INFLUENCE OF DIETARY SPINACH (*AMARANTHUS HYBRIDUS LINN*) AND BITTERLEAF (*VERNONIA AMYGDALINA*) ON THE BLOOD LEVEL OF ORALLY ADMINISTERED CHLOROQUINE IN THE RABBIT

BY

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D E D I C A T I O N

The author proudly dedicates this thesis to his two loving children ADEOLA AYODEJI AYODELE and ADETOLA OLADIMEJI AYODELE.

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D E C L A R A T I O N

It is hereby declared that this work is solely that of JEROME TOLA AYODELE and has not been present any where else for any other qualification.

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Abstract of Thesis . . . Presented to the Faculty of Science Department of Chemistry, Ahmadu Bello University, Zaria in Partial fulfilment of the requirements for the degree of Master of Science (Analytical Chemistry).

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Blood level data are widely employed to avaluate the absorption route of drugs as a function of dosage form, route of administration and similar variables. In the majority of individuals, the biological properties of a drug such as apparent volume of distribution, elimination and activity are usually unchanged so that differences in blood levels are a direct reflection of differences in absorption rate.

Food does influence the absorption of some drugs and this may be due to absorption of drug onto food components chelation of drug by polyvalent metal ions or complexation with proteins in the food, Food is also known to reduce

delay and increase the absorption of some drugs.

Vegetables like African spinach (Tete) and bitter leaf (Ewuro) are commonly consumed and Nigeria being a malaria endemic area, chloroquine in its various forms is also taken frequently* It will therefore be interesting to see what influrence these vegetables may produce on the blood level of chloroquine when orally administered to rabbits feeding on these vegetables.

The animals were fed ad libitum their normal diet containing 25% of the powdered leaves for 7 days with free access to water before oral dosing with chloroquine. A double dose was initially given followed by single doses at 6 and 24 hours after.

Following the last oral dose blood samples were obtained through vein puncture, hemolyzed and assayed spectrophotometrically for chloroquine.

Results obtained showed higher initial blood levels in those rabbits fed on bitter leaf and spinach compared to the control. The peak in the animals fed on normal diet, bitter leaf and spinach were observed at 3, 2, and 4 hours respectively. The peak in the animals fed on spinach was one hour delay compared to the peak in the animals fed on normal diet. The levels at the eighth hour were almost the same as the initial

blood level.

It was also noticed that the presence of bitter leaf has resulted in an early peak which how-ever is not significantly different in magnitude as compared to the control which peaked at 3 hours. Nevertheless the total amount of ehloroquine passing into the blood was lower in the animals fed on bitter leaf.

With respect to rabbits fed on spinach there was a delay in the peak. , The peak was not significantly different from that of the control when compared. However the total amount of chloroquine in the blood was higher than in the control. In terms of maintaining high chloroquine blood level, spinach diet appears to be superior to normal fed and more so to bitter leaf.

CHAPTER 1

I N T R O D U C T I O N

There are problems in the estimation of drug concentrations in the body. The ideal sample for analysis in probably plasma water from arterial blood but this cannot be obtained as a routine practice. Data are often presented simply as blood levels presumably derived from analysis of the whole blood samples.

Blood Level

A therapeutic agent usually must attain some minimum concentration at its site of action before it elicits a given pharmacologic response. At apparent distribution equilibrium there is frequently a corresponding minimum drug concentration in the blood which is referred to as the therapeutic blood level or the minimum effective concentration of the drug for a specified therapeutic purpose.

Blood level data are widely employed to evaluate the absorption rate of drugs as a function of dosage form, route of administration and similar variables. In the majority of individuals, the biological properties of a drug such as apparent volume of distribution, elimination and activity are usually unchanged so that differences in blood levels are a direct

reflection of differences in absorption rate.

The ultilization of blood levels as criteria for the comparative absorption efficacy and activity of a series of derivatives or homologs is not that simple and such data can be misinterpreted. The various compounds, despite basic structural similarity may differ considerably in distribution pattern, biologic half-life and therefore activity. In such cases blood level can be interpreted rationally only if the dissimilarities above are considered oconcurrently. Many drugs are bound partially to plasma proteins and therefore exist in the blood in part as free drug and in part as drug protein complex. Only the free drug can diffuse to other tissues since the protein bound portion can not pass across blood vessel wells in healthy individuals. Consequently the greater the degree of protein binding, the smaller the amount of drug which is available to extra-vascular sites since the driving force for diffusion from blood stream to other tissues is the concentration gradient diffusible drug.

The study of blood levels in animals presents problems. Apart from devising a sensitive technique early in the life of an experimental drug, one must know exactly what the method measures.[1]

The study (of blood levels) may not be a simple 'one off'

measurement, the level should be monitored throughout the test as so many factors like sex difference, pregnancy, alteration in absorption, enzyme induction inhibition, overloading of metabolic pathways and/or accumulation of compound in certain tissue can cause variations which could radically alter our judgment of the safety of a compound. Since food and most drugs are taken orally it will not be surprising the presence or absence of food and the type of food would have some influence on the efficacy of a drug.

Diet - Drug Interaction

Food may influence drug absorption more directly because of absorption of drug onto food components. An example is the chelation of drugs by polyvalent metal ions such as calcium, magnesium or complexation with proteins. However the absorption of tetracycline has been shown to increase in the presence of the neutral fat tripalmitin. This effect of tripalmitin appears to be associated with the removal of calcium. Solid or semi-solid food may act as a mechanical barrier preventing drug movement toward the mucosal surface of the gastro-intestinal tract and may indirectly inhibit drug absorption depending on the digestive capacity of gastro-intestinal secretions.

Cases have been reported in the literature of food reducing, delaying, increasing or having no effect at all on the absorption of idifferent drugs. The majority of studies demonstrate an inhibitory effect and there are sufficient examples i.. all cases as in examples below.[2]

Presence/Absence of food

Among drug products whose rate and extent of absorption appear to be inhibited to varying degrees by the presence of food are penicillins and pivampicillin. The peak serum penicillin levels were reduced by food and the overall serum levels were again prolonged when results in non-fasted individuals were compared to the fasted ones.[3] Pivampicillin, and antibacterial agent, is normally given to patients with food to minimize gastro-intestinal irritation, so the influence of food on its bioavailability is important. Its absorption was not affected when it was dosed with a light uncooked meal but a distinct reduction in serum levels was reported when the drug was administered with a breakfast of eggs and dairy products.[4]

Although the separation of drugs into reduced and delayed categories is somewhat arbitrary, in some cases it may be based on the behaviour of particular drugs and drug formulations. Drugs whose absorption may be delayed by food

include aspirin effervescent and potassium ion. The absorption of aspirin from the conventional tablets appears to be slightly reduced by food but absorption from effervescent tablets appears to be slightly delayed with serum levels from fasted and non-fasted subjects approaching similar values. The effervescent one may reduce the effect of food by increasing the gastric emptying time and as well preventing precipitation of aspirin in the stomach.[5]

The bioavailability of potassium ions from a solution and from slow release tablets was examined by Ben et al. They suggested that slow absorption of potassium ion in the presence of food and its slow rate of elimination produced less flunctuation in total body stores of potassium than when the drug was doses on empty stomach. Since the object of potassium therapy is to replenish and maintain body stores at a desired level, better therapeutic results was obtained when administered with food.[6]

Prednisone falls into the category of those few drugs whose absorption may not be affected by food. Tembo et al showed that prednisone plasma level determined showed no differences between fasted and non fasted treatments in plasma blood levels

at each sampling time; peak plasma levels and at times of peak.[7]

Drug and Specific Classes of food.

The various classes of food carbohydrate, protein, fats vitamins and minerals affect the blood levels of some orally administered drugs.

Carbohydrate

Some carbohydrates have been known to affect the blood levels of drugs like benzy penicillin and barbiturates and are known to complex with certain other drugs. For example starch and sucrose diets potentiate the lethal reaction of benzy penicillin in albino rats.[8] A high intake of various sugars and other carbohydrates increases the duration of sleep induced barbiturates. In both cases the carbohydrate diet resulted in high blood level being maintained for longer times indicating that patients on high carbohydrate diets might require lower dosages of these drugs than the usual prescriptions.[9]

Potato and arrow root starches form complexes with salicyclic acid, benzoic acid and many of its salts. These interactions have been reported to be due to certain attractive forces between them. Such complexes when formed in the gastrointestinal tract will not be absorbed thus resulting in low blood level of the drugs.[10]

Fats.

The gastric secretion and gastric motility are inhibited by fats and fatty acids. The concomitant ingestion of a fat soluble drug and a fatty diet will result in prolonged stay of the drug in the gastro-intestinal tract thereby increasing the absorption of the drug. For example absorption of griseofulvin has been shown to be increased by high fat meals but not by high protein or carbohydrate meals in normal individuals.[11] Essential fatty acids in the diet alter the rates of drug detoxification and induce alterations in activity of the hepatic microsomal enzymes which are responsible for metabolism of many drugs.[12]

Proteins

The most oustanding deficient component in diets from developing countries is protein. A dietary reduction in either quality or quantity of protein causes depression of hepatic microsomal mixed function oxidase activities. Protein deficiency decreases the microsomal reaction rates *in vitro* for a variety of oxidative pathways with substrates such as phenolbarbital, but an increase in the disappearance *in vivo* of pentobarbital.[12]

Vitamins

Rats fed on a diet deficient in vitamin B - complex slept for longer period after oral administration of pentobarbital than

those rats on normal diet. Certain vitamin diet also affect the metabolism of a flavonoid glucoside. Booth et al also indicated that patients on anticoagulant therapy such as dicoumarol should not take food items containing vitamin K.[14]

Minerals

The metabolism in vitro and in vivo of aniline and aminopyrine were significantly reduced in magnesium deficient rats but normal metabolism was restored in rats fed with magnesium containing diets.[15]

Specific foods

Certain types of food also influence the blood level of orally administered drugs in one way or the other. For example cheese, beer and wine, coffee, coladrinks yorghurt and bannanas are all known to contain amine precaussors and in general combination therapy with mono-amine oxidase inhibitors are reported to be dangerous.[16] They are liable to cause severe headache, intracranial bleeding, circulatory bleeding, severe hypertension and death. The interaction between these foods and mono-amine oxidase inhibitor drugs do not usually occur in the gastro-intestinal tract but after absorption due to the presence

of catecholamine in the foods. The mono-amine oxidase normally metabolises catecholamines resulting in the accumulation of catecholamines in the body. The ingested catecholamine from these foods increase the level of Catecholamine in the body to the extent that hypertension results.[17]

Vegetable - Drug Interactions

Some vegetables are known to interact with drugs but very little is known about some indigenous species like spinach (Amaranthus Hybridus Linn) and bitter leaf (Vernonia Amygdalina).

Several indoles present in brussels sprouts and cabbage stimulate intestinal drug metabolism in rats fed on either dried brussels sprouts or dried cabbage in a nntritionally semisynthetic diet. Vegetables and other dietary constituents can stimulate the intestinal metabolism of drugs and thereby alter their biological effects.[20] A number of edible plants including alfalfa, a constituent of purina rat chow, brussels sprout, cabbage and turnips are inducers of benzopyrene hydroxide activity in rat small intestine.[21] Rats fed on brussels sprouts in nutritionally complete semisynthetic diet metabolized hexobarbital and 7-ethoxy coumarin more rapidly than those fed on

the diet devoid of these vegetables. The small intestine from rats fed cabbage in the semisynthetic diet showed an increased rate of metabolism of the substrates and was concluded that constituents of cabbage and brussels sprouts are capable of stimulating the activity of intesti-nal drug metabolizing enzymes.

Amaranthus hybridus linn (Spinach)

The local names of amaranthus hybridus are African Spinach, spinach amarathus, bush green, spinach green, Tete (Yoruba) and Alyafu (Hausa).

The amaranths are used in various parts of the world as potherbs particularly the wild amaranths known as strawberryblites. They are good fodder plants and have cooling as well as nutritive properties. The Chinese attribute to the amaranths the property of increasing the intellect.

Vernonia amygdalina (bitter leaf)

Bitter leaf Ewuro (Yoruba); Shiwaka (Hausa) is an erect branched shrub or small tree 2-4.5 metres high, abundant in grassland throughout the tropics. The leaves which are up to 30cm long are alternate simple and normally rather irregularly toothed on petioles up to 30 cm long.[23]

It is the most generally recognised as the Yoruba chewstick, the root and twig being widely used. It is the most valued of the tooth cleaners and it is chewed mostly as a stomach tonic and appetiser. The Hausas use it mixed with rednatron medicinally for gastro-intestinal troubles.[24]

The bitter leaves are used in soup after removing most of the bitter constituents. They are antiscobic and a digestive tonic. A form of the special food called in Hausa '<u>fate fate</u>' is made from the leaves along with butter etc; This may be taken by men merely as food but women in the belief that it renders them sexually more attractive. The leaves are rubbed on the body for itching conditions, parasitic diseases, ring-worm etc; or a cold infusionis used as a wash. During the puerium women drink a decoction of the leaves to

affect the milk and act as a vicarious preventive of worms in the infant. The leaves are rubbed on the breast to assist weaning.

In French Guinea, the leaves are used as febrifuge and as a tonic after fever, while in Ghana a decontion of the leaves is a cough medicine. A purgative enema is prepared from the macerated leaves through a cloth and pepper/spices added. For horses the leaves are crushed in water and given for internal disorders with mucous discharge from the nose. For worms they are added as a tonic to horse food with bran natron etc. The bark of the roots and stems are very bitter and more astringent. In Angola it is used for fever with diarrhoea while in Abyssinia the leaves are rubbed inside honey jars to add bitterness or a laxative property.[24]

The liquid plus the dirty white foams obtained during washing and squeezing of the leaves is used for curing open wounds and the plant is believed to have some curing effects on urinary tract diseases.[25]

Chemical composition of vernonia amygdalina and amaranthus hybridus linn.

To further appreciate the various uses to which those vegetables have been put to, it may be necessary to deal with their chemistry.

Vegetables have been of more importance to the botanists with regards to classification, agronomists with

regards to their nutritive value than they have caught the attention of chemists. For example the composition of many vegetables including the amaranthus and the bitterleaf still remains very much a mistery to the chemists.

However plant extracts of vernonia amygdalina are known to contain alkaloids which are present in small quantities but their structures have not yet been trully eluciddated. The presence of alkaloids in this species denotes possible possession of pharmacological activity.

Vernonia amygdalina (family compositae) is a young family rich in the so called secondary metabolites and in substances involved in life processes of the so-called primary metabolites a classical example of a primary metabolite in them is polyfructane (Inulin), a characteristic constituent of compositae. This completely replaces starch as a reserve poly saccharide. Essential oils, terpenoids, acetylenic compounds, phytomelans, caffeic acid, their esters and flavonoids are all typical secondary products in the family. The alkaloids are present in large numbers in a series of tribes but they are in very small quantities and their structures have not been sufficiently elucidated. The seneciones tribe are exceptional for the occurrance of numerous alkaloids of the necine type.[26]

More than 1,000 species of the family compositae have been investigated and all contain several different

types of acetylenic compounds especially those with 10, 13, 14 and 18 carbonatoms in a molecule and sesquiterpene lactones are abundant. They are the characteristic secondary metabolites of the family whose specific functions; if any, are not yet known. In view of the large number it is possible that a series of compositae plants has its own endemics sequiterpenic lactones, inspite of the fact that the number of the known basic types is limited.

From the survey on the frequency of occurance of sesquiterphenic, vernonia amygdalina is shown to contain mostly lactones of the germacrane series; the so called germacranolidesand to a lesser extent elemonolides.[26]

The amaranthus (spinach) and various of its species contain varying amount of alkaloids, tannin and saponins. An interesting feature of the amaranths is the presence of various endogenous levels of acetycholine in different plant organs with less of the acetycholine in the root.[25]

Malaria

Malaria, a disease characterized by successive chills, fever and sweats is the most widespread parasitic disease in Africa and indeed the whole world. It is acclaimed that at least one-third of the world population suffers from it. It is a worldwide problem of greater economic, social and political consequences.[27] Malaria is found throughout the whole of the country, infact Nigeria is a hollow endemic malaria country. It is most found in the southern part because of its vegetation and swampy nature.

In the north it is common where there are dams or standing water. Rice and sugar cane farms are breeding grounds for the primary host the mosquito.[28]

Malaria is both chronic, acute and a drain on human resources. It is prevalent in the rural areas which are virtually neglected in terms of health facilities. The poor treatment of people in these areas causes human and economic wastes, lowering production and the dynamo of production.

To reduce its mortality grip particularly among the children in the age group 2-6 years and to reduce its anaemic chronic ill health effect there is need for its control and total eradication.

For effective control of malaria, the following measures are essential, treatment of human infection with antimalaria prophylactic drugs, protection of human beings from exposure to bites of mosquitoes, preventing breeding grounds for mosquitoes by elimination of stagnant water, the use of insecticides, the use of mosquito pathogens and consideration of vector behaviour and human behaviour.

To date no successful immunologic process that provides total protection against malaria is known. The most ideal drug would be an inexpensive palatable, long acting, non-toxic compound that is prophylactic and curative without inducing resistance and without after effects.

Chloroquine

Chloroquine is an antimalaria drug found effective against avian and the common human malaria. It is almost

completely absorbed from the gastro-intestinal tract and the peak plasma concentration is reached in one to three hours with blood levels falling off rather rapidly after administration is stopped. It is usually administered orally but may also be given intravenously in divided doses over the period of therapy..

Investigation with tissue extracts from different animals indicate the basic extracts of tissues consist predominantly of the parent drug over the wide range of tissue levels from a few micrograms to one milligram of base per gram of tissue. A survey of the distribution of chloroquine in monkey and white rat showed that the highest concentration was in the liver but in rabbit and dog the highest concentration is in the kidney and spleen respectively.[29] The relatively high localization in the liver may be an important factor in its usefulness for the treatment of hepatic amebiasis.

Chloroquine is used for the control of acute attacks of vivax malaria and for suppressive purposes. The drug is neither a prophylactic nor a radical curative agent in vivax malaria. In regions where plasmodium falciparum is generally sensitive to chloroquine, it is markedly effective in terminating acute attacks of this malaria and

usually brings about complete cure in this type of malaria. Although not useful in intestinal amoebiasis, chloroquine is an effective agent in the treatment of amebic hepatitis. Clinical response is usually as prompt and complete as response to emetine. Since chloroquine is well tolerated, it has been routinely used in amebiasis without demonstrable hepatic involvement. Chloroquine has been employed with some success in giadiasis, tapeworm infection etc. It is quite effective in the treatment of photoallergic reactions. The drug has actions on the heart and may be used as an antiarrhythmic when the standard drug cannot be used.

Although the toxicity of chloroquine is quite low the dose employed for the therapy of an acute malaria attack may cause mild transient headache, visual disturbances, gastro-intestinal complaints (nausea, vomiting, dizziness etc) and puritus. Prolonged chronic medication for suppression of the disease causes a few significant toxic reactions and the drug occassionally must be discontinued because of intolerance.

Corneal opacities, blurred vision and retinal damage from prolonged treatment with high doses also occur.

Convulsions occasionally occur on the intensive dose schedule employed for the treatment of amebiasis. An account of chloroquine uses, is well documented by Goodman and Gilman.[17]

Purpose of the Project

Nutritional state of animals have been known to influence markedly drug blood levels and there is a wealth of evidence from numerous studies with experimental animals that the quantity and quality of the ingested food influence their susceptibility to infections as well as the severity of their course. In man the evidence for a similar relationship is less clearcut, due to the difficulties in carrying out controlled experiments with human beings and thus much of the evidence is circumstantial and based on epidemological studies and experimental animals.

African Spinach (Amaranthus hybridus linn) commonly called Tete among the Yorubas, Alyafu in Hausa and bitterleaf (Vernonia amygdalina) (Yoruba, Ewuro, Hausa, Shiwaka) serve as part of soup ingredients of many Nigerians. On the other hand with the advent of total elimination war on mosquitoes and malaria parasites, chloroquine in its various forms is consumed by Nigerians as a safe guard and remedy against

malaria attack. It would therefore be interesting to investigate what influence these vegetables may produce on the blood level of orally administered chloroquine when the rabbit feeds on these two vegetables. The primary aim of the project therefore was to investigate the influence of each of the African spinach (henceforth referred to as spinach) and bitterleaf on the blood level of orally administered chloroquine. It is hoped that results obtained would offer useful information on the following.

Whether patients on chloroquine therapy should be encouraged or discouraged to feed on either of these vegetables and whether a change in the dose of chloroquine would be required as a result of a diet of either vegetable.

CHAPTER II

ASSAY OF CHLOROQUINE

The increasing use of antimalarial drugs in large field campaigns emphasized the need for improvement in existing assay methods and/or the development of new techniques that could be utilized in the field for checking the blood levels of these drugs.

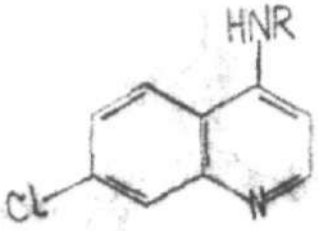
The concentration of a drug in blood, plasma or serum is the best indicator available of the concentration of the drug in the whole body and of concentrations at sites of action. It is not necessarily equal to any of these concentrations but since the majority of drug distribution in tissues is by reversible physicochemical processes, it is reasonable to suppose that these concentrations are systematically related to concentrations in blood, plasma and serum which are themselves closely related to each other.[30]

Measurement of drugs in whole blood is often technically difficult because of purification difficulties, additionally, the process by which drug molecules are localized in red cells, have not been fully investigated.

Review of Methods of Analysis

Chloroquine and several of its metabolites have been determined on a number of occasions using various methods. For example, Titus et al. isolated the desethyl (A) Compound from the urine of human volunteers using counter current distribution method.[31] Kuroda has also isolated four metabolites of chloroquine from human tissues using a combination of paper chromatography and ultra-violet spectroscopy. In addition to the desethyl compound of Titus et al. were the bisdesethyl (B); the carbinol (C) and the 4-amino -7- chloroquine (D) derivatives.[32]

Mcchesney using flourescence technique confirmed the above findings and in addition found traces of the 4' aldehyde (E) and the 4' carboxyl derivatives (F). By his method of analysis, he listed the amount of determinable excretory products as 70% chloroquine, 23% desethyl chloroquine; 1-2% bisdesethyl and the others as trace degradation products. In addition he reported that about one third of the administered chloroquine was unaccounted for and remains obscure in this very complex biotransformation for the drug.[33]



Compound	R
Chloroquine	$-CH(CH_3)(CH_2)_3N(C_2H_5)_2$
A	$-CH(CH_3)(CH_2)_3NHC_2H_5$
B	$-CH(CH_3)(CH_2)_3NH_2$
C	$CH(CH_3)(CH_2)_3OH$
D	$-H$
E	$-CH(CH_3)(CH_2)_2CHO$
F	$CH(CH_3)(CH_2)_2COOH$

Various other methods have since been developed for the analysis of blood level of chloroquine.

Identification of chloroquine by Spot tests

In the approximate period of two decades following World War II, there has been an increase in the use of chloroquine for mass prophylaxis of malaria that it became necessary to have relatively simple methods to ensure that the drug was taken regularly. Tests of this type usually employ Hcl/$KclO_3$ or 25% H_2SO_4, Chlorinated lime; H_2SO_4/$KclO_3$ on biological extracts and methyl orange, eosin yellow and complex with copper/ cobalt on pure drug or urine samples. Other tests utilize various reagents to react either with the pure drug or the drug in a dosage for [34, 35, 36].

Extraction Methods.

Among the various extraction procedures described in literature are those of Leo Kazyat and Anne Robinson et al. described below.

Leo Kazyat Procedure

A drop of saturated potassium hydroxide (KOH) was added to each sample of blood; plasma, red cells or

urine specimen. The specimen was then tested for alkalinity with Hydrion PH paper. More KOH was added if necessary. Each samples was extracted with chloroform by shaking the extraction vessel for 5 minutes. Then the solvent and aqueous layer were allowed to separate completely. The chloroquine now in the solvent (chloroform) was re-extracted with hydrochloric acid by shaking the extraction vessel for 3 minutes to insure complete recovery of the chloroquine from the solvent. When the aqueous acidic phase has separated and cleared its absorbance was determined spectro photometrically.[37]

Ann Robinson et al Procedure

5ml of blood was suspended in sodium hydroxide solution (20%W/V) and was heated on a boiling water bath. The cool hydrolysates were extracted with chloroform. The chloroform extract was washed free from alkaline and the bases were extracted into hydrochloric acid. Direct extraction of body fluid Specimen 5ml of the specimen was made alkaline with strong ammonia solution and extracted with chloroform. Basic substnaces were recovered by extraction of the chloroform with dilute hydrochloric acid.[38]

Spectrophotometric Assay

It has been observed that the ultraviolet (uv)

spectra of chloroquine base and phosphate salt are similar in O.IN or O.OIN HCl. Absorption maxima are often observed at 343, 328, 256 and 22 nm. Measurements are most favourably made at 343 nm where absorption is most intense and least affected by interferring substances in the biological sample.[32]

The chloroquine base is obtained by ether or chloroform extraction of an alkaline homogenate ofthe biological sample. After the separation of interferring materials, the base is in turn extracted into a solution of O.IN or O.OIN or HCl and quantitatively determined by measuring its UV absorption.

Waaret using spectrophotometric method, found that the concentration of chloroquine phosphate of an adult male 4 hours after the ingestion of 0.5g. was 3.5 µg per 5ml of blood. Grundmann adopting the procedure of Monnet determined the tissue distribution of chloroquine in the rabbit.[29] Various other workers have used the method for determining chloroquine in tissues, blood and urine.[32; 33, 39.40]

Quantitiatively, this technique may have some limitations as applied to biological materials because of the impurity of the extracted samples and

the sensitivity for high concentrations of the material; however it is quite easy to calculate maximum and minimum molar absorptivity values, peak ratio values and mu values. This kind of data provide additional information for the identification of a given drug or chemical family, for example its metabolites. The instrument has the accuracy of determining Ug concentration of the sample.

Spectrofluorimetric Analysis

Flourometric procedures have been extensively utilized for the quantitative determination of chloroquine in biological materials. In the early days when instrument was not sufficiently advanced an additional irradiation step was required to convert the chloroquine to a more intense flourophore. With the advent of the highly sensitive spectrophoto-flaurometers utilizing the xenon arc source and monochromators, the chloroquine fluorescence can be measured directly.

The feasibility and applicability of fluorometry to the analysis of drugs from biological materials has been demonstrated. Brodie et al. found that the fluorescence of chloroquine in PH.9.5 borate buffer after isolation from biological specimen may be stabilized by the addition of

cysteine. The sample is then irradiated with UV light and the measurement made using a suitable fluorometer. The sensitivity of the procedure is about 0.1. microgram (μg) [33, 41, 42, 44].

Gravimetric Analysis

Parikh reported the quantitative estimation of chloroquine as chloroquine-silicatungstate precipitate (ppt). In this method chloroquine phosphate or sulphate in various dosage forms was dissolved in water, heated with concentrated hydrochloric acid and to this mixture was added aqueous solution of silicotungstic acid. The solution was boiled, filtered, washed, dried and weighed. The proposed compound of the precipitate was ($S_1\ O_2$ .12 WO_3. $2(C_{18}\ H_{26}\ N_3\ Cl)$. $2H_2O$). By use of appropriate gravimetric factor the various salts of chloroquine could be determined.[45]

Non-aqueous titration

Chloroquine phosphate can be titrated with acetous 0.IN perchloric ($HClO_4$) acid. The titration may be carried out manually with crystal violet as indicater or determined potentiometrically. The titration is rapid although non-selective. This non specificity is no draw back, so long as good identification tests are adopted.

Wu et al. have reported the determination of eleven antimalarial drugs using this titrimetric procedure in which a powdered sample of 5 milli equivalent weight was dissolved in normal hydrochloric acid and then made alkaline with 6N. NaOH; then shaken with chloroform and one gram of tragacanth. The resulting solution was filtered and was titrated with O.IN acetous $HClO_4$ to blue or green with crystal violet as the indicator. In this case each ml of O.IN $HClO_4$ is equivalent to 25.79mg of chloroquine phosphate.[46]

Chromatographic Analysis

Paper chromatigraphy. This is a very useful aid in the separation and identification of chloroquine. Though the development of thin layer chromatography has provided the advnatages of speed, sensitivity and resoltuion in many cases; paper chromatography has the merit of simplicity and is capable of handling much greater quantities of material. With the aid of paper chromatogram, microgram amounts of chloroquine can easily be detected in extracts of tissues and body fluids. For example Goldbaum et al. used iodoplatinate reagent to visualize the chloroquine which in turn may be eluted

off the paper and the spot quantitated by other methods.[47]

Thin layer Chromatography (TLC). This has become the single most important tool for the detection, identification and routine screening of urine and other biological materials for drugs and their metabolites. The method essentially has allowed for rapid, sensitive detection of compounds and especially permits the use of reagents for detection (that is acids and alkalis) that normally destroy paper.

Many methods utilising TLC were developed over the past few years for the detection of chloroquine and its metabolites from biological materials.[34]

The basic procedure requires that the basic substances from the acid solutions used for assay be recovered into chloroform and the solvent evaporated. The residue are then chromatographed on silica gel plates with chloroform-ethanol ammonia (80:20:1) by volume as the solvent. Potassium iodoplatinate solution is used to detect basic substances on the chromatograms. Chloroquine Rf (o.75-o.80) and the metabolite (Rf o.30) gave brown colour reaction.[48]

Gas Liquid chromatography (GLC). The following procedures have been demonstrated to be applicable to the GLC

examination of chloroquine base. Vanden Henvel and many other workers have all reported the detection of the drug in microgram amounts from biological samples after solvent extraction. Holtzam has also shown that as little as 5 nonogram. (5×10^{-9} g) quantities of chloroquine base could be detected using electron-capture. The conditions reported however are for pure drug but may be of potential value in the analysis of the substance in biological materials. 49,50,51.

Miscellaneous methods of analysis

Ronshdi and Shafik have reported the determination of chloroquine phosphate by titration with anionic surfoctant dioctyl sodium sulfo succinate (Aerosol.O.T) using dimethyl yellow as indicator. They also employed complexometric method using bismuth complexonate to precipitate the chloroquine base followed by titrating the liberated ethylene diamine tetra acetic acid (EDTA) with zinc sulphate.

Various chloroquine phosphate salts have been determined using ammonium reinckate. The insoluble reinckate salt formed at PH 1 is removed and the amount of excess reagent in the filtrate is measured colorimetrically. The difference in absorbance between the sample and blank and the standard and blank represent the absorbances of the sample and standard solutions respectively. 53, 54.

Experimental

Extraction of chloroquine from blood

A general method for the extraction of chloroquine (base, salt and metabolites) from biological materials usually involves adjustment of the PH of the solution (to between PH 8 and 10) using saturated solution of KOH, NH_3 and subsequent extraction with an organic solvent (heptane, ether, chloroform etc). The modified form of Leo Kazyat procedure used in this work is described below.

2mls of blood sample containing some chloroquine was strongly alkalinized using strong ammonia solution (2ml 27% W/W). The specimen was tested for alkalinity with Hydrion PH paper and more ammonia solution was added as necessary. The alkalinized blood sample was then, heated for an hour in a boiling water bath. After cooling, it was extracted with two 20ml portions of chloroform by shaking the separatory funnel for 5 minutes. The solvent and the aqueous phases where allowed to separate completely.

The chloroquine base was re-extracted from the chloroform layer into 4ml 0.1N hydrochloric acid. To ensure complete transfer of the free chloroquine base into the acid, the separatory funnel was shaken for three minutes.

The 0.1N acid containing the free chloroquine base as its chloride salt was transferred to a clean dry quartz cuvette of path length 1cm.

The absorbance of the chloroquine was determined on Unicam Ultra-Violet Recording Spectrophotometer model sp. 8,000.

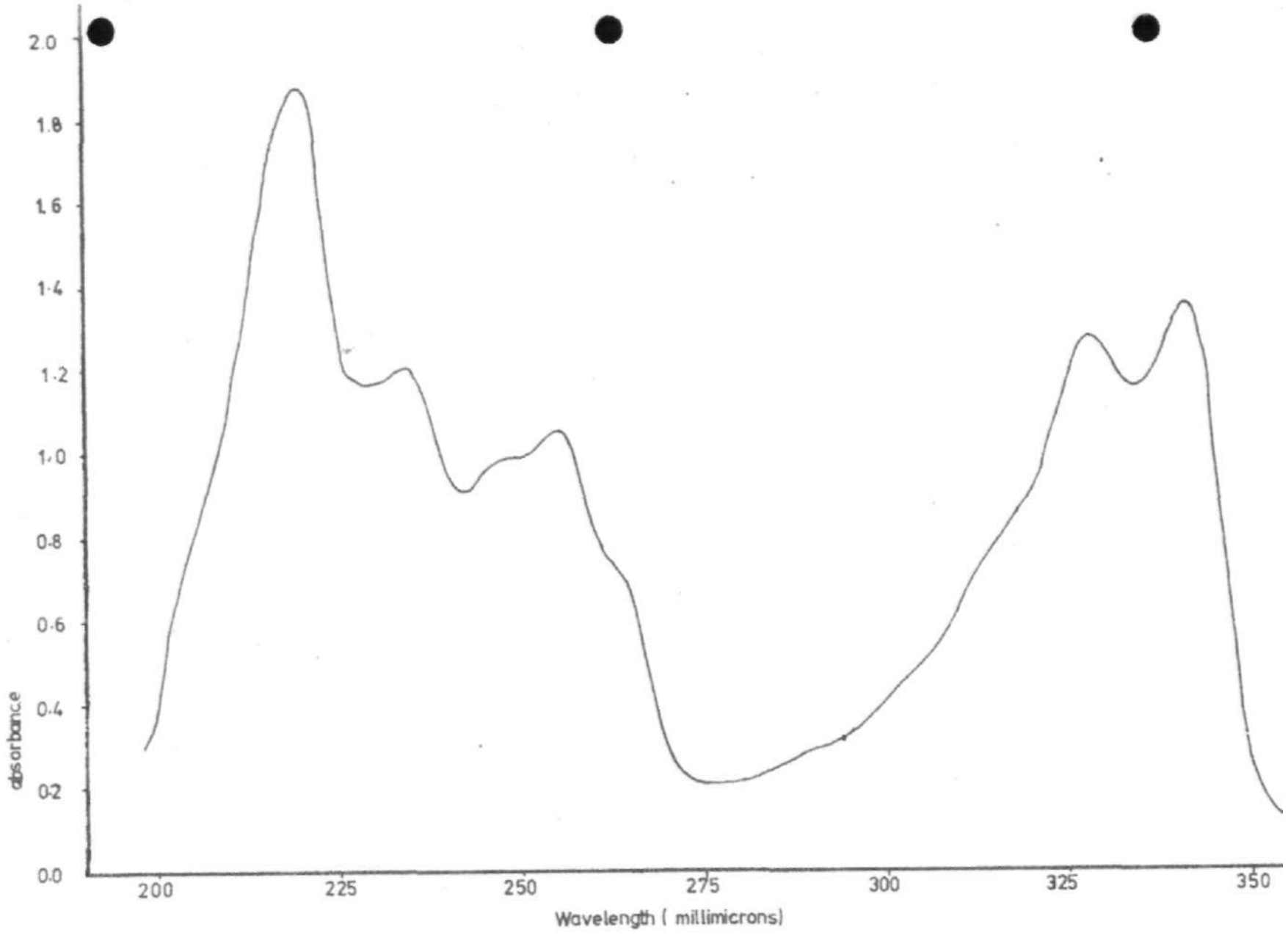
A typical absorption spectrum of chloroquine in 0.IN hydrochloric acid recorded on this instrument is shown in fig. 1.

Preparation of Calibration Curve

Chloroquine stock solution was prepared by dissolving 161.3 (3.13×10^{-4} mmol) of chloroquine phosphate in a litre of 0.IN hydrochloric acid to give 100 mg of free chloroquine base.

From this stock solution; working standard of 0.5 - 20 micrograms per millititre (µg/ml) were prepared by dilution with 0.IN hydrochloric acid. With this a calibration curve relating micrograms free chloroquine base to absorbance was set up using the Unicam Ultra-Violet recording spectrophotometer Model sp. 8,000. The absorbance was read at 343 nanometer (nm).

The data of the absorbance values are as presented in table 1. The absorbance of the standard solutions versus concentration of free chloroquine in µg/ml was ploted.(fig.2)



A typical spectrum of Chloroquine base in 0·1N Hydrochloric Acid

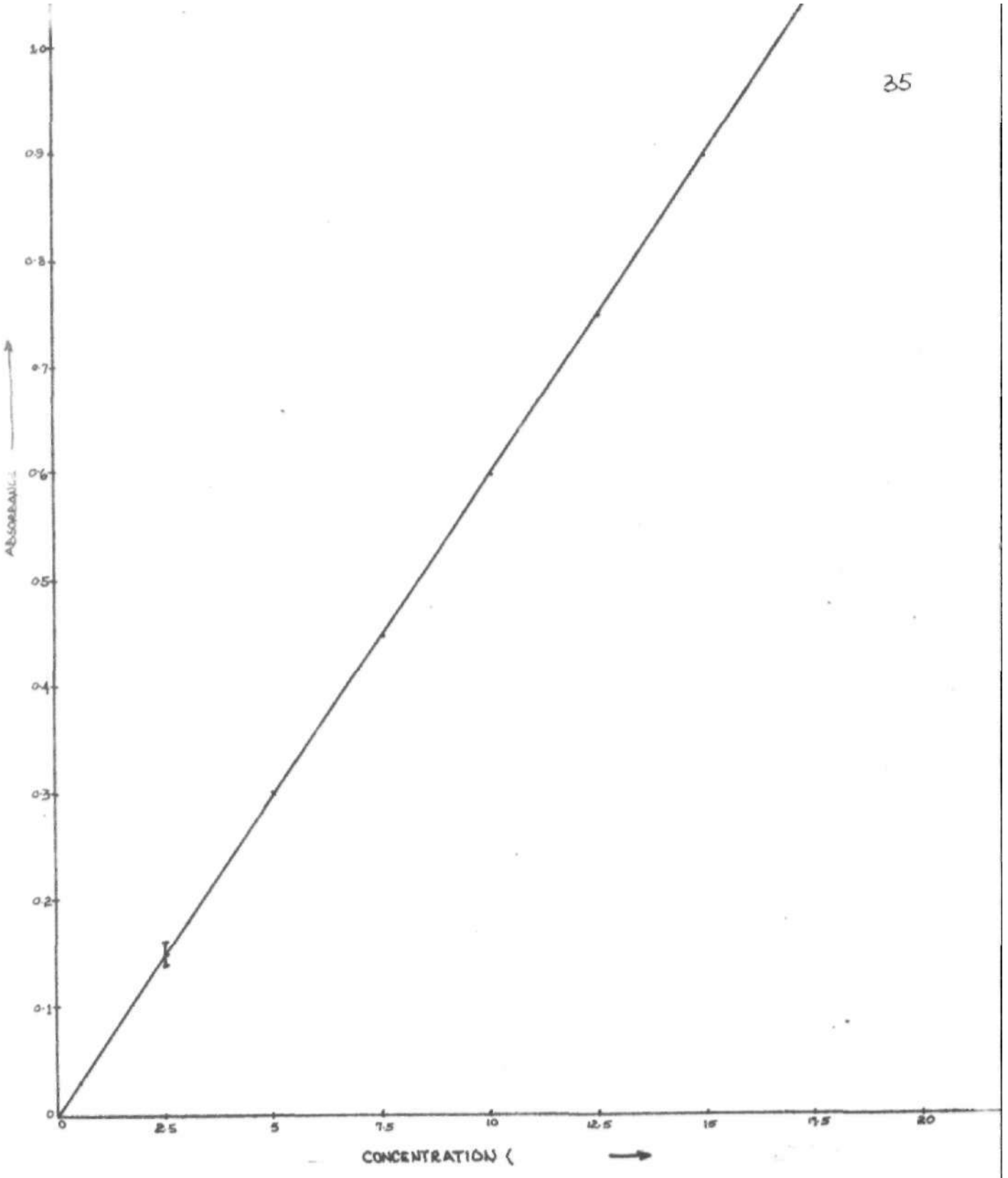
FIG 1

Calibration Curve for free Chloroquine base

Concentration ($\mu g ml^{-1}$)	Absorbance
0.50	0.03±0.00
2.50	0.15±0.003
5.00	0.30±0.002
7.50	0.45±0.002
10.00	0.60±0.003
12.50	0.75±0.03
15.00	0.90±0.004

The values above represent the mean and the standard error of the mean (SEM) for seven sets of readings.

TABLE 1



CALIBRATION CURVE FOR FREE CHLOROQUINE BASE (STANDARD)

FIG 2

Aqueous Extraction of free chloroquine base

Standard stock solution of chloroquine was prepared by dissolving 161.3mg of chloroquine phosphate to give 100mg of pure free chloroquine base in a litre of distilled water.

From this stock solution, working standards with concentration range of 0.5 - 20 µg/ml were passed through extraction procedure as described below.

To 5mls of the working standards were added 3mls of 0.1N sodium hydroxide (NaOH) solution and this was then extracted with successive quantities each of 20mls chloroforms until there was a complete extraction. The combined chloroform extracts were then washed with successive quantities, each of 10mls of water until the washings were no longer alkaline to titan yellow paper. Any chloroquine dissolved in the combined water used for the washings was extracted with 25mls of fresh chloroform and the latter was added to the initial combined chloroform extracts. Finally the chloroquine was extracted from chloroform into 5mls of 0.1N hydrochloric acid.

With the chloroquine now extracted into 0.1N HCl, a calibration curve relating micrograms of free chloroquine base to absorbance at 343 nm was set-up

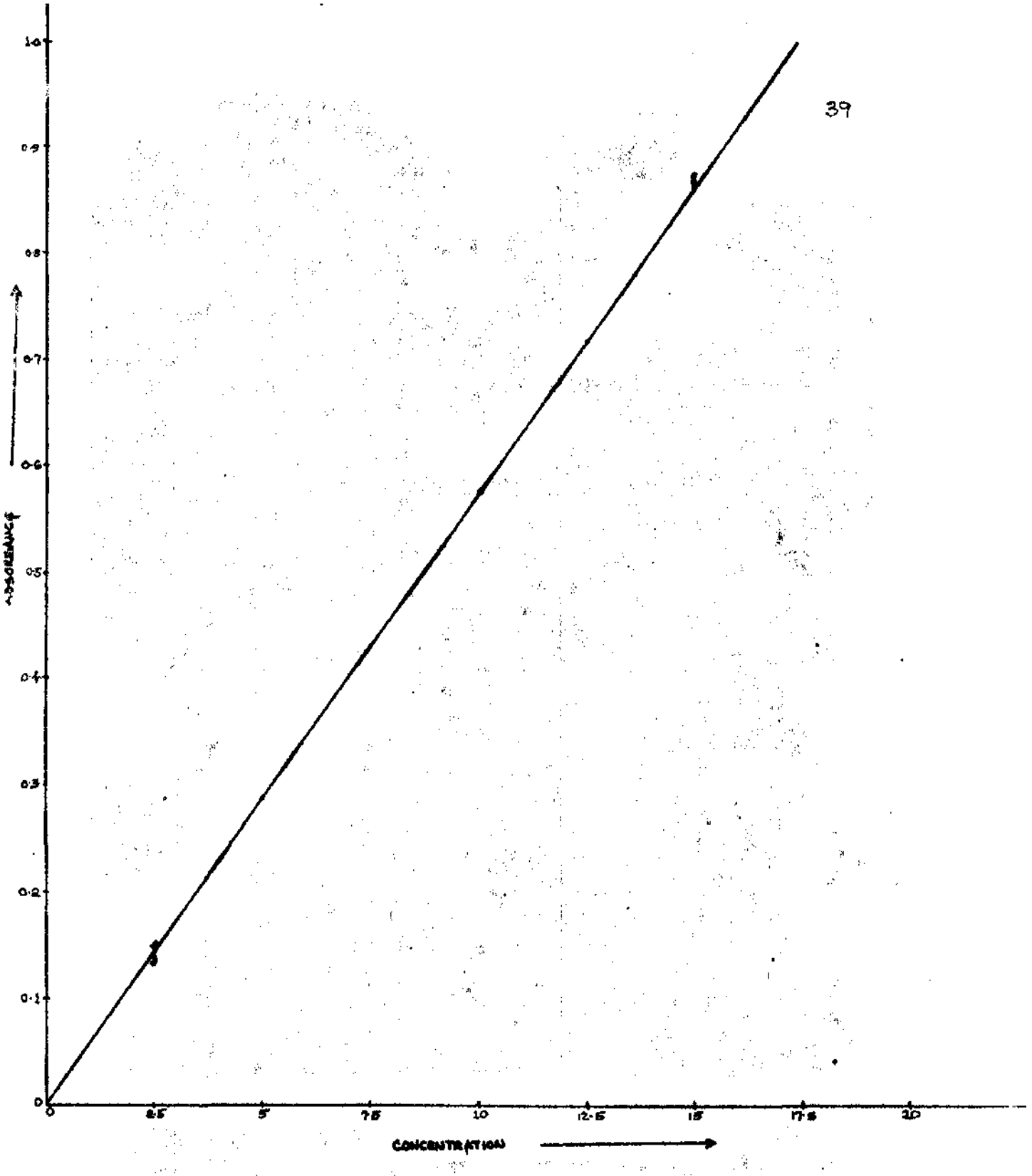
using spectrophotometer sp. 8,000, table 2 and fig. 3

Calibration Curve for aqueous extraction of free chloroquine base

Concentration µg/ml	Absorbance
0.50	0.03±0.00
2.50	0.14±0.008
5.00	0.29±0.001
7.00	0.29±0.001
10.00	0.58±0.003
12.50	0.72±0.002
15.00	0.87±0.005

The values represent the mean and SEM; for seven sets of experimental readings.

TABLE II



CALIBRATION CURVE FOR FREE CHLOROQUINE BASE EXTRACTED FROM AN AQUEOUS SOLUTION

FIG 5

Extraction of chloroquine from blood

161.3mg of chloroquine phosphate (100mg of chloroquine base) was added to 100mls of blood. This was thoroughly agitated for five minutes. 10mls of this blood sample was then passed through the extraction procedure described on page ...31..... The extracted chloroquine was recovered from chloroform into 0.1N hydrochloric acid in a 100ml volumetric flask and the volume was made to the mark.

From this stock solution (of 100 µg/ml) working standards with concentration range from 0.5-20 µg/ml were prepared by dilution with 0.1N HC1. With this a calibration curve relating concentration of free chloroquine base to absorbance was set-up spectrophotometrically (table 3 and fig. 4).

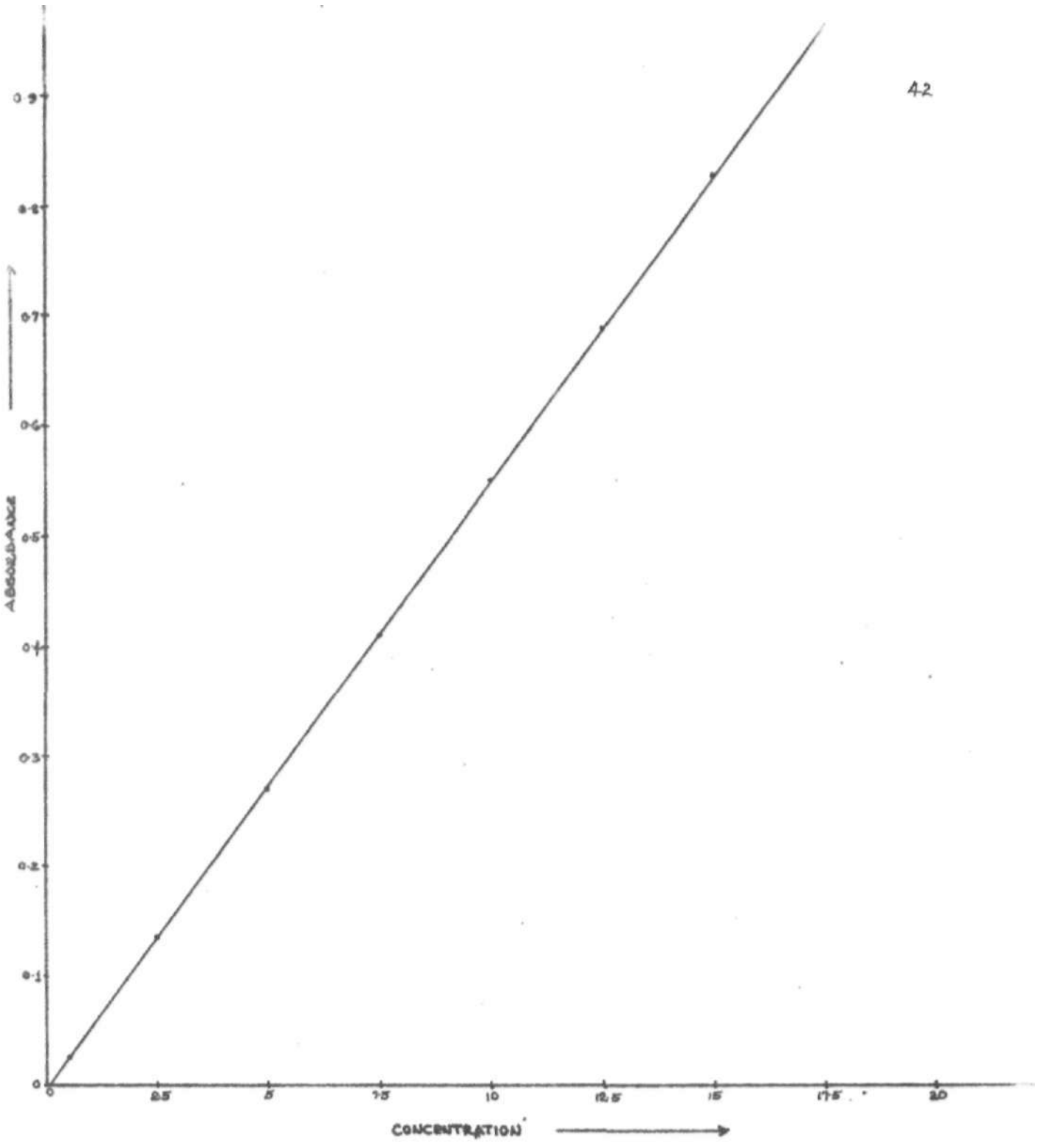
The calibration curves for the free chloroquine base and chloroquine following aqueous and blood extracts are shown together in Fig. 5.

Calibration curve for free chloroquine base extracted from base

Concentration µg/ml	Absorbance
0.50	0.028±0.001
2.50	0.136±0.003
5.00	0.270±0.001
7.50	0.410±0.009
10.00	0.55± 0. 12
12.50	0.69+0.019
15.00	0.83±0. 02
17.50	0.97±0.013
20.00	0.98±0.015

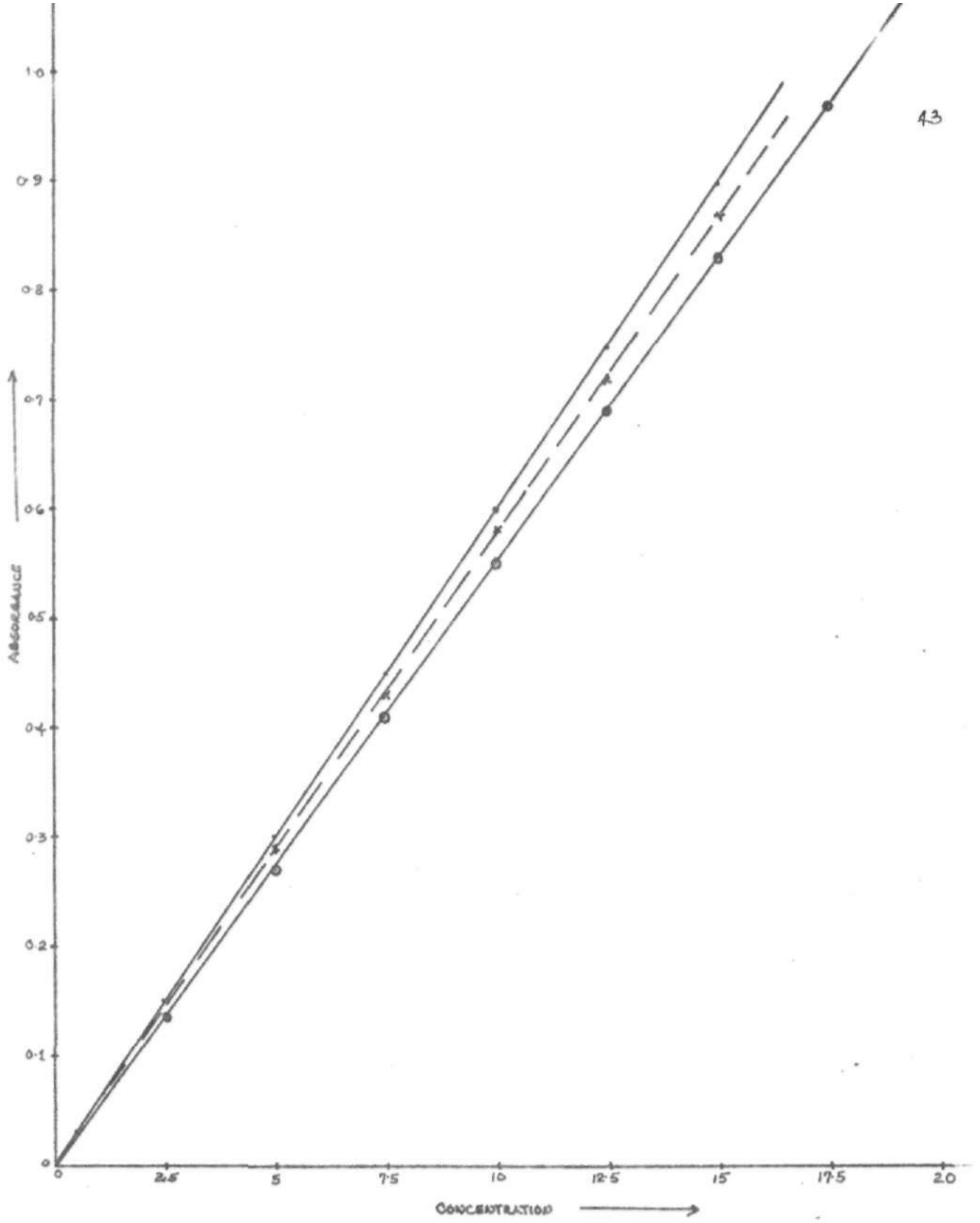
The values represent the mean and SEM. for nine sets of experimental readings.

TABLE III



CALIBRATION CURVE FOR FREE CHLOROQUINE BASE EXTRACTED FROM BLOOD

FIG. 4



COMPARISON OF CALIBRATION CURVES FOR : FREE CHLOROQUINE BASE (STANDARD) (—●—●—)

CHLOROQUINE FROM AQUEOUS EXTRACT (—×—×—)

CHLOROQUINE FROM BLOOD EXTRACT (—●—●—)

FIG. 5

CHAPTER III

ANIMAL STUDIES

In this present study, 18 albino white rabbits weighing 1.97±0.58kg were obtained from the animal house attached to the Faculty of Pharmaceutical Sciences and from out breed stations. After arrival at the laboratory, the rabbits were freely fed a nutritionally complete diet obtained from livestock feeds limited Kaduna.

The Crystalline Chloroquine phosphate, obtained from the Pharmaceutical and Quality Control Unit; Institute of Health, Ahmadu Bello University, Zaria, was used in this study.

All weighings were carried out on Gallenkamp mettler model H51 AR. The grinding of the animal feeds and dried vegetable leaves were carried out using Kenwood Chopper. The mixing of the Vegetable and feed powders were done on Erweka AR 400.

The absorbance of the pure chloroquine; the aqueous and blood extracts were recorded spectrophotometrically over the range 220-360nm. The chloroquine concentration was determined on the basis of the absorption at 343nm.

Treatment of the Feed

The feeds on arrival in the laboratory were grinded into fine powders using a mechanical grinder.

Bitter leaf

Bitterleaf shrubs were freely grown around the staff quarters at the Ahmadu Bello University Campus. This vegetable received no treatment with insecticides and other sprays. The wet leaves were allowed to dry at room temperature in the laboratory. They were grinded in a Kenwood Chopper and sieved to obtained fine powder. The animals were fed their normal diet containing 25% bitterleaf for seven days prior to dosing with chloroquine. During this period they had free access to water.

Spinach.

The Spinach was purchased in bulk from a local farmer along Zaria-Jos road, where it received no treatment with insecticides and other sprays. The leaves were subjected to similar treatments as the bitter leaf above.

Administration of Chloroquine to the Rabbit

A single dose of chloroquine was calculated on the basis of 50mg chloroquine phosphate per kilogram weight of the animal.[20] The weighed chloroquine was

dissolved in 5ml of water and transferred into the syringe. The drug was then administered to the rabbits using a stomach tube. The container was further rinsed twice with 3ml each of water and this was transferred into the syringe and the dosing was repeated. A double dose was given initially to each rabbit but a single dose each was administered 6 and 24 hours later.

Bleeding commenced immediately following the administration of the twenty fourth hour dose. The first 2mls blood sample collected using heparinized syringe, represented the zero hour sample. 2mls blood samples each were collected at 1,2,3,4,5,6 and 8 hours following the initial sample.

Blood Level Studies

The techniques of marginal earvein puncture and incision of the marginal earvein or of the central artery of the ear were employed at obtaining blood samples in rabbits.[57]

To obtain the blood clot free; the syringes used were each wetted with a drop of heparin. The blood samples were later stored in a deep freezer for at least five days.[55]

Assay of the blood Samples Obtained

The frozen, heparinized blood samples were hemolyzed with 2mls of distilled water. They were strongly alkalinized with strong ammonia solution. They were heated for an hour in a water bath to release protein bound chloroquine. After cooling the chloroquine content was assayed as described in chapter II. The absorbance of each sample was determined spectrophotometrically and by interpolation the concentration of chloroquine in µg/ml was determined after correction for dilution.

A graph of average blood chloroquine (µg/ml) versus time (in hours) in seven rabbits fed on normal diet was plotted, table IV and Fig. 6.

In a similar manner blood samples of animals fed on bitter leaf were taken and assayed and the results are presented in table V and Fig. 7. Blood samples of rabbits fed on spinach were treated in a similar manner and the results obtained are presented in table VI and Fig. 8.

CHAPTER IV

RESULTS AND DISCUSSION

It will be noted in chapter III that blood level studies were carried out for three different groups of rabbits. These groups are the rabbits feeding on normal diet (the control) and those fed on similar feed to which either bitterleaf or spinach has been added.

Results

The results obtained on determining the blood level of chloroquine in the three groups of rabbits after oral administration are presented in tables IV, V and VI. These results are for the samples taken after the twenty fourth hour dose as described in chapter III. Since all the blood levels of chloroquine were above 4µg/ml, the [illegible] was started at 4 µg/ml on the Y-axis.

In all of the three different groups of rabbits particular attention was paid to the initial blood level immediately following the last dose, the time the peak value was achieved and the magnitude of this peak value. Attention was also focussed on the disappearance of the chloroquine from the time of peak to the time

of the last observation. The total amount of chloroquine reaching the blood was determined by measuring the area under each curve using a planometer.

Rabbits fed on normal diet (the Control)

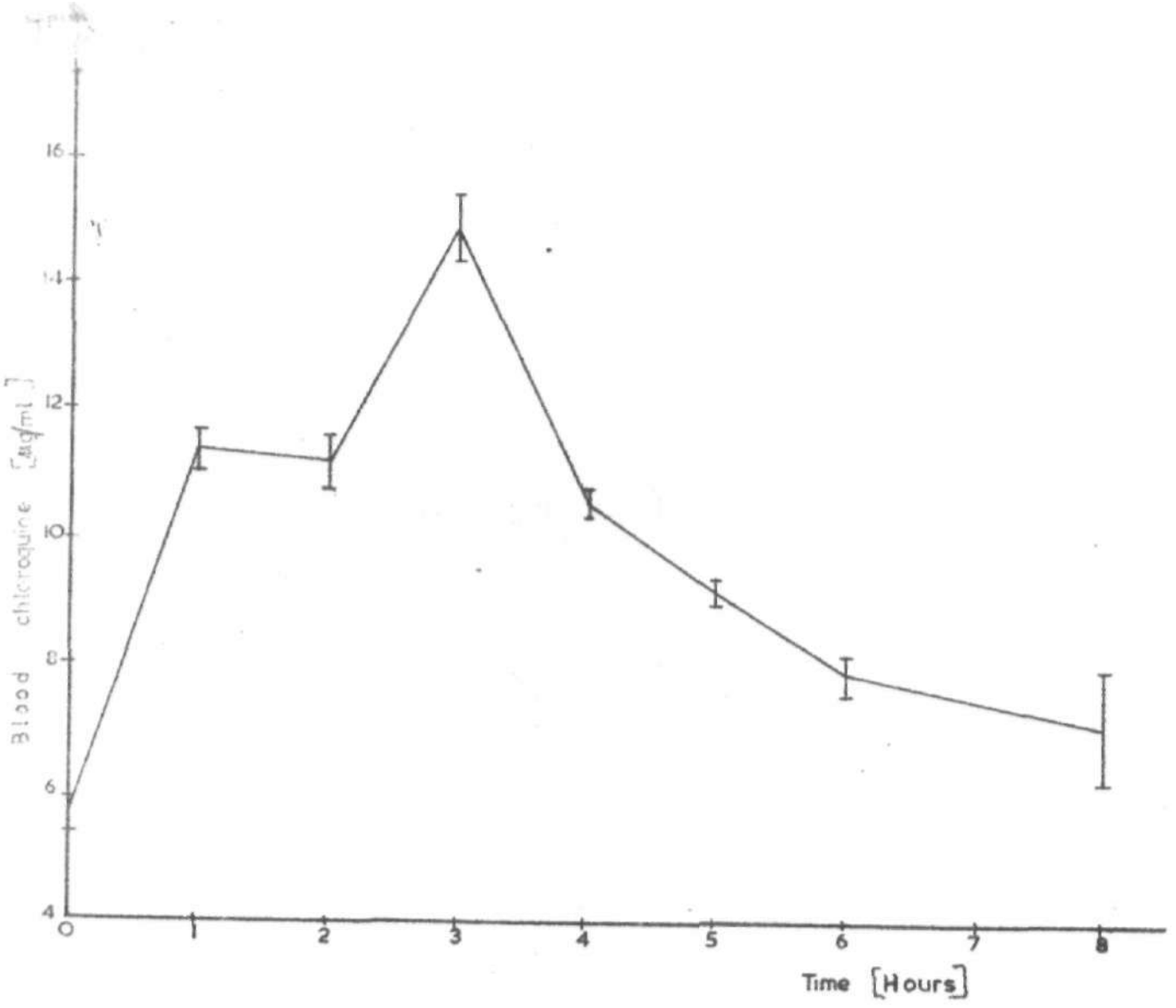
The chloroquine blood level following oral administration in seven rabbits is presented in table IV. There was a gradual increase from the zero hour blood level up to 3 hours. The highest peak value (14.9±0.50 µg/ml) was recorded at 3 hours (Fig. 6).

There was a gradual decrease after 3 hours through the eight hour of the experiment. In fact at 8 hours the blood level value was almost the same as the initial blood level. The area under the curve was found to be 272.07±8.6 cm^2

Average blood chloroquine in seven rabbits fed on Normal diet

Time (Hours)	Concentration µg/ml
0	5.70±0.25
1	11.36±0.30
2	11.22±0.49
3	14.90±0.50
4	10.64±0.22
5	9.19±0.25
6	7.90±0.38
8	7.11±0.88

TABLE IV



Average bloodlevels of chloroquine in seven rabbits fed on normal diet.

FIG.6

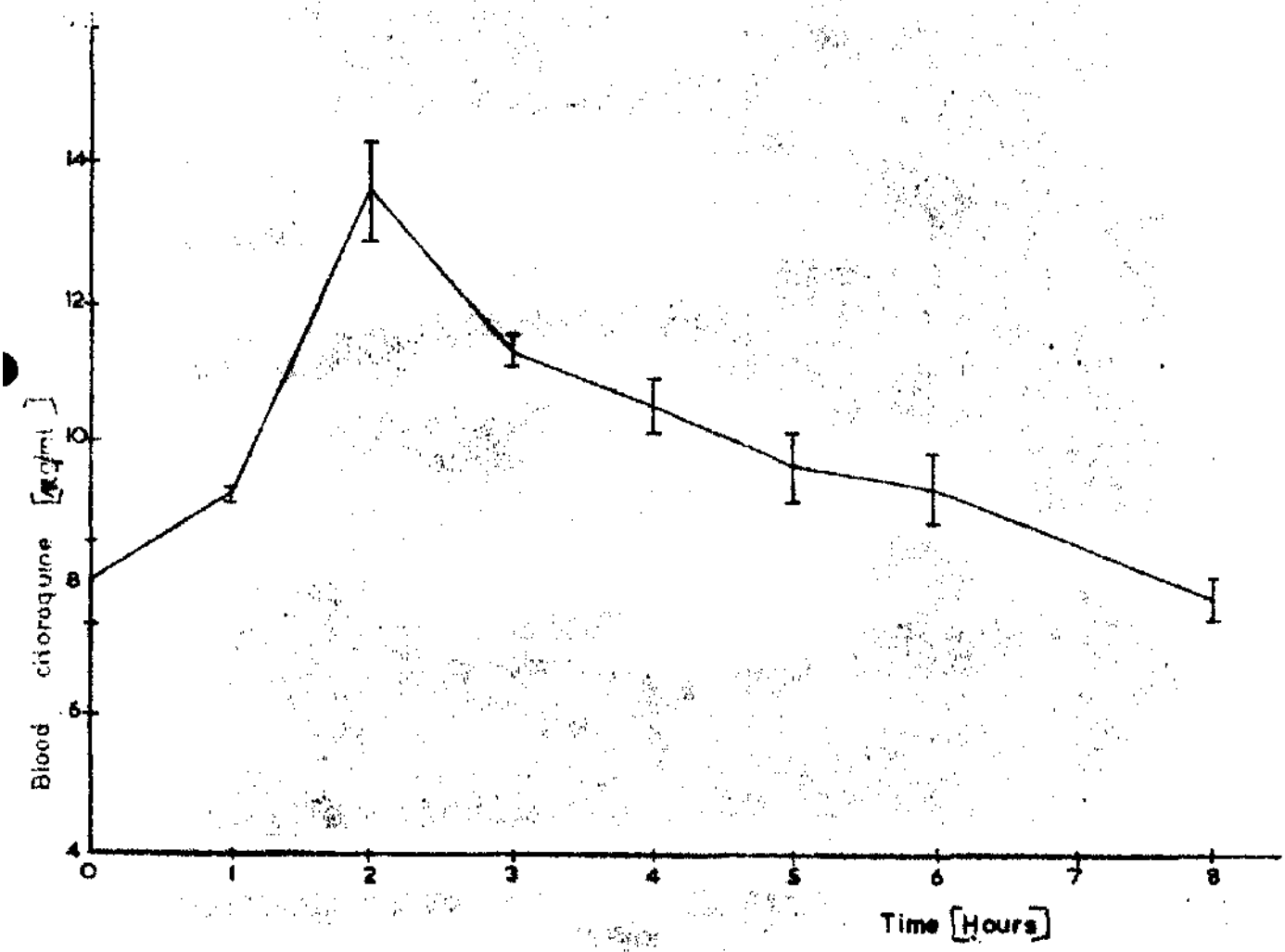
Rabbits fed on bitter leaf diet

The results obtained in six rabbits fed on a diet containing 25% mixture bitter leaf are presented in table V and Fig. 7. There was a rather sharp increase from the zero hour blood level to the Peak at 2 hours (13.61 ± 0.75 µg/ml). However a gradual decrease was observed from 2 hours through 8 hours when the experiment was over. At 8 hours after the last dose the chloroquine level had already returned to the initial blood level. The area under the curve was found to be 214.80 ± 21.4 cm^2.

Average Blood Chloroquine Level in Six rabbits fed on normal diet containing bitter-leaf

Time (Hours)	Concentration µg/ml
0	7.93 ± 0.67
1	9.20 ± 0.14
2	13.61 ± 0.75
3	11.30 ± 0.25
4	10.52 ± 0.41
5	9.60 ± 0.50
6	9.30 ± 0.56
8	7.70 ± 0.30

TABLE V

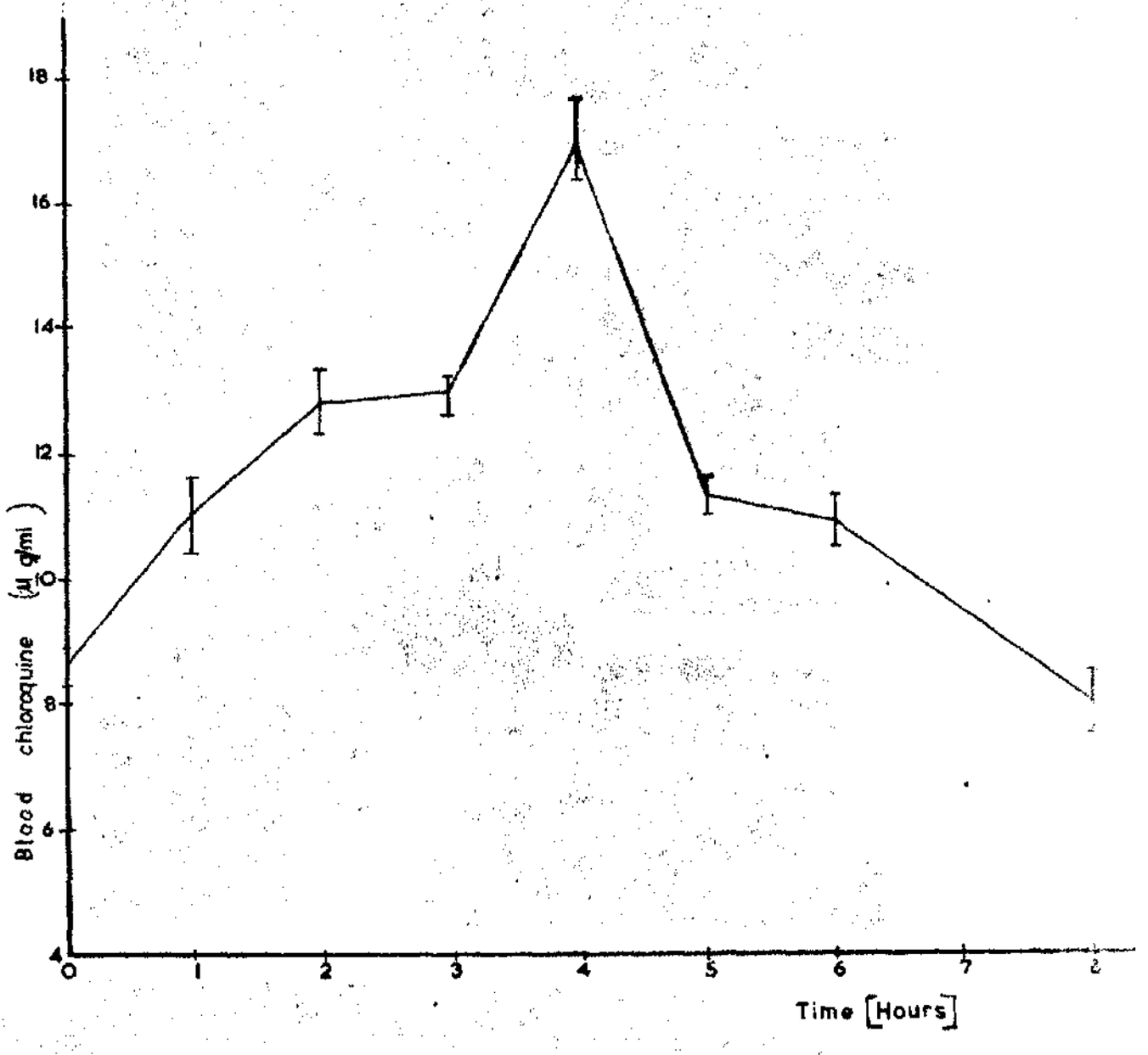


Average bloodlevel of chloroquine in six rabbits fed on a normal diet contai
bitter leaf.

FIG. 7

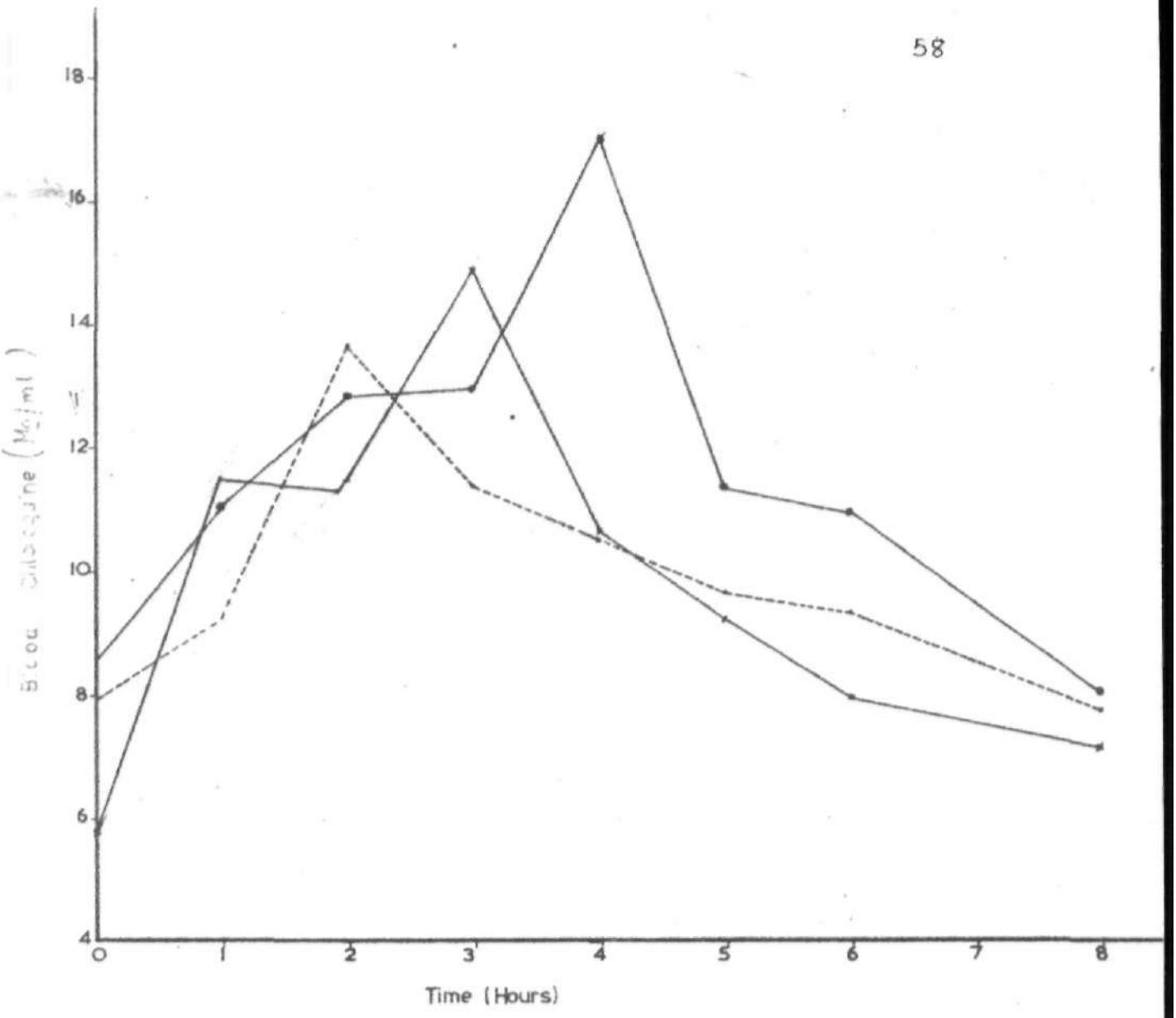
Rabbits fed on Spinach diet

The chloroquine blood level in five animals fed on a diet containing 25% spinach is presented in table VI. There was a gradual increase in the chloroquine blood level from zero to 2 hours. The blood level remained unchanged through the third hour but this was followed by a rather sharp rise to the peak at 4 hours. (16.9 + 0.60 μg/ml; Fig. 8). Unlike the gradual increase from zero to 2 hours a sharp decrease in the chloroquine blood level was observed between 4 and 5 hours followed by a gradual decrease from 5 through 8 hours. The blood level at 8 hours after the last dose had returned to a level a little lower than the initial blood level. The area under the curve was found to be 288.80 ± 31.7 $cm.^2$



Average bloodlevel of chloroquine in five rabbits fed on normal diet containing spinach

FIG-8



Mean amount of Chloroquine extracted from blood of rabbits fed on normal diet (*——*) bitter leaf (•– – – –•) and Spinach (•——•)

FIG. 9

Discussion

In order to fully appreciate the significance of the different results obtained in the three different groups of rabbits, the curves for these three groups are shown together in Fig. 9.

Initial blood level

This represents the blood level which was maintained by the doses administered prior to the booster dose. The zero hour blood level for the rabbits feeding solely on normal diet was found to be lower than in those feeding on bitter leaf and spinach ($P < 0.01$; $P < 0.025$) respectively). However there was no significant difference in the initial blood levels between the two groups of animals feeding on either vegetable. It appears therefore that in the presence of bitter leaf and spinach these two groups of animals maintain higher blood levels of chloroquine than the control.

Time and magnitude of Peak

Time of Peak. In rabbits feeding on a normal diet, the peak was at 3 hours while the peak in those animals fed no bitter leaf diet was at 2 hours (an hour earlier than in the control). For those rabbits fed on spinach diet the peak was at 4 hours - an hour delay when compared to the time of peak in the control.

The early peak in rabbits fed on bitter leaf compared to the control could not be easily explained. It may due in part to decrease gastric emptying time and rapid movement through the gastro-intestinal tract.

The delay at attaining the peak in those rabbits fed on spinach when compared to the control may be due to increase in stomach emptying time.

Magnitude of Peak. The values of the magnitude of peak are presented in tables IV, V and VI for the three groups of rabbits. From a casual observation at fig. 9 it would appear as if rabbits fed on spinach diet have a higher peak value compared to those fed on normal diet while rabbits fed on bitter leaf have the lowest value. However there is no significant difference between the peak blood level of chloroquine for the rabbits feeding on normal diet and bitter leaf on one hand; and the control and those fed on spinach on the other hand. Nevertheless the rabbits fed on spinach have higher peak levels than those fed on bitter leaf ($P < 0.05$).

From the foregoing the various peaks at 2,3 and 4 hours represent the peak for the groups of rabbits fed on bitter leaf, the normal diet only and spinach respectively. It will therefore be interesting to see how the peak value for one group of animals compares with the corresponding blood levels of the other two groups at the same hour.

The values at 2 hours for the rabbits fed on bitter leaf (the peak blood level) was found to be higher than the value for the group fed on normal diet ($P < 0.025$).

However this value at 2 hours was not significantly different from those fed on spinach (Fig. 9). This thus indicates that animals fed on spinach maintained an equally high level of chloroquine at 2 hours.

The blood level for animals fed on normal diet at 3 hours (the peak value) was higher than the value for those animals fed on bitter leaf ($P < 0.005$) but not significantly different from the blood level of rabbits fed on spinach, diet maintained equally high blood levels at this hour.

The blood level for rabbits fed on spinach at 4 hours (the peak value) was higher than for those fed on bitter leaf and the normal diet. ($P < 0.005$, $P < 0.05$ respectively). Thus rabbits feeding on spinach maintained a high blood level of chloroquine from zero through 4 hours. The blood levels in the cases of rabbits feeding on normal diet and those feeding also on bitter leaf are comparatively not consistent.

Overall Chloroquine intake.

Having found that there is no significant difference between the peaks for the group of rabbits fed on normal diet and those fed on bitter leaf on one hand and those fed on normal diet and the group on spinach diet on the other; it was therefore thought necessary to determine the total amount of chloroquine that has passed into the blood stream. This is

important as it would give some information on the influence of these vegetables on the absorption of chloroquine from the gastro-intestinal tract.

The area under the curve in the group of rabbits fed on normal diet was found to be higher than that of the group fed on bitter leaf ($P < .0.05$). Also the area under the curve of the group fed on spinach was higher than that of the control ($P < .0.025$).

It is interesting to note that at the eight hour when the last observation was made; the blood level values obtained for each group were not significantly different from each other and were almost the same with their corresponding initial levels.

Conclusion

It was noticed that the presence of bitter leaf in the diet of rabbits has resulted in an early peak of chloroquine blood level. However this is not significantly different in magnitude when compared with the animals fed on normal diet (peak at 3 hours). The total amount of chloroquine passing into the blood stream during the period of observation was lower in the animals fed on bitter leaf than in the control.

With respect to the rabbits fed on spinach there was an hour delay in the peak compared with the control. The peak was not significantly different from that of the control;

nevertheless the total amount of chloroquine reaching the blood was higher than in the control. Another important observation was that even though the peak blood level of chloroquine was achieved earlier in both the control and the animals fed on bitter leaf, rabbits fed on spinach maintained equally high levels at these various hours. Therefore a high chloroquine blood level was maintained for a longer period in the animals fed on spinach. In terms of maintaining high chloroquine blood level, spinach diet appears to be superior to normal feed and more so to bitter leaf.

From the present study one can not say whether a change in dosage of chloroquine will be necessary as a result of diet of bitter leaf or spinach. However the influence of these vegetables on the blood levels of orally administered chloroquine in the rabbits has triggered off further research in this direction and I am glad to note that further work by other researchers in this area is already in progress.

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AUTOBIOGRAPHICAL SKETCH

Jerome Tola Ayodele was born at Ikerin-Opin, Irepodun LGA Kwara State of Nigeria into a middle class Christian family on July 15, 1952.

He received his early education at Idera Community School, Idera-Opin 1960-1962; Saint Brigid's Catholic School, Osi 1963-1965; Saint John's College Kaduna 1966-1972. He obtained his West Africa School Certificate (WASC) in December 1970 and Higher School Certificate (HSC) in December, 1972.

He later taught General Science to form II and IV students for eight months (January - August 1973) at Okene Teachers College Okene. In the same year he proceeded to Ahmadu Bello University Zaria where he graduated in Chemistry (Second Class Lower Division) in June 1976. Immediately after the graduation, he went on National Youths Service Corps and was deployed to Ogun State (NYSC/ABU 1976/2905). During his primary assignment, he was posted to Abeokuta Girls Grammar School where he taught Forms III, IV and V Chemistry. His secondary assignment during the service was

two-fold; building a post-office at Orile Ilugun and the last part of the assignment was in the construction of a 5 Kilometre road linking a small village to the Abeokuta-Ibadan road.

In the fall of the year 1977 Tola enrolled for his Master of Science degree in analytical Chemistry. During this period he was on a part-time employment of the Department of Chemistry as a demonstrator to the undergraduate students particularly the Veterinary Medicine, B.Sc. III Combined and B.Sc. III Biochemistry honours students.

Tola is a participant in many social, cultural and religious activities. During his college days he was a treasurer of the Legion of Mary Society (1969-1970); and President (1971-1972). He was also a member of the school choir, photographic club; Red Cross Society etc. He is a foundation member and PRO of Egbe Basiri Society Ikerin - Opin; member and Publicity Secretary of Odu-Ofa Klub etc. His interests include reading, listening to indigenous and foreign lyrics and Photographing.

Tola is a Keen-Footballer and Lawn Tennis player. He's married with two children.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality as a thesis for the degree of Master of Science (Analytical Chemistry).

J. Ayoola Owoyale
Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate in scope and quality as a thesis for the degree of Master of Science (Analytical Chemistry).

Kilpral Singh
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This thesis was submitted to the Faculty of Science, Department of Chemistry, Ahmadu Bello University, Zaria, and was accepted as partial fulfillment of the requirements for the degree of Master of Science (Analytical Chemistry).

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