.43±0.46	48.8±2.3	22.6±1.4	21.4±1.2	8.1±1.2
	T2	14	4.2±1.4	98.1±1.0	40.62±4.51	9.90±1.46	5.20±0.56	0.99±0.25	81.1±5.5	5.24±0.48	46.8±1.5	22.3±2.6	23.3±2.0	7.5±1.4

C = Control
T1 = Treatment commenced 6 hours after infection
T2 = " " 48 " " "

TABLE 5D

Statistical comparison of the effect of chloroquine on some serum biochemical values in female mice 6 days after infection with Plasmodium berghei

STUDENT'S 'T' VALUES

Days after inoculation	Sample	Parasitemia	Temperature	Glutamic pyruvate transaminase	Leucine amino peptidase	Alkaline phosphatase	Total bilirubin	Glucose	Total protein	Albumin	Total Alpha	βGlobulin	γGlobulin
6	$\frac{C}{T_1}$)	24.8^{xx}	4.1^{xx}	14.0^{xx}	7.5^{x}	1.0'	2.3'	4.9^{x}	1.1'	2.2'	0.1'	3.6^{x}	1.8'
6	$\frac{C}{T_2}$)	21.5^{x}	3.7^{xx}	10.0^{xx}	5.0^{x}	0.1'	1.6'	3.1^{x}	0.8'	1.5'	0.08'	3.4^{x}	0.1'

C = Control

T1 = Treatment commenced 6 hours after infection.

T2 = " " 48 " " "

'Not significant.

x Significant at 5%

xx Significant at 1%

Experiment 2b.

Effect of Chloroquine on Plasmodium gallinaceum in chicks

Procedure.

In this experiment the method of Coatney et.al reported by Wisologle (1946) was used. Eighty six-days old chicks were inoculated intravenously with about 25 million parasitised erythrocytes. Forty of these chicks were left as untreated controls and the other 40 chicks were treated with 2.0mg chloroquine base per kilogram body weight once daily starting about four hours after inoculation. On the day of infection and at 3 days intervals, 5 chicks from each group were examined for their body temperatures and percentage parasitemia before they were killed and their pooled sera analysed as before. The experiment was repeated three times.

Results. Table 6 shows the results of this experiment. From the statistical comparison of the biochemical values, it was observed that chloroquine had - significant curative effect on serum glutamic pyruvate transaminase, leucine amino peptidase, and glucose in addition to its effect on parasitemia. The protein fractions were not as significantly affected as they were in the mice.

Conclusion

Serum level of glutamic pyruvate transaminase, luecine amino peptidase and glucose can be used to assess the curative effect of antimalarial agents in chicks.

Conclusion

Serum level of glutamic pyruvate transaminase, leucine amino peptidase and glucose can be used to assess the curative effect of antimalarial agents in chicks.

TABLE 6A

Effect of Chloroquine on Plasmodium gallinaceum infection in chicks

Days after inoculation	Sample	No.of chicks	Parasitemia %	Temperature °F	Glutamic pyruvate transaminase Int-Unit/ml	Leucine amino peptidase mu/ml	Alkaline phosphatase King armstrong units/100ml	Total bilirubin mg/100ml	Glucose mg/100ml	Protein mg/100ml	Albumin	Total alpha globulin	BGlobulin	Globulin
											Percentage composition			
0	Normal	15	-	105.6±0.8	7.8±1.4	56.11±2.72	7.41±0.72	0.84±0.21	215.4±6.2	4.31±0.25	50.7±4.8	17.1±2.0	13.8±2.6	19.0±3.2
3	Normal	15	-	105.5±0.8	8.04±0.90	49.36±2.14	6.93±0.82	0.75±0.49	221.5±7.8	4.20±0.41	53.2±3.4	18.6±3.9	13.1±3.5	16.2±5.8
	C	15	-ve	106.1±2.6	8.83±1.55	61.77±4.20	7.52±1.48	0.94±0.31	199.3±6.7	3.79±0.44	38.5±5.0	23.9±3.1	11.6±2.7	28.8±2.5
	T	15	-ve	105.8±1.5	6.64±2.61	58.26±3.28	6.11±1.34	0.82±0.23	206.7±1.9	4.02±0.31	34.0±8.9	21.6±2.5	12.4±2.2	31.6±4.7
6	Normal	15	-	105.6±0.6	7.71±1.23	51.29±3.31	7.10±0.99	0.90±0.36	219.8±8.5	4.28±0.37	52.6±3.5	18.6±2.4	10.7±4.0	20.1±3.0
	C	15	64.4±8.2	104.9±2.1	1728±3.25	89.50±6.70	6.44±1.26	2.41±0.82	181.9±8.1	3.14±0.56	33.2±4.9	25.4±2.6	11.9±3.8	31.0±4.2
	T	15	5.2±1.4	105.3±1.1	1042±1.52	60.62±2.5	6.97±0.74	1.03±0.33	204.4±5.6	3.97±0.22	41.1±2.6	21.5±1.5	9.6±2.7	27.5±2.3
9	Normal	15	-	105.5±0.3	7.53±0.81	53.45±2.21	6.85±0.50	0.86±0.59	220.7±5.7	4.30±0.24	49.0±6.5	19.5±2.5	11.4±4.3	20.1±2.5
	C	13	22.4±6.9	103.5±2.6	2836±5.22	102.5±10.90	5.83±1.18	3.14±1.08	175.3±9.2	3.45±0.40	38.5±6.6	23.1±2.9	12.5±2.8	29.8±5.9
	T	15	2.1±1.5	105.1±0.7	9.45±2.34	65.61±4.22	6.64±0.92	1.05±0.61	205.2±6.4	3.92±0.21	46.7±3.5	20.4±2.0	10.4±3.3	22.5±3.5

C = Infected Control

T = Infected treated chloroquine.

TABLE 68

Statistical comparison of the effect of chloroquine on chicks 9 days after infection with Plasmodium malariae

STUDENT'S 'T' VALUES

Day	Sample	Parasitaemia	Temperature	Glutamic pyruvate transaminase	Leucine amino peptidase	Alkaline phosphatase	Total bilirubin	Glucose	Total protein	Albumin	Total alpha globulin	Beta globulin	Gamma globulin
9	C / T	4.6^{xx}	2.1^{x}	5.6^{x}	5.5^{x}	0.9$^{\cdot}$	1.0$^{\cdot}$	4.6^{x}	1.8$^{\cdot}$	1.8$^{\cdot}$	1.3$^{\cdot}$	0.8$^{\cdot}$	1.8$^{\cdot}$

C = Infected control

T = Infected treated chloroquine

$^{\cdot}$Not significant

^{x}Significant at 5% level.

^{xx}Significant at 1% level.

INVESTIGATION 3

Effect of extracts of some medicinal plants used locally for malaria on infection of Plasmodium berghei in mice.

Experiment 3a

Suppressive and curative effects of some plant extracts on Plasmodium berghei infection in mice.

This set of experiments was designed to find both suppressive and curative effects of each plant extract using a combination of the procedures of Thurston (1953) and Peters (1970). In this method mice were given the extracts of the plants under study at different stages in the development of the infection. Suppressive drugs are effective only when treatment is started very early in the infection whereas curative drugs are effective even when treatment is started after the infection has already been well established.

Procedure.

In this set of experiments, 10 litters of mice consisting of at least 5 males were selected. They were placed in 5 groups of 10 mice, each group comprising a member of the 10 litters. They were infected as usual with one million parasitized erythrocytes. One group of 10 mice was left as untreated control.

The second group was treated with 300/ug chloroquine orally daily while the other three groups were given 1ml of the test plant extract prepared traditionally as described in chapter three, twice daily treatment commencing , 24 and 48 hours respectively after inoculation and continued till the end of the experiment. The percentage parasitemia and body temperature of each mouse were followed daily. The results of such experiments using the six plant extracts under investigation are recorded in tables 7 to 12.

Results.

Table 7 shows the result of the effect of water extract of Azadirachta indica leaves popularly known as 'Dogoyaro' on the infection in mice. The results show that the extract does not possess suppressive or curative action on Plasmodium berghei in mice as the levels of parasitemia in the groups treated with the extract were not different from those of the controls whereas chloroquine was able to reduce the level significantly. Also, the extract has no beneficial effect on the temperatures of the mice. From the number of mice which survived till the end of the experiment, it would seem that the extract did not delay the onset of death of the animals.

Also it hardly reduced the number of mice which died.

Tables 8 to 12 show the results of the experiments with the other extracts. The results also show that the water extracts of leaves of Morinda lucida, Alstonia boonei, mixtures A and B and the alcoholic extract of the bark of Enantia chlorantha prepared as used traditionally, and in the proportions described in detail in chapter three did not affect the levels of parasitemia, temperature and the number of mice that survived until the eighth-day significantly when compared with the controls.

Conclusion

The results of these experiments show that none of the 6 plant extracts had anti-malarial activity on Plasmodium berghei infection in mice at concentrations relative to those normally used by man.

TABLE 7

Effect of water extract of Azadirachta indica on temperature and % parasitemia in male mice infected with Plasmodium berghei

Days after inoculation	Infected control			Infected + chloroquine			Infected + extract of Azadirachta indica								
							6hours after inoculation			24hours after inoculation			48hours after inoculation		
	No. of Mice	Temperature of	Parasitemia %	No. of Mice	Temperature of	Parasitemia %	No. of Mice	Temperature of	Parasitemia %	No. of Mice	Temperature of	Parasitemia %	No. of Mice	Temperature of	Parasitemia %
1	10	98.8±0.3	3.8±0.7	10	98.8±0.5	-ve	10	98.8±0.2	2.5±1.7	10	98.9±0.5	2.9±1.9	10	98.8±0.3	3.0±1.4
2	10	98.9±0.7	8.6±2.4	10	98.6±0.4	1.1±0.3	10	98.8±0.9	9.1±2.1	10	98.8±0.4	8.7±1.2	10	99.1±0.7	10.1±2.7
3	10	98.2±0.6	20.4±4.1	10	98.7±0.6	1.8±0.7	10	98.0±1.0	15.4±2.3	10	98.3±0.7	19.8±4.1	10	98.2±0.5	21.5±7.2
4	9	97.4±1.8	39.7±5.2	10	98.3±0.6	2.7±1.1	10	97.9±1.1	35.8±8.7	10	97.5±0.8	30.4±11.5	9	97.2±1.3	40.3±13.8
5	9	96.2±2.5	53.6±8.8	9	98.5±0.2	3.5±1.2	8	96.4±1.8	51.9±7.1	8	96.7±1.9	53.6±8.5	9	95.9±2.0	58.4±11.2
6	6	94.5±1.1	68.2±14.3	9	98.6±0.3	1.4±0.7	5	95.0±2.1	63.3±9.4	6	95.2±2.1	60.9±9.8	5	94.1±1.7	70.5±5.6
7	2	<94	52.4±4.6	9	98.8±0.4		3	94.1±0.8	59.4±3.1	2	<94	60.2±7.5	3	<94	51.4±4.6
8	1	<94	40.0	9	98.7±0.3	Ll	0	-	-	2	<94	36.8±5.2	0	-	-

The water extract was obtained from 320gm fresh leaves per litre of water. Each mouse was given 1ml of the extract twice daily.

TABLE 7

Effect of water extract of Morinda lucida on temperature and % parasitemia in male mice infected with Plasmodium berghei

Days after inoculation	Infected control			Infected + chloroquine			Infected + extract of Morinda lucida								
							6hours after inoculation			24hours after inoculation			48 hours after inoculation		
	No.of Mice	Temperature oF	Parasitemia %	No.of Mice	Temperature oF	Parasitemia %	No.of Mice	Temperature oF	Parasitemia %	No.of Mice	Temperature oF	Parasitemia %	No.of Mice	Temperature oF	Parasitemia %
1	10	98.7±0.2	4.8±0.6	10	98.8±0.1	-ve	10	98.8±1.0	2.1±1.0	10	98.5±0.7	1.5±0.6	10	98.8±0.3	2.5±1.3
2	10	98.8±0.1	10.8±1.4	10	98.6±0.4	2.0±0.5	10	98.5±0.7	6.1±1.7	10	99.1±0.5	5.6±2.2	10	98.7±0.4	6.2±0.9
3	10	99.1±1.4	27.5±3.5	10	98.3±0.6	4.7±0.5	10	98.3±0.5	23.5±2.9	10	98.6±1.2	21.4±3.7	10	99.0±0.8	24.1±2.3
4	10	98.0±1.9	43.9±8.7	10	98.5±0.5	1.6±0.3	10	97.4±1.2	45.3±6.5	9	97.9±1.3	41.3±5.5	10	98.4±1.5	45.7±6.2
5	8	96.9±1.8	60.5±10.2	10	98.6±0.2	1.3±0.4	10	96.6±1.5	53.6±9.2	7	96.4±0.9	58.4±12.6	8	96.1±2.2	65.4±12.4
6	4	95.2±2.1	65.8±8.0	10	98.6±0.6		5	94.7±0.8	72.4±7.1	5	95.1±1.1	66.9±9.5	4	96.6±1.0	70.8±7.3
7	2	<94	52.0±4.9	10	98.5±0.3		3	<94	68.4±11.5	2	94.3±0.3	47.5±6.6	3	<94	55.6±7.5
8	2	<94	41.7±6.1	10	98.7±0.4		1	<94	42.8	0	-	-	1	<94	51.2

The water extract was obtained from 540 fresh leaves per litre of water. Each mouse was given 1ml of the extract twice daily.

TABLE 9

Effect of water extract of Alstonia boonei on temperature and % parasitemia in male mice infected with Plasmodium berghei

Days after inoculation	Infected control			Infected + chloroquine			Infected + extract of Alstonia boonei								
							6hours after inoculation			24hours after inoculation			48 hours after inoculation		
	No.of Mice	Temperature °F	Parasitemia %	No.of Mice	Temperature °F	Parasitemia %	No.of Mice	Temperature °F	Parasitemia %	No of Mice	Temperature °F	Parasitemia %	No.of Mice	Temperature °F	Parasitemia %
1	10	98.8±0.5	5.8±0.7	10	98.6±0.1	0.9±0.6	10	98.7±0.3	2.7±1.0	10	98.9±0.2	3.6±1.4	10	98.7±0.5	4.1±0.8
2	10	99.3±0.7	8.6±1.4	10	98.8±0.5	2.9±0.5	10	98.8±0.5	7.9±1.2	10	99.1±0.4	10.8±2.7	10	98.9±0.7	11.4±3.6
3	10	98.7±0.7	20.4±4.2	10	98.5±0.5	3.8±0.9	10	98.4±0.2	29.6±5.7	10	98.9±0.4	30.4±6.9	10	98.5±0.6	28.5±8.3
4	10	98.2±2.1	59.7±8.2	10	98.6±0.2	1.4±1.0	10	97.7±1.1	44.5±10.3	9	98.5±1.5	47.3±13.0	9	97.5±1.2	51.4±8.5
5	7	96.1±1.6	65.5±10.2	8	98.6±0.6	2.3±1.5	8	96.4±0.8	61.5±8.1	6	96.1±1.8	68.2±9.3	8	95.8±0.8	70.6±9.5
6	4	94.6±2.0	72.9±10.2	8	98.7±0.3	1.2±0.9	8	95.1±0.6	76.4±13.5	5	95.3±2.5	59.3±8.4	6	95.1±1.6	68.3±10.2
7	2	95.1±1.6	43.9±7.8	8	98.5±0.5	<1	5	94.2±1.3	61.6±9.6	1	<94	63.4±0	3	94.5±1.1	52.1±9.5
8	1	<94	39.4	8	98.6±0.3		1	94.6±0	58.7±	0	<94		2	<94	43.2±8.1

The water extract was obtained from 400gm of fresh leaves per litre of water. Each mouse was given 1ml of the extract twice daily.

TABLE 10

Effect of alcoholic extract of Enantia chlorantha on temperature and % parasitemia in male mice infected with Plasmodium berghei

Days after inoculation	Infected control			Infected + chloroquine			Infected + extract of Enantia chlorantha. 6hours after inoculation			24hours after inoculation			48 hours after inoculation		
	No.of Mice	Temperature C°	Parasitemia %	No.of Mice	Temperature C°	Parasitemia %	No.of Mice	Temperature C°	Parasitemia %	No.of Mice	Temperature C°	Parasitemia %	No.of Mice	Temperature C°	Parasitemia %
1	10	99.1±0.4	1.9±0.9	10	98.8±0.2	-ve	10	99.2±0.6	2.6±0.6	10	98.7±0.3	2.1±2.0	10	98.8±0.1	1.8±1.1
2	10	98.9±0.6	6.3±1.2	10	98.8±0.1	1.0±0.5	10	98.5±0.5	5.4±2.2	10	98.5±0.3	6.2±3.7	10	98.7±0.5	8.6±3.2
3	10	98.0±1.0	15.7±3.8	10	98.4±0.5	2.5±0.4	10	97.6±1.8	14.9±1.8	10	97.0±0.9	18.4±5.4	10	97.5±1.2	16.3±3.6
4	8	96.7±2.8	31.9±6.6	10	98.3±0.3	3.8±0.9	10	96.3±1.5	28.5±7.2	10	96.5±1.2	29.0±11.7	9	96.6±1.7	36.2±.7
5	8	95.9±1.4	52.6±6.4	10	98.6±0.6	1.1±1.4	7	95.5±2.5	49.6±8.5	6	95.2±1.6	58.7±9.8	6	96.1±2.3	54.2±13.7
6	6	95.2±1.6	61.4±7.2	10	98.9±0.1	1.0±0.3	5	94.7±1.3	50.2±11.0	5	94.3±0.7	68.3±5.2	6	94.6±1.2	59.4±14.5
7	4	<94	51.2±3.4	9	98.8±0.3	1	4	<94	57.7±6.1	4	<94	44.5±15.2	3	94.1±1.5	50.3±5.2
8	2	<94	33.5±6.0	9	98.7±0.5	1	0			2	<94	39.1±4.0	0		

The residue obtained from 250gm of bark per litre of alcohol was redissolved in 1 litre of water. ½ ml was given to each mouse twice daily.

TABLE 11

Effect of water extract of a mixture of plants Mixture A on temperature, and parasitemia in male mice infected with Plasmodium berghei

Days after inoculation	Infected control			Infected + chloroquine			Infected + extract of mixture A								
							6hours after inoculation			24hours after inoculation			48 hours after inoculation		
	No.of Mice	Temperature oF	Parasitemia %	No.of Mice	Temperature oF	Parasitemia %	No.of Mice	Temperature oF	Parasitemia %	No.of Mice	Temperature oF	Parasitemia %	No.of Mice	Temperature oF	Parasitemia %
1	10	98.6±0.7	2.0±0.5	10	98.7±0.2	-ve	10	98.8±0.2	1.8±0.8	10	98.8±0.4	2.0±1.2	10	98.7±0.2	1.1±1.6
2	10	98.1±0.5	4.6±1.2	10	98.6±0.4	-ve	10	98.0±0.9	4.0±2.5	10	98.4±0.6	4.7±2.5	10	98.3±0.6	4.4±0.7
3	10	97.7±0.7	14.3±2.3	10	98.6±0.4	1.8±0.2	10	97.8±0.9	11.9±6.5	10	98.1±1.2	10.3±5.4	10	98.2±1.0	13.1±6.8
4	10	96.9±1.3	25.6±10f	10	98.8±0.3	2.8±0.7	9	96.5±1.4	29.4±8.1	10	97.8±0.8	21.9±5.1	9	97.1±0.5	25.3±5.7
5	10	95.2±1.9	43.4±8.5	10	98.8±0.1	3.7±1.2	9	96.1±0.8	48.1±16.2	8	96.0±2.0	41.1±9.3	9	95.9±1.6	38.2±8.9
6	8	94.4±0.7	55.7±6.3	10	98.9±0.2	1.1±0.7	6	94.9±1.5	51.4±9.4	7	95.5±1.4	60.3±8.8	7	94.5±2.4	56.1±7.5
7	6	<94	59.2±11.1	9	98.8±0.4	<1	3	94.7±1.7	66.7±8.2	4	<94	57.2±6.1	3	<94	61.3±9.1
8	3	<94	48.8±6.6	9	98.8±0.2	<1	1	<94	58.2	2	<94	42.5±3.3	1	<94	54.5

The water extract was obtained from a mixture of plants whose composition was stated on page 71. Each mouse was given 1ml of the extract twice daily.

TABLE 12

Effect of water extract of a mixture of plants Mixture B on temperature and percentage parasiteria in male mice infected with Plasmodium berghei

Days after inoculation	Infected control			Infected + chloroquine			Infected + extract of mixture B								
							6hours after inoculation			24hours after inoculation			48hours after inoculation		
	No.of Mice	Temperature oF	Parasitemia %	No.of Mice	Temperature oF	Parasitemia %	No.of Mice	Temperature oF	Parasitemia %	No.of Mice	Temperature oF	Parasitemia %	No.of Mice	Temperature oF	Parasitemia %
1	10	99.0±0.2	3.4±1.8	10	99.8±0.4	-ve	10	98.7±0.5	3.2±0.8	10	98.8±0.4	3.0±1.6	10	98.7±0.5	3.0±2.1
2	10	98.6±0.2	8.4±1.2	10	98.6±0.3	2.1±1.3	10	98.7±0.6	7.3±2.1	10	98.1±0.8	8.7±2.7	10	98.8±0.3	7.8±1.6
3	10	98.1±0.5	18.6±3.6	10	98.7±0.2	5.2±1.0	10	97.8±1.1	16.6±4.4	10	97.7±0.5	15.4±7.6	10	97.6±0.5	16.9±2.9
4	9	97.2±0.6	30.4±8.6	10	98.6±0.5	3.6±1.7	8	97.5±2.6	27.4±5.8	10	96.8±1.6	31.1±8.5	8	96.9±1.4	33.4±4.6
5	9	96.9±1.4	58.9±11.4	10	98.8±0.5	2.2±0.8	8	96.8±1.3	56.6±9.2	8	96.0±3.1	51.2±8.2	7	96.2±2.3	61.4±7.7
6	6	95.5±2.1	75.4±10.1	10	98.7±0.3	<1	5	95.0±1.6	68.7±6.6	5	95.2±1.4	71.9±11.0	7	95.7±1.7	75.9±11.9
7	3	95.1±0.9	68.2±6.6	10	98.8±0.2	<1	2	94.7±0.5	55.1±4.5	4	94.1±0.7	65.1±8.2	3	95.0±0.5	59.1±7.2
8	-	-	-	10	98.7±0.5	<1	1	<94	30.6-	2	<94	49.9±3.6	1	96.6±0	50.7±0

The water extract was obtained from a mixture of plants whose composition is stated on page 71. Each mouse was given 1 ml of the extract twice daily.

Experiment 3b

Clinical and biochemical effects of some plant extracts on Plasmodium berghei infections in mice.

In an earlier experiment it was established that the extracts of the plants under investigation at very high concentrations had no suppressive action on the level of parasitemia in the mice. Thus water extracts of leaves of Azadirachta indica, Morinda lucida and Alstonia boonei (prepared by the method of Berg et. al. described in details in Chapter three) of concentrations varying from 1Kg/litre to 4Kg/litre and these extracts concentrated in vacuo up to 100 times had no suppressive effect on the levels of parasitemia in infected mice. The results of these preliminary experiments apart from indicating that the extracts were ineffective as anti-malarial agents also showed that they were not potent poisons as they did not affect the time of death and the number of infected mice which died significantly. It was therefore considered important to find out the action of these extracts on some selected biochemical values in the serum of both normal and infected mice in an attempt to find out their action on the metabolism of the animals.

Procedure.

Each experiment involved the use of 16 litters of male mice, each litter containing 7 male mice. While one litter of 5 mice was used to determine the normal values for the various tests, on the day the experiment was started, the other 15 litters were divided into 3 sets of 5 litters. One set was used for determinations on the 2nd day, another for the 4th day and the third for the 6th day after inoculation. Each set of 5 litters was again subdivided into 7 groups of 5 mice. Three groups in each set were inoculated with normal mice erythrocytes while the other four groups were inoculated with one million parasitized erythrocytes. Of the infected mice, one group of 5 in each set was left as untreated control, another was given 300/ug chloroquine daily. The other two groups were treated with two concentrations of the extract under observation. Of the uninfected mice one group was left as the normal control while the other two groups were given the two concentrations of the extracts given to the infected groups.

At two days interval the mice were examined for their

body temperatures and parasitemia before all the mice in each group were killed. Their pooled sera were analysed for their biochemical values. Their liver and spleen, the two most adversely affected organs in malaria, were preserved in formalin. Stained sections of these organs were examined. The plant extracts used had the following concentrations.

1. Agadirachta indica extract A_{31} was prepared from 1Kg of fresh plant material per litre. This extract concentrated 100 times gave A_{32}.

2. Morinda lucida extract M_1 was prepared from 1Kg of fresh plant material per litre. This extract concentrated 100 times gave M_2.

3. Alstonia boonei extract Als_1 was prepared from 1Kg of fresh plant material per litre. This extract concentrated 100 times gave Als_2.

4. Enantia chlorantha extract E_1 was prepared from 500gm of dried and powdered bark per litre of locally brewed alcohol. The residue obtained after evaporation of the solvent was redissolved in water. It was then concentrated 100 times to give E_2.

5. Extracts of Mixtures A and B were prepared from double the weight of the various components indicated in details in Chapter three in 2 litres of water to give Mix A_1 or Mix B_1 respectively. These extracts concentrated 100 times gave Mix A_2 and Mix B_2 respectively.

The results of these experiments are expressed graphically in figs 5 to 10 in which the following general abbreviations were used for convenience.

N = Normal uninfected untreated control.

INFC = Infected untreated control.

INF + CHLO = Infected treated with chloroquine.

INF + X = Infected treated with the extract under investigation.

N + X = Normal uninfected given the extract under investigation.

Fig. 5A.

Effect of water extract of leaves of Azadirachta indica on parasitemia, temperature and some biochemical values of serum constituents in normal mice and mice infected with Plasmodium berghei.

Fig 5A

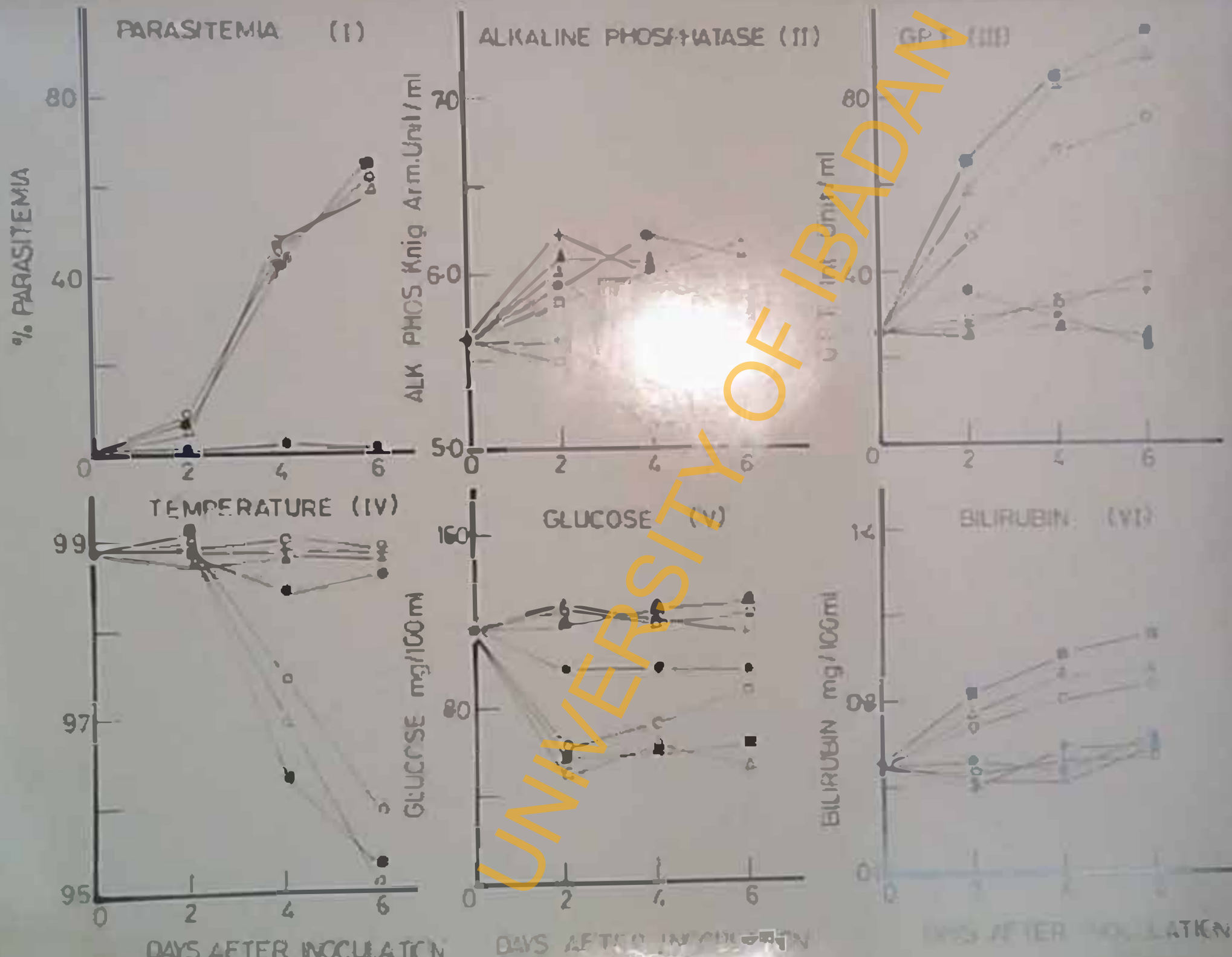
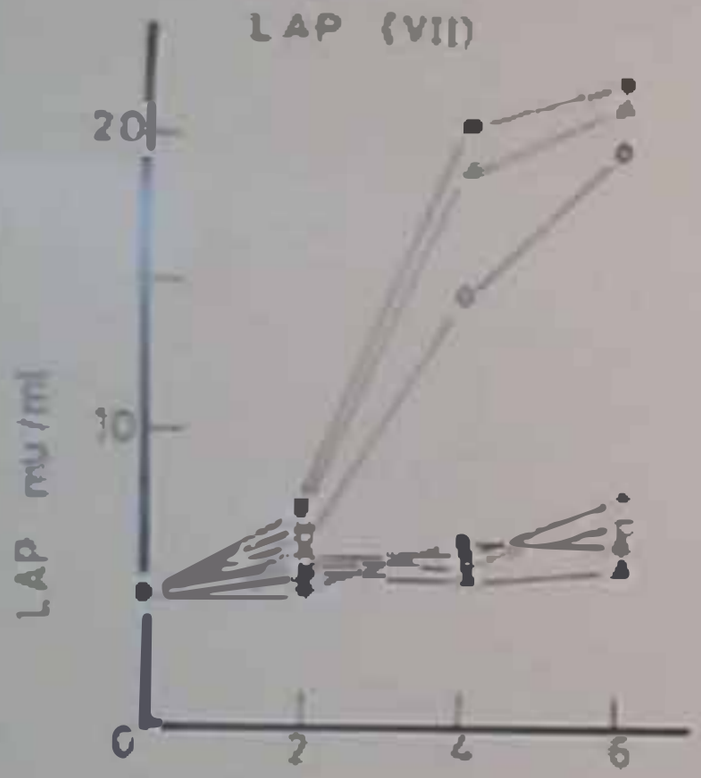
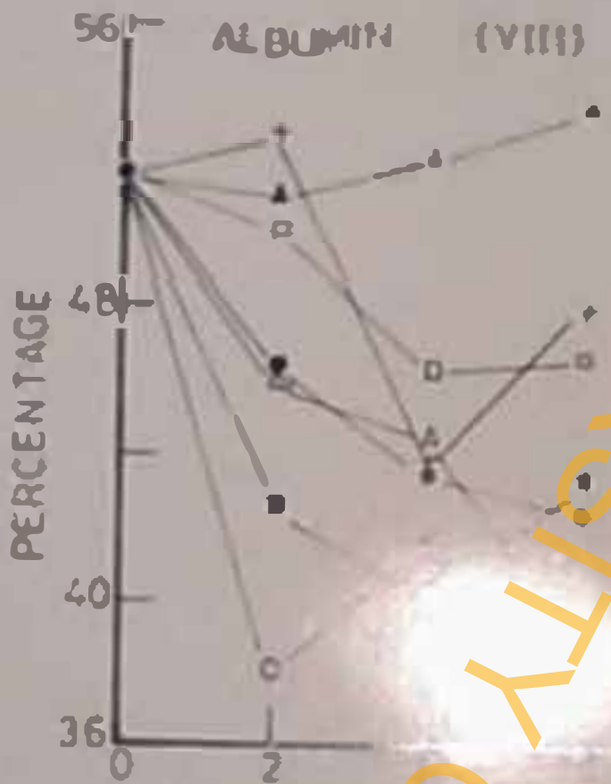


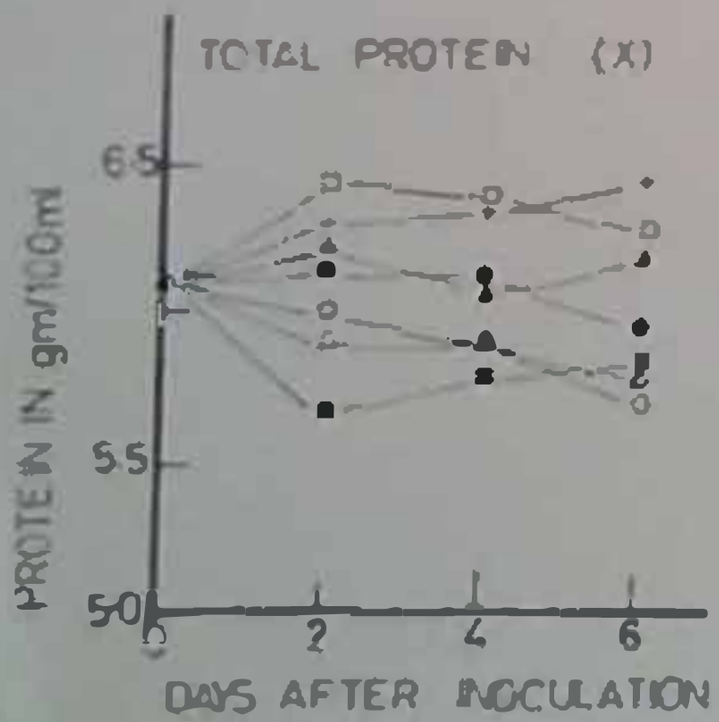
Fig. 5B.

Effect of water extract of leaves of Azadirachta indica on some biochemical values of serum constituents in normal mice and mice infected with Plasmodium berghei.

Fig 5B







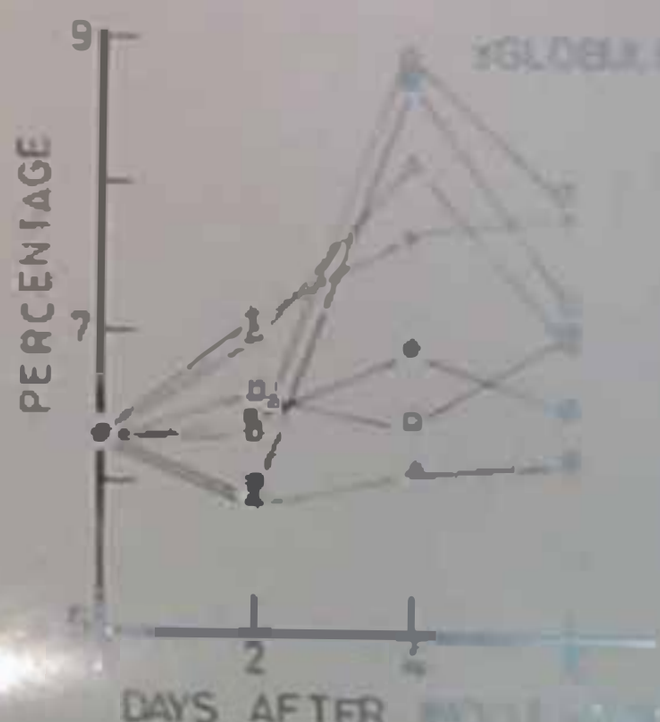


Fig. 6A.

Effect of water extrac

on parasitemia, temperature

serum constituents in norm

Plasmodium berghei

Fig. 6A.

Effect of water extract of leaves of Morinda lucida on parasitemia, temperature and some biochemical values of serum constituents in normal mice and mice infected with Plasmodium berghei

Fig 6A

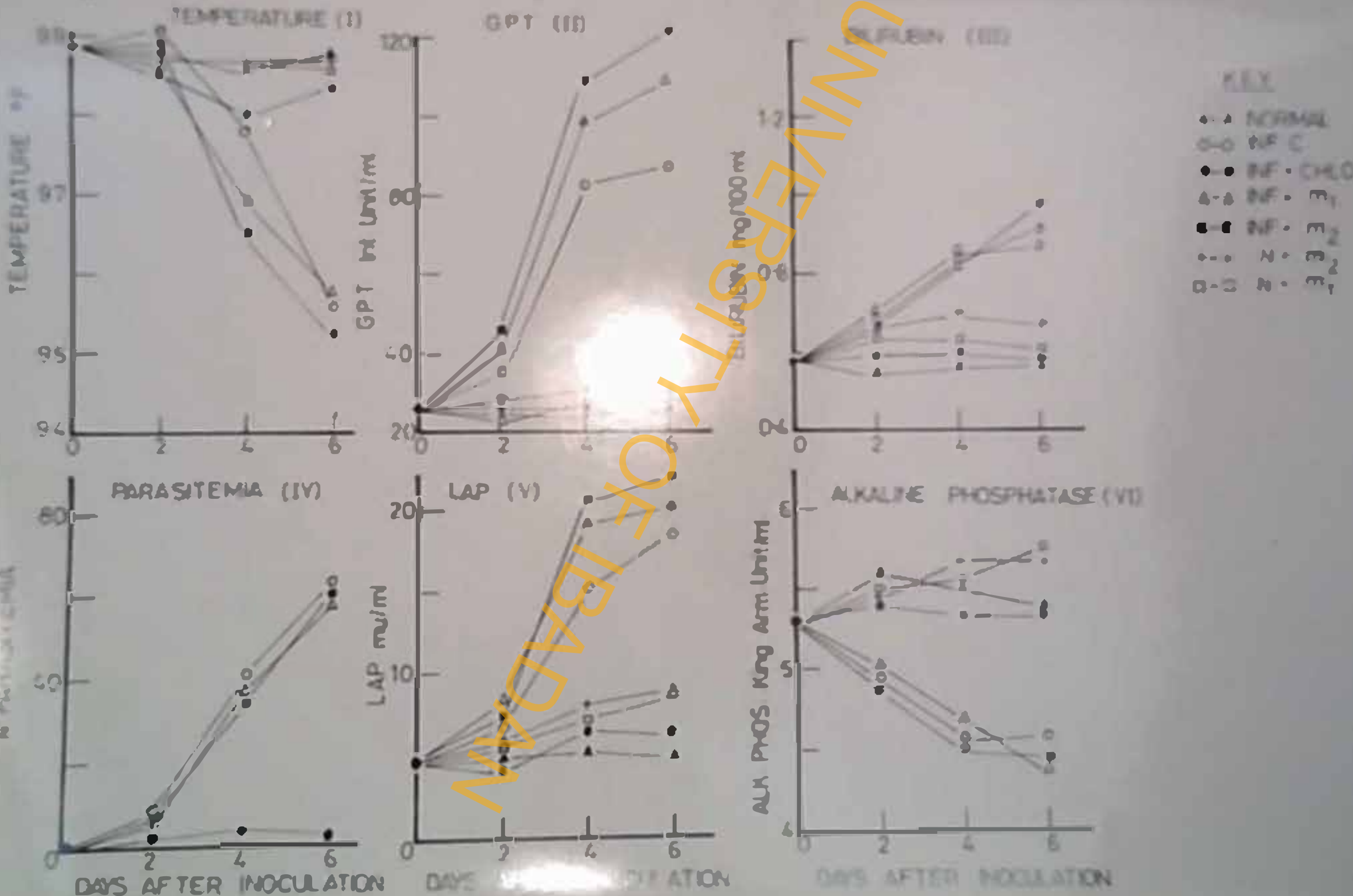
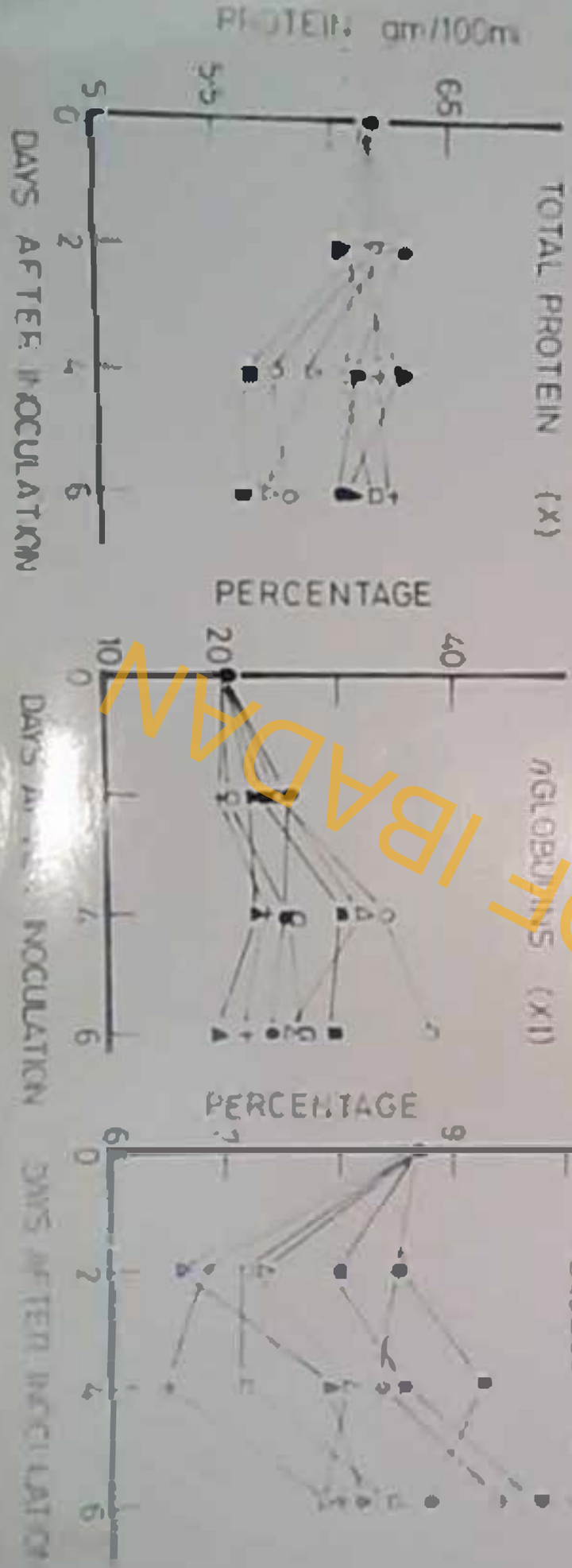


Fig. 68.

Effect of water extract of leaves of Morinda lucida on some biochemical values of serum constituents in normal mice and mice infected with Plasmodium berghei.



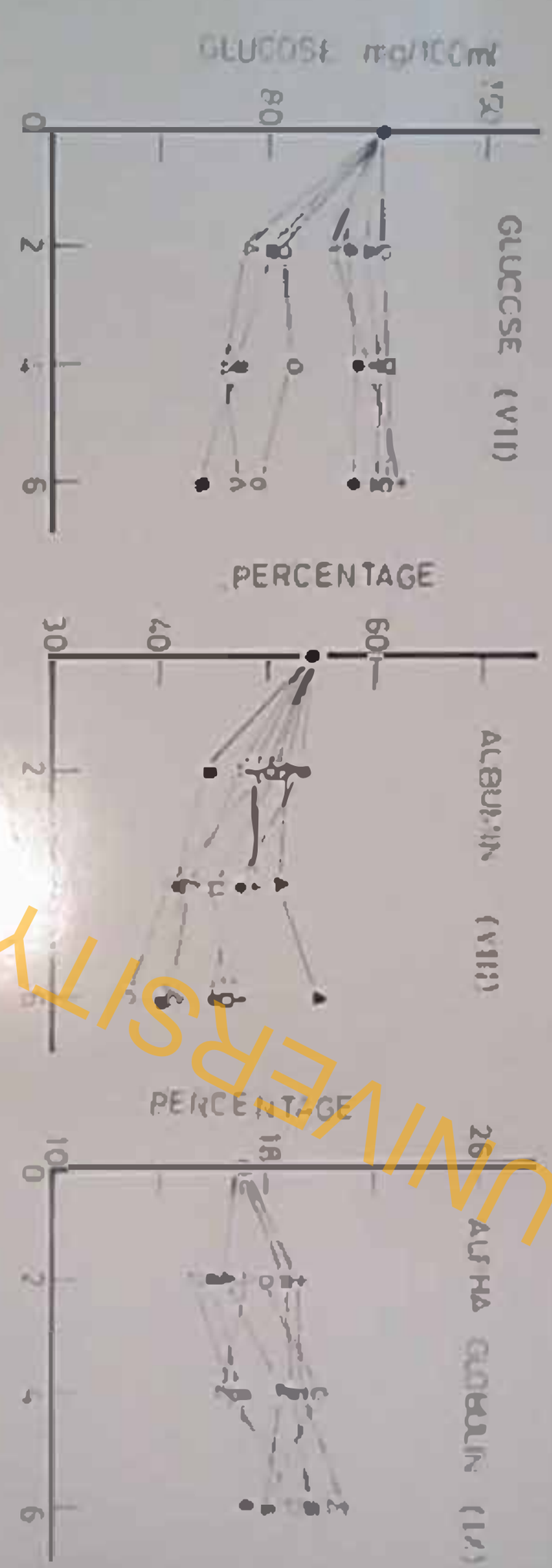
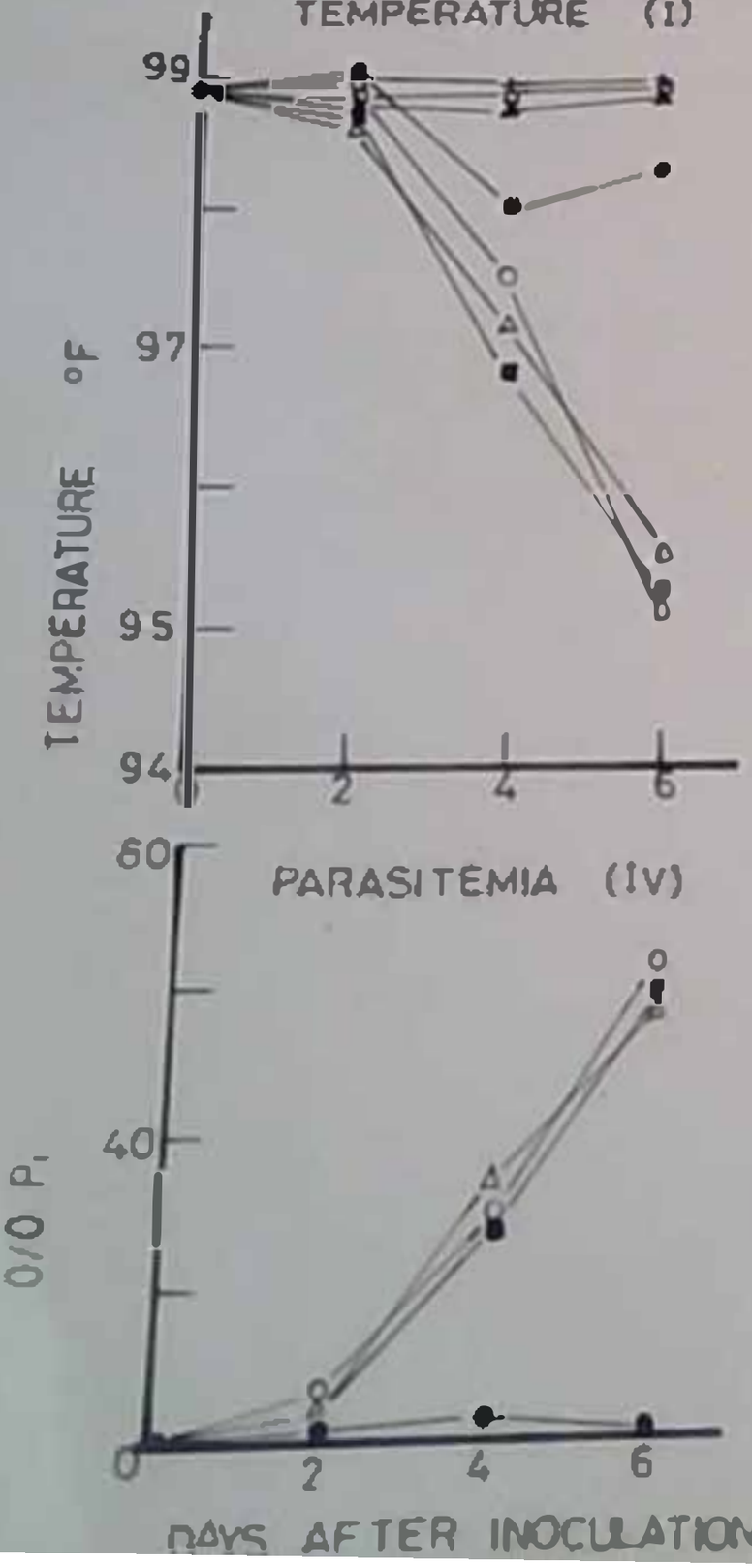
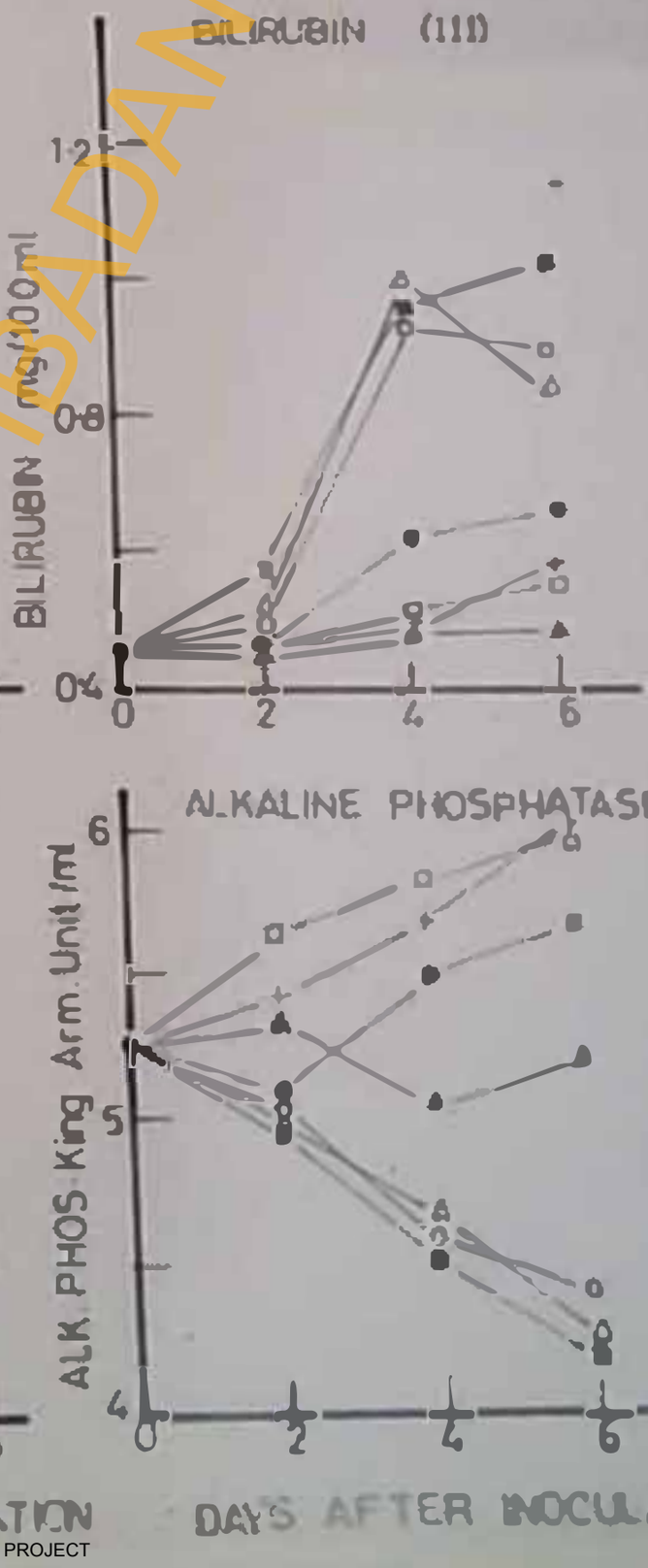


Fig. 7A.

Effect of water extract of leaves of *Alstonia boonei* on parasitemia, temperature and some biochemical values of serum constituents in normal mice and mice infected with *Plasmodium berghei*.

Fig 7A





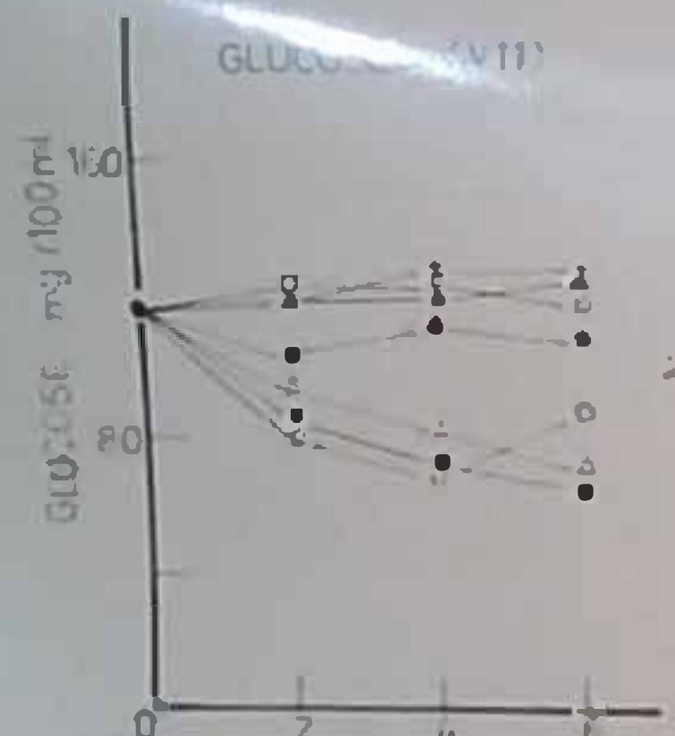
KEY

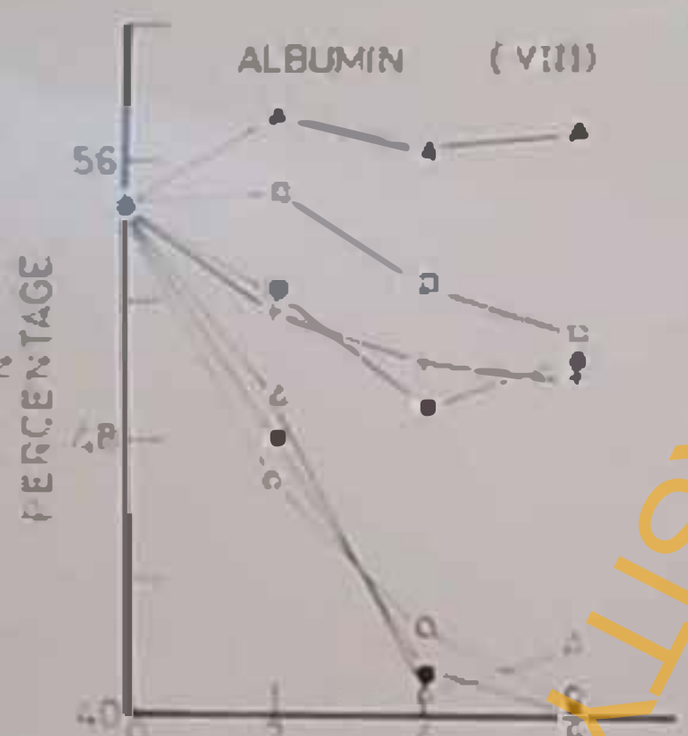
- ▲ - ▲ NORMAL
- o - o INF C
- ● - ● INF . CHLO
- ▲ - ▲ INF . Als_1
- ■ - ● INF . Als_2
- +--+ N . Als_2
- □ - □ N . Als_1

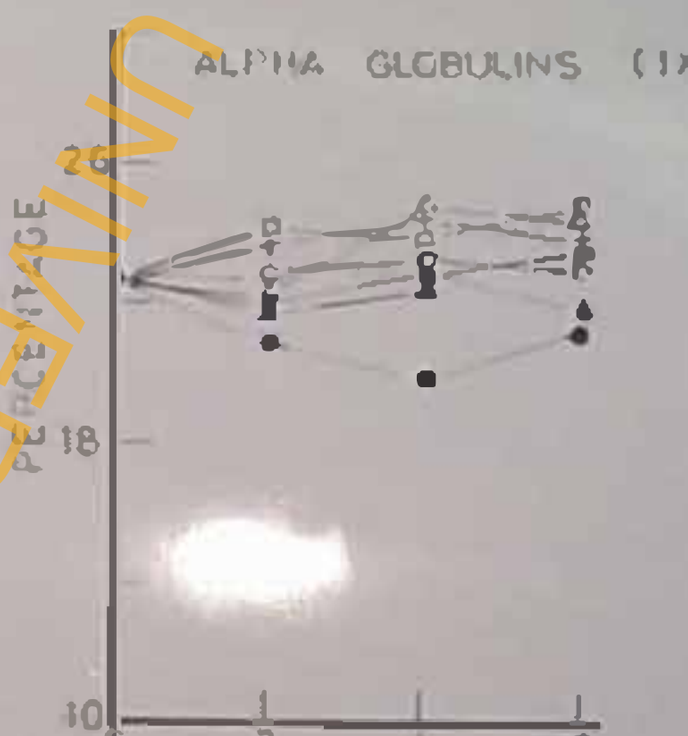
Fig. 78.

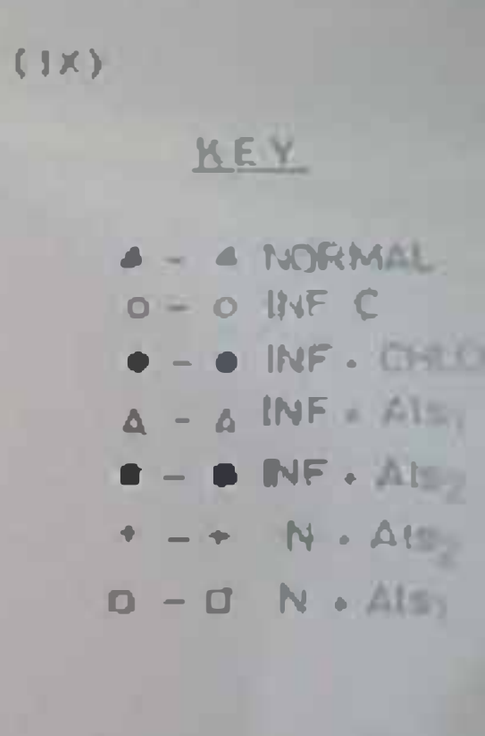
Effect of water extract of leaves of Alstonia boonei on some biochemical values of serum constituents in normal mice and mice infected with Plasmodium berghei.

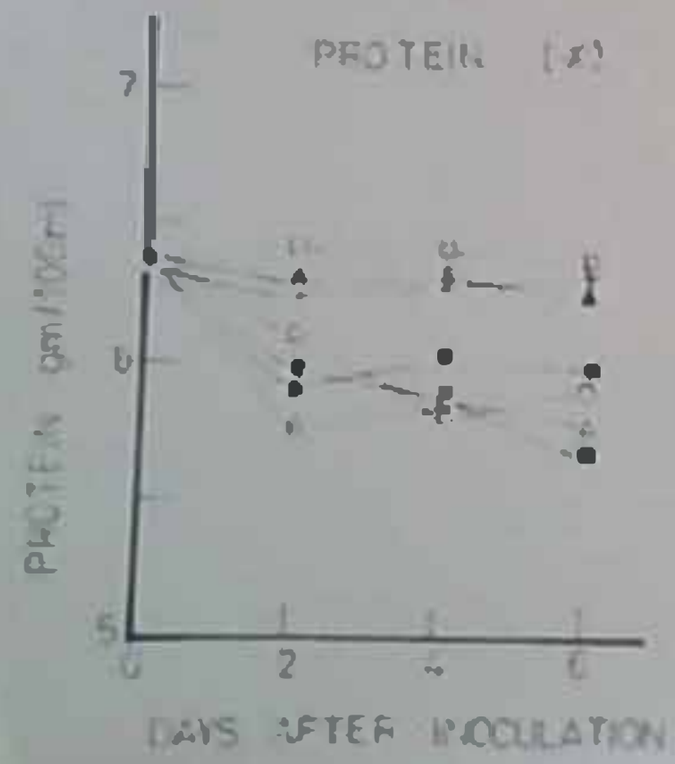
Fig 7B

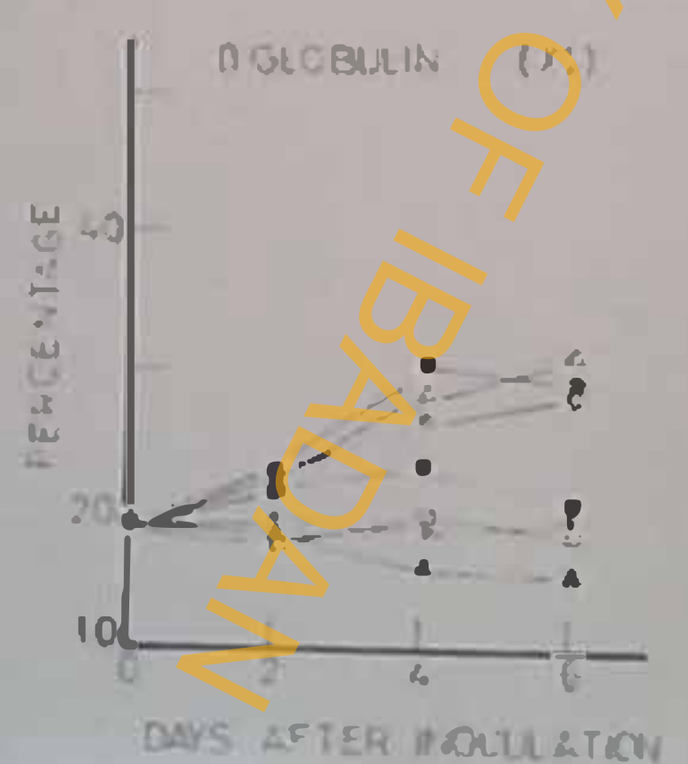












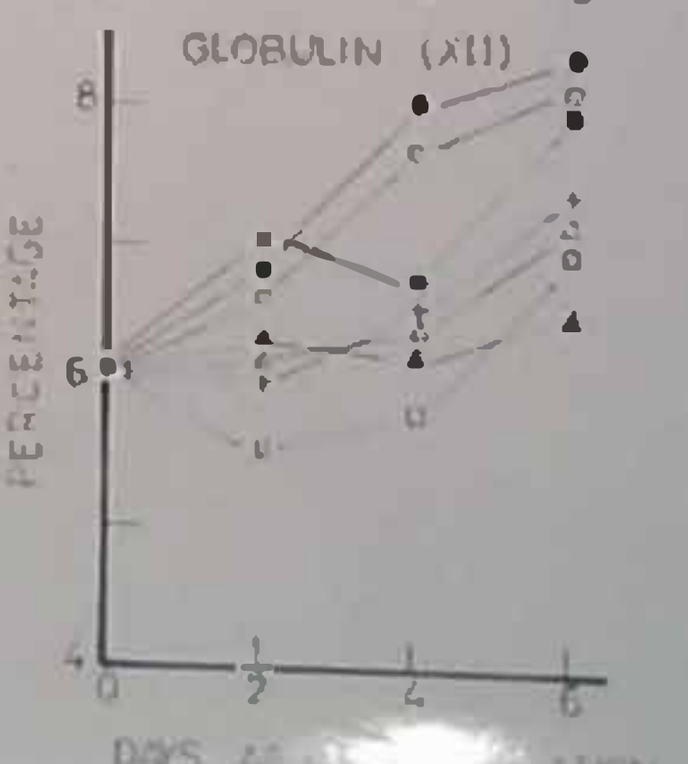


Fig. 8A.

Effect of alcoholic extract of bark of Erantia citrantha on parasitemia, temperature and some biochemical values of serum constituents in normal mice and mice infected with Plasmodium berghei.

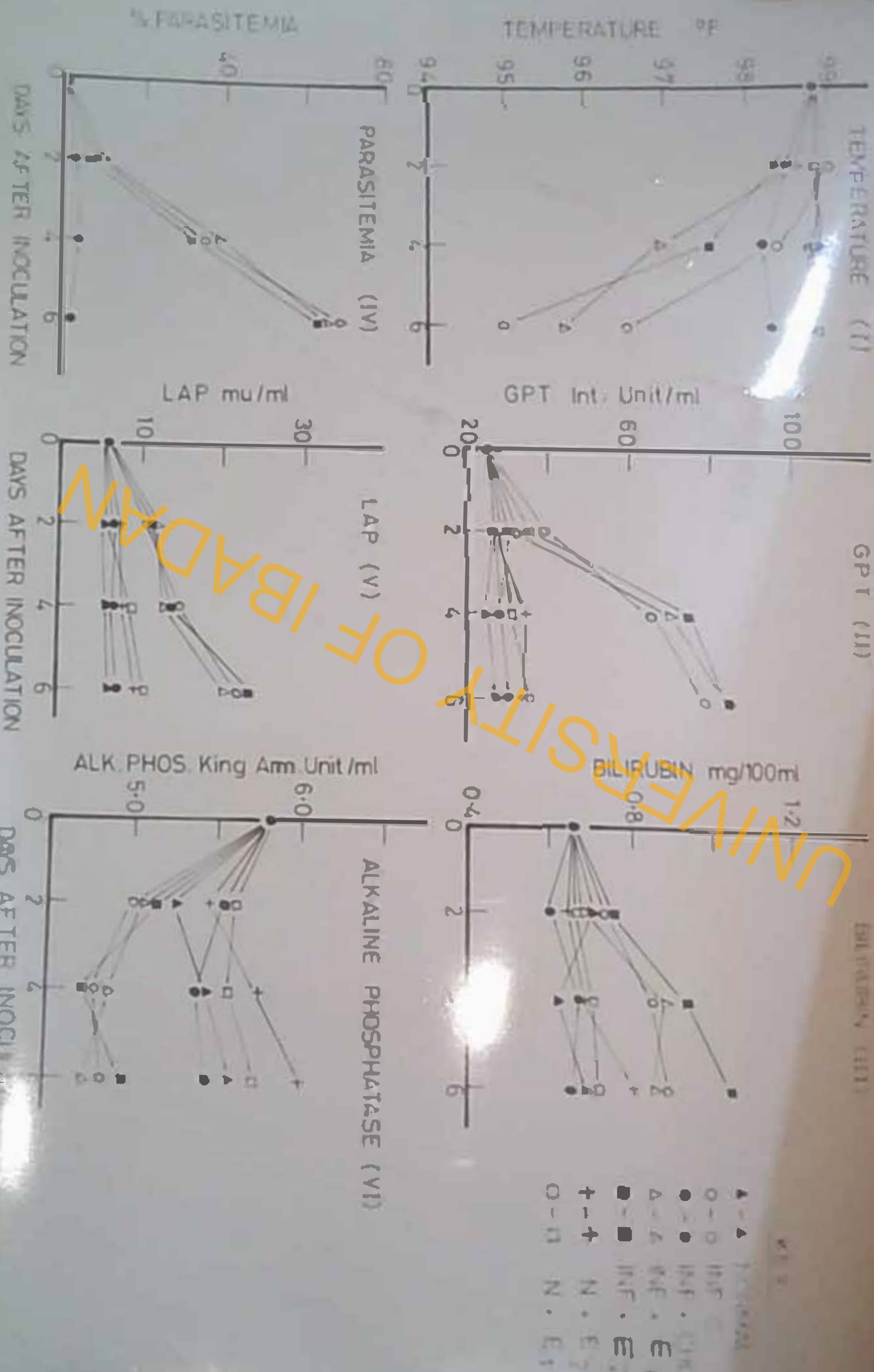
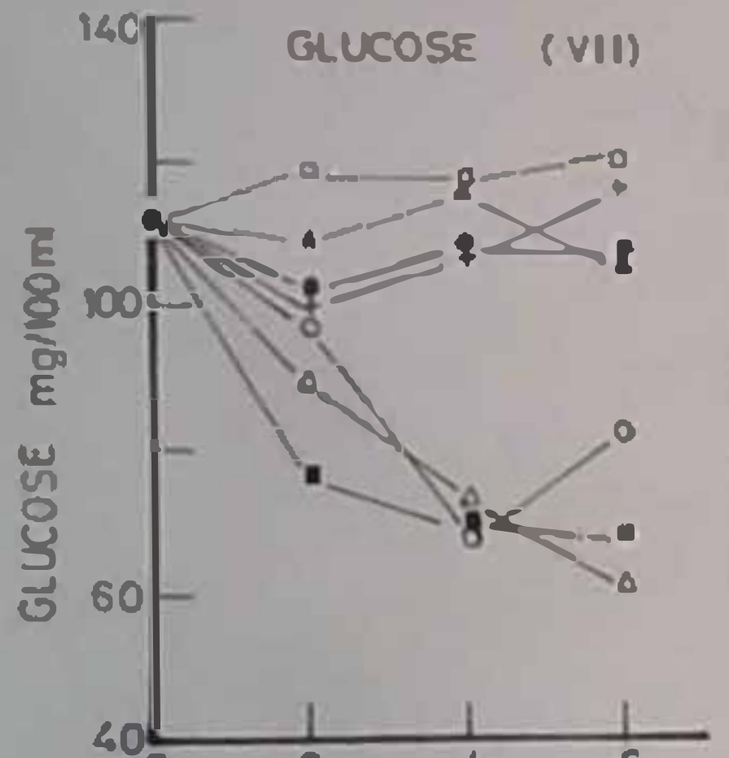
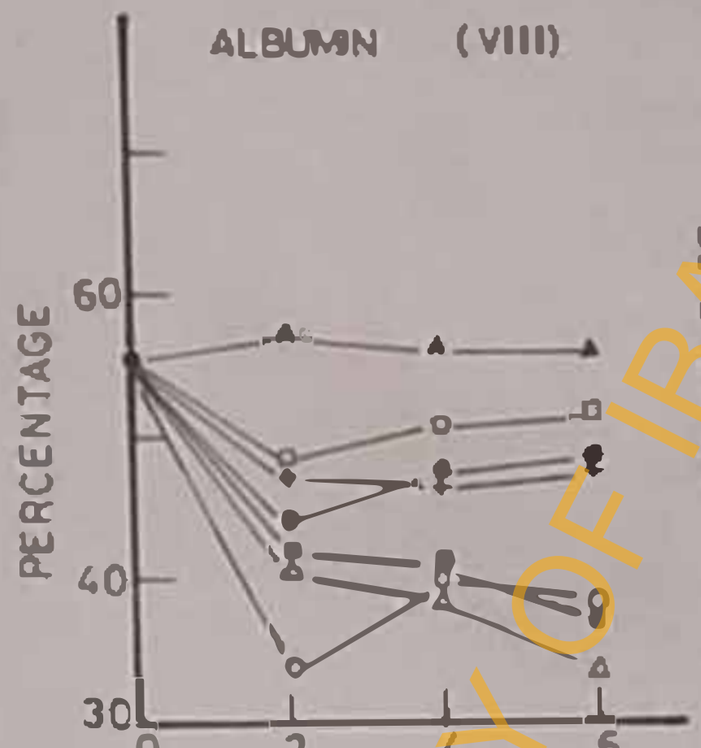


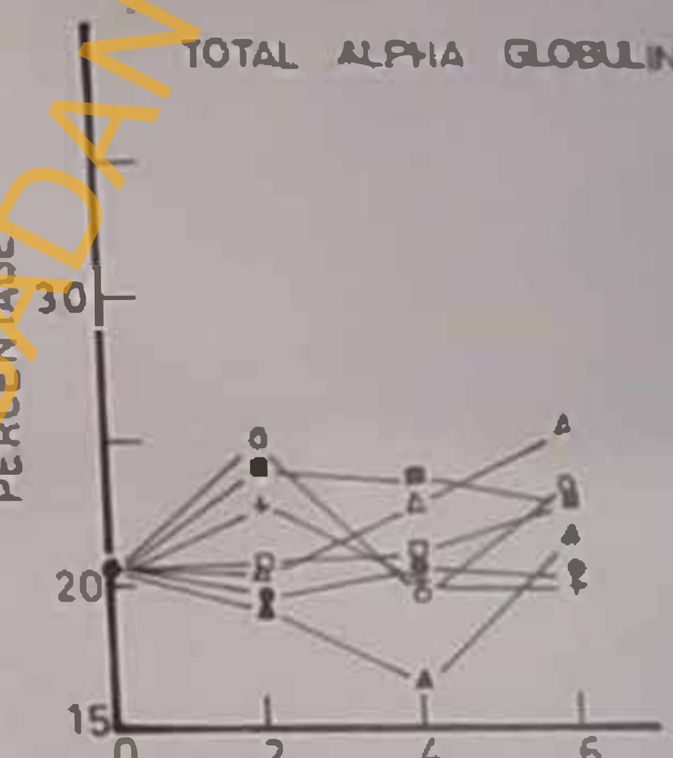
Fig. 88.

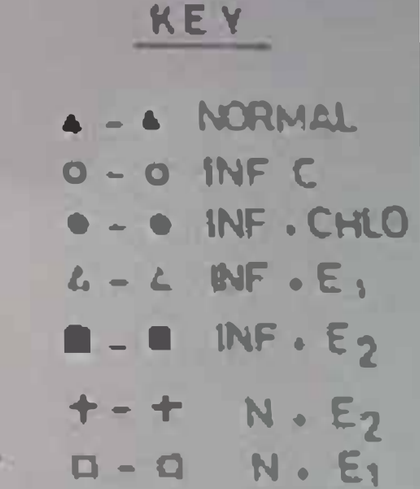
Effect of alcoholic extract of bark of Enantia chlorantha on some biochemical values of serum constituents in normal mice and mice infected with Plasmodium berghei.

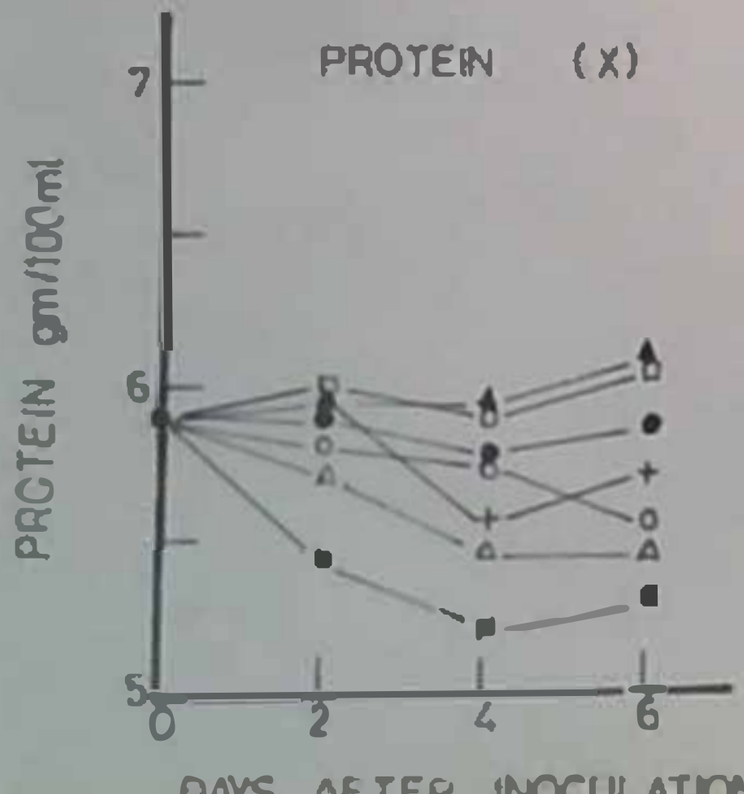
Fig 8B

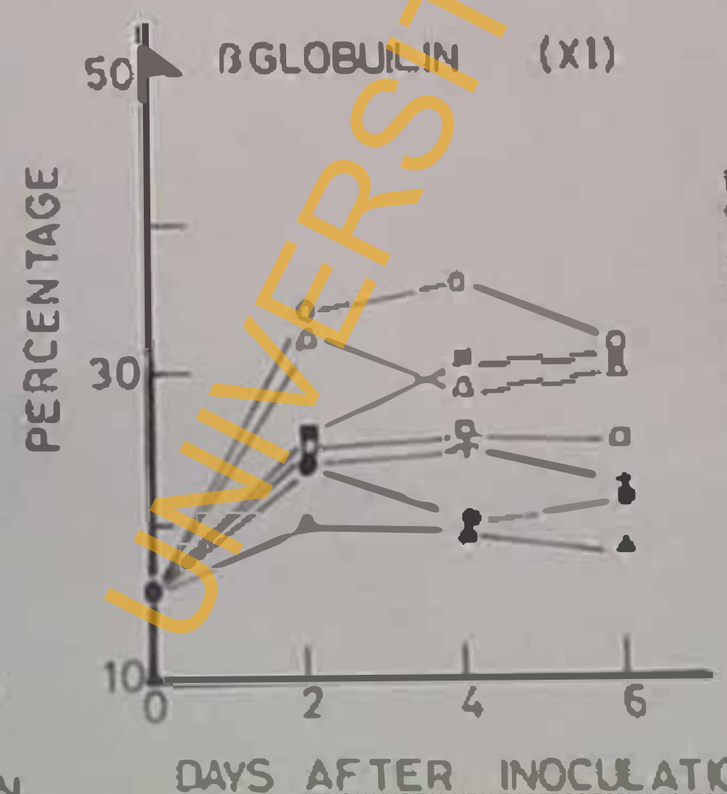












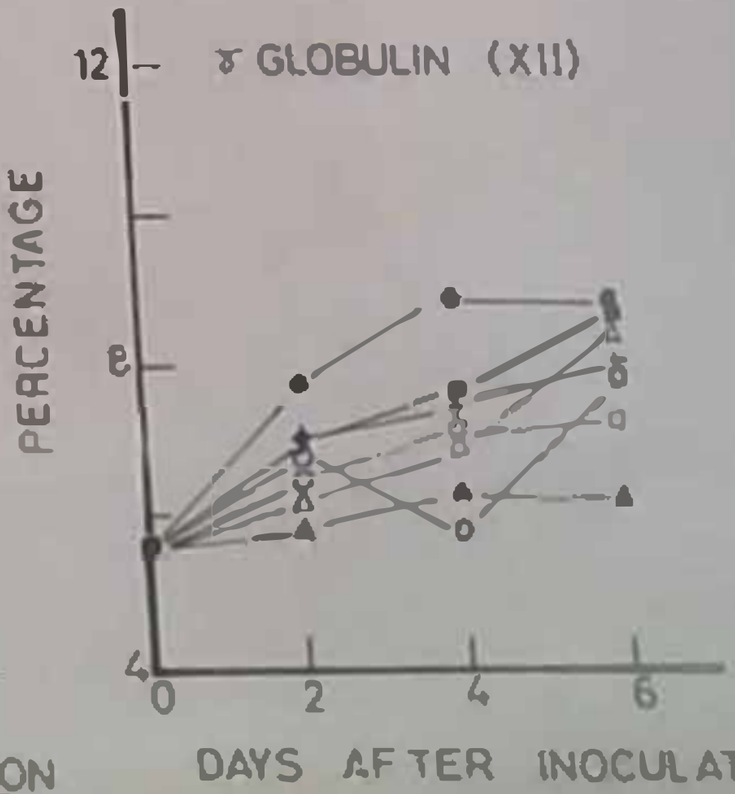
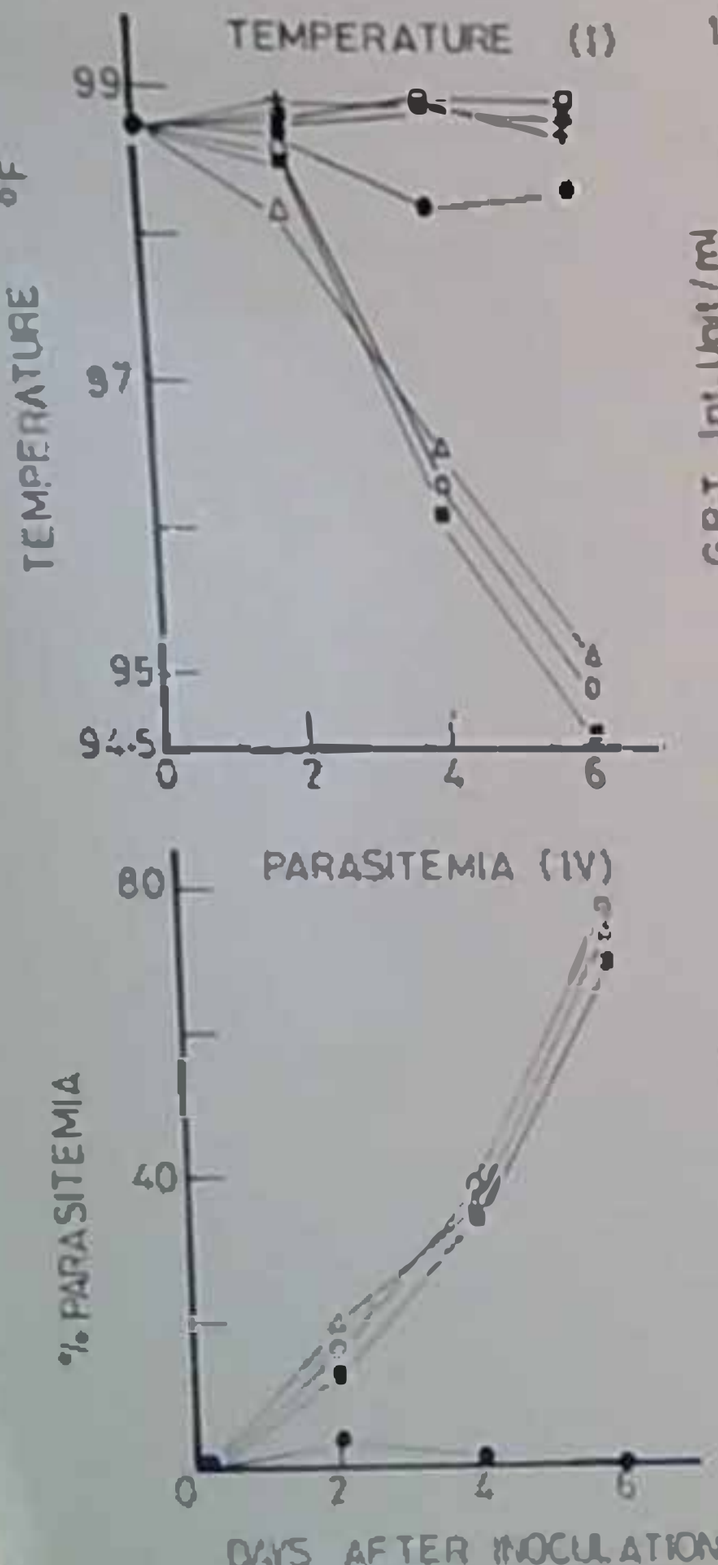
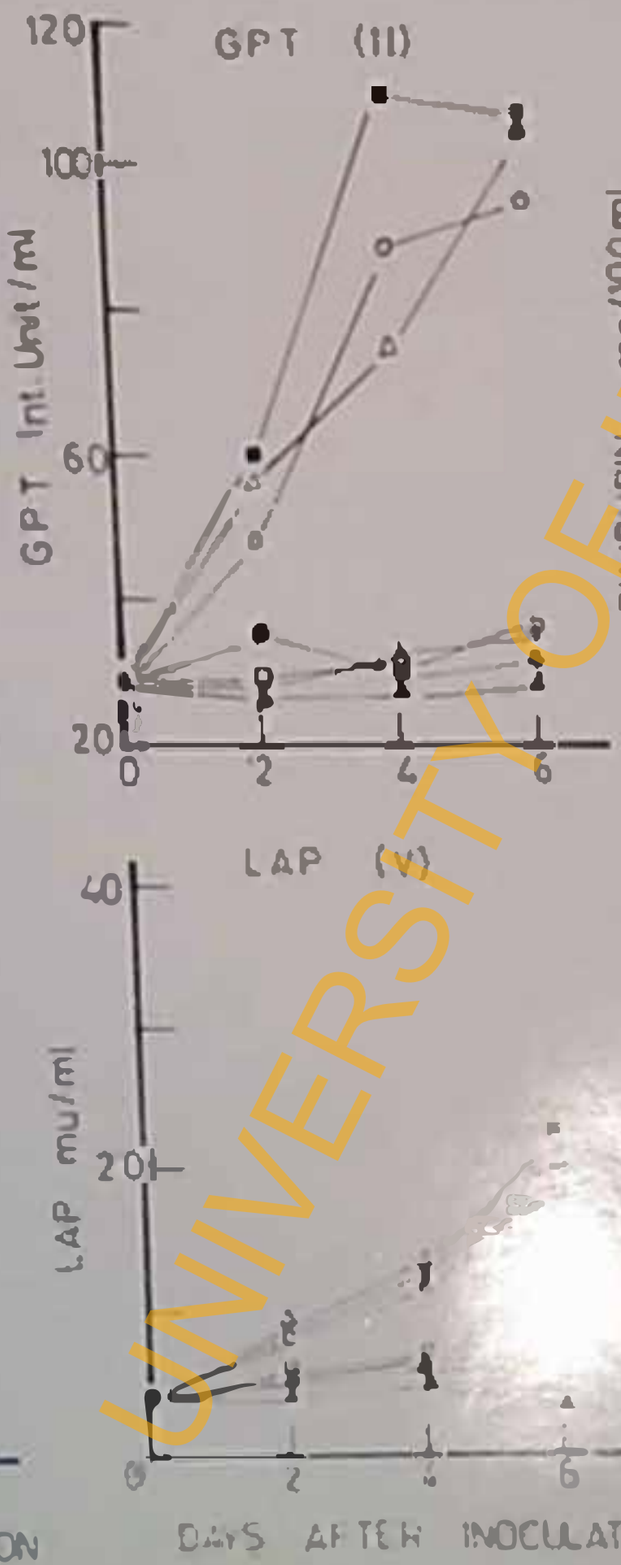


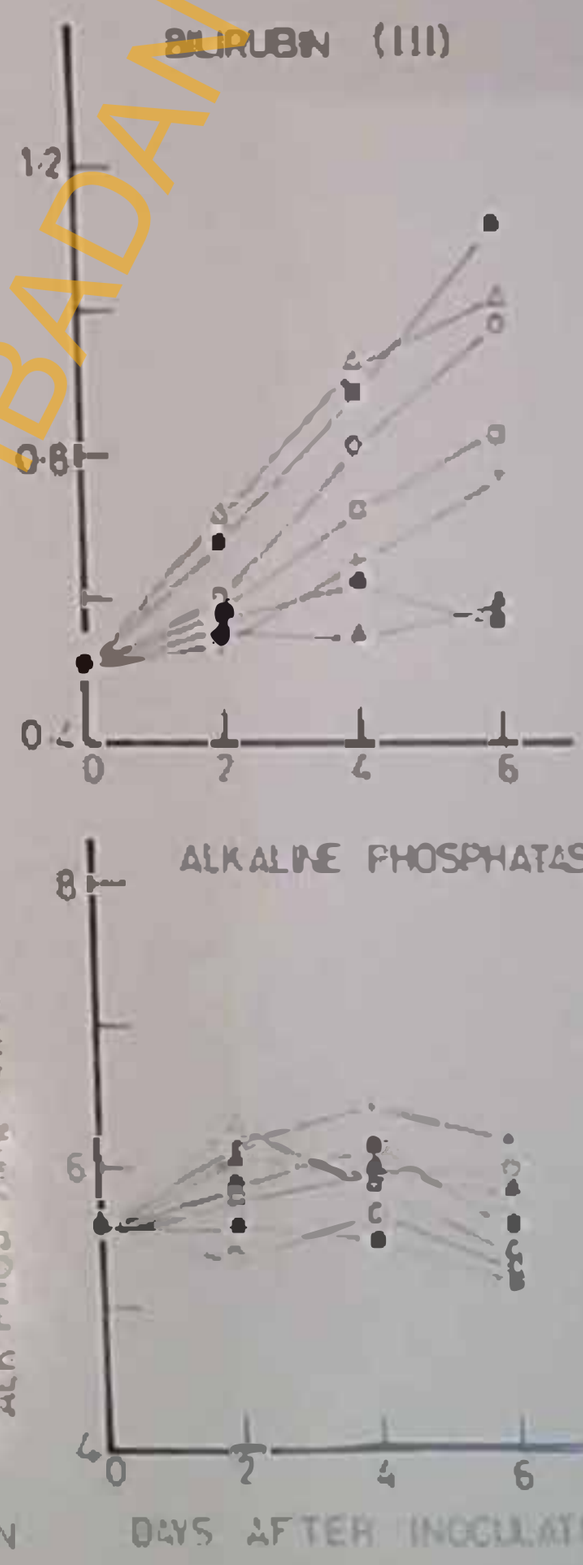
Fig. 9A.

Effect of water extract of a mixture of plants (Mix 1) on parasitemia, temperature and some biochemical values of serum constituents in normal mice and mice infected with Plasmodium berghei.

Fig 9A







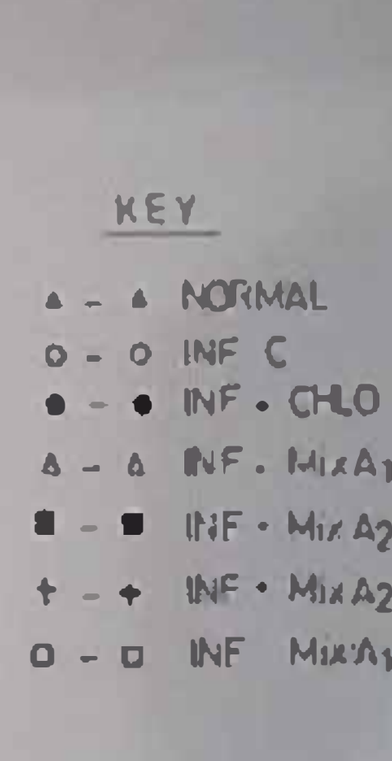


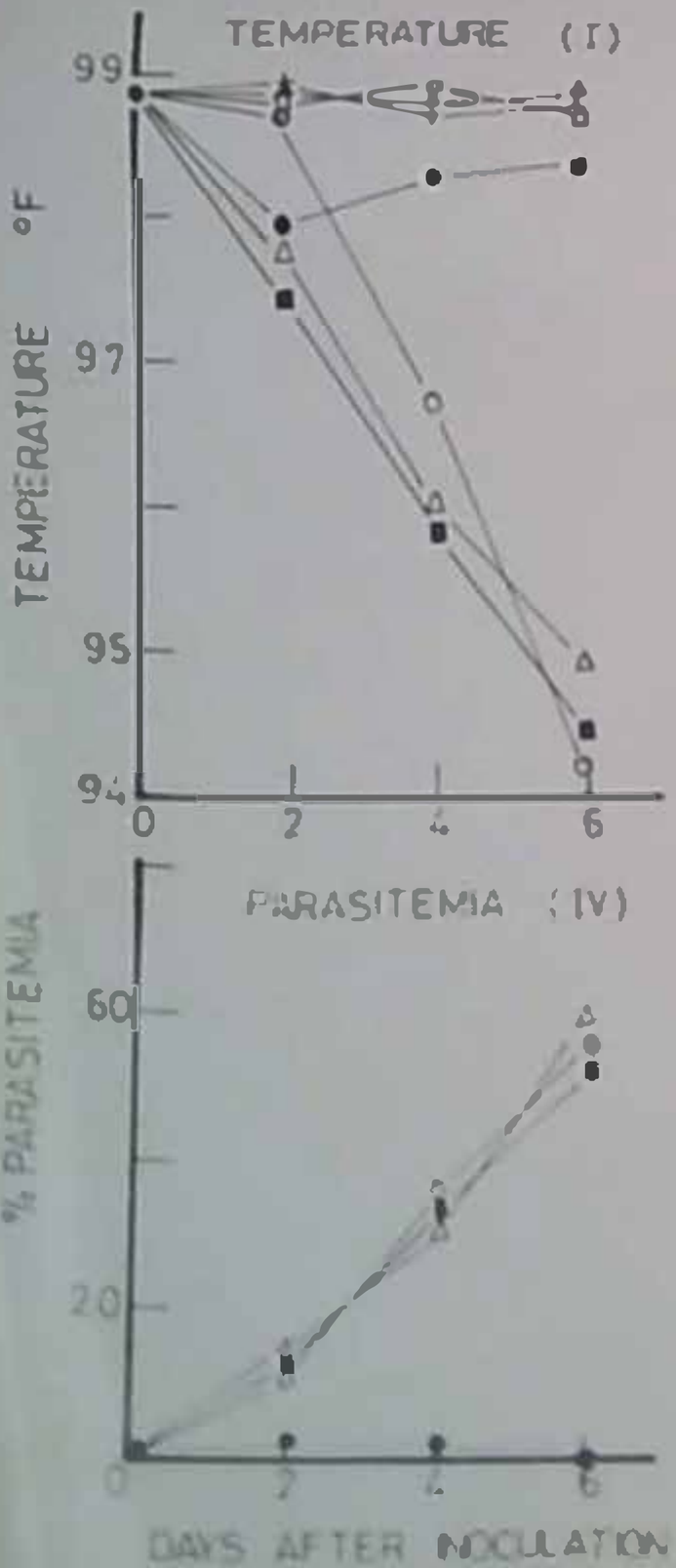
Fig. 9B.

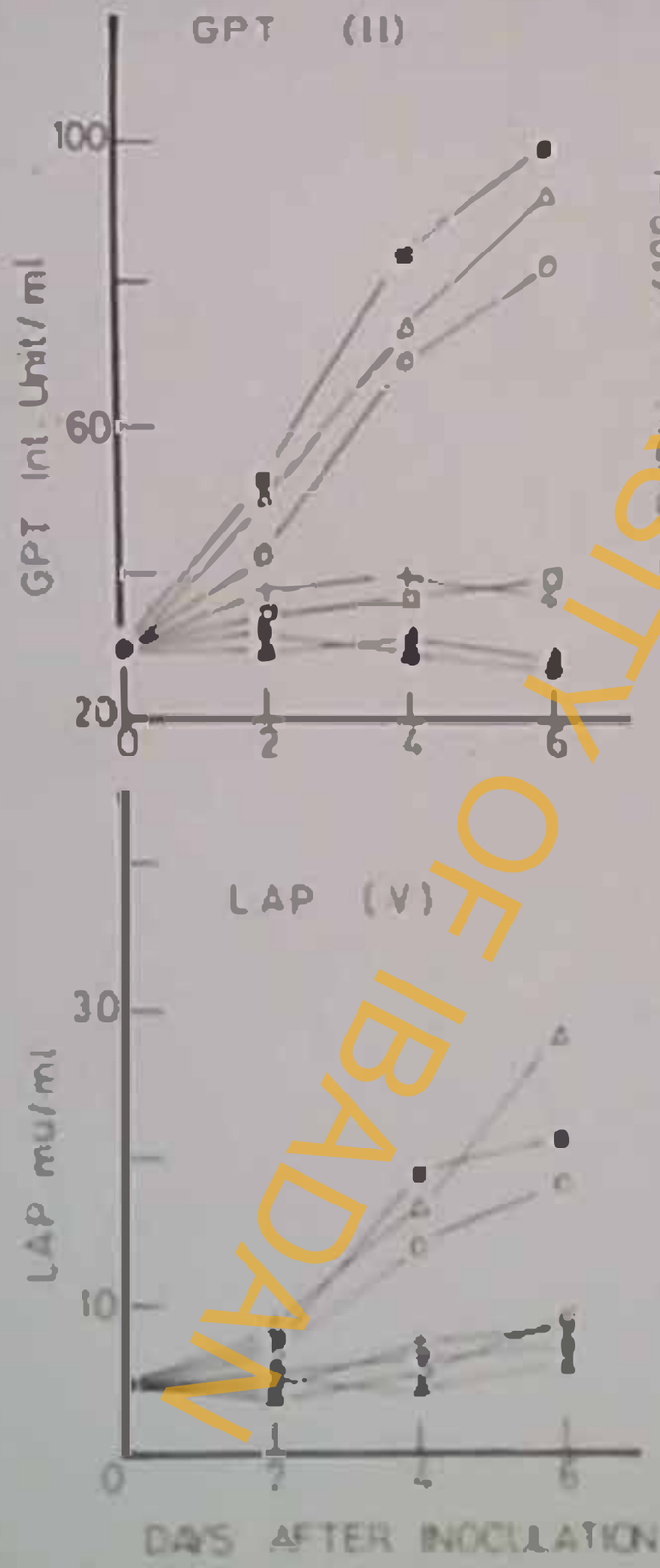
Effect of water extract of a mixture of plants (M.A) on some biochemical values of serum constituents in normal mice and mice infected with Plasmodium berghei.

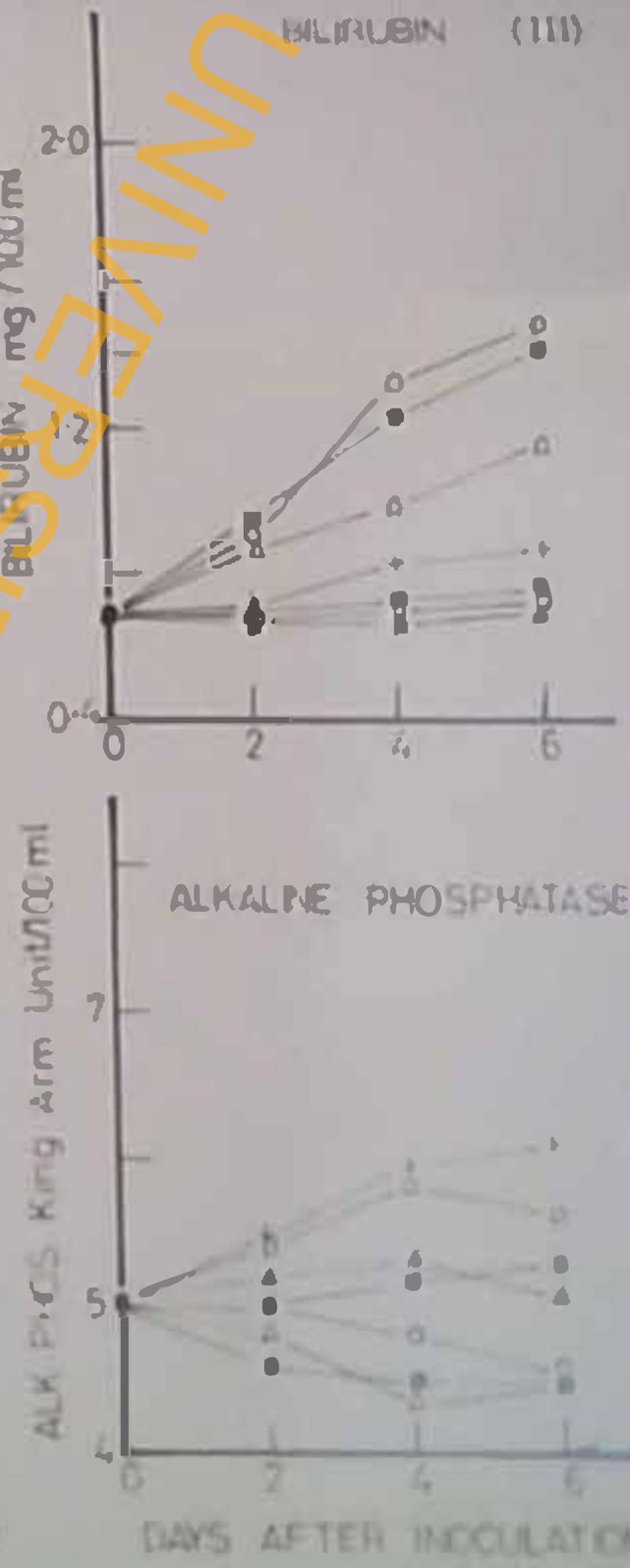
Fig. 10A.

Effect of water extract of a mixture of plants (Mix 3) on parasitemia, temperature and some biochemical values of serum constituents in normal mice and mice infected with Plasmodium berghei.

Fig 10A







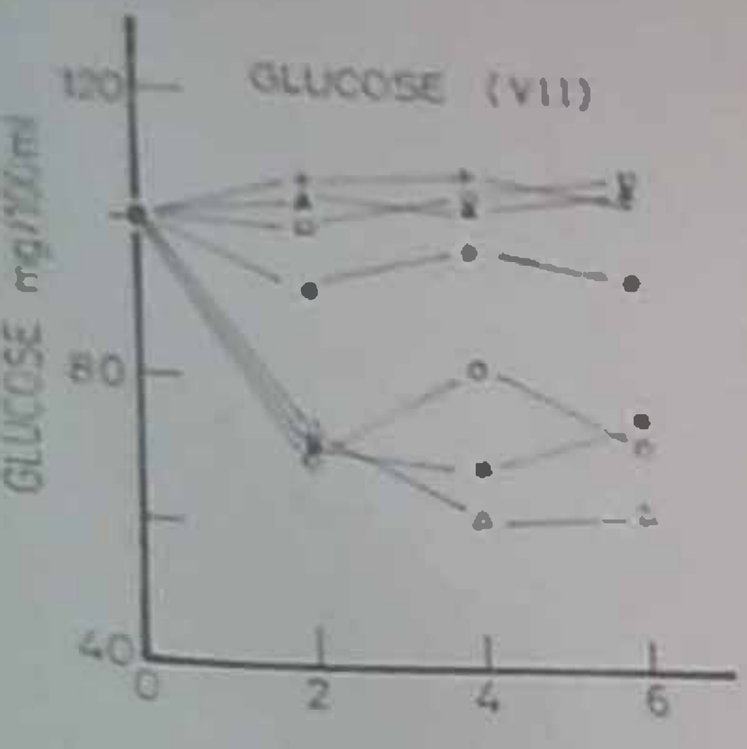
KEY

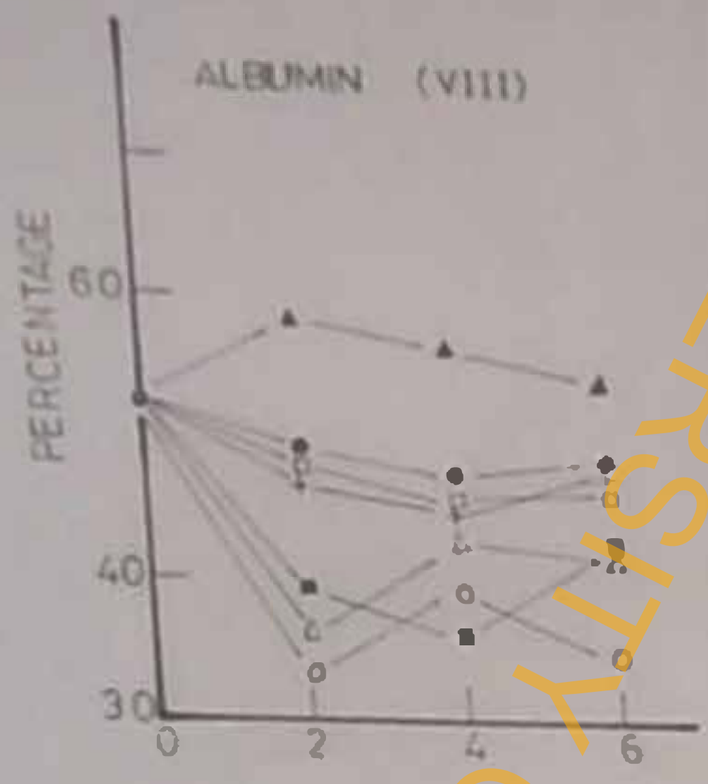
▲ - ▲ NORMAL
○ - ○ INF C
● - ● INF · CM10
△ - △ INF · Mix B1
■ - ■ INF · Mix B2
† - † N · Mix B2
□ - □ N · Mix B1

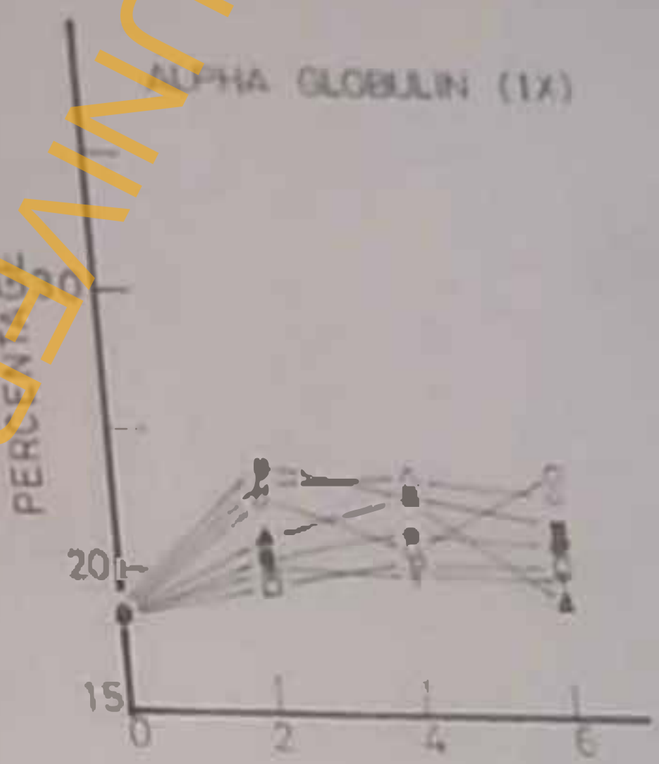
Fig. 108.

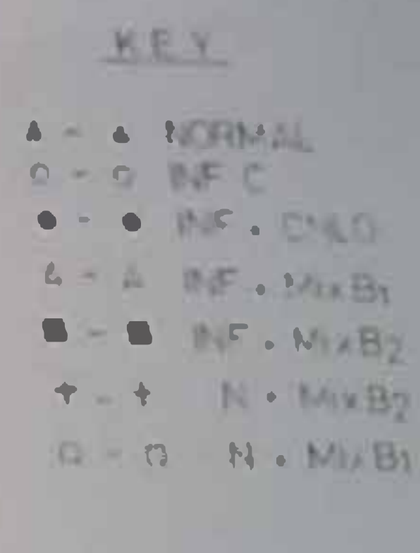
Effect of water extract of a mixture of plants (Mix B) on some biochemical values of serum constituents in normal mice and mice infected with *Plasmodium berghei*.

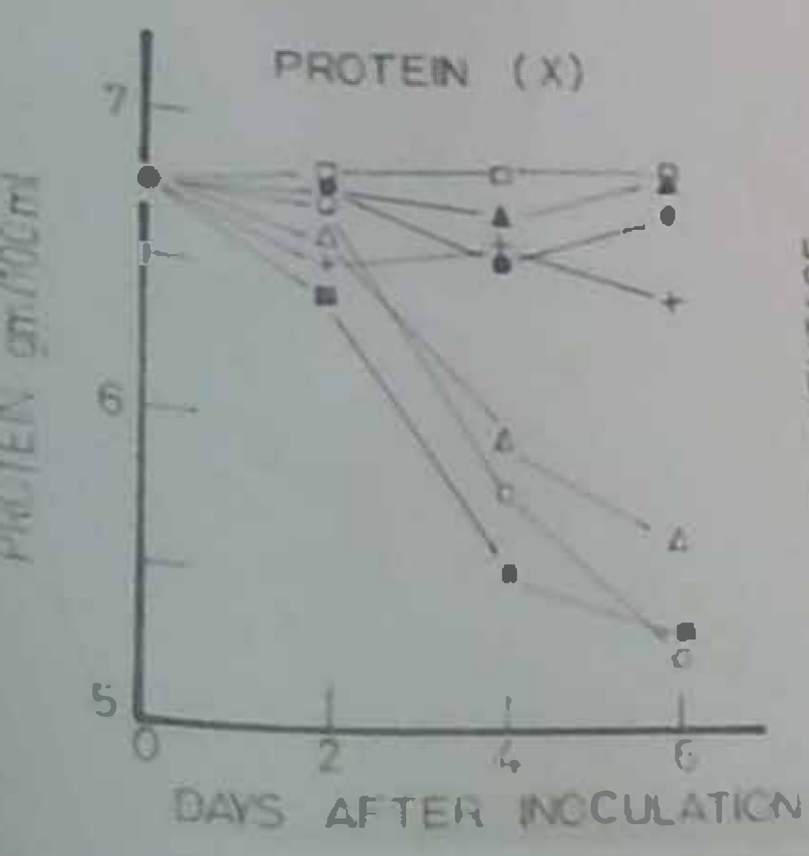
Fig 10B

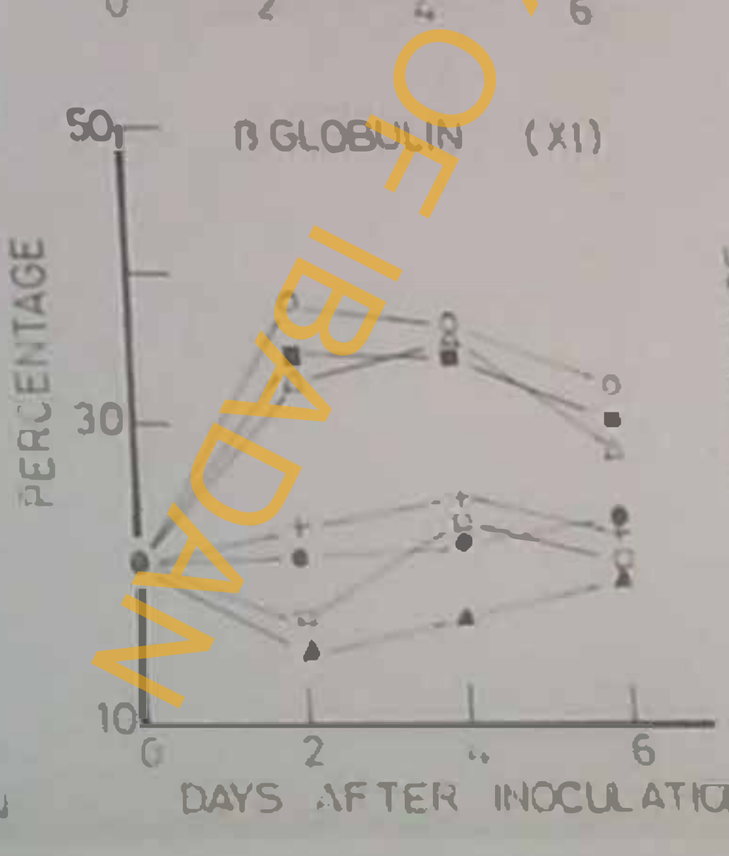


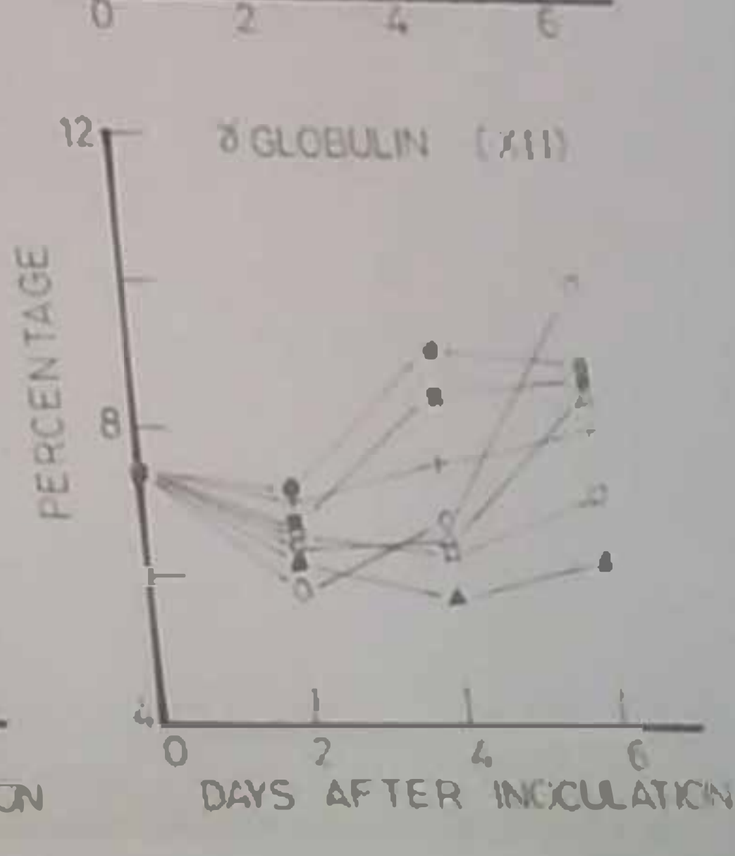












Results.

The results of six experiments using the six plant extracts under investigation are expressed graphically in Figures 5 to 10.

In all experiments, chloroquine, the reference drug significantly reduced the level of parasitemia and abnormalities in the serum biochemical values associated with the infection.

On the progress of parasitemia in mice, none of the 6 extracts suppressed parasitemia in infected mice as the levels of parasitemia were not significantly different from those of the controls (Fig 5A IV, 6AIV, 7AIV, 8AIV, 9AIV, 10AIV).

None of the extracts had beneficial effect on the temperatures of infected mice as there were no differences in the rate of fall of body temperature in those that received the extracts and the controls. They also did not alter the body temperatures of normal mice (Figs 5AI, 6AI, 7AI, 8AI, 9AI and 10AI).

None of the extracts had beneficial effects on the state of the liver in the infected animals as none of them suppressed the abnormalities in the values of some serum

biochemical constituents associated with the infection. Instead some of the extracts appeared to aggrevate the effect of the infection on the state of the liver. Raised values were observed in the serum levels of glumatic pyruvate transaminase and lucoine amino peptidase in infected mice given water extract of Azadirachta indica, Morinda lucida and Mixtures A and B when these values were compared with those of the infected controls in each experiment. For example, by the 6th day after infection and treatment with water extracts of Morinda lucida (Fig 6/II) there were elevations in the values of glumatic pyruvate transaminase in the infected mice given the extract from 87.1 units in the control to 109.7 and 121.4 units in the infected mice given the water extract and its concentrate (100times). Water extracts of Azadirachta indica and Mixtures A and B had similar effects on this enzyme.

Raised value of the activities of these enzymes (GPT and LAP) are known to indicate hepatic dysfunction. Elevation in infected animals given the extracts can therefore be interpreted to mean that the state of the liver has become worse. This is hardly surprising in view of the fact

that the liver is the sight of metabolism of foreign substance in the body. By giving infected mice these extracts which in the first place do not prevent damage of the liver by malarial parasites, the already weak liver is now given the addition responsibility of metabolising the foreign substances contained in the extracts. This probably overworks and weakens the liver furthor and makes it more susceptible to the infection.

Alternatively the components of the extracts may be toxic to the liver in which case raised enzyme activity should be observed in the normal mice given the extracts. Results showed that the six extracts produced only small elevations in the serum activities of these enzymes, at the concentrations in which they were used. The extracts can therefore not be considered to be actively toxic to the liver of normal mice.

The serum alkaline phosphatase activity was not significantly elevated by any of the extracts. This points to the fact that there is no obstruction in biliary excretion.

Total serum bilirubin values were not significantly raised when infected and normal mice were given these

extracts showing that there was no jaundice accompanying the observed liver dysfunction.

Glucose levels in malarial infections are usually low compared with normal values. In these experiments Morinda lucida, Azadirachta indica and Mixture B have depressed the serum glucose even further in infected mice given the extract. This observation confirms the earlier observation that these extracts have adverse effects on the liver which is the sight of glucose regulation.

Total serum protein values did not vary significantly in infected mice from values obtained in the controls. The protein fractions however presented a more complicated picture. Mice infected and given the extracts showed no improvement in their protein pattern for in all cases lower levels of albumin were observed whereas in mice treated with chloroquine higher albumin levels were observed.

It was also observed that in all cases slightly decreased albumin values were obtained in normal mice given the extracts.

Histological studies on liver did not reveal degenerations in normal mice. Infected mice showed changes which

were consistent with the infection. This include congestion of the liver sinusoids by infected cells and necrosis, Plate I.

Conclusion.

The 6 plant extracts under study have no beneficial effect on infected mice as they neither suppressed the infection nor improved the liver dysfunction associated with the infection.

The extracts are not very toxic to the normal animals but could be considered toxic to infected animals.

INVESTIGATION 4

Clinical and biochemical effects of some plant extracts on *Plasmodium gallinaceum* infection in chicks.

Procedure

Six-days old chicks infected with *Plasmodium gallinaceum* were divided into four groups of 15 chicks. One group was left as untreated control, another group was given chloroquine 0.1mg/Kg body weight daily and the other two groups were given 1ml of two concentrations of each plant extract twice daily starting 4 hours after inoculation. Three groups of 15 chicks were inoculated with normal chick erythrocytes. One group was left as normal control while the other two groups were given 1 ml of the two concentrations of the plant extract under study. At three days intervals, 5 chicks from each group were selected. After their body temperatures and percentage parasitemia had been recorded all the chicks in one group were killed and their pooled sera analysed for some biochemical values.

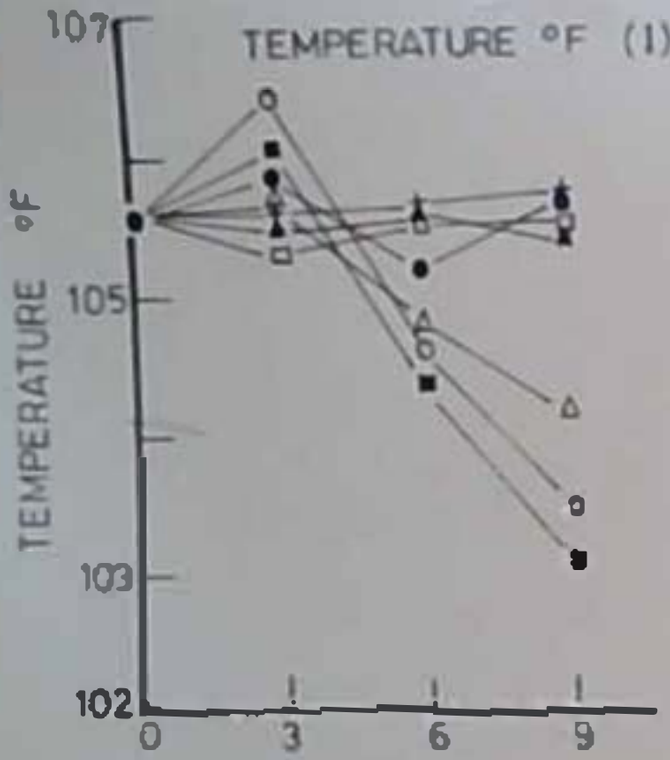
Livers and spleens from these chicks were preserved and sections were stained for observations.

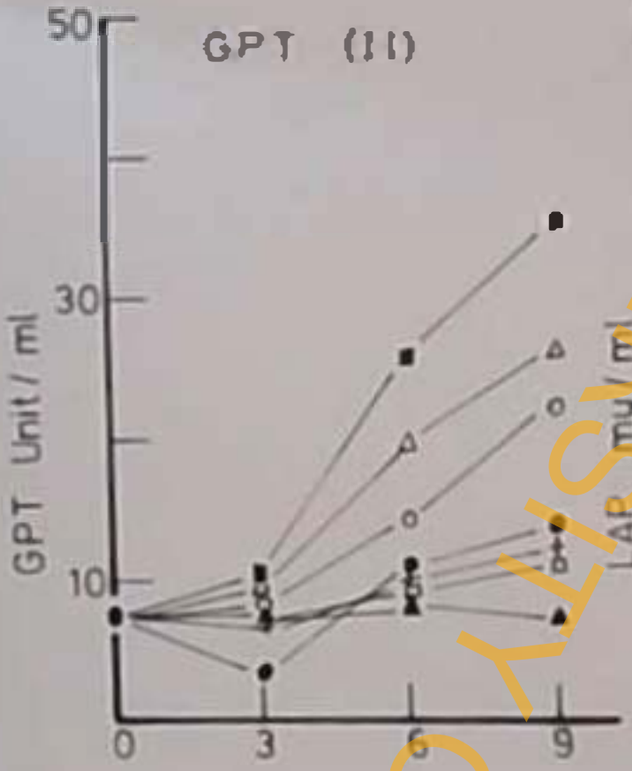
The same plant extracts used in the previous experiment with *Plasmodium berghei* were used.

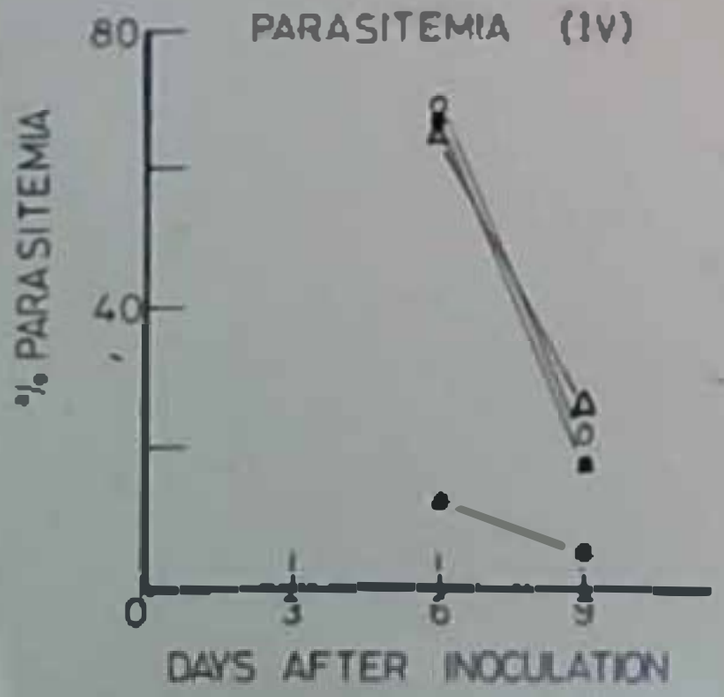
Fig. 11a.

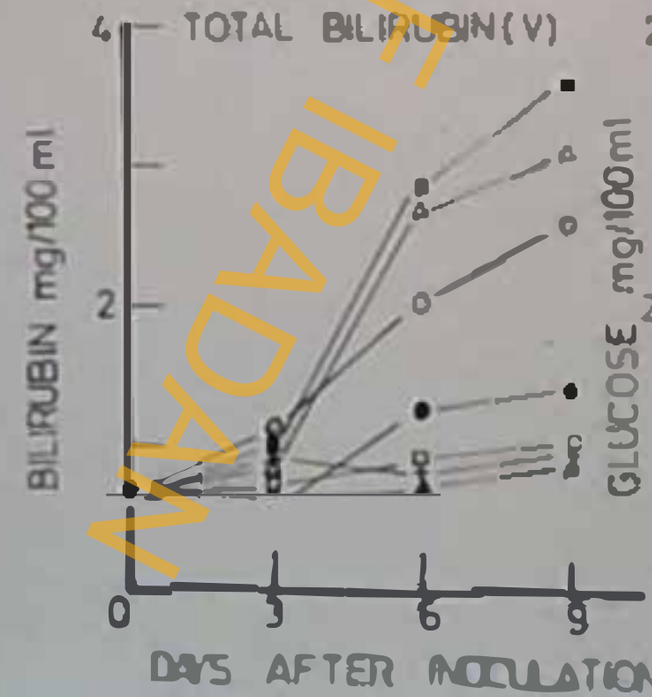
Effect of water extract of leaves of Azadirachta indica on parasitemia, temperature and some biochemical values of serum constituent in normal chicks and chicks infected with Plasmodium gallinaceum.

Fig. 11A









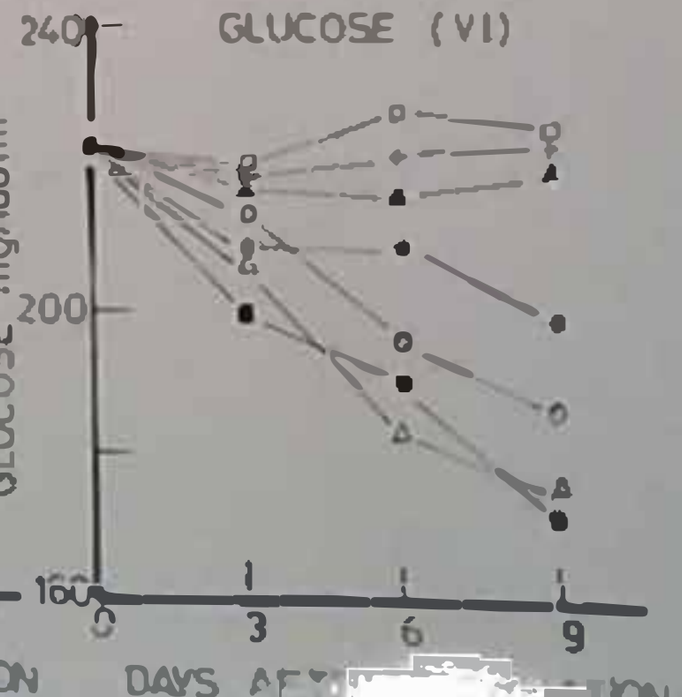
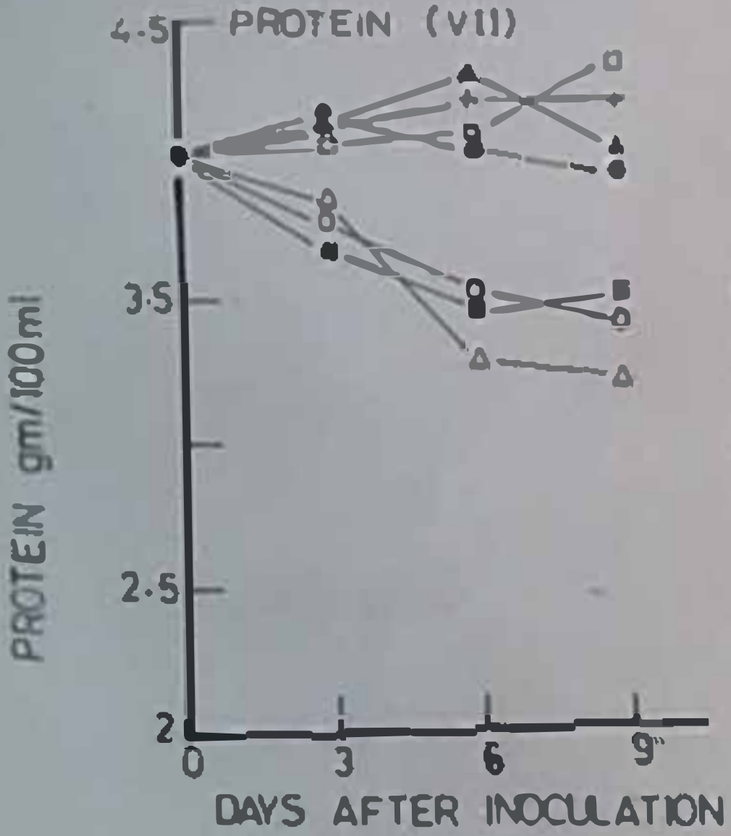
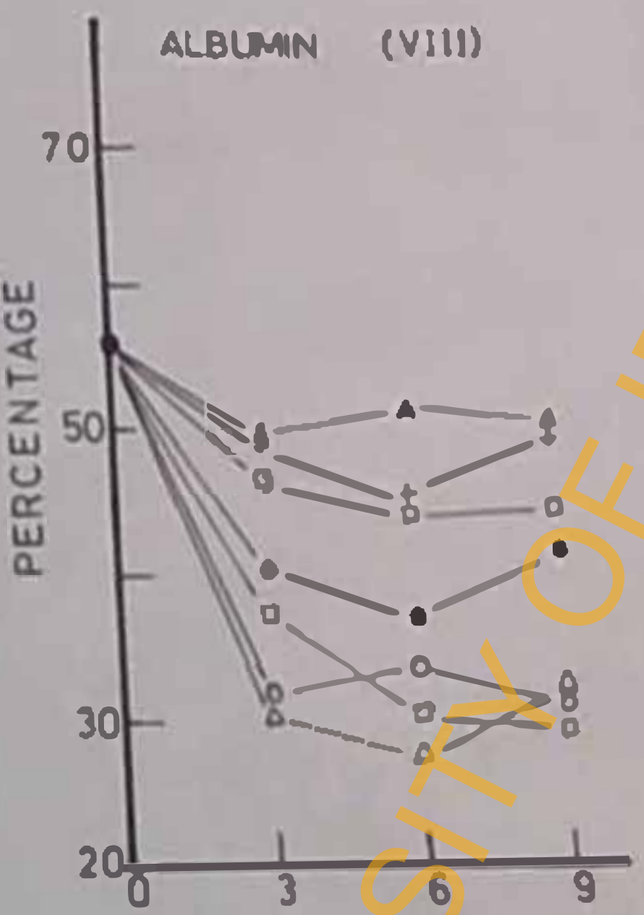


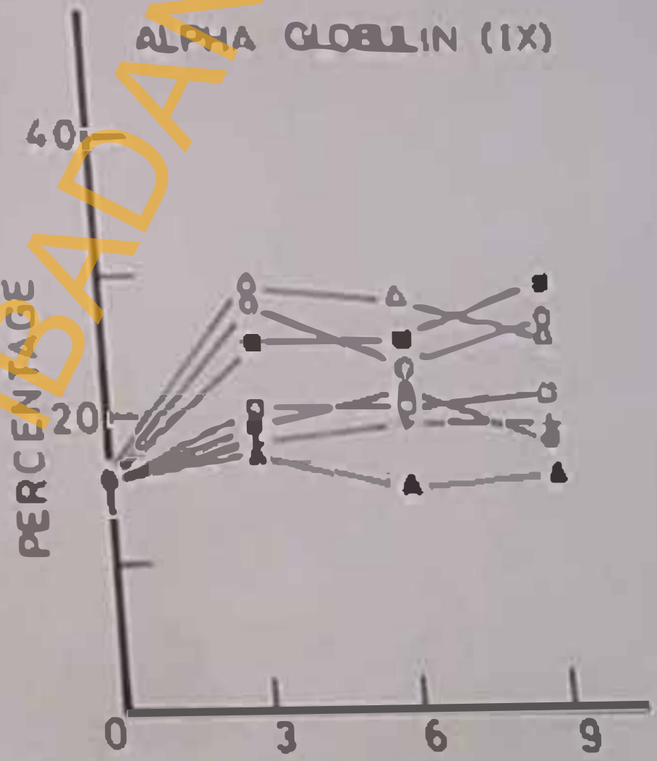
Fig. 11B.

Effect of water extract of leaves of Azadirachta indica on some biochemical values of serum constituent in normal chicks and chicks infected with Plasmodium gallinaceum.

Fig 118

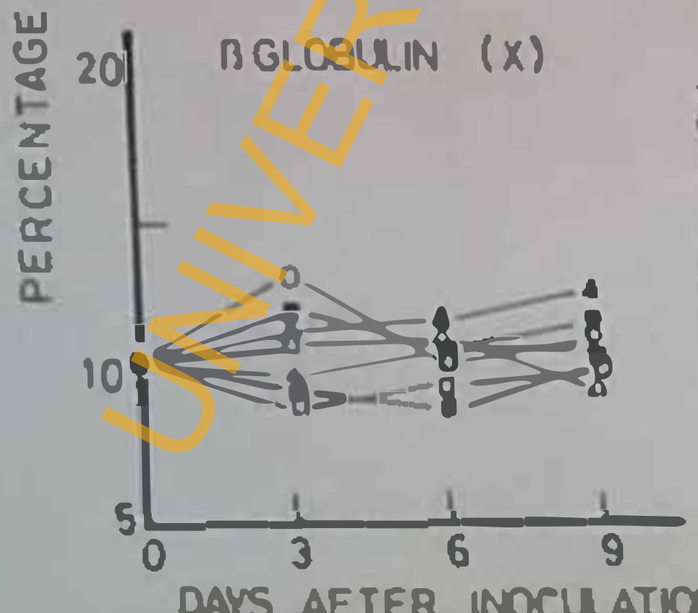






KEY

▲ - ▲ NORMAL
○ - ○ INF C
● - ● INF + CHLO
▲ - ▲ INF + Az_1
■ - ■ INF + Az_2
✝ - ✝ N + Az_2
□ - □ N + Az_1



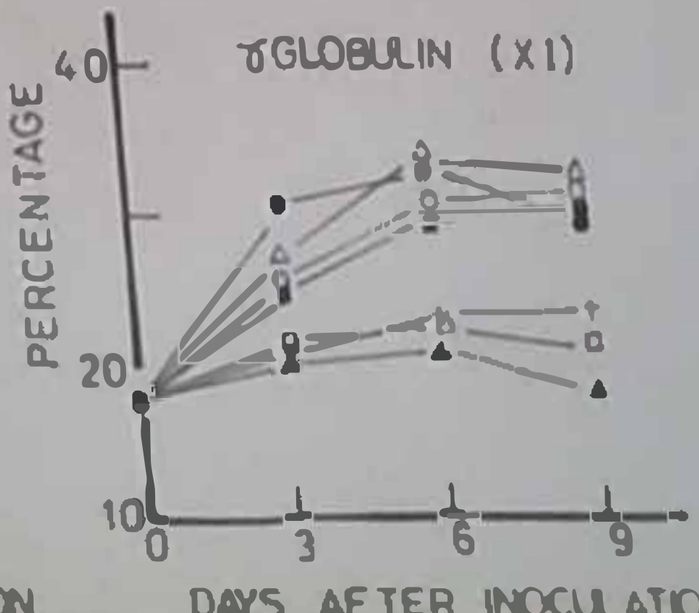
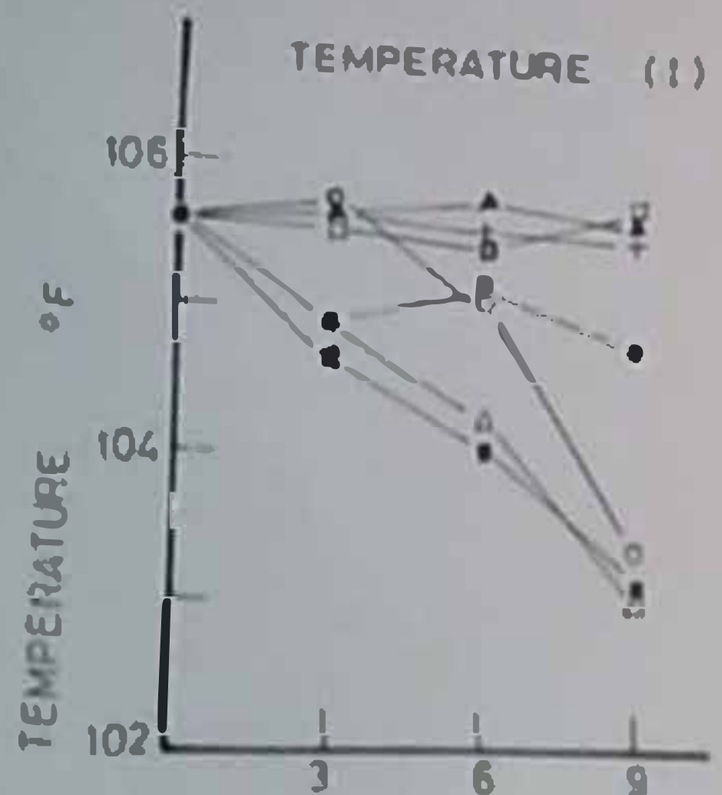
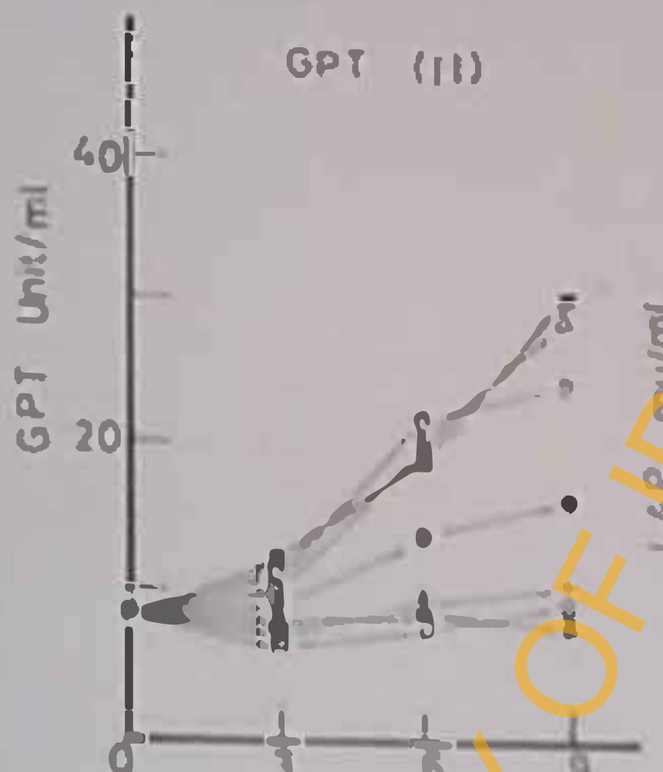


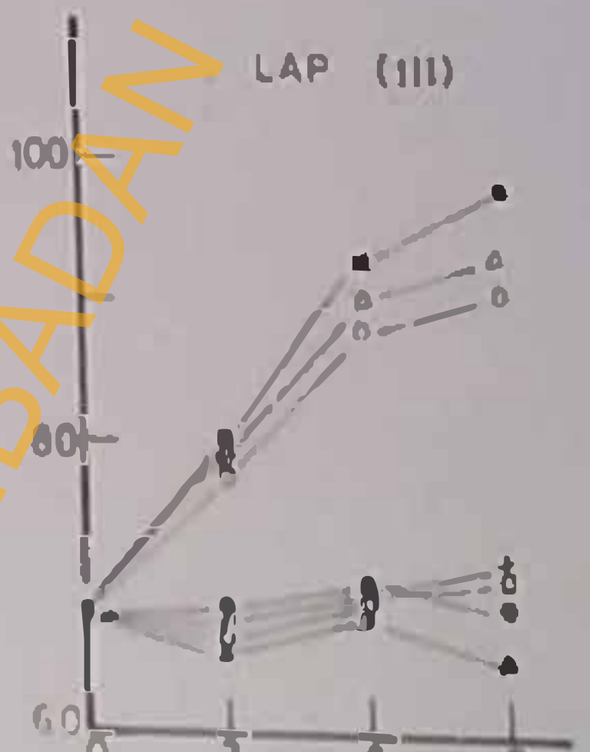
Fig. 12A

Effect of water extract of leaves of Morinda lucida on parasitemia, temperature and some biochemical values of serum constituent in normal chicks and chicks infected with Plasmodium gallinaceum.

Fig 12A

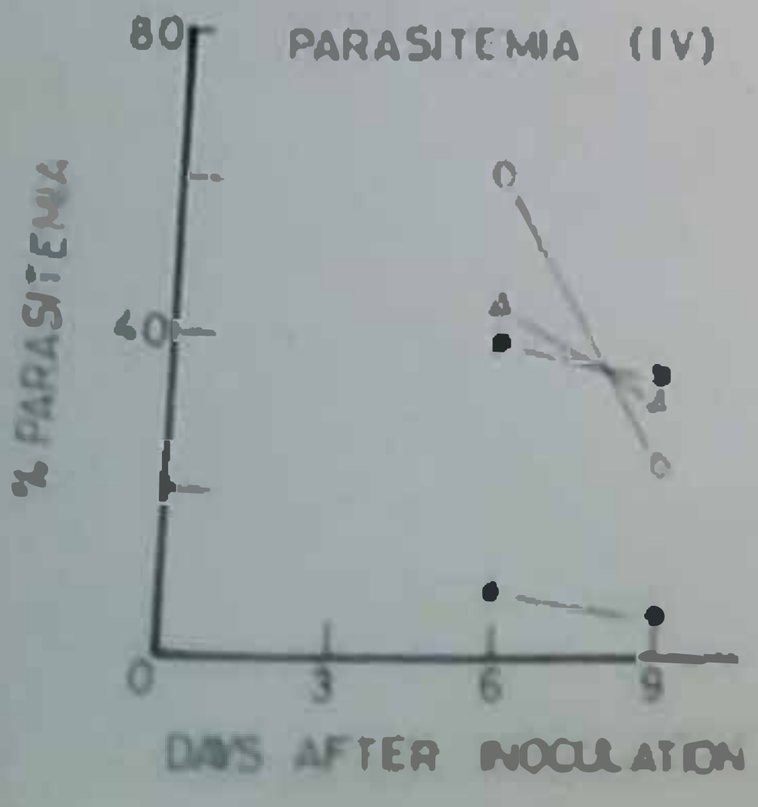


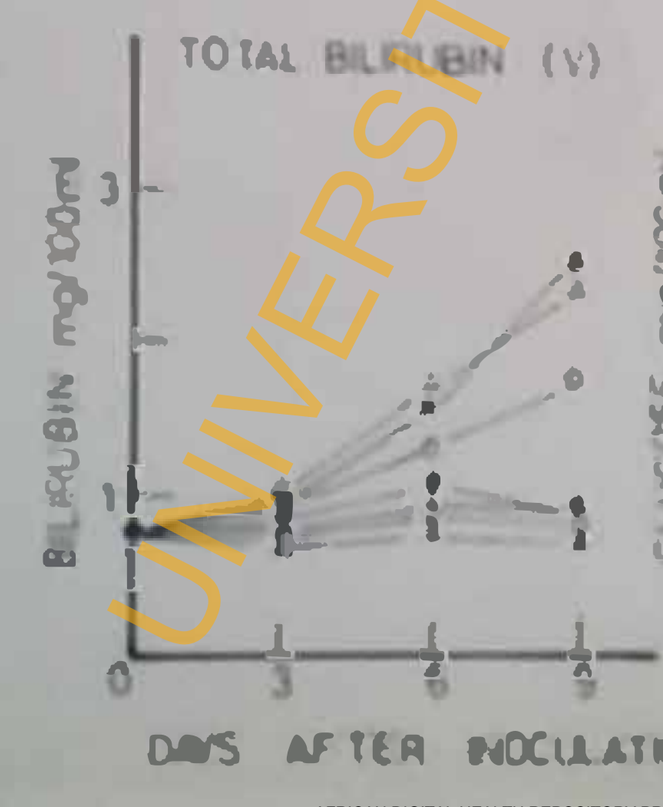




KEY

- ▲ - ▲ NORMAL
- o - o INF C
- ● - ● INF • CHLO
- ▲ - ▲ INF • M1
- ■ - ■ INF • M2
- ◆ - ◆ N • M2
- □ - □ N • M1





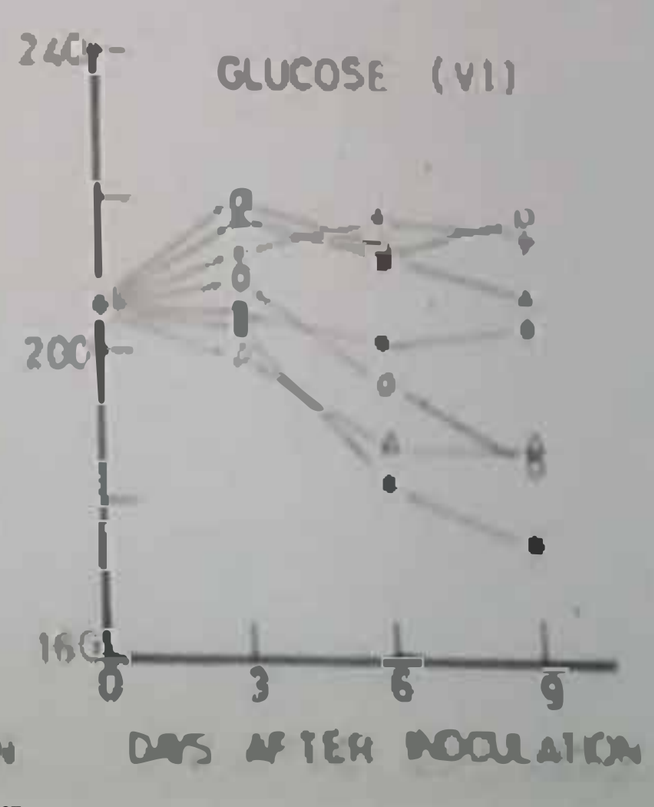


Fig. 12B.

Effect of water extract of leaves of Morinda lucida on some biochemical values of serum constituent in normal chicks and chicks infected with Plasmodium gallinaceum.

Fig 12B

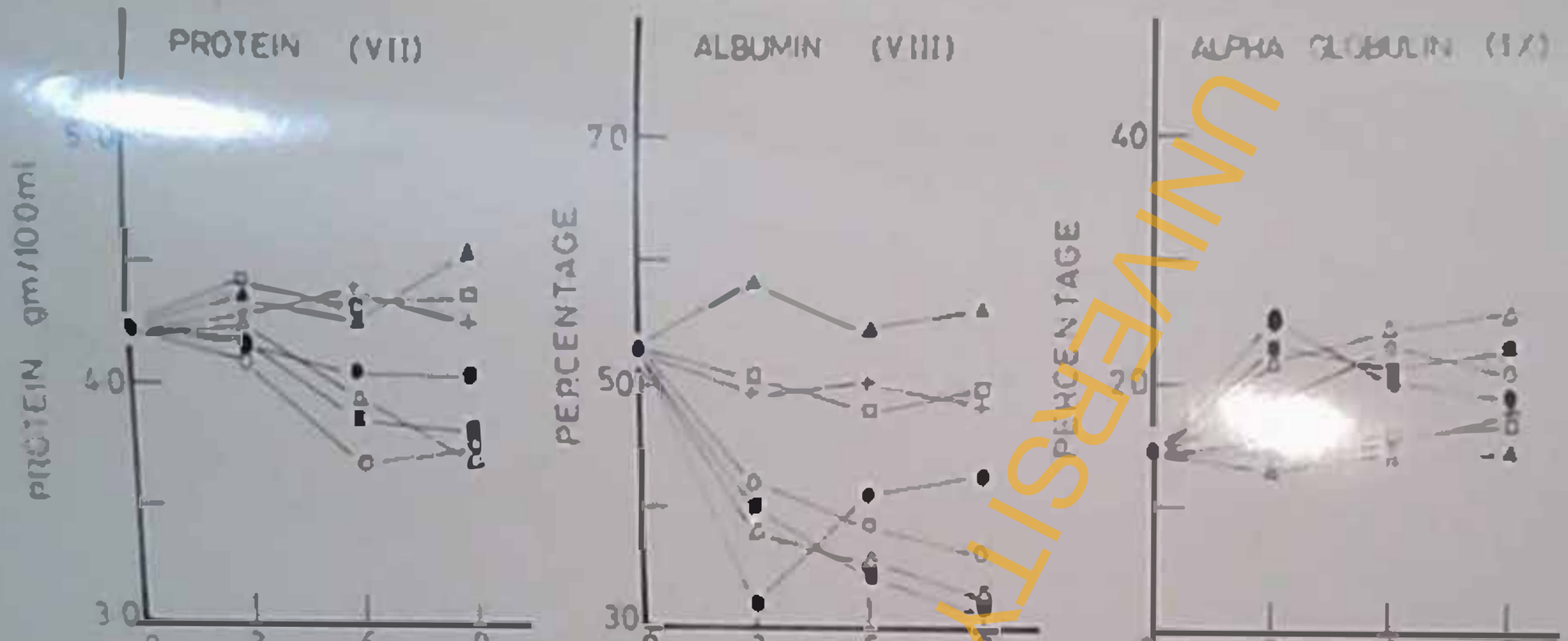
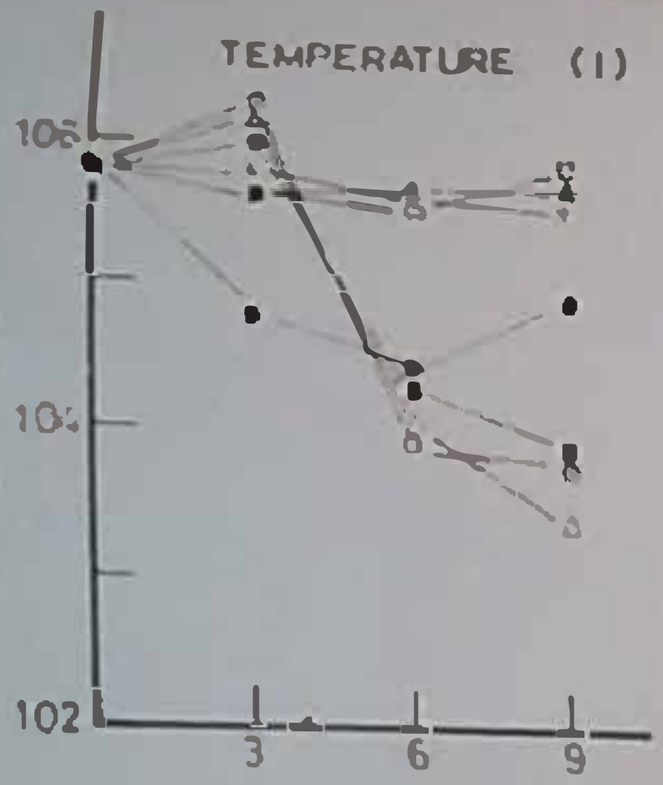
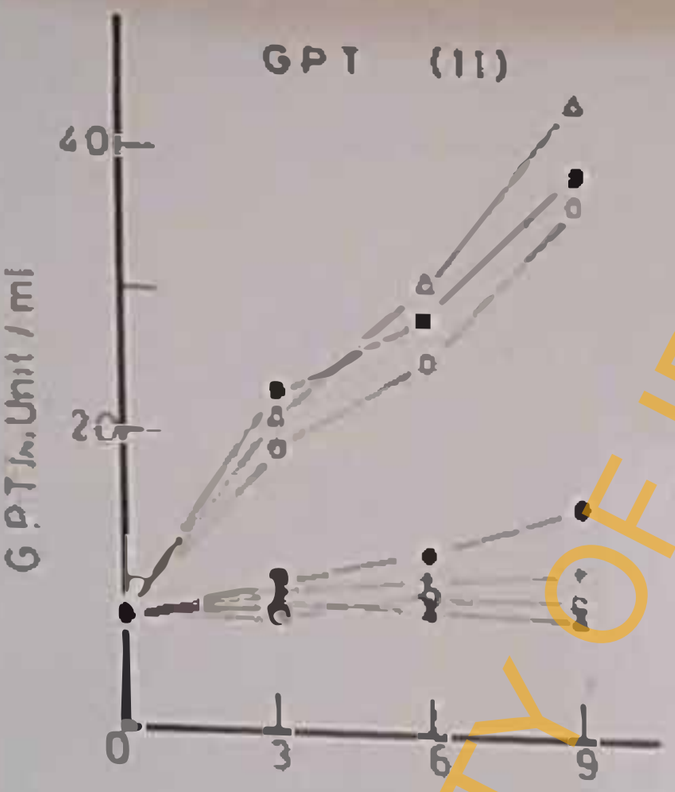


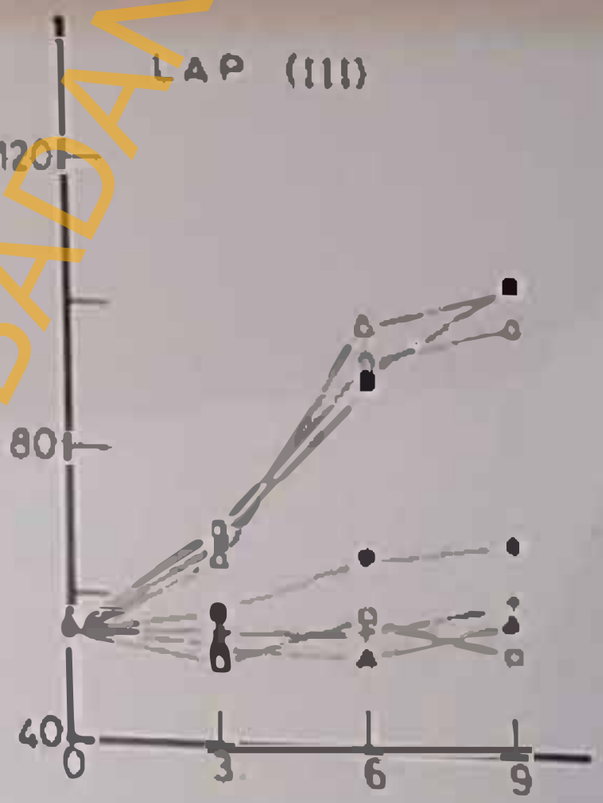
Fig. 13A.

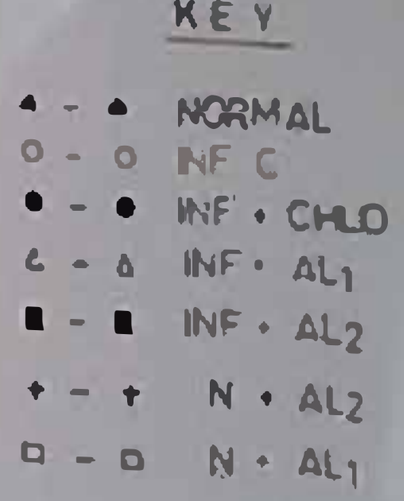
Effect of water extract of leaves of Alstonia boonei on parasitemia, temperature and some biochemical values of serum constituent in normal chicks and chicks infected with Plasmodium gallinaceum.

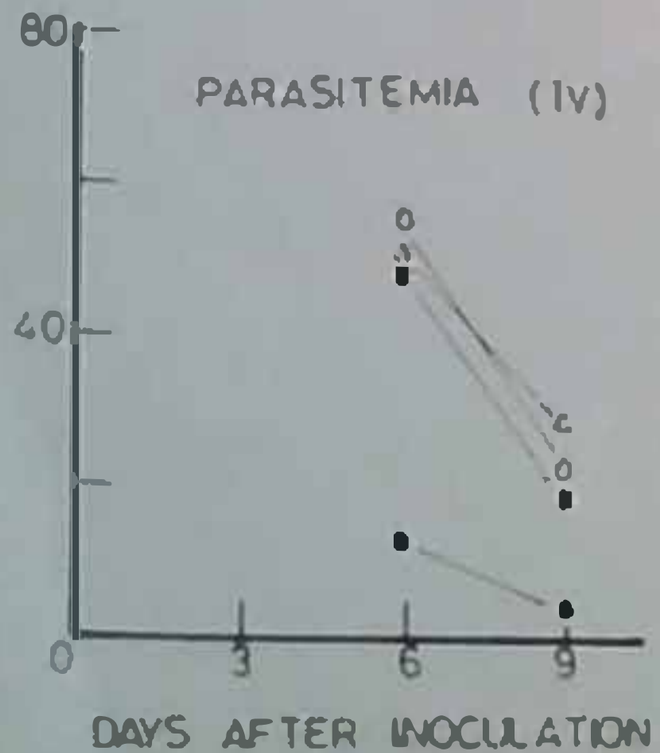
FIG 13A

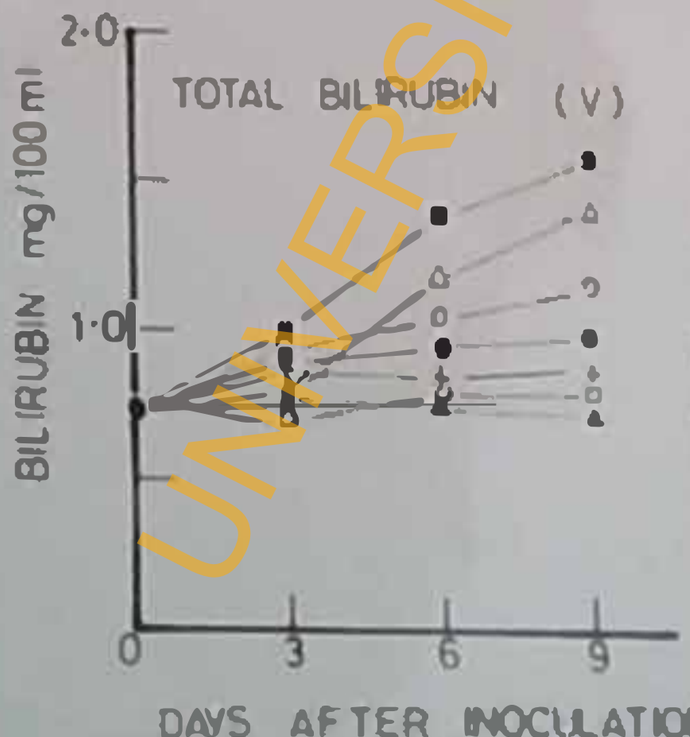












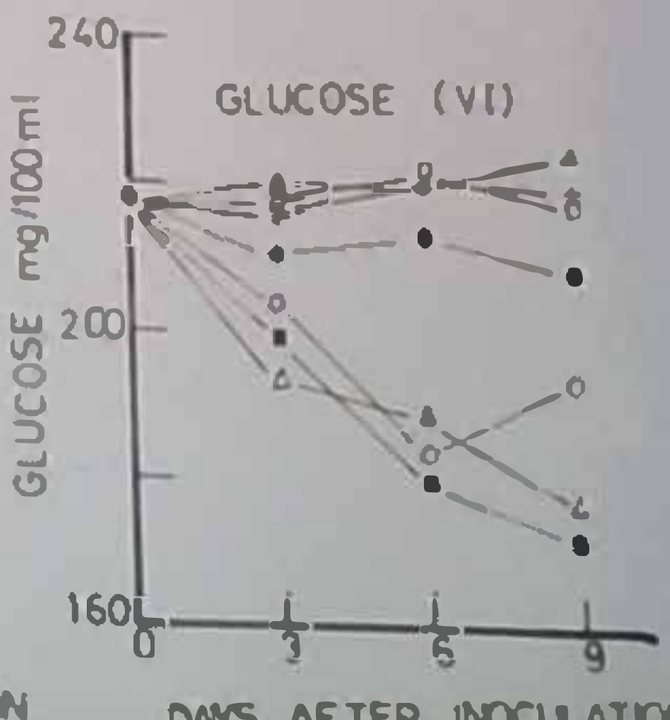


Fig. 13B.

Effect of water extract of leaves of Alstonia boonei on some biochemical values of serum constituents in normal chicks and chicks infected with Plasmodium gallinaceum.

Fig 138

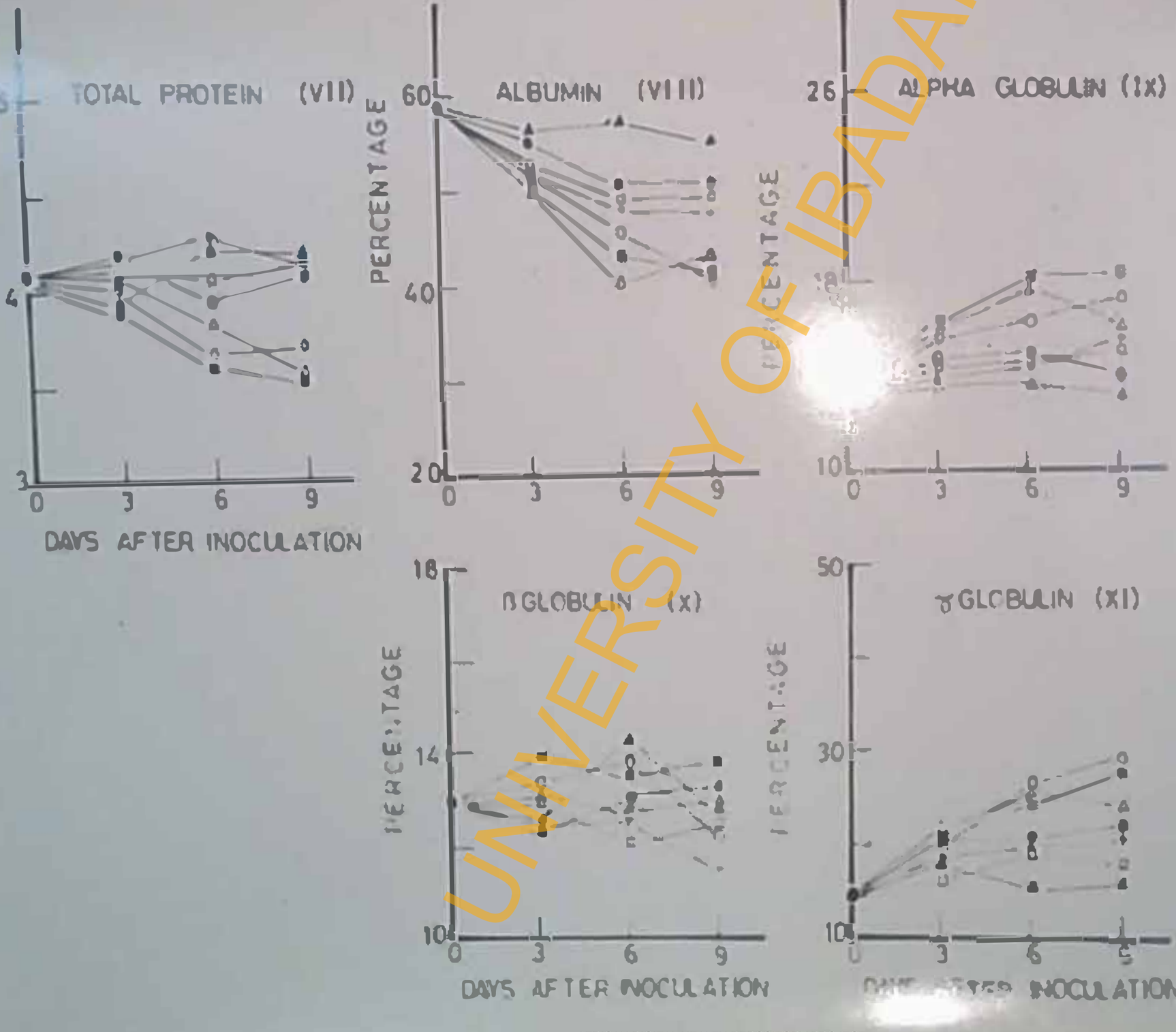


Fig. 14f.

Effect of alcoholic extract of the bark of Enantia chlorantha on parasitemia, temperature and some biochemical values of serum constituent in normal chicks and chicks infected with Plasmodium gallinaceum.

Fig 14A

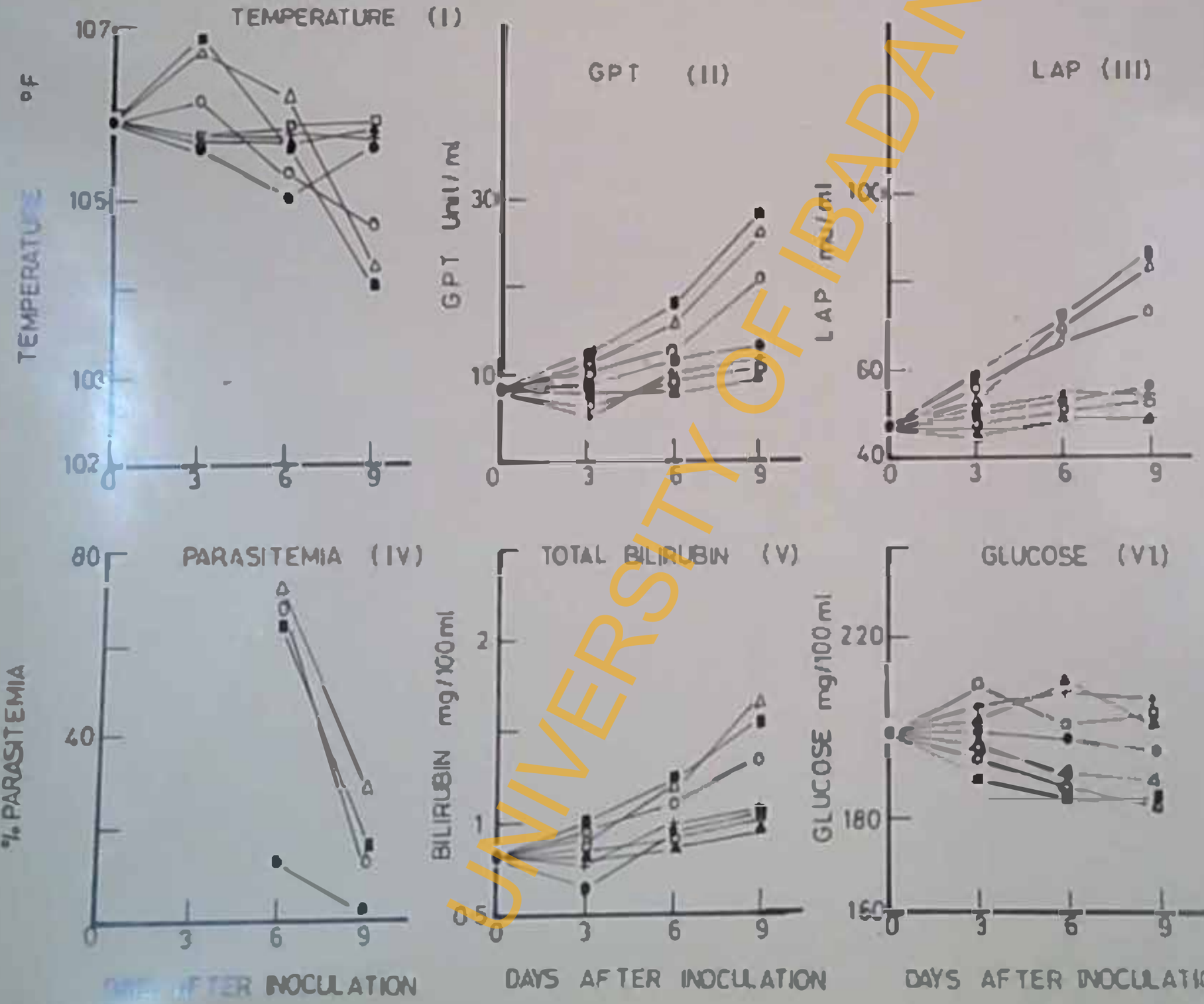
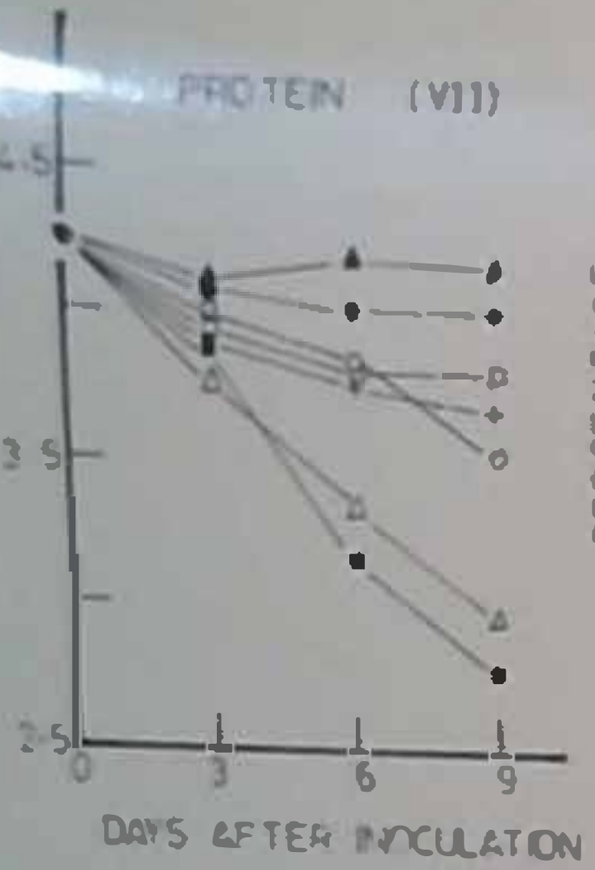
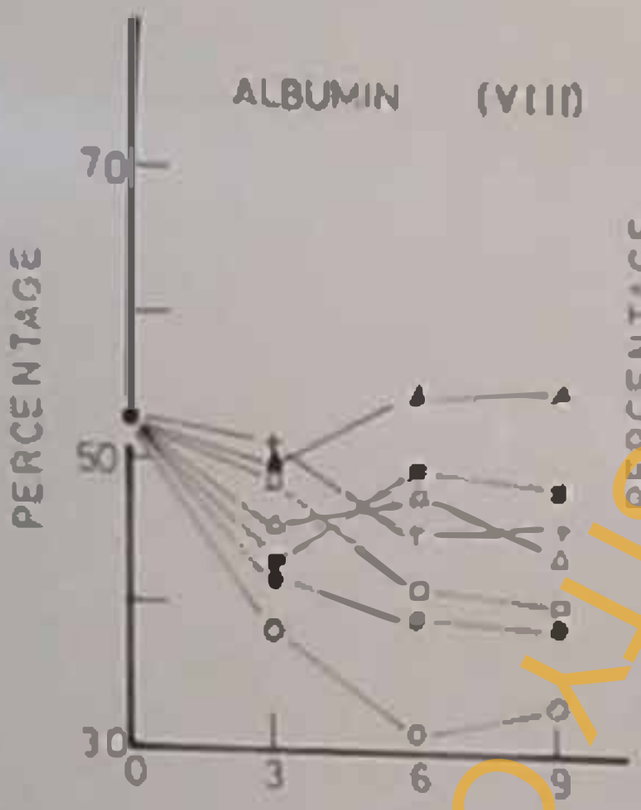


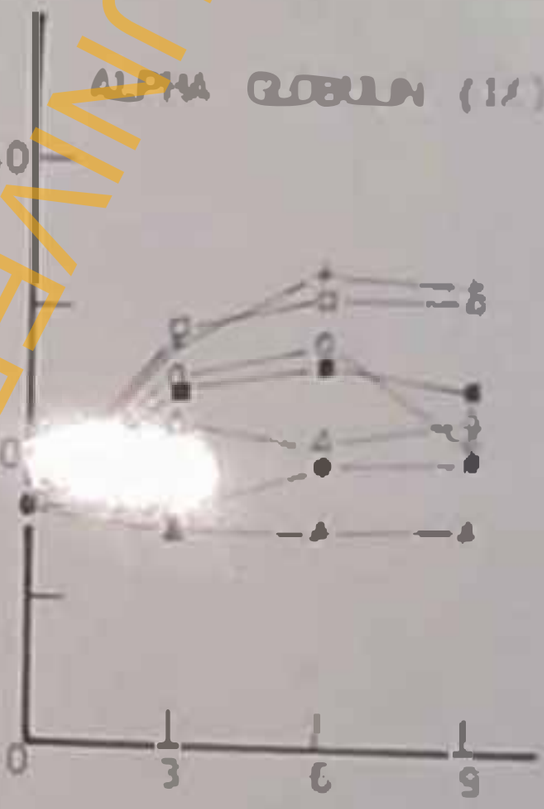
Fig. 148.

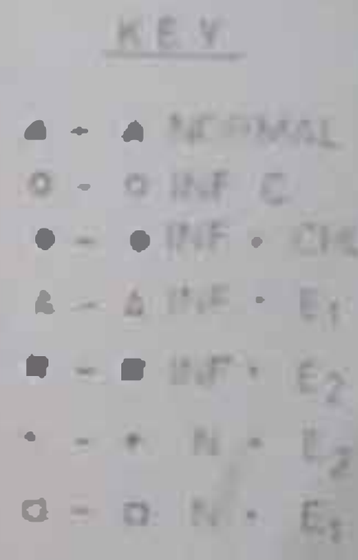
Effect of alcoholic extract of the bark of Enantia chlorantha on some biochemical values of serum constituent in normal chicks and chicks infected with Plasmodium gallinaceum.

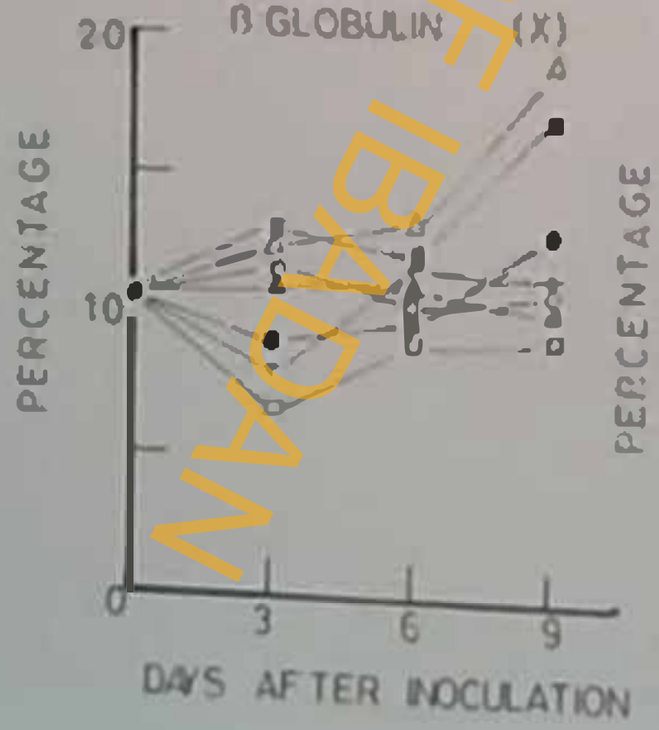
Fig 14B

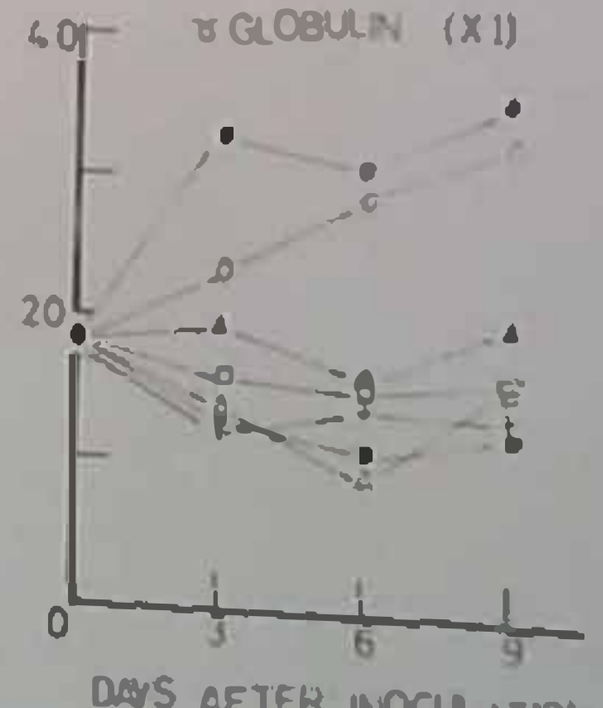












Results

Figures 11 to 14 show the results of experiments with some plant extracts.

Spermacoce chlorantha appeared to cause an increase in the body temperature of chicks early in the infection (Fig. 14A1) while Morinda lucida would appear to have lowered the temperature early in the infection (Fig. 12A1) when these observations are compared with the controls.

Only Morinda lucida produced a suppressive action on the level of parasitemia (Fig. 12AIV). Compared with chloroquine this effect is not very significant.

The four extracts tried had no beneficial effect on the serum biochemical values studied as none of them suppressed increases in serum Glutamic Pyruvate Transaminase, Leucine Amino Peptidase and total bilirubin or prevented the depression of serum glucose in the infected chicks which received the extracts. Instead they would appear to have aggravated the infection as manifested by elevated serum Leucine Amino Peptidase, Glutamic Pyruvate Transaminase and bilirubin by most of the extracts.

Abnormally high values were not observed in normal chicks which were given these extracts.

Total serum protein was not adversely affected by most of the extracts. But Enantia chlorantha caused depression of total protein value in both normal and infected chicks when compared with the controls (Fig. LAB VII).

Protein fractions show a number of variations, the most consistent being a decrease in the albumin fraction in both infected and normal chicks given these extracts. Enantia chlorantha differed considerably from the others in that in addition to depressed albumin it also caused large elevations in the alpha globulin fractions and decreases in the γ-globulin fractions in normal chicks (Fig. LAB XI). In the infected chicks this effect was masked by the effect of the infection but significantly lower γ-globulin fractions were observed compared with the controls.

Conclusion.

Extract of Morinda lucida suppressed Plasmodium gallinaceum infection in chicks slightly but it does not improve the state of health of the chicks.

Most of the extracts aggravated the infection in chicks by causing increased abnormalities in serum biochemical values. Extracts of Enantia chlorantha affected serum protein pattern more adversely than the other plant extracts.

Plate I

Liver of a mouse infected with Plasmodium berghei showing A, infected cells in the sinusoid. B malaria pigments.

INVESTICATION 5

Studies of the effect of some plant extracts on Plasmodium berghei and Plasmodium gallinaceum in vitro.

The test plant extracts in these experiments were the extracts of the two plants which were considered suitable for further investigation. Azadirachta indica was chosen on the basis of its popularity amongst the local people and Morinda lucida because of the apparent suppressive action on chick malaria. The extracts used were the water extracts prepared by the method of Ferg et. al. (1962) in chapter three in which 1kg of fresh plant material was macerated and boiled in several changes of water. The water extract was combined and concentrated to a smaller volume under reduced pressure. From this concentrate were obtained the petroleum ether extract, Chloroform/methanol extract and Chloroform extract using methods already described in details in Chapter three. The yields of the three fractions in both plants are given below.

	Azadirachta indica	Morinda lucida
Pet ether extract.	250mg/Kg.	400mg/Kg.
Chloroform/Methanol extract	500mg/Kg.	200mg/Kg.
Chloroform "	120mg/Kg.	50mg/Kg.

Experiment 5a.

Effect of some plant extracts on infectivity of Plasmodium berghei in vitro.

Procedure.

Mouse erythrocytes infected with Plasmodium berghei were incubated for 6 hours with the various extracts under investigation using a modification of the method of Rieckmann et al. (1968). The incubation period was reduced from 24 hours to 6 hours because of the considerable loss of viability of Parasites observed in the control cultures after 24 hours. Other methods of cultivations tried were not reproducible.

In this method citrated blood collected by heart puncture from infected mice having about 30% of their erythrocytes Parasitized was adjusted with physiological saline to contain about 5.0×10^6 red blood cell per cumm. The blood was swirled in a flask containing glass beads for about 5 minutes to defibrinate it.

1 ml aliquots of the blood was pipetted into sterile flat bottomed screw cap bottles containing 5mg glucose which was added to the tubes in 0.1ml solution. To the control was added 0.5ml of 0.1NHCl adjusted to pH 6.0-8.0. Each of the extract was tested in two bottles. They were dissolved in 0.1NHCl and adjusted to pH 6.0-8.0 with NaOH. The bottles and their contents were shaken gently to mix the contents and they were then incubated at 38°C for 6 hours in a water bath. At 0 hour and after 6 hours of incubation, the viability of the parasites was tested by injecting 0.1ml of the content of the control tubes into 5 clean mice. Also the viability of the parasites exposed to the drugs was tested in clean mice after 6 hours incubating. The mice were examined daily for four days for percentage parasitemia.

Results.

The results of experiments with extracts of both plants on Plasmodium berghei are shown in tables 13 and 14. Table 13 shows that chloroquine had some suppressive action on the parasite in-vitro when the level of parasitemia in the mice in this group is compared with the control 6 hours after incubation. None of the extracts used had any suppressive

action. Also Table 14 shows that the extracts of Morinda lucida had no effect on the infectivity of parasites exposed to them.

Conclusion.

Extracts of Azadirachta indica and Morinda lucida have no effect on infectivity of Plasmodium berghei in-vitro.

TABLE 13

Effect of extracts of *Azadirachta indica* on infectivity of *Plasmodium berghei* in-vitro.

Extract	Concentration of drug	Percentage Parasitaemia: Days after inoculation 1	2	3	4
Control Cur	-	4.8±1.6	10.5±2.6	36.2±5.0	58.1±7.2
" 6 hrs.	-	2.8±1.2	10.8±3.1	26.1±4.8	47.7±8.8
Chloroquine	0.25mg	0.6±0.7	3.8±1.6	10.2±2.7	27.7±3.4
Ester A	½ml of 1% litre	2.9±1.4	12.4±2.7	25.5±6.3	45.8± 7.2
"	½ml of A x 100	2.1±1.3	8.6±3.4	23.7±5.0	43.3±5.4
Pet ether	2.50mg	2.3±1.5	10.7±2.5	26.0±4.2	39.9±6.1
"	250mg	1.9±1.5	9.5±30	25.5±3.7	42.9±4.4
$CHCl_3$/MeOH	5.00mg	2.6±0.9	11.4±5.1	30.2±4.2	48.6±5.3
"	500mg	3.0±1.6	13.6±4.4	21.3±5.2	45.0±6.3
$CHCl_3$	1.00mg	1.7±1.4	10.8±3.5	23.5±1.7	41.2±4.8
"	100mg	2.9±1.2	12.6±2.8	29.4±3.5	48.9±5.7

TABLE 14

Effect of extracts of Morinda lucida on infectivity of Plasmodium berghei in vitro.

Extract.	Concentration of drug	Percentage Parasitaemia: Days after infection 1	2	3	4
Control Otu	-	5.7±1.5	20.9±3.1	42.2±4.8	57.5±6.1
" 6 hrs.	-	2.6±1.0	11.7±2.8	23.8±4.5	43.5±4.6
Chloroquine	0.25mg	1.2±0.9	2.6±1.0	8.9±2.3	19.5±2.5
Water M	½ml of 1kg/litre	2.2±1.4	8.9±3.2	22.6±4.4	43.2±6.1
"	½ of M X100	1.9±1.3	10.8±2.7	20.4±3.8	40.1±5.2
Pet ether	4.00mg	2.5±1.2	10.1±3.3	23.5±4.1	42.2±4.5
"	4.00mg	2.0±1.4	9.9±2.6	22.1±3.5	41.2±4.6
$CHCl_3$:MeOH	2.00mg	3.2±1.0	12.4±4.2	25.4±5.1	48.8±5.3
"	200mg	2.4±0.7	10.8±2.2	23.3±2.7	40.4±3.2
$CHCl_3$	0.50mg	2.1±1.6	9.4±3.5	20.5±3.1	45.6±4.0
"	50mg.	2.8±1.2	12.3±4.3	24.1±2.9	41.1±3.6

Experiment 5b

Effect of some plant extracts on infectivity of *Plasmodium gallinaceum* in vitro

Progedure.

A modification of the method of Taylor et. al. (1951) was used. Parasitized blood was obtained from infected chicks while normal blood was obtained from uninfected chicks by heart puncture. To each sterile 25ml conical flask were introduced 3.9ml citrated normal chick blood; 0.1ml of penicillin in isotonic saline containing 5,000 units of sodium penicillin G; 0.5ml of parasitised blood diluted to contain about 5×10^6 parasitised erythrocytes; and 0.5ml of the test plant extract or chloroquine dissolved in 0.1NHCl and adjusted to pH 6.0–8.0 with NaOH before adding it to the flasks. Each plant extract was tested in two separate flasks. Two flasks to which 0.5ml of 0.1 NHCl (adjusted to pH 6.0 to 8.0 with NaOH) was added were used as controls. The flasks were then incubated in a water bath at 38°C for 6 hours with continuous shaking. The incubation period was reduced to 6 hours because of loss of viability obtained during 24 hours incubation of controls.

The number of viable parasites in each control was estimated at the beginning and at the end of 6 hours incubation by inoculating 4 six-days old chicks with 0.2ml of the content of each flask. The other cultures containing the test extracts were also tested in clean chicks. The inoculated chicks were examined daily until parasites were detected in their blood.

Results

Tables 15 and 16 show the effect of the extracts. From table 15 it was observed that none of the extracts of *Azadirachta indica* used delayed the propatent period but suppressed the level of parasitemia in clean chicks when compared with the control. Chloroquine did not only prolong the pre-patent period by about 24 hours it also reduced the percentage parasitemia. The slightly lower percentage parasitemia observed in the pot other extract of this plant was not found significant when subjected to Student's 't' test.

Extracts of *Morinda lucida* also did not have effect on viability of the parasites.

Conclusion.

Extracts of *Azadirachta indica* and *Morinda lucida* do not alter infectivity of *Plasmodium gallinaceum* in vitro.

TABLE 15

Effect of some extracts of *Azadirachta indica* on infectivity of *Plasmodium falciparum* after 6 hours incubation in vitro.

Extract	Concentration of drug in 5ml	Percentage Parasitemia: Days after inoculation				
		5	6	7	8
Control 0 hr.	-	11.7±0.6	42.8±5.9	69.4±10.1	91.7±11.4	-
Control 6 hrs.	-	-ve	3.6±0.7	34.7±5.4	49.1±6.1	66.2±8.4
Chloroquine	0.2mg	-ve	-ve	2.7±1.0	3.8±1.4	12.4±2.7
Water A	½ml 1kg/litre	-ve	3.6±1.3	11.7±3.1	23.3±6.5	44.5±7.6
Water	½ml 1kg x 100	-ve	2.9±1.8	18.1±5.9	30.4±6.2	53.5±9.1
Pet ether	2.50mg	-ve	3.7±1.5	12.4±3.6	26.5±7.7	49.3±8.6
"	250mg	-ve	2.3±0.1	15.5±4.7	29.1±6.1	45.5±7.0
CHCl3/MeOH	5.00mg	-ve	3.5±1.9	13.7±5.4	22.8±6.6	52.6±8.1
"	500mg	-ve	4.1±2.0	12.5±4.8	31.7±8.5	44.8±6.1
CHCl3	1.00mg	-ve	3.0±1.6	17.0±3.9	20.6±4.1	38.0±8.6
"	100mg	-ve	4.5±1.9	18.3±3.2	28.5±5.2	43.1±6.9

TABLE 16

Effect of some extracts of Morinda lucida on infectivity of Plasmodium gallinaceum after 6 hours incubation in vitro.

Extract	Concentration of drug in 5ml	Percentage Parasitemia: Days after inoculation +5	+6	+7	+8	+9
Control 0 hr.	-	18.6±2.9	36.1±4.6	63.[illegible]	76.0±14.4	
Control 6 hrs.	-	-ve	3.6±1.1	27.3±5.7	[illegible]6.1	41.3±8.2
Chloro-quine	0.02mg	-ve	-ve	-ve	3.1±1.4	8.2±2.6
Water	0.5ml 1kg/ litre	-ve	2.9±2.4	25.5±4.4	57.2±8.4	65.6±9.5
Water	0.5ml x100	-ve	3.5±1.8	32.6±[illegible]	55.5±3.5	59.7±10.1
Pet ether	4.00mg	-ve	3.0±1.4	21.3±2.7	46.6±4.9	48.5±5.6
Pet ether	400mg	-ve	2.5±1.6	24.7±1.4	58.2±7.8	49.6±7.1
$CHCl_3$/ $MeOH^3$	2.00mg	-ve	5.3±1.8	28.0±4.5	49.2±6.2	37.1±4.2
"	200mg	-ve	3.2±1.6	21.3±8.7	52.5±7.1	57.5±5.2
$CHCl_3$	0.50mg	-ve	4.0±1.9	25.3±3.7	45.0±5.8	48.3±4.4
"	50mg	-ve	3.4±2.0	27.8±4.1	49.5±3.7	51.5±8.3

Experiment 20

Effect of some plant extracts of lipid synthesis by Plasmodium gallinaceum and Plasmodium berghei in vitro.

Procedure.

Infected and normal blood samples were collected from both infected and normal mice and chicks respectively as described in chapter three. The blood samples were washed and their red blood cell counts and percentage parasitemia were determined. Into each sterile test tube were introduced 3ml Kreb's phosphate buffer containing 0.04m glucose; 2.5ml of infected or normal blood; and 0.5ml of chloroquine or plant extract under observation. The drugs were dissolved in 0.1 NHCl and adjusted to pH 6 - pH8 with NaOH. To the controls, 0.5ml of 0.1 NHCl which was also adjusted to pH6-pH8 was added. The tubes were then incubated at 37°C for 4 hours with continuous shaking. At the beginning of the incubation and 4 hours later, 2ml aliquots of each sample was withdrawn and analysed for the free fatty acid content using the method of Dole described earlier. Another 0.5ml aliquots of each sample was withdrawn at the beginning and end of the 4 hours incubation and analysed for the total phospholipid content using the method of Bassir described earlier.

Each test drug was tested in triplicate.

TABLE 17

Effect of some extracts of Azadirachta indica on the synthesis of lipid 'in vitro' by Plasmodium berghei.

Extract	Concentration of drug in 6ml	Total Phospholipid $\mu g/10^{10}$ RBC	FFA $\mu eq/10^{10}$ Red Blood Cell
Normal control	1 -	3.75±0.69	0.085±0.014
Infected control	-	10.48±1.13	0.72±0.11
Chloroquine	0.25mg	3.40±0.27	0.29±0.08
Water	0.5ml of 1kg/litre	9.82±1.22	0.69±0.21
Water	0.5ml of 1kg/litre x 100	12.61±1.44	0.71±0.34
Pet ether	2.50mg	11.35±1.42	0.83±0.25
"	250mg	8.71±1.66	0.83±0.17
$CHCl_3$/MeOH	5.00mg	10.84±2.12	0.70±0.13
"	500mg	14.12±1.81	0.75±0.26
MeOH	0.50mg	10.85±0.74	0.93±0.11
"	50mg	9.77±1.03	0.75±0.19

TABLE 18

Effect of some extracts of Morinda lucida on lipid production in vitro by Plasmodium berghei

Extract	Concentration of drug in 5ml	Total Phospho-lipid mg/10^10 RBC	PP/ mg/10^10 Red Blood Cell
Normal control	-	4.84±1.02	0.03±0.019
Infected "	-	15.31±1.81	0.67±0.18
Chloroquine	0.25mg	4.80±0.74	0.18±0.05
Water	0.5ml of 1kg/litre	15.84±1.03	0.65±0.15
	0.5ml of 1kg/litre x 100	13.[illegible]	0.63±0.24
Pet ether	4.00mg	14.95±0.91	0.71±0.12
	40.0mg	13.18±1.25	[illegible]
CHCl3/MeOH	2.00mg	13.72±2.45	[illegible]
	200mg	12.14±1.68	0.71±0.12
MeOH	5.50mg	14.62±1.80	0.59±0.19
	50mg	13.25±1.77	0.62±0.21

TABLE 19

Effect of some extracts of Azadirachta indica on lipid production in vitro by Plasmodium gallinaceum

Extract.	Concentration of drug in 5ml	Total Phospho- mg/10^{10} RBC	^{c}PA µeq/10^{10} Red Blood Cell
Normal control	-	8.51±1.66	0.90±0.14
Infected "	-	20.55±0.84	1.63±0.24
Chloroquine	0.02mg	9.63±0.72	0.84±0.32
Water	0.5ml 1kg/litre	19.65±0.82	1.81±0.44
"	0.5ml 1kg/litre x 100	18.92±1.15	1.66±0.28
Pet ether	2.50mg	21.80±1.09	1.34±0.36
"	250mg	21.06±0.54	1.56±0.45
Chloroform/ MeOH	5.00mg	19.51±0.98	1.74±0.28
"	500mg	20.42±1.41	1.60±0.31
Chloroform	1.00mg	18.14±1.05	1.73±0.46
"	100mg	20.26±1.24	1.65±0.17

TABLE 20

Effect of some extracts of Morinda lucida on lipid production in vitro by Plasmodium gallinaceum

Extract	Concentration of drug in bml.	Total Phospholipid mg/1010 RBC	FFA µeq/10^{10} Red Blood Cell
Normal control	-	5.90±0.58	0.63±0.08
Infected "	-	14.86±0.76	1.25±0.14
Chloroquine	0.02mg	7.25±0.46	0.83±0.21
Water	0.5ml 1kg/litre	13.44±0.70	1.50±0.42
"	0.5ml 1kg/litre x 100	14.59±0.35	1.04±0.53
Pet ether	4.00mg	14.15±0.44	1.19±0.22
"	400mg	12.60±0.52	1.34±0.36
Chloroform/ MeOH	2.00mg	13.95±0.83	1.16±0.26
"	200mg	11.74±1.21	1.16±0.50
Chloroform	0.50mg	14.81±0.79	1.28±0.37
"	50mg	14.65±0.85	1.42±0.68

TABLE 20

Effect of some extracts of Morinda lucida on lipid production in vitro by Plasmodium falciparum

Extract	Concentration of drug in blood	Total Phospholipid mg/1010 RBC	FFA µeq/10^{10} Red Blood Cell
Normal control	-	5.80±0.58	0.63±0.08
Infected "	-	14.86±0.76	1.25±0.14
Chloroquine	0.02mg	7.25±0.46	0.83±0.21
Water	0.5ml 1kg/litre	13.44±0.70	1.50±0.42
"	0.5ml 1kg/litre x 100	14.59±0.35	1.04±0.53
Pet ether	4.00mg	14.15±0.44	1.19±0.22
"	400mg	12.60±0.52	1.34±0.36
Chloroform/ MeOH	2.00mg	13.95±0.83	1.16±0.26
"	200mg	11.74±1.21	1.16±0.50
Chloroform	0.50mg	14.81±0.79	1.28±0.37
"	50mg	14.65±0.85	1.42±0.68

Result.

Table 17 and 18 show the effects of the extracts on Plasmodium berghei. In both cases chloroquine reduced the amount of phospholipid synthesised in vitro significantly from 20.55 mg/10^{10} Red Blood Cell in those with chloroquine. This difference is significant at 10% level and it can therefore be concluded that potent antimalarial drugs interfere with lipid synthesis by Plasmodium berghei.

From table 17 the pet ether extract (250mg/5ml suspension) produced the highest reduction in the amount of Phospholipid synthesised. When subjected to 't' test this value was not significant. It can therefore be concluded that this extract of Azadirachta indica is not a potent antimalarial agent.

Table 18 shows the result of the extracts of Morinda lucida on Plasmodium berghei. None of the extracts used had significant effect on the amount of lipid synthesised by this parasite.

Tables 19 and 20 show the results of extracts of both plants on Plasmodium gallinaceum. No extract of both plants was observed to suppress the amount of lipids synthesised significantly.

Conclusion:

The results show that the various extracts of Azadirachta indica and Morinda lucida had no significant suppressive action on the lipid production of both Plasmodium berghei and Plasmodium gallinaceum in vitro.

INVESTIGATION 6

Clinical trial in man of water extracts of Morinda lucida and Azadirachta indica.

Morinda lucida which was the only plant showing some anti-malarial property in chicks and Azadirachta indica, the most popularly used plant for malaria fever were selected for clinical trial on some patients in the University Health Centre under the supervision of the Deputy Director of the Health Centre.

Procedure.

Children between the ages of 5 years and 15 years who reported in the health centre with fever had their blood examined for malarial parasites. Children with positive parasite counts were hospitalised and treated with water extracts of the above named plants prepared as used traditionally. Blood samples for some biochemical studies were taken at the beginning of the trial and at two days interval during the trial. Temperatures of the patients were recorded three times daily. The patients were treated three times daily with the water extracts prepared by boiling 500gm fresh leaves of Azadirachta indica in 1 litre

of water. Each patient was given about 100ml of this extract three times daily.

In cases where the condition of the patient deteriorated within 48 hours, chloroquine was immediately administered.

Owing to difficulties encountered in this trial only four patients were tried on each extract.

Results.

Figures 15 and 16 show the effect of the extracts on the temperatures of the patients. The figures showed that both extracts had no beneficial effect on the body temperature of the patients. When chloroquine was administered the Patients generally had their body temperatures lowered and parasites cleared from their blood.

After 48 hours of therapy no parasites were detected in the blood films of one patient given Aquilaria agallocha and only one out of the three who had the extract of Moringa oleifera for two days. Patients who gave negative parasite counts were not clinically better than the others as they still had high temperatures and were generally unwell.

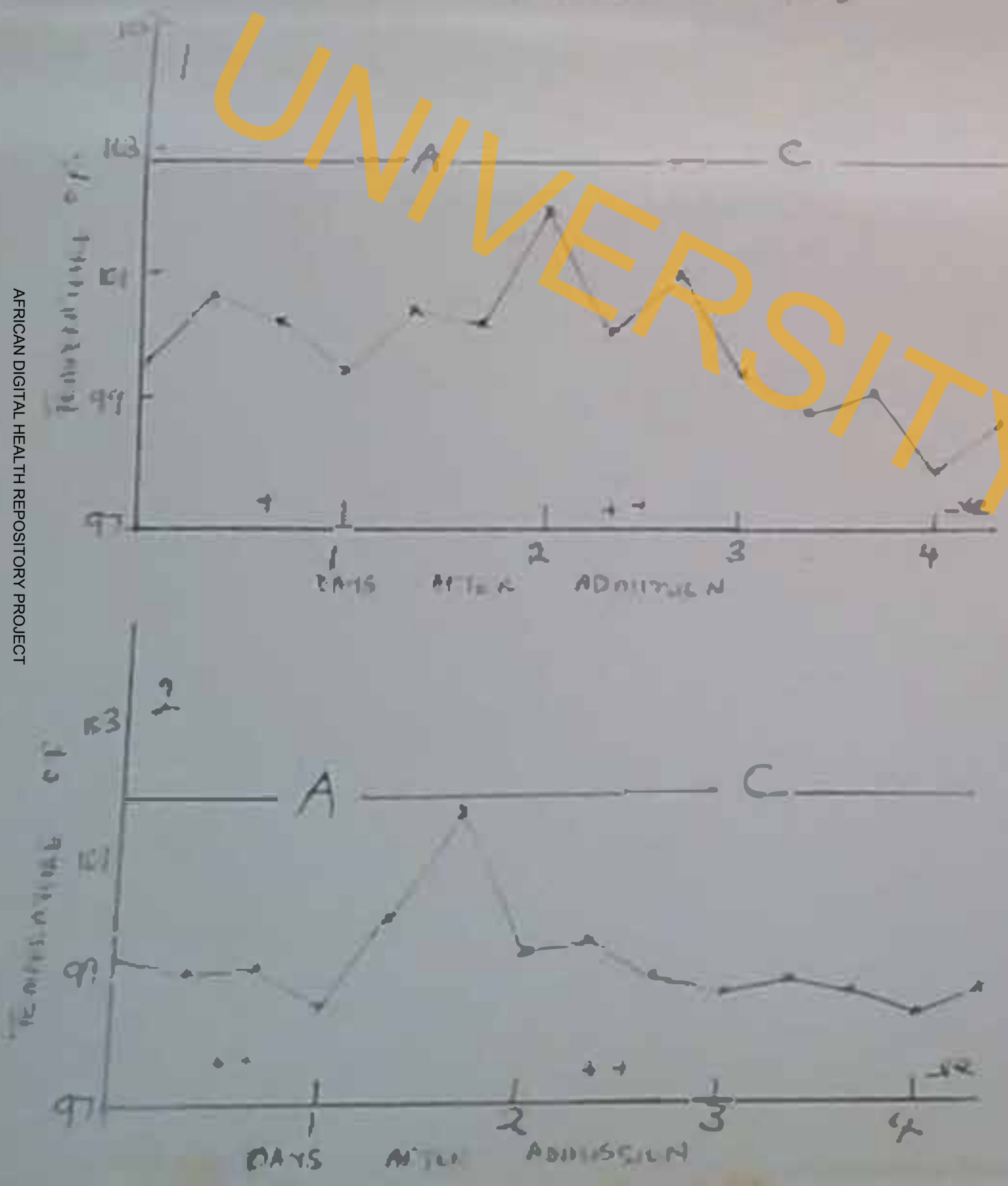
Variations in the serum biochemical values were

inconsistent for while some patients showed increases in their serum biochemical values others showed a decrease (Tables 21 and 22). But it was observed that in all cases lower levels of albumin were found. This is consistent with observations of earlier workers who have also reported lower albumin levels in human malaria.

Conclusion.

Extracts of Azadirachta indica and Morinda lucida had no beneficial effect on the temperature and general clinical picture of the patients.

FIG 15 EFFECT OF WATER EXTRACT OF AZADIRACHTA INDICA ON THE TEMPERATURE OF SOME MALARIAL PATIENTS.



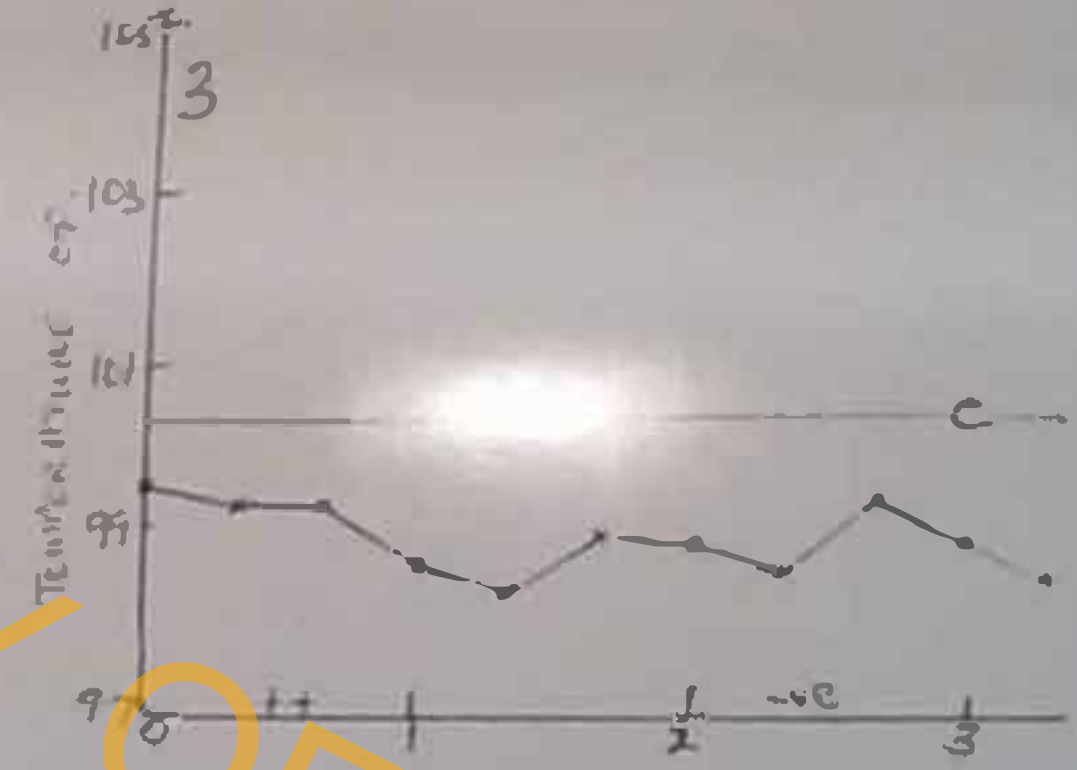


Fig 16 Effect of water extract of Azadirachta indica on the malarial patients.

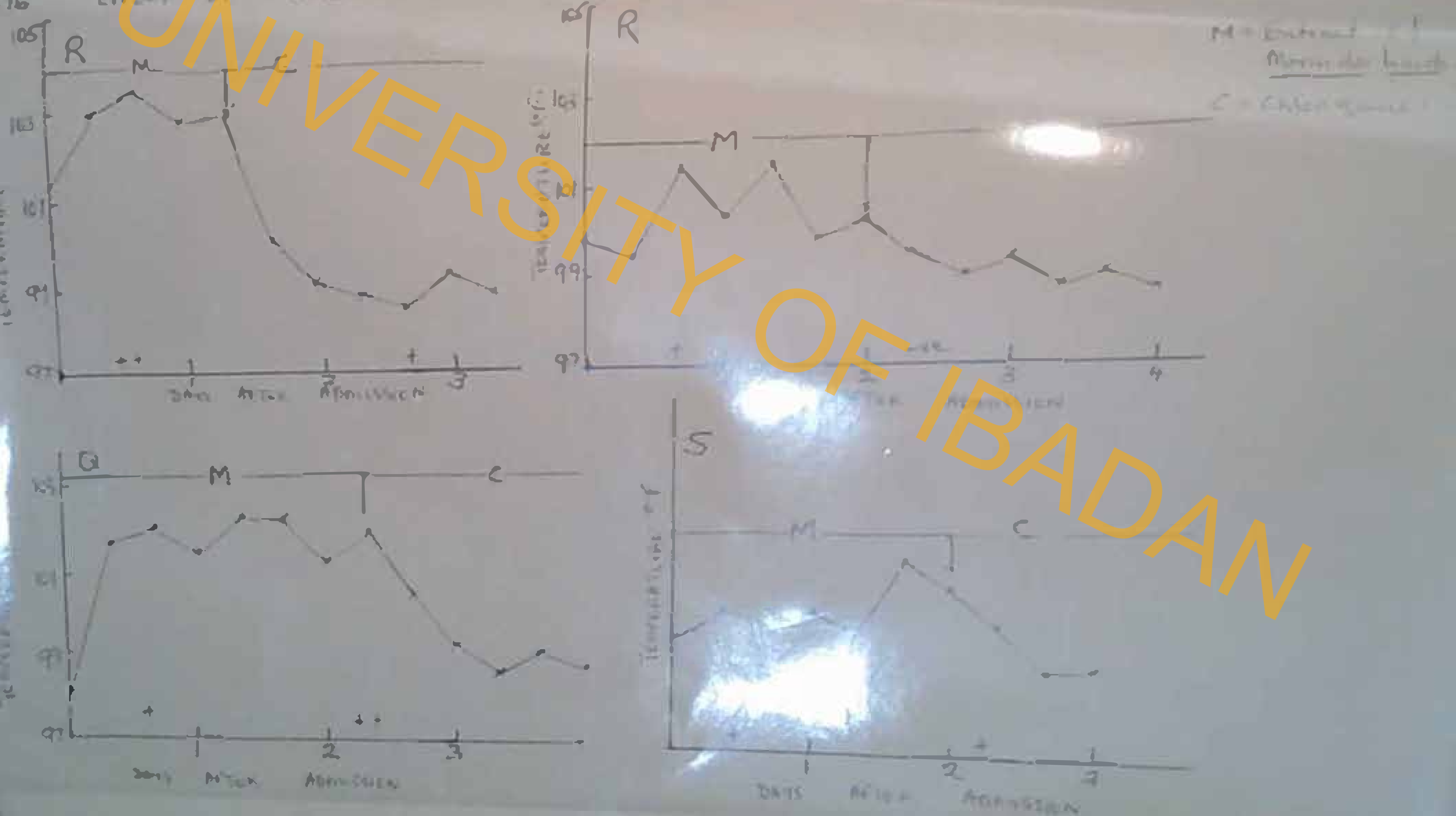


TABLE 21

Effect of water extract of Azadirachta indica leaves on some biochemical values in some malarial patients

Day	Patient	Sex	Age	Parasitemia	Temperature F	G.P.T. IntUnit/ml	L.A.P. mu/ml	Total bilirubin mg/100ml	Glucose mg/100ml	Total protein mg/100ml	Albumin %	Total alpha %	Beta Globulin %	Gamma Globulin %
0	1	F	11.5	+	99.8	17.2	9.72	1.05	94.2	7.1	46.6	13.5	20.5	22.4
	2	M	10	++	99.2	20.1	8.10	0.86	72.8	7.5	45.8	14.4	18.2	21.6
	3	F	12	+	99.8	16.81	6.48	0.84	88.5	8.6	47.5	13.7	19.2	19.0
	4	F	9	++	99.4	18.5	8.66	0.57	115.3	7.6	41.9	14.8	23.1	20.2
	X	F	7	Normal	98.8	8.5	13.4	0.60	128.6	6.8	51.7	7.3	21.0	20.6
2	1	F	11.5	++	102.0	18.5	9.48	0.86	100.5	7.0	45.5	13.7	21.6	20.2
	2	M	10	+	99.3	22.4	9.38	0.88	83.2	8.1	45.6	14.1	19.5	22.0
	3	F	12	-ve	99.0	16.2	8.35	1.08	97.2	8.6	46.4	12.9	23.6	17.1
	4	F	9	+	99.3	16.8	8.8	0.79	104.6	7.2	43.1	13.9	24.2	18.8
	X	F	7	Normal	98.5	8.8	12.15	0.75	106.7	6.6	52.9	8.8	20.2	19.9
	1	F	11.5	-ve	98.7	18.6	9.87	0.94	92.6	7.2	44.5	13.1	21.4	23.3
	2	M	10	-ve	98.6	20.7	9.51	0.71	89.5	7.7	46.3	14.0	21.4	19.3
	X	F	7	Normal	98.6	8.1	15.2	0.44	112.4	6.5	52.5	8.6	19.6	20.2

+ indicates the presence of 1 parasite per field in a thick blood film.

++ " " " " 2 or more parasites per field etc.

TABLE 21

Effect of water extract of Azadirachta indica leaves on some biochemical values in some malarial patients

Day	Patient	Sex	Age	Parasitaemia	Temperature	G.P.T. IntUnit/ml	L.A.P. mu/ml	Total bilirubin mg/100ml	Glucose mg/100ml	Total protein mg/100ml	Albumin %	Total alpha %	βGlobulin %	γ Globulin %
0	1	F	11.5	+	99.8	17.2	9.72	1.05	94.2	7.1	46.6	13.5	20.5	22.4
	2	M	10	++	99.2	20.1	8.10	0.86	72.8	7.5	45.8	14.4	18.2	21.6
	3	F	12	+	99.8	16.81	6.48	0.84	88.5	8.6	47.5	13.7	19.2	19.0
	4	F	9	++	99.4	18.5	8.66	0.57	115.3	7.6	41.9	14.8	23.1	20.2
	X	F	7	Normal	98.8	8.5	13.4	0.60	128.6	6.8	51.7	7.3	21.0	20.6
2	1	F	11.5	++	102.0	18.5	9.48	0.86	100.5	7.0	45.5	13.7	21.6	20.2
	2	M	10	+	99.3	22.4	9.38	0.88	83.2	8.1	45.6	14.1	19.5	22.0
	3	F	12	-ve	99.0	16.2	8.35	1.08	97.2	8.6	46.4	12.9	23.6	17.1
	4	F	9	+	99.3	16.8	8.8	0.79	104.6	7.2	43.1	13.9	24.2	18.8
	X	F	7	Normal	98.5	8.8	12.15	0.75	106.7	6.6	52.9	8.8	20.2	19.9
	1	F	11.5	-ve	98.7	18.6	9.87	0.94	92.6	7.2	44.5	13.1	21.4	23.3
	2	M	10	-ve	98.6	20.7	9.51	0.71	89.5	7.7	46.3	14.0	21.4	19.3
	X	F	7	Normal	98.6	8.1	15.2	0.44	112.4	6.5	52.5	8.6	19.6	20.2

+ indicates the presence of 1 parasite per field in a thick blood film.

++ " " " " 2 or more parasites per field etc.

TABLE 22

Effect of water extract of Morinda lucida on some serum biochemical values in some malarial patients

Day	Patient	Sex	Age	Parasitemia	Temperature of	G.P.T. IntUnit/ml	L.A.P. mu/ml	Total bilirubin mg/100ml	Glucose mg/100ml	Total protein mg/100ml	Albumin %	Total alpha %	β-Globulin %	γ-Globulin %
0	P	F	9	++	101.4	17.9	9.8	1.08	108.2	7.2	48.2	13.3	23.4	21.5
	Q	M	10	+	98.2	19.8	7.6	1.31	94.5	6.8	43.7	16.2	16.5	23.6
	R	F	8	+	99.7	15.2	8.3	0.82	112.0	7.7	46.0	12.4	22.1	19.7
	S	F	8	+	99.5	18.6	12.4	0.90	86.5	7.3	48.2	11.8	16.2	22.4
2	P	F	9	+	98.9	17.7	8.3	0.90	106.4	7.3	48.0	13.6	24.4	19.5
	Q	M	10	++	102.1	14.4	8.2	1.61	81.5	7.0	40.4	16.8	21.5	21.4
	R	F	8	+	98.9	16.1	11.2	0.64	95.3	7.5	45.5	11.2	25.6	20.0
	S	F	8	-ve	99.0	18.8	9.4	2.41	101.6	7.6	48.7	12.6	19.8	21.4
	Q	M	10	-ve	98.8	15.2	8.6	0.94	92.7	7.0	42.5	17.7	22.5	20.1

\+ indicates the presence of one parasite per field on a thick blood film.

++ " " " " two or more parasites per field on a thick blood film.

CHAPTER FIVE

DISCUSSION

Effect of malarial infection on experimental animals.

Plasmodium berghei, a rodent malarial parasite, has been found to produce fatal infections in most strains of white mouse although the severity of infection varies with the species of mice. In susceptible strains the course of infection is usually characterised by a brief acute attack associated with a rapidly progressive parasitemia which leads to death (Brigg et.al. 1960). Antimalarial drugs like chloroquine and paraquine when administered early can suppress or cure mice of this infection (Cox et. al. 1954: Jacobs et. al 1963 and Thurston, 1950).

Similarly *Plasmodium gallinaceum* infection in chicken is fatal unless the infected chicks are treated with anti malarial drugs (Curd 1943, Jaswant Singh et. al. 1952)

Malarial infection is accompanied by changes in the body temperatures of the hosts. These changes are not consistent in all species for in mice for instance, *Plasmodium berghei* infection is accompanied by a fall in the body temperature of the mice (Schneider, 1968) while in man it is generally accompanied by high fever

Malarial infections in man and other animals are accompanied by some biochemical changes in the host caused either by direct action of toxins released into the circulation of the host by the parasites or by the reaction of the host to the infection. For example it has been suggested that the red cell destruction in malarial infections involves not only direct rupture of the red cells by emerging merozoites but also some damage to non-parasitized red cells by toxins (Luckerman 1964). This situation is said to lead to anaemia that are out of proportion to the levels of parasitemia in the host. Evidence for the existence of such toxins is based on demonstration by in-vitro techniques of the ability of infected plasma to alter normal cell physiology and bring about osmotic fragility (Berh, 1969; Herman, 1969). Also the presence of toxins has been demonstrated by Riley and Wagun (1960) who showed that serum of Plasmodium berghei infected mice inhibit oxidative phosphorylation in isolated mouse liver mitochondria. This action probably accounts for the destruction of liver tissue observed in this infection and leads to the observed liver dysfunction.

Results of experiments with mice and chicks showed considerable variations in the serum protein during the course of an untreated infection. In both cases insignificant decre ses were observed in serum total protein values but marked decreases were observed in serum albumin levels with increases in the globulins in mice and

globulins in chicks. Similar changes have been reported in mice infected with Plasmodium berghei by Briggs et.al. (1960) and Sadun et.al. (1966); and in chicks infected with Plasmodium gallinaceum by Rao and Cohly (1953) and monkey infected with Plasmodium falciparum.

The presence of an abnormality in the plasma proteins indicates that some pathological or physiological factors are present and are responsible for this condition. Peterman (1950) has given the usual conditions which cause abnormalities in serum or plasma proteins as acute inflammation, chronic inflammation and proliferative processes, liver and biliary disorders. He also reported that the observed abnormalities are only a reflection on the state of the subject under study and not indicative of a specific disease. In liver diseases, decreased serum proteins, especially albumin, is usually observed and this is because the liver is responsible for the synthesis of most plasma proteins, especially albumins. Thus in malarial infections, the observed decrease in the serum albumin can be associated with hepatic damage in this disease. Any drug which is capable of preventing this liver damage should therefore affect the serum proteins.

The observed variations in serum glucose levels in this study are similar to those reported by earlier workers. In mice there was usually a significant decrease in serum glucose level confirming

the observations of Sodun et al. (1965). In chicks slight but insignificant decreases were also observed.

Malarial parasites are known to utilize glucose in considerable quantities for energy production (Conedella 1960). Lower serum glucose levels in this disease can therefore be associated with high parasitemia. This high demand for glucose by the parasites can lead to loss of glycogen in the liver. Singer (1954) has reported that cytoplasmic condensation occurs in liver cells in mice infected with Plasmodium berghei. He has suggested that this condition may represent a depletion of fat, glucose and other storage materials from the cells and that these are not replaced during the infection. The observed lower serum glucose in this infection can therefore be a direct reaction of the host to the infection.

The changes observed in the serum levels of glutamic pyruvate transaminase (GPT), and leucine amino peptidase (LAP) are likely to be associated with the hepatic damage present in this infection as raised values are generally associated with hepatic damage (Reed 1965). Only slight variations were observed in serum alkaline phosphatase and total bilirubin, showing that there was no obstruction of the bile duct in this disease.

Malarial infections in these experimental models are therefore

accompanied by measurable clinical variations like temperature, some serum biochemical values (glutamic pyruvate transaminase, leucine amino peptidase, serum proteins and glucose.)

Effect of anti-malarial drugs on experimental infections.

Malarial infections in experimental models respond to anti-malarial drugs and these models were infact used in screening programmes for the discovery of potent anti-malarial drugs (Wiselogle 1945-1946; Thurston, 1953). Two such convenient models are the Plasmodium berghei - mouse system the Plasmodium gallinaceum - chick system.

Tables 5a and 5c show the effect of chloroquine, the standard drug in this project, on the progress of Plasmodium berghei infection in male and female mice and table 6 shows its effect on Plasmodium gallinaceum in white leghorn chicks. From the tables it can be seen that chloroquine suppressed infections in these animals as evidenced by the significantly lower levels of parasitemia in treated animals compared with the untreated controls. Also it prevented the fall in body temperatures of both mice and chicks that is usually observed in this disease.

Associated with this decrease in the level of parasitemia were reduced serum levels of glutamic pyruvate transaminase, leucine amino peptidase and bilirubin and increased levels of

glucose in the animals treated with chloroquine compared with the infected controls. The protein fractions had not returned to normal proportions but they were closer to the normal values than those in the untreated controls. Therefore this drug in addition to improving the clinical symptoms of the animals, also suppressed the serum biochemical abnormalities observed in the controls.

Effect of local plant extracts on experimental infections

Tables 7 to 12 show the effect of some locally used plant extracts on malarial infection in mice starting at different times in the course of infection. None of the six extracts prepared traditionally had any suppressive or curative action on the Plasmodium berghei infection in mice at the concentrations in which they were used. These concentrations were relative on weight basis to that which will normally be used by man. In another investigation it was observed that none of the extracts was effective against this disease in mice even at concentrations equivalent to 100kg plant materials per litre. In both cases the extracts had no beneficial effect on the temperatures of the mice as temperatures in those treated with the extracts were not different from those of control mice. Therefore these extracts had no effect on the clinical symptoms of Plasmodium berghei infection in mice.

Their effects on some serum biochemical values were investigated Figures 5 to 10 show the results of experiments using the six plant

extracts under investigation. These figures showed that none of the extracts had any beneficial effect on the biochemical values investigated as chloroquine did. Infact some of the extracts would appear to aggravate the abnormalities associated with the infection, as shown by raised serum values of glutamic pyruvate transaminase, and leucine amino peptidase by Morinda lucida and the MixturesAandB in infec ed mice compared with controls.

In normal uninfected mice only slight and insignifi ant increases were observed in the serum levels of both enzymes, (glutomic pyruvate transaminase and leucine amino peptidase) and no changes in serum glucose, bilirubin and total proteins, although slight decreases were observed in the albumin fractions in all cases.

It can therefore be concluded that these extra s had no beneficial effect on the infected mice as they did not improve the clinical and biochemical symptoms of infected mice. The results would also seem to suggest that the extracts could be toxic to the infected animals although they are rel tively non-to ic to healthy animals.

In the chick malaria, however, it was found that Plasmodium gallinaceum infection is slightly suppressed by ater ex ract of Morinda lucida at a high concentration. The suppression was not

significant when compared with that produced by Chloroquine. Apart from this slight reduction in level of parasitemia the extract does not seem to be of any other benefit to the chick as it did not suppress the abnormalities that accompanied the infections. Rather it seemed to aggravate the effect of infection as it caused raised values of serum glutamic pyruvate transaminase and leucine amino peptidase in the infected chicks above those in the infected controls. In normal chicks only slight increases in these enzymes were observed, even after 5 days of therapy.

All the other extracts had no suppressive action on Plasmodium gallinaceum in chicks and did not improve the biochemical abnormalities. Enantia chlorantha behaved differently from the other extracts in this model in that it was the only extract which affected serum proteins significantly It caused a decrease in the total protein level as well as decreases in the albumin and globulin fractions and large increases in the alpha globulin fractions in both normal and infected chicks. These observation would seem to suggest that the extract could cause nephrosis in chicks although observations on the kidney did not reveal any degeneration of this organ.

In chick malaria therefore, only water extract of Morinda lucida had slight suppressive action.

The differences in the reactions of Plasmodia berghei and gallinaceum infections to these extracts are not unexpected for it is known that different species of parasites react differently to chemotherapy. Plasmodium berghei for instance has been reported to respond poorly to some useful drugs and is hypersensitive to others. Thus it has been shown by Thurston (1950) that this parasite is only half as sensitive to the action of quinine as Plasmodium gallinaceum and to sulfonamides and sulfones (Thompson et. al. 1967)

These differences in response to antimalarial drugs could be due to several factors of which the rate of absorption, metabolism and excretion are a few. Schmidt (1971) (quoted from Thompson and Corbel 1972) has suggested that the poor response of Plasmodium berghei to chloroquinine was evidently because mice have only limited capacity to metabolise the parent drug to its active metabolites. Therefore the apparent difference between the action of Morinda lucida extract on Plasmodia berghei and Gallinaceum could be due to one of these reasons.

Reports of other workers on some of these plants do not agree with the results of our experiments. For instance, Spencer et. al. (1947) in his survey of plants for anti-malarial activities found water and alcoholic extracts of the barks of Alstonia constricta, Azadirachta indica and Mangifera indica ineffective

against avian malaria. The major alkaloid of Alstonia congensis, echitamine, has been reported to possess no antimalarial properties (Goodson and Henry 1925). Wiselogle (1945-1946) reported that an unspecified component of the bark of Morinda lucida has a suppressive action against Plasmodium lophurae in duck while most extracts of Alstonia constricta and Alstonia scholaris were found inactive against this avian model.

Although in this investigation most of the plant extracts have been found inactive against both rodent and avian malarial infections, some of them are useful medicinally in other ways. For instance most Morinda species are known to possess tannins, methylanthraquinones and alizarine derivatives (Oliver, 1960). Also Adesogan (1973) reported the isolation of two anthraquinols - oruval and oruvalol - and ten anthraquinones from the stem of Morinda lucida obtained locally from trees in Ibadan and Akure in the Western state of Nigeria. Medicinally anthraquinones are used as purgative and laxatives. This plant can therefore be useful as a source of laxatives and purgatives and these side effects might be responsible for the benefit derived from the use of this plant.

Enantia chlorantha is known to contain the alkaloid berberine (Oliver 1960). The acid sulphate of this alkaloid which could be

used for indigestion, diarrhoea, malaria and sickness in pregnancy has been used successfully in curing oriental sore by injecting it into the sore (British Pharmacopoea index 1944).

Azadirachta indica has been found useful medicinally. One of its constituent nimbidin, is used medicinally as a bitter principle (Indian Pharmacentical Codex 1954). A locust phagorepellent, meliantriol, has recently been isolated from the seed oils of both Azadirachta indica and its closely related plant Melia azedarach (Takunle, 1973). In addition powdered dried leaves of this plant are reported able to protect beans from weevil attack. There is no doubt, therefore, that this plant possesses some beneficial medicinal value to man although its antimalarial property is doubtful.

Effect of extracts of Morinda lucida and Azadirachta indica on some malarial patients

From the observations made on a few hospitalized malarial patients, it is unlikely that the water extracts of both plant possess the acclaimed antimalarial properties, as children treated with these extracts responded badly to therapy. However no valid conclusions can be drawn from this study mainly because of the small number of patients involved and the short duration of the

trials. This was so because malaria is a deadly disease which must be handled with utmost caution. Secondly the results are difficult to interprete because the patients came in at different stages in the infection and they possessed different levels of immunity to this disease. In all cases, the patients have had several cases of the disease.

Figure 15 shows that one patient (No. 3) out of the 4 given water extract of Azadirachta indica showed no positive parasite in his blood smear after 48 hours of treatment while patients Nos 1 and 4 showed increases in the level of parasitemia and patient No. 2 showed neither an increase nor a decrease. The extract could have been responsible for the clearance of parasite in this patient; but this patient showed no improvement in other criteria studied for he still had a high temperature of about 99.6°f and was generally unwell. The apparent clearance of the parasite could also be attributed to the immune response of the patient to this disease for it is known that patients with high immunity to the disease ever come the infection without medication.

None of the patients treated with Moringa lucida extract had their blood cleared of the parasite before the administration of chloroquine even after 72 hours of treatment (Figure 16 (No. 3). Also there were some fluctuations in the body temperatures.

The precise benefit derived from the use of these infusions is difficult to evaluate since they do not improve either the clinical or biochemical state of the patients. The most obvious conclusion from this study was that these infusions were not as potent as chloroquine because patients who did not respond to therapy with these infusions became well when treated with chloroquine.

The use of these plants for medicinal purpose appears to be dictated by their bitter tastes. Perhaps this is because most orthodox anti-malarial drugs have bitter tastes. Bitter principles in increasing the flow of saliva and gastric juice increase the appetite and improve digestion thus acting as general tonics in cases of debility and lack of appetite. Malarial infection usually causes both debility and lack of appetite. Some of these locally used plants are known to contain bitter principles. For instance Azadirachta indica contains nimbidin, Sarcocephalus esculentus contains berberine, Morinda lucida contains some tanins and anthraquinones which have bitter tastes. It is therefore possible that these extracts act by improving the appetite of the patients who if they can eat enough good food will have enough strength to combat the debilitating effect of this disease.

Some people in addition to drinking the hot infusion also

both in the hot extract and inhale the vapour from it. These processes make the patient sweat profusely and as a result may lower his body temperature giving him a temporary relief from some of his symptoms.

In some cases the infusions act as purgatives and laxatives as well as diuretics in which case the frequent removal of these waste products from the body also helps to lower the temperature of the patient. Morinda lucida which has some anthraquinones might be effective in this way as anthraquinones are known to be effective as purgatives and laxatives.

It is therefore possible that the action of some of these plant extracts are simply symptomatic and not anti-parasitic as one would expect

Effect of drugs on malarial parasites in-vitro

The mode of action of anti malarial drugs varies with the drug and the species of parasites but generally their antimalarial properties are associated with the interference of some vital processes in the parasite. For example chloroquine has been shown to bind strongly to nucleoproteins in-vitro and it has been suggested that this action is a possible mechanism by which it interferes with cellular processes in the malarial parasite

bath in the hot extract and inhale the vapour from it. These processes make the patient sweat profusely and as a result may lower his body temperature giving him a temporary relief from some of his symptoms.

In some cases the infusions act as purgatives and laxatives as well as diuretics in which case the frequent removal of these waste products from the body also helps to lower the temperature of the patient. Lorinda lucida which has some anthraquinones might be effective in this way as anthraquinones are known to be effective as purgatives and laxatives.

It is therefore possible that the action of some of these plant extracts are simply symptomatic and not anti-parasitic as one would expect

Effect of drugs on malarial parasites in-vitro

The mode of action of anti malarial drugs varies with the drug and the species of parasites but generally their antimalarial properties are associated with the interference of some vital processes in the parasite. For example chloroquine has been shown to bind strongly to nucleoprotoins in-vitro and it has been suggested that this action is a possible mechanism by which it interferes with cellular processes in the malarial parasite

(Irvin et. al. 1949). More recently Schellenberg and Vallery (1961) have shown that chloroquine, quinine and quinacrine but not pentaquine can inhibit the incorporation of P^{32} labelled phosphate into DNA or RNA by Plasmodia gallinaceum and berghei. Also pentaquine, primaquine and pamaquine all bind to native DNA resulting in inhibition of DNA functions. These processes are suggested to be partly responsible for their antimalarial actions.

Some antimalarial drugs are known to interfer with oxygen uptake in-vitro by the parasite. For example Shiffer et. al. (1966) showed that chloroquine at [illegible] can inhibit Plasmodium berghei from utilizing oxygen. Peters et. al (1968) have also reported that pamaquine at a concentration of 10^{-5} molr reduced oxygen uptake by 55% in-vitro by Plasmodium berghei.

Certain inhibits processes like lactate formation from glucose in Plasmodium gallinaceum under aerobic and anaerobic conditions (Silverman et. al. 1964), and it produces some inhibition on the glycolytic enzymes of Plasmodium gallinaceum (Lynch and Irvin 1965)

Several other biochemical processes in the parasite are interfered with by anti-malarial drugs and the anti-malarial properties of individual drugs may involve one or more of these processes.

In our study, effect of extract on infectivity of parasites after exposure to the extracts and their ability to synthesise lipids from glucose were studied as these criteria of action seem to be generally applicable to most anti-malarial drugs whose active agents are not metabolites of the drugs.

Results of effects of extracts of Azadirachta indica and Morinda lucida on Plasmodium berghei and Plasmodium gallinaceum in-vitro are shown in tables 37, 38, 39 and 40.

None of the extracts used reduced the viability of both parasite species exposed to them at very high concentrations compared with the action of chloroquine. Also both parasite species were found to produce significant quantities of lipids from glucose in the presence of these extracts while chloroquine reduced the amount synthesised significantly.

In one of our experiments it was found that extracts of both plants did not inhibit the oxygen uptake in-vitro by Plasmodium berghei while chloroquine produced about 50% inhibition under the same conditions. It would therefore seem that these plant extracts possess no valuable anti-malarial action on both parasite species in-vitro.

In an attempt to extend these in-vitro studies to human

malarial parasites it was found out that most patients who roported for fever in hospitals had very low percentage parasi-temia; usually below 10% parasitemia was obtained and only in isolated cases had parasitemia above this recorded. With such low levels of parasitemia the quantity of lipids synthesized in-vitro was so insignificant as to make it difficult to use this method to evaluate the action of the extracts. An attempt to transfer human malarial parasites to local primates also failed as the species used did not show high parasitemia in their peripheral blood. If it had been possible to infect those primates with human species, then it would have been possible to obtain the parasites at a density suitable for such in-vitro investigations.

From the results of the investigation with the various plant extracts in-vivo in experimental animals and man, it can be concluded that there was specie variation in the response of the test systems to the extracts. Plasmodium-gallinaceum - chick system appeared to be the most sensitive to these extracts for it is the only system in which some definite (though insignificant) anti-malarial action was observed.

Further work might be done on Moringa lucida by isolating specific components and trying these on avian

plamodia; but other plant extracts do not seem to require extensive studies as they apparently are ineffective in the two species tried.

Summary.

The water extracts of leaves of Azadirachta indica (Dogonyaro), Morinda lucida, Alstonia boonei, and Mixtures A and B and the alcoholic extract of Quantia chlorantha have been found to possess no anti-malarial properties against Plasmodium berghei infections in mice. Only the water extract of Morinda lucida was found to possess some anti-malarial action against Plasmodium gallinaceum infection in chicks.

These plant extracts had no beneficial effect on the clinical symptoms of the infection as they neither reduced the extent of parasitaemia nor improved the deterioration of the body temperatures of animals given the extracts.

In addition to this, the extracts did not correct the serum biochemical abnormalities observed in infected animals as chloroquine did. Instead some of the extracts, especially the water extracts of Morinda lucida, Azadirachta indica and Mixtures A and B seemed to aggravate the effect of the infection in infected mice and chicks by causing an elevation of serum levels of GPT and IAP above those observed in the control animals.

In the normal mice and chicks most of the extracts were not

hopato toxic at the concentrations in which they were used. But the alcoholic extract of Phantis chlorantha would appear to be slightly toxic to chicks as it was responsible for a decrease in total serum proteins and decreases in the albumin and γglobulin fractions with a significant increase in the alpha globulin fraction. These observations would seem to suggest that the extract could cause nephrosis in the chick.

Malarial patients responded very poorly to the use of water extracts of Azadirachta indica and Morinda lucida. Extended clinical trials will be necessary to draw a valid conclusion but in the few cases studied, there was no improvement in the clinical symptoms of the patients.

In in-vitro experiments extracts of Morinda lucida and Azadirachta indica had no effect on the viability of Plasmodium berghei and Plasmodium gallinaceum. They also had no effect on their ability to produce lipids from glucose in-vitro as chloroquine does.

CONTRIBUTION TO KNOWLEDGE

This study has made the following contributions to knowledge about chemotherapy of malaria.

1. The water extract of leaves of the plant Morinda lucida, which has a slight suppressive action on Plasmodium gallinaceum infection in chicks, was found to be ineffective against Plasmodium berghei infection in mice and would seem to be ineffective against human malaria. Also the water extracts of the leaves of the plant Azadirachta indica, were ineffective against malaria in mice, chicks and man, while extracts of leaves of the plant Alstonia boggei, bark of the plant Enantia chlorantha and mixtures of various plant materials have no antimalarial property against malaria in mice and chicks.

2. The plant extracts were not toxic to normal animals but were toxic to infected animals as they aggravated the serum biochemical abnormalities which generally accompany malaria infection.

3. In vitro, extracts of leaves of Morinda lucida and Azadirachta indica had no effect on the infectivity and lipid production of Plasmodium berghei and Plasmodium gallinaceum.

REFERENCES

Adesogan, E.K. (1973). Anthraquinones and Anthrauinolo from Morinda lucida. The biogenetic significance of Oruwal and Oruwalol. Tetrahedron 29: 4099-4102.

Alving et. al. (1948). The clinical trial of eighteen analogues of pamaquine (Plasmochin) in vivax malaria, (chesson strain). J. Clin. Invest. 27: 34.

Alving, A.S., Arnold, J., and Robinson, D.H , (1952). Status of Primaquine. Mass therapy of subclinical vivax malaria with Primaquine. J. Amer. Med. Ass. 149: 1558.

Anpino, J.F. (1964). Clinical Chemistry, Principles and Procedures. 3rd Edition. 1964 135-140p

Bahr, G.F. (1969). Quantitative cytochemistry of malaria infected erythrocytes (P. berghei, P. chabaudi, and P. vinckei). Milit. Med. 134: 1013-1025.

Bailey, L. (1962). Techniques in protein chemistry.

Baker, F.J., Silverton, R.E. and Luckcock, E.D. (1957). An introduction to medical laboratory technology London. Butterworth and Co Ltd. 276-283'.

Bassir. O. (1971). Handbook of Practical biochemistry. 55p

Black, R.H. (1946). The effect of antimalarial drugs on *Plasmodium falciparum* (New Guinea strains) developing in vitro. *Trans. R.Soc. trop. Med. Hyg.*; *40*, 163-170.

Blacklock and Southwell. A Guide to Human Parasitology. 8th Ed. H.K. Lewis & Co. Ltd. 12-19.

Box, E.D., Gingrich, D.W. and Celaya, B.L. (1954). Standardisation of a curative test with *P. berghei* in white mice. *J. Inf. Dis.* *94*: 78-83.

Boyd, M.F. and Proske, H.O. (1941). Observations on the blood proteins during malaria infections in. *J. trop. Med.*, *21*: 245.

Bray, R.S. (1957a). Studies on malaria in chimpanzees, II *Plasmodium vivax*. *Am. J. trop. Med. Hyg.*: 6, 514.

——— (1957b) Studies on malaria in chimpanzees IV. *Plasmodium Ovale*. *Am. J. trop. Med. Hy.*: 6, 638.

——— (1958) Studies on malaria in chimpanzees. VI *Laverania falciparum*, *Am. J. trop. Med. Hyg.*: 7, 20-24.

——— (1959) Pre-erythrocytic stages of Human malaria parasite. *Plasmodium malariae*. *Brit. Med. J.* *II*: 679.

Briggs, M.T., Garza, B.L. and Box. E. D. (1960). Alterations of serum protoins in mice acutely and chronically infected with Plasmodium berghei. Expt. Parasite. 10: 21-27.

Brody. J. A. and Dunn, F.L. (1959). Malaria surveillance in the United States, 1958: Am. J. trop. Med. 8: 635.

Bruce - Chwatt, L.J. (1967). Clinical trial of antimalial drugs. Trans. R. Soc. trop. Med. Hyg. 61: 412-424

Buttle et. al. (1938). The action of the cinchona and other alk aloids in bird malaria. Biochem. J. 32. 47-58.

Conodella, R.J. (1968). Lipid synthesis from glucose carbon by Plasmodium berghei in vitro. Am. J. trop. Med. Hyg. 17. 660-664.

Conodella, R.J. and Zaro, L.H. (1967). Automated mass screening of compounds for antimalarial activity. Automation in analytical chemistry 281-285p. Technicon symposium. Technicon corporation New York.

Conodella, R.J., Jarrell J.J. and Zaro, L.H. (1969). Plasmodium berghei: Production in vitro of free fatty acid. Expt. Parasit. 24. 130-136.

Coatney, G.R. and Roudabush, M.(1949). A catalogue of the species of the genus Plasmodium, and index of their host. In "Malariology" 29p

Coatney G.R. and Greenberg, J. (1952). The use of antibiotics in the treatment of malaria. Ann. N.Y. Acad. Sci. 55, 1085.

Coatney et. al. (1953). Survey of antimalarial agents. Publ. Hlth. Monogr. No 9: 1-322

Coggeshall, L.T. (1938) The cure of Plasmodium knowlesi malaria in rhesus monkeys with sulfanilamide and their susceptibility to reinfection. Am. J. trop. Med. 18: 715-721

Coggeshall, L.T. (1940) The selective action of sulfanilamide on the parasite of experimental malaria in monkeys in vivo and in vitro. J. exp. Med. 71: 13-20

Covell et.al. (1955) Chemotherapy of malaria WHO Monograph Ser. NO. 27, Geneva.

Crollman, A. (1962). Pharmacology and Therapeutics. Henry Kimpton, London.

Curd, F. H. S. (1943) The activity of drugs in the malaria of man, monkeys and birds. Ann. trop. Med. Parasit. 37: 115-143.

Darrow, E. M., Gingrith, W.D. and Prine J.H. (1952). The effect of antibiotics on experimental malaria (Plasmodium cathemerium and Plasmodium berghei). Am. J. trop. Med. Hyg. 1: 927 - 931.

Davey, D. G. (1944) Biology of the malaria parasite in the vertebrate host. Nature. 153: 110

——— (1946a) Concerning exo-erythrocytic forms and the evidence for their existence in human malaria. Trans. Roy. Soc. trop. Med. Hyg. 40: 171

——— (1963) Chemotherapy of malaria. Biological basis of testing methods. In "Experimental Chemotherapy" Academic Press, New York and London. 487-517p

Diggens. S. M. and Gregory, K. (1969). Comparative response of various rodent malarias to chemotherapy Trans. R. Soc. trop. Med. Hyg. 63: 7

Dole V.P. (1956). A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J. Clin. Invest. 35: 150-154.

Dolo, V. P. and Emeroon, K. (1945). Electrophoretic changes in the plasma protein patterns of patients with relapsing malaria J. Clin. Invest. 24: 644

Fakunle, C. O. (1972). Ph.D. Thesis, Ibadan. Chemistry of Melia azedarach and Azadirachta indica.

Falco, E. A. et. al. (1951) 2:4 - diaminopyrimidines - a new series of antimalarials. Brit. J. Pharmacol. Chemother. 6: 185-200.

Fushina, A. (1969). Ph.D. Thesis, Ibadan Extractives from Azadirachta indica and Khaya senegalensis.

Ferg, P.C. et. al. (1962). Pharmacological screening of West Indian Plants J. Pharmacy and Pharmacology 14: 9.

Findlay, C. M. (1951) "Recent Advances in Chemotherapy" Vol II. Churchill, London. 597p

Fulton J. D. and Macgraith, B. C. (1948). Physiological pathology of malaria in "Manual of Malariology". Saunders, Philadelphia.

Garnham, P.C.C. (1948) Exoerythrocytic schizogony in Malaria. Trop. Dis. Bull. 45: 831.

Garnham, P.C.C. (1966) "Malaria parasites and other haemosporidia". Blackwell Scientific Publications, Oxford.

———, Bird, R.G. and Baker, J.R. (1955). The pre-erythrocytic stage of Plasmodium ovale. Trans. Roy. Soc. trop. Med. Hyg. 49: 158

Gieman, Q. M., Siddiqui, W.A. and Schnell, J.V. (1966). In vitro studies on erythrocytic stages of plasmodia. Milit. Med. 131: 9(Suppl). 1015 - 1025

——— (1969) Biological basis for susceptibility of Aotus trivirgatus to species of plasmodia from man. Milit. Med. 134: 780 - 786.

Goodson, J.A. and Henry, T.A. (1925). Echitamine. J. Chem. Soc. 127: 1640 - 1648.

Goodwin, L. G. (1949). Response of Plasmodium berghei to antimalarial drugs. Nature, London 164: 1133

Greenberg, J., Taylor, D. and Josephson, E.S. (1951). Studies on Plasmodium gallinaceum in vitro. II The effects of some 8-amino quinolines against the erythrocytic parasites. J. Infec. Dis. 88: 103

Henry, T.A. (1925). Echitamine and other alkaloids of Alstonia. J. Chem. Soc. 127: 1184.

Herman, R. (1969). Osmotic fragility of normal duck erythrocytes as influenced by extracts of Plasmodium lophurae infected cells and plasma. J. Parasit 55: 626-632.

Hess, B. (1963). Enzymes in blood plasma. Academic Press.

Hocking, G.M. (1959). "Pakistan Medicinal Plants II" Academic Press. London.

Huff. C. G. (1949) Life cycle of malaria parasites with special reference to the newer knowledge of pre-erythrocytic stages. In "Malariology". Saunders, Philadelphia. 54p

——— and Coulson, F. (1944). The development of Plasmodium gallinaceum from sporozoite to erythrocytic trophozoite. J. Infec. Dis. 75: 231 - 249.

Hutchinson J. and Dalziel . J.M. (1962). 'Flora of West Tropical Africa'. Second edition. Crown Agents for Overseas Government and Administration, London.

Irvin, J.L. and Irvin, M.E. (1949). The interaction of antimalarials with Nucleic Acids. Science 110: 426.

Jacobi, K. (1965) Die standardisierung der chemotherapie der Hagetier malaria (Plasmodium berghei) an Mäusen - Mausoon. Z. Tropenmed. Parasit. 16: 258 - 268

Jacobs. R.L., Alling, D. W. and Cantrell, E.F.(1963). An evaluation of antimalarial combinations against Plasmodium berghei in the mouse. J. Parasit. 49: 920-925

James, S.F. and Tate, P. (1938) Exoerythrocytic schizogony in Plasmodium gallinaceum. Parasit. 30: 128

Jang. C.S. et. al. 1948 Pharmacology of Ch'ang Shan (Dichroa febrifuga) a Chinese Antimalarial herb. Nature (london) 161: 400

Jaramillo-Arango (1950) "The conquest of Malaria" 29. Hienemann, London.

Jaswant Singh, Brau, P.C. and Ray, A. P. (1952). Screening of antimalarials against P. gallinaceum in chicks. Part I. Preliminary studies. Indian J. Malar. 6: 145-148.

Josephson,E.S. et. al. 1953. Further studies on the effect of antimalarial drugs against erythrocytic forms of Plasmodium gallinaceum in vitro. J. Infec. Dis. 93: 257.

Koepfli, J.B., Mead J.F. and Brockman, J.A. Jr. (1947). An alkaloid with high antimalariol activity from Dichroa febrifuga. J. Amer. Chem. Soc. 69: 1837

Konopka, E.A., Goble, F.C and Donovan J.S. (1966). Sex of host as a factor in protozoal chemotherapy. Abntracts 3rd Int. pharmac. meeting Sao Paulo. 212.

Kremers, E. (1931). The chemistry of cinchona, historically considered. Proc. Celebrations 300th Anniversary of the first recognised use of cinchona. St. Louis, 139.

Kretschmar, W. (1965) The effects of stress and diet on resistance to Plasmodium berghei and malarial immunity in the mouse. Annls. Soc. belge. Med. trop. 45: 325 - 344.

Krishnaswami, A.K., Satya, P. and Ramakrishnan, S. P. (1954). Studies on Plasmodium berghei. Vincke and Lips 1948. XV Acquired resistance to sulphadiazine Indian J. Malar. 8: 9 - 18

Kudo, R.R. (1966) Protozoology Charles C. Thomas. Springfield. U.S.A. 717p

Lambo, J. O. (1974). The healing powers of herbs. A paper read at the Pan African Conference on Medicinal Plants

and the Relationship between Traditional and Modern Medicine.

Lorch, E. and Coy, K.F. (1966). Photometric "Titration" of FFA with the Technicon Auto Analyser. Analytical Biochem. 16: 244-256.

Lunn, J.S. et. al. (1966). Changes in antibody titres and serum protein fractions during the course of prolonged infection with vivax or with foloiparum malaria. Am. J. Trop. Med. Hyg. 15: 3

Marshall, P.B. (1945). The absorption of cinchona alkaloids in the chick and its relationship to antimalarial activity. J. Pharmacol. Exp. Ther. 85: 299.

Marshall, E.K. Jr., Litchfield, J.T.Jr. and White, H.J. (1942) Sulfonamide therapy of malaria in ducks. J. Pharacol. Exp. Ther, 75: 89-104.

McIlroy, R.J. (1950). The plant glycosides, Arnold, London.

Mitra, C.R. (1963) "Neem", Indian Central Oil Seed Committee. Hinayatnagar, Hyderabad.

Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153: 375-380

Oliver, B. (1960) Medicinal Plants in Nigeria. Published as a private edition by the Nigerian College of Arts, Science and Technology, 1960.

Peters, W. (1963). Bartonellosis and malaria in the albino mouse. Proc. 7th Int. Congr. trop. Med. Malar. Rio do Joneiro 5: 81

——— (1965a) Drug resistance in Plasmodium berghei Vincke and Lips 1948. Chloroquine resistance. Expt. Parasit. 17: 80-89

——— (1965b) Competitive relationship between Erythrozoon coccoides and Plasmodium berghei in the mouse. Expt. Parasit. 16: 158-166

——— (1967a) Rational methods in the search for antimalarial drugs. Trans. R. Soc. trop. Med. Hyg. 61: 400-410.

——— (1967b) Chemotherapy of Plasmodium chabaudi infection in albino mice. Ann. trop. Med. Parasit. 61: 52-56.

——— (1968) The chemotherapy of rodent malaria, I. Host-parasite relationship part I. The virulence of infection in relation to drug resistance and time elapsed since isolation of the "wild" strain. Ann. trop. Med. Parasit. 62: 230-245.

——— (1970) Chemotherapy and Drug Resistance in Malaria. Academic Press, London and New York.

Peterman, M.L. (1960). The Plasma Proteins Vol. II. Academic Press, New York. 310p

Phifer, K.O., Yielding, K.L., and Cohen, S.N. (1960) Investigation of the possible relation of ferriheme acid to drug resistance in P. berghei. Expt. Parasit. 19: 102-109.

Porter, J.A. Jr. and Young, M.D. (1967). The transfer of Plasmodium from man to the carcaset, aquinus geoffroyi. J. Parasit. 53: 845-846.

Polot, H. (1966) In vitro cultivation of erythrocytic forms of Plasmodium knowlesi and Plasmodium berghei. Milit. Med. 131 (Suppl). : 1026-1031.

Polot, H and Barr, C. F. (1968). DNA, RNA and Protein synthesis in erythro cytic forms of P. knowlesi. Am. J. trop. Med. Hyg. 17: 672-679.

Rao, R.R. and Cohly, H.H. (1953). Microelectrophoretic study on serum protein from normal and malarial chicken infected with Plasmodium gallinaceum. Current Sci. 22: 204-205

Blockman, K. H. et. al. (1968). Effects of chloroquine, quinine and cycloquanil upon the maturation of asexual erythrocytic forms of two strains of P. falciparum in vitro. Amer. J. trop. Med. Hyg. 17: 661-671

Blotman, S. and Frankel, S. (1957). A colourimetric method for determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminases. Amer. J. Clin. Path. 28: 56-63.

Riley, H.V. and Deegan, T. (1960). The effect of Plasmodium berghei malaria on mouse-liver mitochondria Biochem. J. 76: 41-46.

Rollo, I.M. (1952). Dorapric. Experimental chemotherapy. Trans. R. Soc. trop. Med. Hyg. 46: 474-484

Russell, P. B. (1960) Medicinal Chemistry. Wiley, New York 815p

Russell, P.F. et. al. (1963). Practical Malariology. London, Oxford University Press.

Sadun, E. H., Williams, J.S. and Martins, L.K. (1965a) Pathophysiology of Plasmodium berghei infection in mice. Expt. Parasit. 17: 277-286.

------(1965b) Biochemical changes induced in mice infected by infections with the plerocercoid larva of cestode. J. Parasit. 51: 532.

——— (1966) Serum biochemical changes in malaria infections in man, chimpanzees and mice. Milit. Med. 131: 9(Suppl). 1094-1106.

Schollenberg, K.A. and Coatney, G.A. (1961). The influence of antimalarial drugs on Nucleic Acid synthesis in Plasmodium gallinaceum and Plasmodium berghei. Biochem. Pharmacol. 6: 143-152.

Schmidt, L.H. (1956). The alkaloids. Vol 5. Academic Press, New York. 141p

Schmidt, L.H. and Coatney, G.R. (1955). Review of investigations in malaria chemotherapy. Amer. J. trop. Med. Hyg. 4: 200-216

Schneider, M. D. (1968). Characteristics and cross resistance patterns of chloroquine resistant. Plasmodium berghei infection in mice. Expt. Parasit. 23: 22-50.

Schneider. J. (1954). Plasmodium berghei and chemotherapy. Indian J. Malar. 8: 275-279.

Schneider, J., Decourt, Ph. and Montezin. C. (1949). Sur l'utilisation d'un nouveau Plasmodium (Plasmodium berghei) pour l'etude et la recherche de medicaments anti paludiques. Bull. Soc. Path. exot 42: 449-452.

Sengupta et. al. (1960). Terpenoids and related compounds, constituents of the trunk bark of Melia Azodirachta and the structure of the ketophenol nimbiol. Tetrahedron 10: 45-46

Siddons, L. B. (1953). Screening of antimalarial compounds in mice with Plasmodium berghei infection. Indian J. Malar. 7: 41-52.

Silverman et. al. (1944). The in vitro metabolism of Plasmodium gallinaceum. J. Inf. Dis. 75: 212-230.

Singh J. et. al. (1953) Screening of antimalarials against Plasmodium gallinaceum in chicks. Indian J. Malar. 7: 117.

Singha, S. C. (1965). Medicinal Plants of Nigeria. Nigerian National Press Ltd., Apapa.

Singer, I. (1954). The cellular reactions to infections with Plasmodium berghei in the white mouse. J. Inf. Dis. 94: 241 - 261

Skolton F.S. et. al. (1968). Inhibition of coenzyme Q system by chloroquine and other antimalarials. J. Am. Chem. Soc. 90: 5334-5336.

Speok. J.F. and Evans Jr. E.A. (1945). The biochemistry of the malaria parasite. Mechanism of pyruvate oxidation in the malaria parasite. J. Biol. Chem. 159: 83

Spencer C.F. et. al. (1947). Survey of Plants for anti-malarial Activity Lloydia: 10: 145-174

Stauber, L.A. (1954) Application of Electrophoretic Techniques in the field of Parasitic Diseases. Expt. Parasit. 3: 544-568

Suppan, L. (1931). Three centuries of cinchona. Proc. celebration 300th Anniversary of the first recognised use of cinchona, St. Louis, 29.

Taylor, D. J. et.al. (1951) Studies on P. gallinaceum in vitro. A method for maintainance of the erythrocytic parasite in vitro. J. Infec. Dis. 88: 158

——— (1952). The in vitro activity of certain antimalarials against erythrocytic forms of Plasmodium gallinaceum. Amer. J. trop. Med. Hyg. 1: 132-139

Taylor, H. L. et. al. (1949) The effects of induced malaria, acute starvation and semi-starvation on the electrophoretic diagram of serum proteins of normal young men. J. Clin. Invest. 28: 273

Trager, W. (1967). The different effects of antimalarial drugs on P. lophurae developing intracellularly and extracellularly in vitro. Am. J. trop. Med. Hyg. 16: 15-18

Trout D.L. et al. (1960). Titration of F.F.A. of plasma: a study of current methods and a new modification. J. Lipid. Res. 1: 199-202.

Thompson, P. E. and Beyles, A. (1966). Eradication of Sporythrozoon coccoidoo with oxophonarsine in normal and drug resistant lines of Plasmodium berghei in mice. J. Parasits. 52: 674-678.

——— and Worbel, L. H. (1972). Medicinal Chemistry. A series of Monographs. Vol. 12. "Antimalarial Agents. Chemistry and Pharmacology". Academic Press, New York and London.

——— et. al. (1967). Relations among antimalarial drugs. Results of studies with cycloguanil-, sulfone-, or chloroquine-resistant P. berghei in mice. Am. J. trop. Med. Hyg. 16: 133-145.

Thurston J. P. (1950). The action of antimalarial drugs in mice infected with Plasmodium berghei, Br. J. Pharmacol. Chemother. 5: 409-416

——— (1953). The chemotherapy of Plasmodium berghei I. Resistance to drugs. Parasit. 43: 246-252.

Valoy, H. (1962). Practical Clinical Biochemistry. Academic Press.

Vinke, I. H. and Lips, M (1948). Un nouveau Plasmodium d'un rongeur sauvage du Congo, Plasmodium berghei, n. sp. Annls. Soc. belge. Med. trop. 28: 97-104

Warhurst, D. C. (1966). Bioassay of Plasmodium berghei. Trans. R. Soc. trop. Med. Hyg. 60: 6.

——— and Folwell, R.O. (1968). Measurement of the growth rate of the erythrocytic stages of Plasmodium berghei and comparisons of the potency of inocula after various treatments. Ann. trop. Med. Parasit. 62: 349-360

Warshaw, L. J. (1949). Malaria. The biography of a killer. Rinehart and Company. Inc. New York, Toronto.

Watt, J.M. and Breyer-Brandwijk, C.H. (1962). The Medicinal and Poisonous Plants of Southern and Eastern Africa.

Webster, W. (1964). A New English Dictionary.

Wolldo, D.T., Briggs, N.T. and Sadun, E.H. (1966). Susceptibility to Plasmodium berghei. Parasitological, biochemical and hematological studies in laboratory and wild animals. Milit. Med: 131: 9(Suppl). 859-869

World Health Organization (1972) Report on the Malaria Eradication Programme. Conference Doc A25/39, Twenty-fifth World Health Assembly.

Wisologlo, F. Y. (1946). A survey of antimalarial drugs, 1941-1945 Vol. 1 and 11. Edwards, Ann. Arbor, Michigan.

Yeoli, M. and Most, H. (1965). Studies on sporozoite - induced infection of rodent malaria. The pre-erythrocytic tissue stage of Plasmodium berghei. Am. J. trop. Med.Hyg. 14: 700

——— et.al. (1966) Life cycle and patterns of development of Plasmodium berghei in normal and experimental hosts. Milit. Med. 131: 900-904

Zuckermann, A. (1957). Blood loss and replacement in Plasmodial infection 1. P. berghei in untreated rats of varying age and in adult rats with erythropoietic mechanisms manipulated before inoculation. J. Inf. Dis. 100: 172-206.

——— (1964) Autoimmunization and other types of indirect damage to host cells as factors in certain protozoan diseases. Exp. Parasit. 15: 138-182.