INFLUENCE OF MEALS ON THE BIOAVAILABILITY AND PHARMACOKINETIC PROFILES OF CHLORPROPAMIDE IN MAN

BY

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D E C L A R A T I O N

I hereby declare that the work reported in this thesis was carried out by me under the supervision of Dr. Abdullahi Mustapha, the Head of the Department of Pharmaceutical and Medicinal Chemistry and Dr. Ibrahim Abdu-Aguye the Head of the Department of Pharmacology and Clinical Pharmacy.

It has not been presented in any previous application for higher degree. The works of other investigators are referred to and acknowledged accordingly.

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CERTIFICATION

This thesis entitled **INFLUENCE OF MEALS ON THE BIOAVAILABILITY AND PHARMACOKINETIC PROFILES OF CHLORPROPAMIDE IN MAN** by **MOJIRADE TAIBAT BAKARE** meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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D E D I C A T I O N

Dedicated to My Late

Father Hadji Yusuf Bakare

And

My Late Sister

DR.(Mrs) Atinuke Zikhrat Oyelola

May their souls rest in perfect peace - Amen.

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ABSTRACT

The effects of three different Nigerian meals on the bioavailability and pharmacokinetic profiles of chlorpropamide were studied in healthy subjects and in freshly diagnosed Type II diabetic patients. The batch of chlorpropamide tablets and the meals used were pre—analyzed using official methods- The tablets complied with the B.P. (1988) requirements stated under the monograph for chlorpropamide and the meals contained high carbohydrate contents of 80.5%, 76.0% and 84.5% for maize, cassava and yam flour meals respectively.

Plasma levels of chlorpropamide were determined using HPLC method. In addition, glucose levels were measured by a standard glucose oxidase method (Martinek, 1964). Higher levels of chlorpropamide were observed when the drug was administered in fasting state compared with concomitant administration of drug with maize, and cassava flour meals. Concomitant administration of the drug with yam flour meal however increased chlorpropamide levels in healthy subjects and had no effect on chlorpropamide levels in diabetic patients. Significantly lowered postprandial plasma glucose increments were observed when 250mg chlorpropamide tablet was taken with each meal than when each meal was taken alone, and the glucose levels were even lowered when the drug was taken 30min prior to meal ingestion compared with

when the drug was taken concomitantly with each meal. No significant postprandial plasma glucose increments were observed in healthy subjects.

The pharmacokinetic parameters were calculated by a computer programme KINEMP[1] Administration of chlorpropamide concomitantly with each meal compared with the fasting state affected some absorption parameters (lagtime, tmax, Cmax, ka and tl/2x), the extent of absorption, volume of distribution and the elimination parameters calculated in this study were not significantly affected by any of the meals. Some pharmacokinetic parameters (lagtime, Cmax, tmax, ka, tl/2x and Vd) calculated for the healthy subjects and diabetic patients were significantly different. There was a relationship between the hypoglycaemic responses and chlorpropamide levels attained after administration of the drug to healthy volunteers but no direct relationship was found for the patients. Lower and statistically significant levels of chlorpropamide were detected in saliva compared with the plasma. The saliva and plasma (S/P) ratio was less than unity at each sampling point and there was a correlation between the saliva and plasma concentratons of chlorpropa-mide measured.

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ABBREVIATIONS

B.P.	=	British Pharmacopoeia
^{o}C	=	Degree Centigrade (Celsius)
g	=	Gram
L	=	Litre
MCG	=	Microgramme
UG	=	Microgramme
Mg	=	Milligrame
ML	=	Millilitre
M.Mol.	=	Millimole
Min	=	Minute
h	=	Hour
HCl	=	Hydrochloric Acid
HNO_3	=	Nitric acid
NaOH	=	Sodium Hydroxide
G.L.C.	=	Gas Liquid Chromatography
H.P.L.C.	=	High Performance Liquid Chromatography
U.S.P.	=	United States Pharmacopoeia
pH	=	Hydrogen -ion Concentration
U.V.	=	Ultra Violet

CHAPTER ONE

INTRODUCTION

1.1 Pharmacokinetics

Pharmacokinetics is the study of the rate processes associated with absorption, distribution and elimination of a drug, i.e. quantitating drugs and or metabolite levels in body fluids, tissues and excreta at any point in time from the moment of administration until elimination from the body is complete (Kaplan and Jack, 1980). Clinical pharmacokinetics is a health sciences discipline which deals with the application of pharmacokinetics to the safe and effective therapeutic management of the individual patient (Sadee and Beelen, 1980).

Absorption is the process by which a drug proceeds from the site of administration to the site of measurement within the body. There are several sites at which drugs are commonly administered. These sites may be classified as either intravascular or extravascular. Intravascular administration refers to the placement of a drug directly into the blood, either intravenously or intra-arterially. Extravascular modes of administration include the oral, sublingual, buccal, intramuscular, subcutaneous, and rectal routes. To enter the

blood, drug administered extravascularly must be absorbed: no absorption step is required when a drug is administered intravascularly (Rowland and Tozer, 1980a).

Distribution is the process of reversible transfer of a drug to and from the site of measurement. Once absorbed, a drug is distributed to the various tissues of the body. The rate and extent of distribution are determined by how well each tissue is perfused with blood, the binding of drug to plasma proteins and to tissue components, and the permeability of tissue membranes of the drug (Rowland and Tozer, 1980a).

Elimination is the irreversible loss of drug from the site of measurement. Elimination occurs by two processes, metabolism and excretion. Metabolism is the conversion of one chemical specie to another. Excretion is the reversible loss of the chemically unchanged drug. The two principal organs of elimination, are liver and kidney. The kidney is the primary site for excretion of the chemically unaltered, or unchanged drug. The liver is the usual organ of drug metabolism; however, the kidney and other organs can also play an important metabolic role for certain drugs (Rowland and Tozer, 1980a).

The primary standard of a pharmacokinetic study is based upon the data obtained following intravenous administration. Since the entire dose administered is placed directly into the blood stream following intravenous administration, the pharmacokinetic parameters obtained are unaffected by all the potential rate-limiting and metabolic factors associated with drug absorption following other routes.

In pharmacokinetics, attempts are made to describe a biological event in mathematical terms, usually by developing mathematical models to represent the body as a system of compartments, even though these compartments often have no apparent physiologic or anatomic reality. The one compartment model, the simplest model depicts the body as a single homogeneous unit. This model is particularly useful for the pharmacokinetic analysis of blood, plasma or serum concentrations, salivary and urinary excretion data for drugs which rapidly distribute between plasma and other body fluids and tissues upon entry into the systemic circulation.

To assume the body behaves as a one-compartment model does not necessarily mean that the drug concentrations in all body tissues at any given time are the same. However, a one-compartment model (Fig. 1a) does assume that any changes that occur in the plasma quan-

titatively reflects changes occurring in tissue drug levels. A second criterion for utilizing a one-compartment model is that drug elimination occurs from the body in a first-order fashion (i.e., the rate of elimination of drug from the body at any time is proportional to the amount of drug in the body at that time). The proportinality constant relating the rate and amount is the first-order elimination rate constant which has units of reciprocal time (for example, min^{-1}, hr^{-1}) (Gibaldi and Perrier, 1975a).

Half-life is one of the most useful pharmacokinetic parameters obtained following drug administration. The half-life of a drug is the time required to reduce the post-absorption concentration of a drug in the blood stream by fifty percent. It is calculated by dividing 0.693 by the calculated scope of each exponential phase of multiphasic blood level curve (Wagner, 1980).

Another pharmacokinetic parameter in frequent use is the area under the curve of blood level versus time. The methods of calculating this parameter are discussed by various authors (Koup and Gibaldi, 1980; Wagner, 1980).

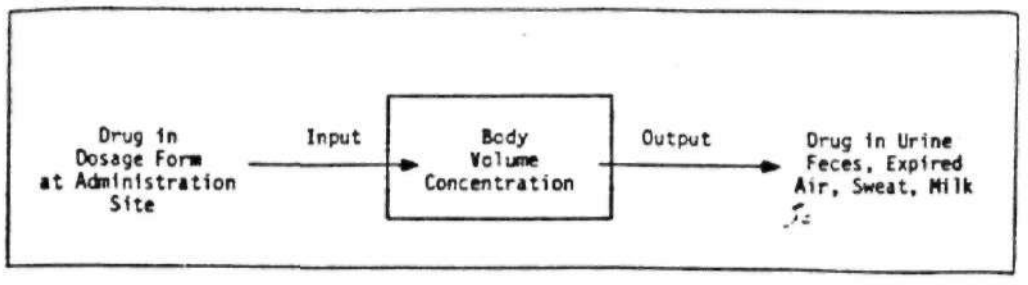


Fig. 1.a Concept of open one - compartment model (from Ritschel, 1980).

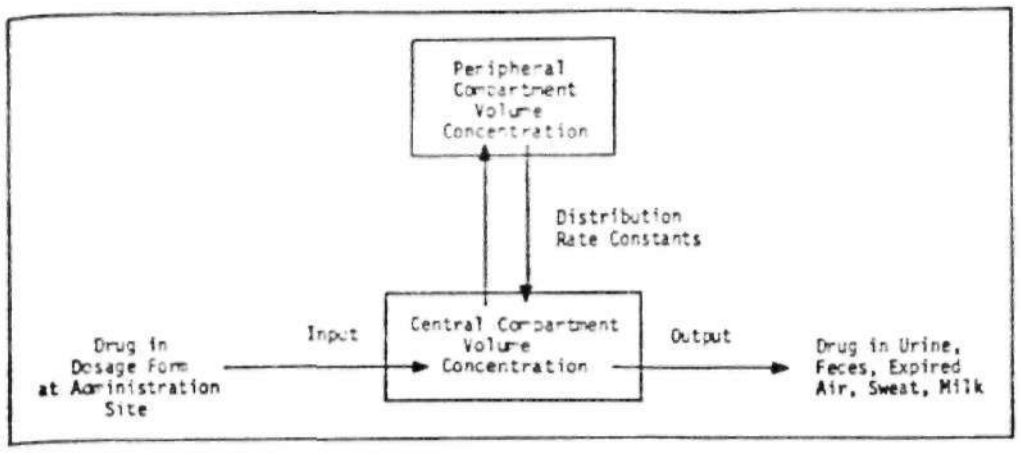


Fig. 1.b Concept of open - two compartment model (from Ritschel, 1980).

The volume of distribution is used to relate the plasma concentration to the amount of drug in the body during the elimination phase. It is calculated by dividing the total amount of drug in the body by the plasma concentration. Total body clearance is a parameter used to quantify drug elimination. Experimentally, total body clearance is calculated by dividing the systemically available dose of a drug by the area under the plasma concentration-time curve produced by that dose. In pharmacokinetic terms, total body clearance is the product of rate constant and volume of distribution. It is the sum of individual clearances of the drug by the various organs and tissues of the body. For a drug that is eliminated solely by renal excretion and hepatic biotransformation, total clearance is the sum of renal and hepatic clearance (Rowland and Tozer, 1980b).

Two compartment Model: When plasma drug levels are measured at early time periods after intravenous administration, an initial distributive phase often can be described kinetically. This distributive phase may last from a few minutes to several hours. The drug entering the body (input) does not instantly distribute (equilibrate) between the blood and those other body fluids or tissues which it eventually reaches (Fig.

1b). The distribution of the drug in blood and other soft tissues, on the other hand occurs at different rates. Eventually a steady state will be reached which terminates the "distribution" phase (Ritschel, 1980).

Three compartment Model: If the drug is distributed to a significant degree in deep depots, such as fat and bone, or is tightly bound in some other tissues, a third compartment may become distinguishable. Thus, the three compartment model embodies models one and two plus an additional tissue compartment (Portmann, 1970).

1.2 Bioavailability

The term bioavailability has been defined as the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed from a drug product and becomes available at the site of action which is normally estimated by its concentration in body fluids, rate of excretion, or acute pharmacological effect (Wagner, 1980). According to this definition, the absorption of an intravenously administered drug is rapid and complete. However, for reasons of convenience and stability, most drugs are administered orally. Therefore, their rate and extent of absorption in an individual are usually not precisely known

(Blanchard and Sawchuk, 1979).

Bioavailability or bioequivalence studies are usually based on measurement of the active drug moiety and/or its metabolite(s) in biological fluids as a function of time. Bioavailability testing in humans usually dictates that the biological fluid sample be limited to blood, urine, or saliva. Drug is administered to subjects who are usually in the fasting state (approximately 12 hours) and who are not permitted food for several hours after receiving drug. Blood samples are then collected after drug administration at a frequency sufficient to permit an estimate of peak plasma or serum concentration of active drug and the total area under the plasma level versus time curve. In most studies where oral dosage forms are compared, the sampling times after each drug administration will be identical. A typical plasma concentration versus time curve that may be obtained after oral administration of a solid dosage form is shown in Figure 2.

Inspection of Figure 2 permits one to observe the major bioavailability parameters - peak plasma concentration (Cmax.), time to achieve peak plasma concentration (tmax.), and area under the plasma level versus time curve (AUC). The first two parameters, Cmax and tmax, are related to and used as measures of the rate

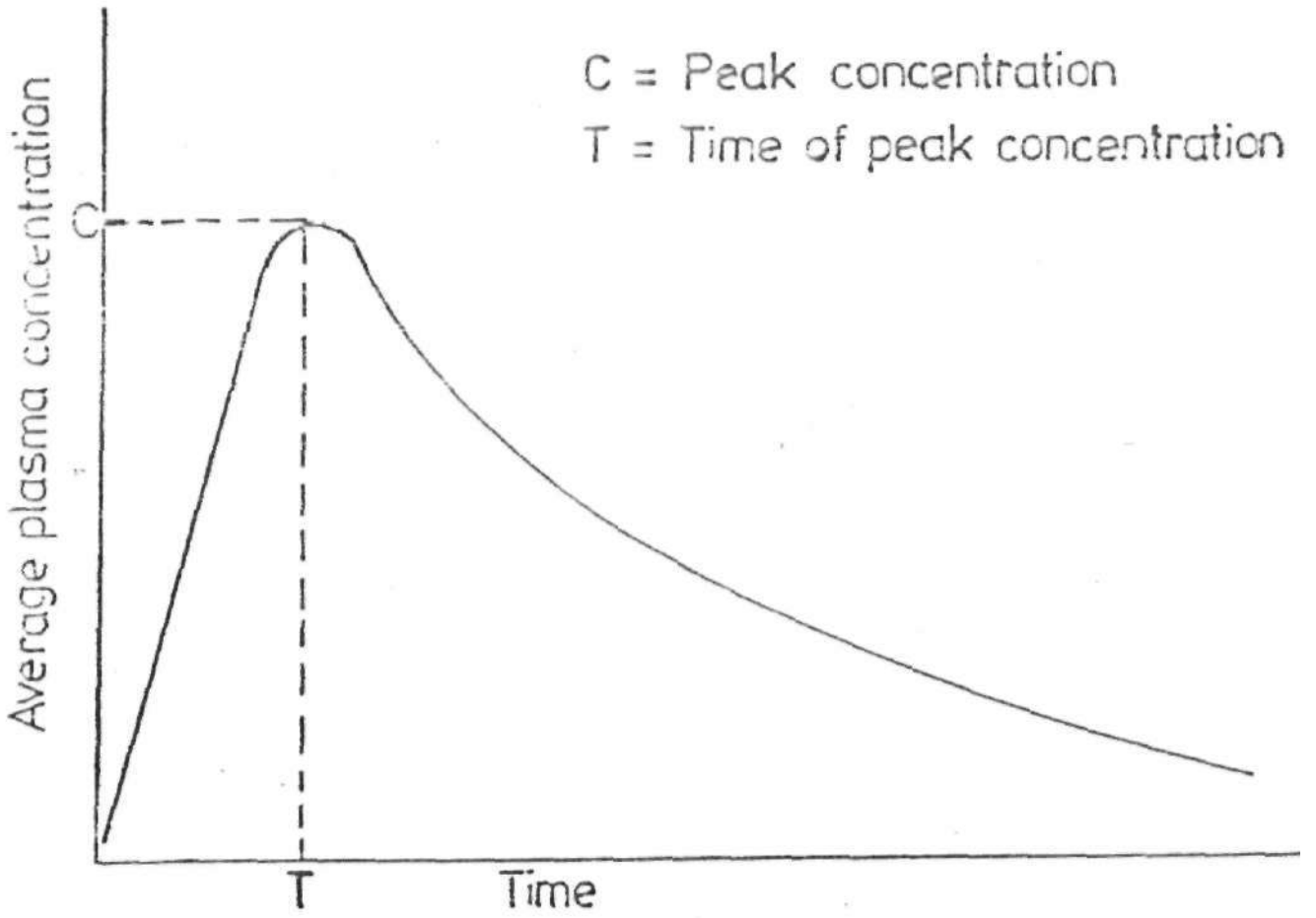


Fig.2 A typical plasma concentration-time curve.

of availability from the dosage form.The extent of absorption from the dosage form is related to the third parameter, AUC.

Determination of the 'absolute' bioavailability of a drug usually involves a comparison of AUC obtained following oral and intravenous administration (for which instantaneous and complete bioavailability is assumed) of the drug. For practical reasons, intravenous administration is often not possible, and in such cases 'relative' bioavailability is determined by comparing the AUC's of the test drug and a secondary reference standard such as an orally administered solution of the drug or a dosage form which has been accepted as a standard (Gibaldi and Perrier, 1975b; Blanchard and Sawchuk, 1979).

There are a number of circumstances which arise making it quite difficult to determine the availability of a drug following administration of a single dose. The plasma levels of certain drugs may be very low such that an assay method sensitive enough to measure these levels may not be available. Other drugs have very long half-lives, and the determination of the total area under a plasma concentration-time curve requires plasma samples to be obtained for excessively long periods of time. Thus it is sometimes desirable to

determine availability based on steady-state plasma drug levels following multiple dosing (Gibaldi and Perrier, 1975b).

For the estimation of availability, determination of AUC is required. The most commonly employed methods for calculating AUC's are the "cut-and-weigh" method, employment of planimeter and application of trapezoidal rule (Gibaldi and Perrier, 1975c).

Some important areas of application of bioavailability are:

a. Determination of those formulation factors that alter the bioavailability of an active ingredient in a drug product or products.

b. Establishing a generic bioequivalence or bioinequivalence of two or more drug products or formulations.

c. Determining the effect of food on the absorption of an active ingredient in a drug product or formulation.

d. Establishing that one drug interferes with the absorption of another drug, and how to avoid the interaction.

e. Determining whether increasing age or specific disease states influence the absorption of an active ingredient in a drug product or formulation.

f. Assessment of the magnitude and variability of the

'first pass' effect with specific drugs after oral administration and the degree to which other routes of administration, such as rectal, nasal or buccal administration, avoid this effect.

1.3 The Clinical Significance of Differences in Drug Bioavailability

A large number of factors have been shown potentially to interfere with drug absosrption. The degree of change in clinical response which may accompany altered drug bioavailability is a function both of the drug's mode of action and also the extent of bioavailability variance. It may be difficult to identify a clinical change with altered drug bioavailability due to the many factors contributing to, and the often subjective nature of assessing clinical response. Nevertheless, several reports have documented actual changes in clinical response which have been associated with bioavailability. Other reports have strongly suggested that changes in the bioavailability of a particular drug would have clinical implications. Most of these compounds have well defined blood or tissue level for therapeutic effect, steep dose-response relationships or narrow therapeutic indices, or have suitable absorption or elimination mechanisms (Blanchard and Sawchuk, 1979). When considering the clini-

cal implications of drug bioavailability changes, whether the cause is a pathological condition, drug interaction, food interaction, product substitution or any other factor, it is appropriate to divide bioavial-ability changes into three major groups: those which have definitely caused changes in clinical response, those which have not been shown to cause clinical changes but are likely to do so, and those which could bring about clinical changes in extreme situations (Blanchard and Sawchuk, 1979).

1. Bioavailability changes which have caused altered clinical response:

Instances where marked clinical changes have been associated with impaired drug bioavailability have been described for Ampicillin (Jordan et al., 1981), Ketoconazole (Mannisto et al., 1982), Rifampicin (Polasa and Krishnaswamy, 1983), Glipizide (Sartor et al., 1982) and Glibenclamide (Wahlin-Boll et al., 1980).

2. Bioavailability changes which are likely to have clinical effects:

If a drug meets any of the aforementioned criteria, it is likely that a change in drug bioavailability will have a measurable clinical effect. Included amongst these drugs are the cardiac glycosides, anticoagulants, antihypertensive agents, anticonvulsants, antineoplastic agents and inorganic ions such as lithium. However, in the majority of cases, levels of these compounds in the body greatly exceeds the minimum required for activity, and small changes in bioavailability are unlikely to be of clinical significance (Blanchard and Sawchuk, 1979).

3. Bioavailability changes which could bring about clinical changes in extreme situations:

A clinical change will occur for all drugs, provided that the change in bioavailability is sufficiently large. The clinical effect may be altered by changes in either the rate or extent of drug absorption (Blanchard and Sawchuk, 1979).

1.4 Factors Influencing Drug Bioavailability

The compressed tablet is the most widely used oral dosage form, and is also most commonly involved in drug bioavailability and bioequivalence problems. This is not suprising owing to the reduction in drug surface area in the compressed formulation and the wide variety of drug forms, tabletting methodologies, excipients and coating that are used.

Absorption may be affected not only by the different dosage forms, but also by similar dosage forms from different manufacturers and different batches of a dosage form from a single source. The most common conventional oral dosage forms, in decreasing order of relative dissolution and absorption rates are solutions, suspensions, capsules, tablets, coated tablets and sustained-released preparations.

Dosage-form related factors which can produce profound differences in bioavailability include formulation and manufacturing variables such as particle size, the chemical form and solubility of the drug; the type and quantity of excipients used; the compaction pressure and other factors (Blanchard and Sawchuk, 1979).

Dosage form properties often account for some modification in the pharmacological activity of therapeutic agents by altering their intrinsic absorption pattern. The schematic diagram in Figure 3 shows drug concentration in the blood as a function of time after oral administration of a fixed amount of drug in three different dosage forms designated I, II, and III. The properties of the dosage forms in this hypothetical example produce a change in the overall absorption rate which is reflected in the blood level-time curve. Activity occurs when the blood concentration reaches the minimum effective drug level (Figure 4). The onset of pharmacological action is significantly earlier with formulation I than with formulation II, and is never attained with formulation III (Blanchard and Sawchuk, 1979). The duration of the clinical effect is often reflected by the length of time that the blood level of drug is equal to or higher than the minimum effective level.

Again it can be seen from the example in Figure 3 that the duration of effect obtained from formulation I is significantly longer than that obtained after equal dose of formulation II. From this general example, it can be seen that dosage form factors have a significant effect on the onset, duration and intensity of

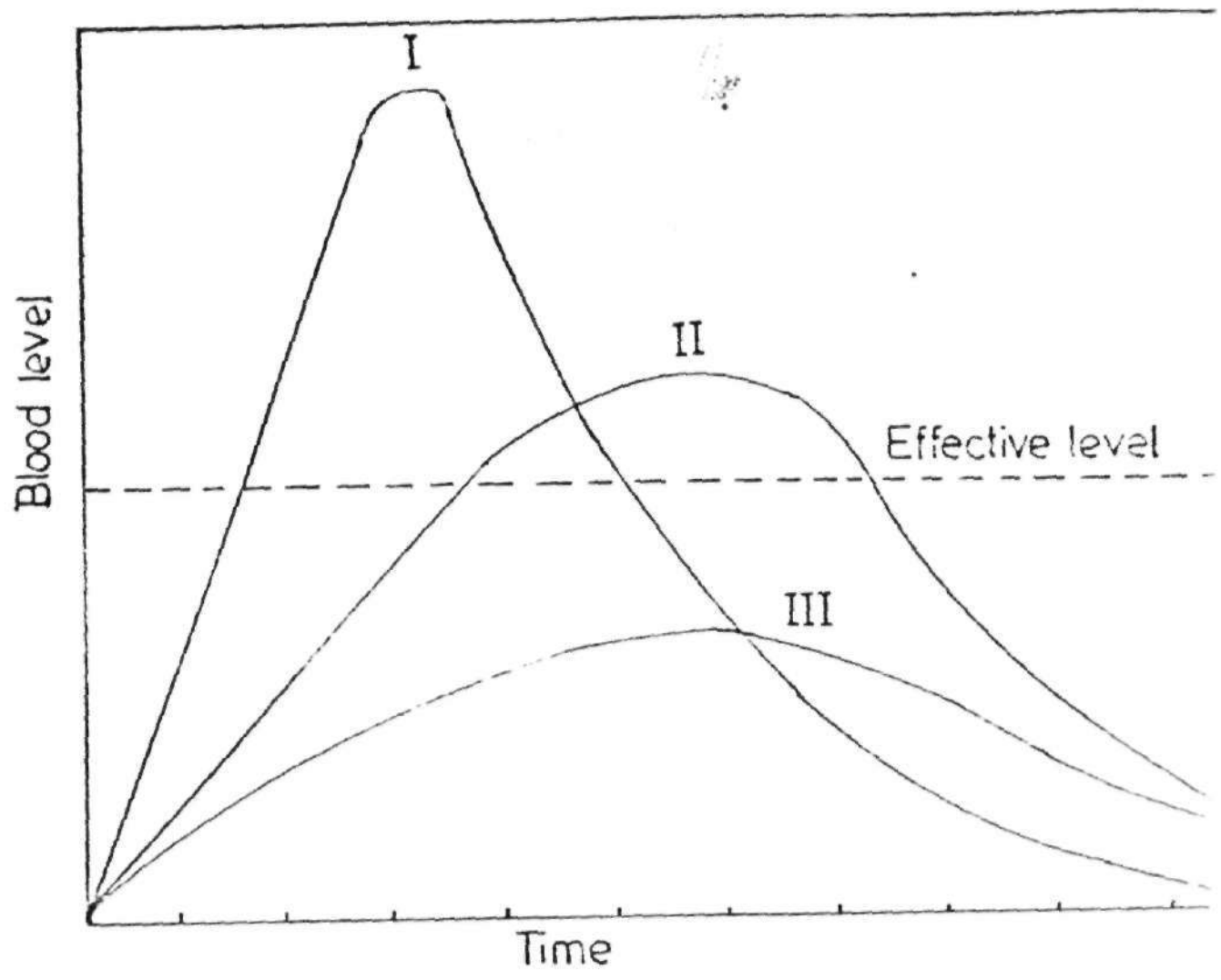


Fig. 3. Blood level-time curves showing possible effects of dosage form on blood levels after oral administration of equal amounts of active agent (from Poole, 1979).

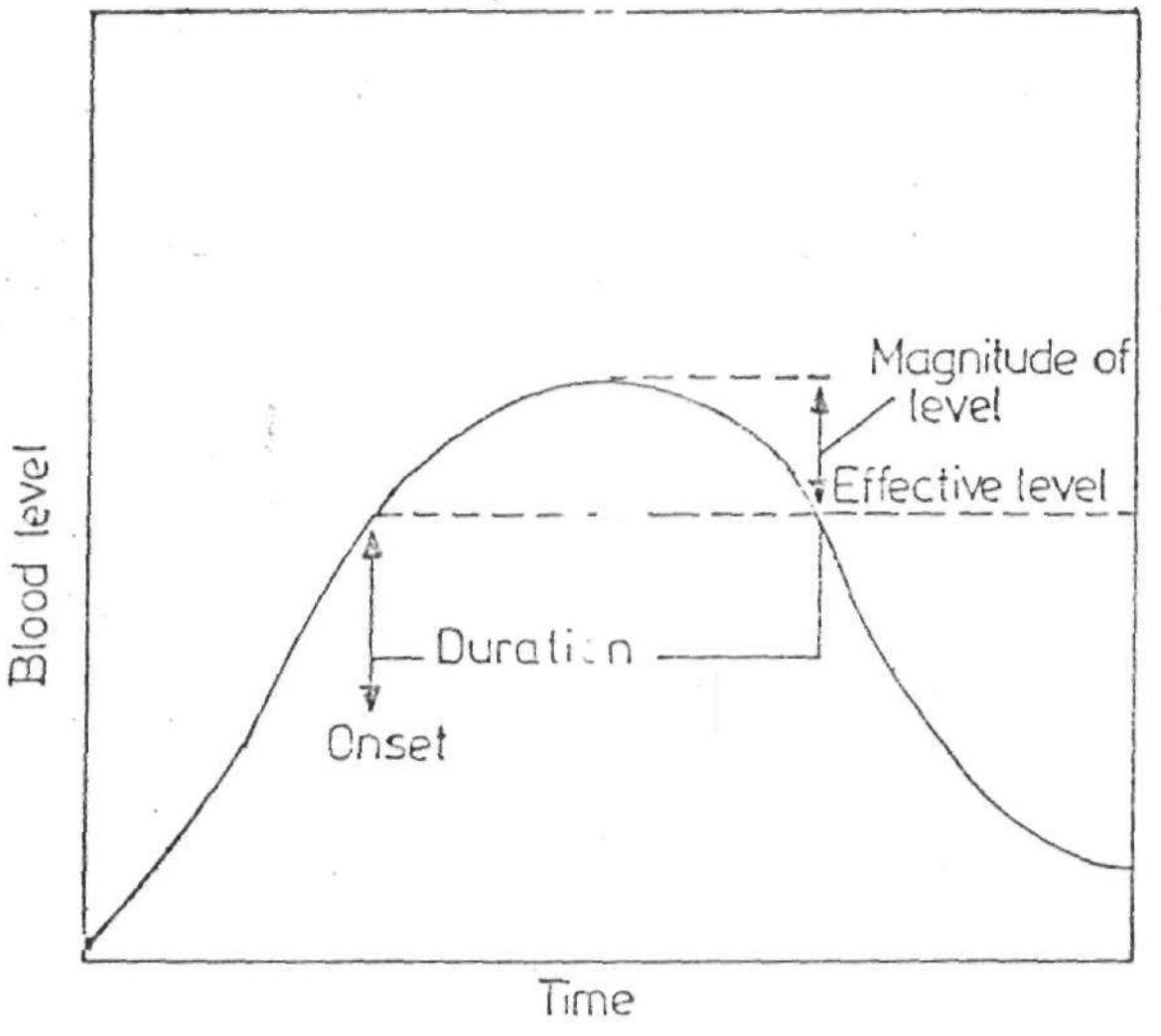


Fig. 4: Relationship of blood level-time curve and pharmacologic effect (from Poole, 1979).

pharmacological action of a particular active component.

The various formulation and dosage form related factors which have been extensively discussed by Poole (1979), Wade (1976) and many other authors include: particle size, crystal form, salt, complex, pH and solubility characteristics. Other factors are excipients and adjuvants as well as manufacturing methods. Poole (1979), showed that bioavailability of some drugs have been improved by particle size reduction.

The crystal form in which a drug is presented to the patient is an important factor influencing the bioavailability of drugs. The crystal form of a drug substance may change because the substance exhibits polymorphism. Polymorphism has been reported to be relatively common, being exhibited by at least one-third of all organic compounds. Apart from the polymorphic forms in which substances may exist, they can also occur in non-crystalline or amorphous forms. The energy required for a molecule of a drug to escape from a crystal in which it is located is very much greater than that required to escape from an armophous powder. Therefore, the amorphous form of a compound is always more soluble than the corresponding crystalline form and may exhibit correspondingly different therapeutic

properties (Poole, 1979). Examples of drugs in which this has been observed in vitro are novobiocin and chloramphenicol.

The property of many organic medicinal compounds to form solvent addition compounds or solvates may also affect their bioavailability. When water is the solvent associated with the drug moiety, the solvate form is called a hydrate. The solubility and rate of solution of a solvate may significantly differ from that of non-solvated form of the drug. Drugs for which significant differences in dissolution rates have been observed include caffeine, theophylline and glutethimide (Poole, 1979).

Complex formation is a mechanism that can influence the biopharmaceutical properties of a drug. Drugs may interact reversibly to form complexes with substances occurring in the body, with other drugs or with pharmacologically inactive components of the pharmaceutical dosage form. Such a drug complex may differ in its physico-chemical properties from the pure drug. For instance, its ability to permeate biological membrane may become altered. Such complexes are thus pharmacologically inactive since dissolution prior to drug absorption usually fails to occur (Poole, 1979).

The effect of complexation on drug absorption depends on the magnitude of the stability constant of the complex. Frequently, there may be little or no effect on the rate of absorption and on the overall bioavailability. However, some drugs which are intrinsically poorly absorbed can be converted to non-absorbable complexes under certain circumstances and their bioavailability is further decreased. Complex formation according to Poole (1979), can be used in formulation of dosage forms in order to increase solubility or stability of drug substances or drug products. The drug complex in such systems is diluted in the biological fluids after administration which results in the dissociation of the complex with little or no effect on the overall absorption properties of the active compound.

The components in a dosage form other than the active drug substance may influence the absorption and subsequently the performance of a drug product. These additives or excipients may either facilitate or inhibit the absorptive process. The importance of excipients in the determination of the final drug effect was clearly demonstrated by an outbreak of phenytoin toxicity in Australia. The toxicity occurred when patients previously maintained in a stable dose of 'Dilantin' capsules in which calcium sulphate had been

used as inert excipient, were given capsules (containing same quantity of active compound) but with the calcium sulphate substituted for lactose as 'inert' excipient. Presumably, the inorganic ion had complexed with phenytoin reducing the absorption (Wade, 1976).

The role of surface active agents is more complex and not always easy to predict. These substances are frequently used to increase solubility or act as emulsificants, but they may either increase or decrease absorption depending on the agent, the quantity, the route of administration, the drug in question and the nature of formulation. It was reported that these surface active agents may change the absorptive surface itself in the small intestine, and that in other cases, the agents promote micelle formation, thus enabling lipid soluble substances to disperse in an aqueous medium (Wade, 1976).

Interaction between drugs and dosage forms may influence the rate and extent of drug absorption. Such interactions may be direct, as in chelation of tetracycline by polyvalent metal ions, or indirect as with the increased rate of acetaminophen absorption due to the stimulating effect of metoclopromide on the stomach emptying rate. However, propantheline reduced the rate of acetaminophen absorption by delaying the

stomach emptying rate (Welling, 1980).

The volume of co-administered fluid may have a considerable effect on drug absorption. Although from a purely physical view-point, drug absorption might be expected to occur more readily from concentrated solutions than from dilute solutions, the reverse appears to be the case. Increased drug bioavailability from dilute solutions may be related to the tendency for a compound to follow net mucosal to serosal fluid flux when hypotonic solutions are introduced into gastrointestinal tract. However, it may be related also to more rapid stomach emptying of large fluid volumes.

Influence of food and specific dietary components on drug absorption has been extensively reviewed by Winstanley and Orme (1989); Hayes and Borzelleca (985). The reviews reflect the complex and unpredictable nature of drug-food interactions, and the difficulty of establishing patterns which may be used as guidelines for optimum drug therapy in relation to food. The presence of food had been shown to decrease, delay, increase and or not to affect drug absorption. Observed effects have been influenced by the type of drug and dosage form, the nature and size of meal, fluid intake, the conditions of the subject and

the time sequence between eating and drug administration.

Although drug bioavailability studies are routinely carried out in healthy human subjects, drugs are administered in practice to patients who may be suffering from a variety of illnesses. Diseases affecting the cardiovascular system, the liver and the gastrointestinal tract may alter circulating drug or metabolite level after oral dosing. Surgical removal of part of the gastrointestinal tract reduces the absorption of most compounds, suggesting that patients who have undergone such procedures may need to be monitored carefully when taking drugs whose absorption efficiency is critical.

CHAPTER TWO

LITERATURE REVIEW

2.1 History and Chemistry of Sulphonylureas

Chlopropamide is one of the sulphonylureas which finds its greatest usefulness in subjects with Type II Diabetes mellitus. Diabetes mellitus is a metabolic disease characterised by hyperglycaemia and eventually glycosuria. It is caused by the inability of tissues to carry out normal metabolism of carbohydrate, fat and protein, due to an absolute or relative lack of insulin. An absolute lack of insulin is found in some diabetic patients who develop the disorder early in life (Juvenile or growth-onset). Relative insulin deficiency is usually found in those diabetics who develop the disease later in life (adult-onset or maturity-onset) (Aguwa, 1986).

Insulin is by no means an ideal treatment for diabetes, the realisation of this fact has led to a great deal of research to develop more effective ways of treating the disease. As a consequence of this urge, two guanidine derivatives, synthalins A and B

were introduced into diabetes therapy in the 1920's. Although they were less toxic than guanidine, they had no therapeutic advantage over insulin. It was shown that prolonged use of these synthetic derivatives could result in renal and hepatic damage and consequently their use was discontinued in early 1930's (Olaniyi, 1989). The hypoglycaemic action of certain sulphonamides was first recognised by Ruiz in 1930 but it was not until 1942 that Johnson and co-workers, working on 5-isopropyl-2-sulphanilamido-1,3,4 - thiadiazole (as a potential antithyroid drug), recognized the hypoglycaemic property. This search led Loubatieres to produce a series of analogues in order to study structure-activity relationships. The first clinically useful drug was produced in 1955 by Frank and Fuchs, a compound originally known as B255, now officially known as carbutamide. Since that time there has been a steady flow of structural modifications which had led to a group of relatively safe and effective oral hypoglycaemic agents being introduced into clinical practice (Olaniyi, 1989).

Chemistry:

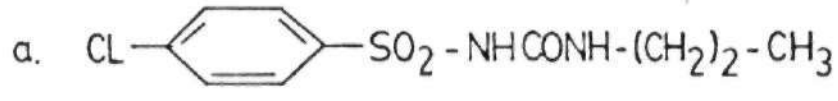
The general structure of the sulphonylureas consist of three portions:-

Sulphonylurea nucleus

Ar----------SO_2--------NHCONH-------R

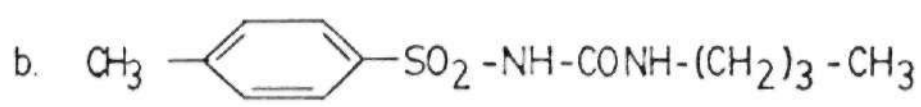
Aryl Moiety Urea Moiety Alkyl group

Variation of the aryl moiety proved most useful. The para-NH_2 group was shown to be unnecessary and replaced by a methyl group to give the drug "Tolbutamide". While being an effective hypoglycaemic agent, tolbutamide was rapidly metabolised by oxidation of the p-methyl group to a carboxyl function and excreted. Replacement of the methyl group by halogen (chlorine) gave a compound with increased potency and longer action. From a series of chloroaryl compounds prepared, the 3-propylderivative was the most effective and was given the generic name of chlorpropamide. Modification of the urea moiety led to loss of activity whilst variation in the alkyl side chain showed that 3 or 4 carbon atoms gave optimum activity. The alkyl group confers lipophilic properties on the molecule. Later development included the introduction of acetohexamide and tolazamide. The structures of chlorpropamide and tolbutamide are shown in Figure 5.

a. 

Chlorpropamide Diabinese (R)

1-[P-Chlorophenyl) Sulphonyl)]-3-Propylurea

b. 

1-Butyl-3(P-tolyl-Sulphonyl) urea

Fig. 5. Structures of (a) Chlorpropamide and (b) Tolbutamide.

2.2 Absorption and Metabolism

Chlorpropamide has long been thought to be almost totally excreted unchanged in the urine (Balant, 1981). However, Brotherton et al. (1969) using chromatographic techniques identified two metabolites (p-chlorobenzene sulphonylurea and p-chlorobenzene sulphonamide) in the urine of diabetic patients. The proportion of the drug metabolised varied in different individuals on long term therapy.

Taylor (1972) identified two additional metabolites, 2-hydroxychlorpropamide and 3-hydroxychlorpropamide from the urine of four diabetic patients receiving 250-400mg of chlorpropamide daily. He reported that the unchanged drug accounted for 95 percent of total drug and metabolite concentrations in Plasma. According to Jackson (1969), it may take as long as 96 hours for 80-90 percent of chlorpropamide to be excreted in the urine and complete elimination of the drug takes 3-7 days.

Chlorpropamide is readily absorbed from the gastro-intestinal tract and is extensively bound to plasma proteins. The half-life in plasma is about 35 hours. It is partly metabolised in the liver to metabolites with some hypoglycaemic activity; the

metabolites and the unchanged drug are excreted in the urine (Martindale, 1989).

2.3 Therapeutic Significance

Chlorpropamide is an oral hypoglycaemic agent useful for the management of hyperglycaemia in Type II diabetics. Hypoglycaemic agents are orally effective in lowering blood glucose levels and are also safe, while being more convenient and more acceptable than insulin to many patients. These agents are not recommended for Type I diabetics, pregnant women, those who have renal disease or those whose hyperglycaemia can be controlled by diet (Aguwa, 1986).

The use of oral hypoglycaemic agents in maturity-onset diabetes suffered a setback after the publication of a study by the University Group Diabetes Programme (UGDP). This was a study commenced in 1961 to evaluate the use of oral agents in the prevention of the long-term complications of diabetes. Several centers involving 12 different clinics were used. About 1000 patients participated in a random double blind study in which diet alone, tolbutamide, insulin and phenformin were compared. The summary of the findings revealed that there were more cardiovascular related deaths in

patients treated with the oral agents than in those on placebos. Those who were treated with diet and insulin had better results. As a result of the study many patients were removed from oral agents and placed on a diet/or insulin treatment. Patients who can be placed on oral hypoglycaemic agents include those who show evidence of pancreatic reserves, that is individuals who require less than 40 units of insulin daily for control. The pancreatic daily insulin output is between 40 and 60 units. Such patients are usually diagnosed after the age of 40 and are not obese. The older the patient at the onset of diabetes the better the chance of satisfactory control with oral hypoglycaemic agents (Aguwa, 1986).

Mechanism of action

The mechanism of anti-diabetic action of sulphonylureas is controversial. While some investigators stress the importance of stimulation of insulin secretion and/or beta cell hyperplasia (beta cytotropic action), others have shown that the chronic antidiabetic activity of sulphonylureas does not result in increased insulin secretion. This suggests that one or more extrapancreatic actions are involved in the anti-diabetic effect of these agents (Lebovitz et al.,

1977).

A direct drug effect on tissue insulin sensitivity has been claimed (Kolterman et al., 1984; Ward et al., 1985). All sulphonylureas used to treat non-insulin-dependent diabetes cause the beta-cell to secrete insulin, probably by binding to the cell surface membranes, reducing the outflow of potassium from the cell and increasing the inflow of calcium. Calcium binds to the protein calmodulin, which mediates micro-filament contraction, and so facilitates the secretion of insulin granules. In vitro experiments the insulin-releasing effects depend on the concentration of drug. There are 1000-fold differences in potency in these in vitro studies between tolbutamide and glibenclamide, but there is no evidence that efficacy differs among the clinically useful sulphonylureas in the systems used (Ferner, 1988).

Sulphonylureas may have extrapancreatic hypoglycaemic effects in addition to their effects on the secretion of insulin. There is evidence for (and sometimes against) actions on insulin receptor numbers or affinity. A body of work now favours the idea that sulphonylureas affect insulin action at a post receptor site, but do not consistently increase the number or affinity of receptors. Hepatic and muscle glucose

uptake or glycogen degradation may be altered, and there continues to be an interest in the ability of sulphonylureas to inhibit ketogenesis, perhaps by inhibiting carnitine acyltransferase (Ferner, 1988).

The observation of a raised plasma insulin to c-peptide ratio has led to the suggestion that decreased hepatic insulin extraction is a direct drug effect (Scheen et al., 1984). Tolbutamide decreases insulin extraction in the perfused rat liver (Marshall et al., 1970). If this is the case, the lesser amount of insulin interacting with and taken up by hepatocytes is relatively more potent in decreasing net hepatic glucose output in the presence of the drug (Taylor and Agius, 1988).

Toxicity

Chlorpropamide has occasionally been implicated in bone-marrow depression. A transient fall in white cell count has been noted, also severe leucopenia, pancytopenia and fatal bone marrow aplasia with agranulocytosis (Jackson, 1969).

Chlorpropamide can cause jaundice but less frequently, usually of less severity and of a different type. In the general run of patients it produces no changes in liver function (Jackson, 1969).

Patients taking chlorpropamide may become sensitive to alcohol. They develop flushing, headache, nausea/vomitting and thoracic discomfort similar to that following disulphiram ("Antabuse$^{(R)}$") within 5-10 minutes of drinking small quantities of alcohol. As many as one-third of the patients may be affected to some extent. The mechanism is not clear. The serum levels of acetaldehyde and 5-hydroxy-indoleacetic acid are apparently not raised (Jackson, 1969). Effects on electrolyte has also been observed and it has been reported that sulphonylureas cause hyponatremia (Nesbel, 1977).

Overdose of Chlorpropamide: More than 500mg of chlorpropamide daily were used in the early trials and produced a high incidence of gastrointestinal side effects; unpleasant taste in the mouth, general malaise, weakness, ataxia and dizziness (Jackson, 1969).

Preparation and Dosage

Chlorpropamide (Diabinese) is marketed in the form of 100-and 250mg tablets. 200mg daily dose will maintain a plasma level of about 10mg per 100ml, while minimum and maximum effective concentrations are achieved with 100 and 500mg, respectively. Higher doses than this are dangerous because of cumulative

elevation of the drug blood levels. Infact, the lower the dose, the safer the drug, so that a starting dose of 250mg or less is recommended. The danger of severe hypoglycaemia from higher doses is greatest in the elderly and mild diabetics (Jackson, 1969).

2.4 Biochemical Basis of Diabetes

Diabetes mellitus is a complex disease characterized by a grossly abnormal pattern of fuel usage - overproduction of glucose by the liver and underutilization by other organs. Diabetes refers to the excessive urination in the disease and mellitus is from Latin, meaning sweetened with honey, and refers to the high level of glucose in the urine. Mellitus distinquishes the disease from diabetes insipidus which is caused by impaired renal reabsorption of water (Stryer, 1988a).

There are two major clinical syndromes of diabetes: One characterized by insulin dependence and early age of onset with weight loss and ketonuria, insulin-dependent diabetes mellitus (IDDM), and the second characterized by a relatively later on-set, insensitivity to insulin and partial insulin deficiency non-insulin-dependent diabetes mellitus (NIDDM) (Taylor and Agius, 1988).

Normally the level of glucose in the blood varies only within narrow limits; it rarely rises above 120mg/100ml or falls below 60mg/100ml. Maintenance of a normal blood sugar level is essential because some tissues, for example the brain, rely on glucose for their metabolism. A fall in blood glucose below a level of about 30mg/100ml causes serious disturbance of mental function which may culminate in coma. Conversely, a rise in blood glucose produces osmotic diuresis and dehydration. There is a mechanism by which the level of blood glucose is maintained within the normal range both after eating a carbohydrate meal and during starvation (Oakley et al., 1975).

Blood sugar rises after a meal containing carboyhydrate, due principally to the breakdown of starch to glucose. The glucose is then absorbed and carried in the portal vein to the liver. In the liver glucose is stored as the polysaccharide glycogen. After an excess of dietary carbohydrate, glucose is synthesized into glycogen. Conversely, in times of shortage of carbohydrate, glycogen is brokendown to glucose and released into the blood stream. The transformation of glucose to glycogen is shown in Figure 6. It is first phosphorylated to glucose-6-phosphate and then converted to glucose-1-phosphate.

The glucose-1-phosphate adds glucose to the end of existing glycogen molecules, thus lengthening the chain. The pathway involved in glycogen breakdown differs however, from the pathway of glycogen synthesis, different enzymes being involved at two key stages in the process: phosphorylase, which produces glucose-1-phosphate from glycogen by adding on phosphate groups and glucose-6-phosphatase which hydrolyses glucose-6-phosphate to give free glucose. Both enzymes are influenced by the activity of hormones. For example, glucagon and adrenaline raise blood glucose by activating phosphorylase which depletes liver glycogen. This activation of phosphorylase involves the intervention of the intracellular messenger substance, cyclic-AMP (adenosine monophosphate). Galactose, fructose and a number of other sugars less commonly present in foodstuffs are converted into glucose or glycogen in the liver (Oakley et al., 1975).

Just as complex carbohydrates are broken down into simple sugars in the gut, proteins are similarly split into amino acids. Amino acids, like sugars, are absorbed into the portal blood stream and carried to the liver. They may also be used to synthesize protein in muscle or kidney. Certain amino acids can be metabolised to glucose. This process is especially important in starvation, which if prolonged, may lead

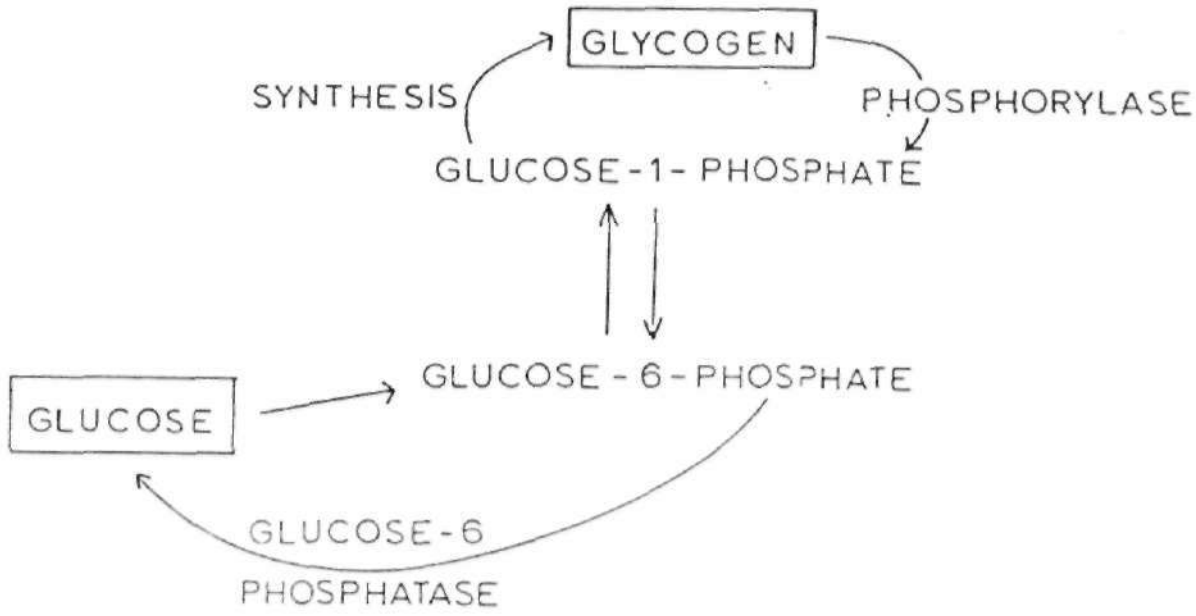


Fig. 6. Synthesis and breakdown of glycogen (from Oakley et al. 1975).

to severe loss of body protein by its conversion into glucose in order to maintain the blood sugar level (Oakley et al., 1975). The low blood sugar level leads to decreased secretion of insulin and increased secretion of glucagon. The dominant metabolic processes are the mobilization of triacylglycerols in adipose tissue and gluconeogenesis by the liver. The liver obtains energy for its own needs by oxidizing fatty acids released from adipose tissue. The concentrations of acetyl CoA and citrate consequently increase, which switches off glycolysis. The uptake of glucose by muscle is markedly diminished because of the low insulin level, whereas fatty acids enter freely. Consequently muscle also shifts from glucose to fatty acids for fuel. The B-oxidation of fatty acids by muscle halts the conversion of pyruvate into acetyl CoA. Hence, pyruvate, lactate and alanine are exported to the liver for conversion into glucose. Proteolysis of muscle protein provides some of these three-carbon precursors of glucose. Glycerol derived from the cleavage of triacylglycerols is another raw material for the synthesis of glucose by the liver. The most important change after about three days of starvation is that large amounts of acetoacetate, 3-hydroxybutyrate and acetone are formed in the liver, and are released in the blood and carried to the brain

(Figure 7). Gluconeogenesis depletes the supply of oxaloacetate, which is essential for the entry of acetyl CoA into the citric acid cycle. At this time ketone bodies become the major fuel of the brain. These changes in fuel usage are accompanied by a rise in the level of ketone bodies in the plasma. The ketone bodies are excreted in the urine, and acetone, which is volatile is also excreted through breathing.

Glycolysis (metabolism of glucose)

Glycolysis is the sequence of reactions that converts glucose into pyruvate with the concomitant production of ATP (Stryer, 1988b). In all cells there is a common system for the metabolism of glucose. In this process the six-carbon molecule is broken down to three carbon substances. Pyruvic acid is the end product of glucose metabolism in mammalian tissues, although under conditions of oxygen lack this may be reduced to lactic acid. The transformation of glucose by this pathways is shown in Figure 8. There are a number of important points of control of glycolysis, one involves the phosphorylation of glucose to glucose-6-phosphate; another involves the further phosphorylation of fructose-1-phosphate to fructose 1, 6-diphosphate; these two phosphorylation reactions, as well as the rate of transport of glucose across the

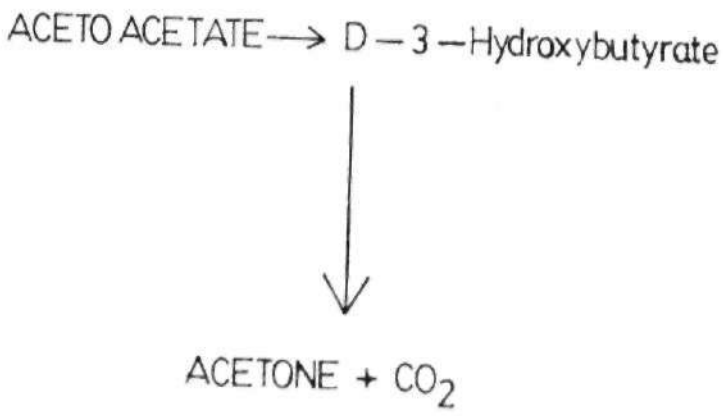


Fig. 7. Relationships of ketone bodies (from Oakley et al., 1975).

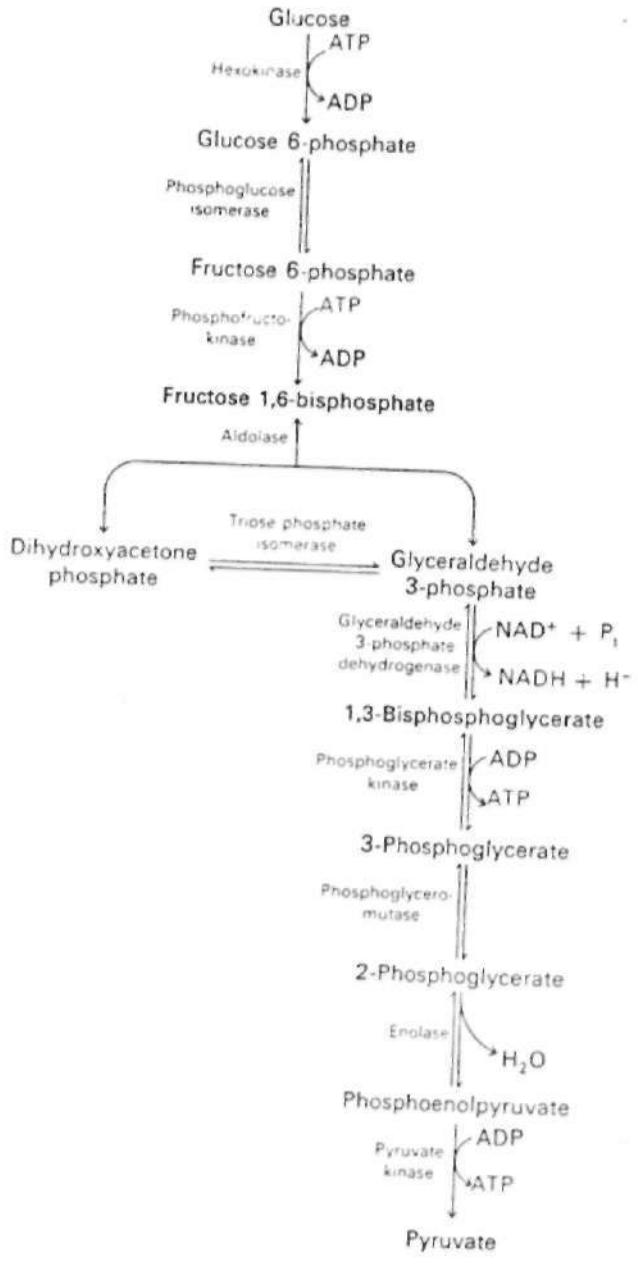


Fig.8. The glycolytic pathway (from Stryer, 1988).

cell membrane, determine the rapidity of glucose flow through the glucolytic pathway. Normally, pyruvic acid is metabolised to give the two-carbon compound, acetyl coenzyme-A, which is then oxidised to carbon dioxide and water. The series of reactions which effect this oxidation was first elucidated by Krebs and is termed the tricarboxylic acid cycle. The cycle involves th addition of acetyl coenzyme-A to oxaloacetic acid to form citrate which is then transformed into a number of other di and tricarboxylic acids (Figure 9). The tricarboxylic cycle normally takes place in the mitochondria which are small intracellular bodies where most of the energy of living cells is derived from oxidative processes. By way of glycolysis and the Krebs cycle there is therefore a complete pathway for the oxidation of glucose to carbon dioxide and water (Oakley et al., 1975).

Gluconeogenesis

Gluconeogenesis is the synthesis of glucose from non-carbohydrate precursors (Stryer, 1988c). The process is important for the maintenance of blood sugar in periods of food deprivation. It is greatly accelerated in diabetes where protein breakdown contributes substantially to the rise in blood sugar (Oakley et al., 1975). The process is also important

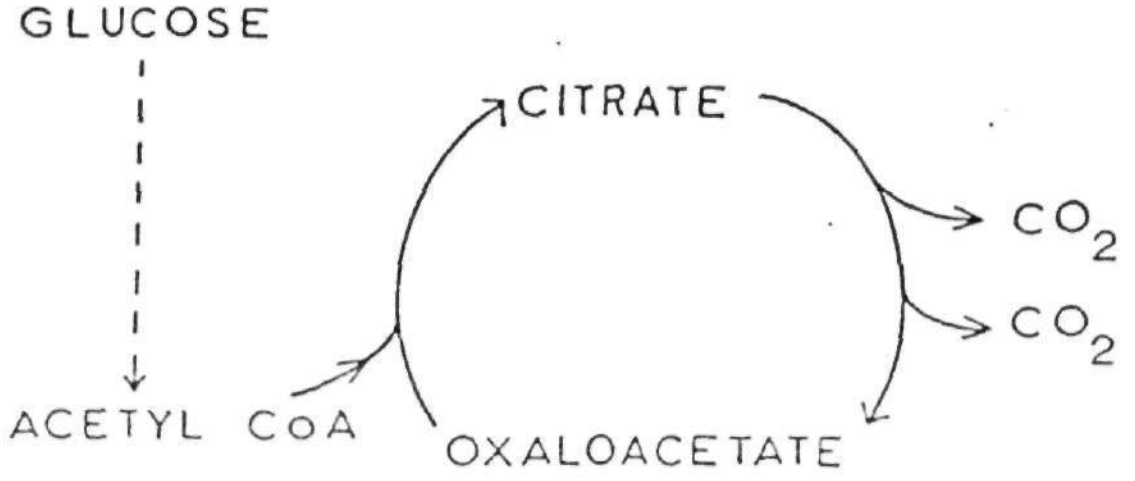


Fig. 9. Tricarboxylic Acid cycle (from Oakley et al., 1975).

during periods of intense exercise (Stryer, 1988c). The gluconeogenic pathway converts pyruvate into glucose. Noncarbohydrate precursors of glucose enter the pathways chiefly as pyruvate, oxaloacetate, and dihydroxyacetone phosphate (Figure 10). The major noncarbohydrate precursors are lactate, amino acids, and glycerol. Lactate is formed by active skeletal muscle when the rate of glycolysis exceeds the metabolic rate of the citric acid cycle and the respiratory chain. Amino acids are derived from proteins in the diet and during starvation from the breakdown of proteins in skeletal muscle. The hydrolysis of triacylglycerols in fat cells yields glycerol and fatty acids. The enzymes of gluconegenesis are located in cytosol. The major site of gluconsogenesis is the liver. Gluconeogenesis also occurs in the cortex of the kidney, but the total amount of glucose formed there is about one-tenth of that formed in the liver because of the kidney's smaller mass. Very little gluconeogenesis takes place in the brain, skeletal muscle, or heart muscle. Rather gluconeogenesis in the liver and kidney helps to maintain the glucose level in the blood so that brain and muscle can extract sufficient glucose from it to meet their metabolic needs (Stryer, 1988c).

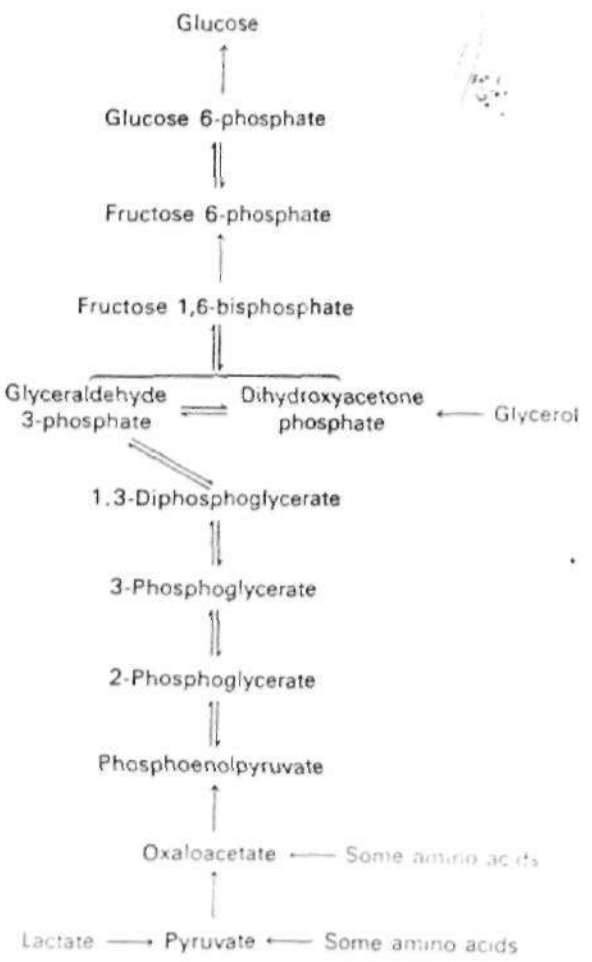


Fig. 10. Gluconeogenic Pathway (from Stryer, 1988).

Fat Metabolism

Fat consists of a combination of fatty acids with glycerol. It is stored in adipose tissue cells subcutaneously, in the omentum and around the viscera. The first stage in fat breakdown is the hydrolysis of neutral fat to give free fatty acids and glycerol. This process is regulated by a number of hormones such as growth hormone, glucagon and adrenocorticoid hormone (ACTH).

In adipose tissue free fatty acids are produced by the action of lipase and transported to other sites where they are further metabolised. While carbohydrate stores provide a source of energy in case of a short term need, fat provides a store of oxidisable material available in conditions of prolonged carbohydrate shortage. Thus fat stores are readily broken down in starvation (Oakley et al., 1975).

Hyperlipidaemia in diabetes mellitus

Lipoproteins are responsible for the transportation of lipids from the intestines as chylomicrons, and from the liver as very low density lipoproteins (VLDL), to most tissues for oxidation and to adipose tissue for storage. Lipid is mobilized from adipose tissue as free fatty acids (FFA) attached to serum albumin. Abnormalities of lipid metabolism occur

at the sites of production or utilization of lipoprotein, causing various hypo- or hyperlipoproteinemias. In diabetes mellitus, insulin deficiency causes excessive mobilization of FFA and underutilization of chylomicrons and VLDL, leading to hypertriacylglycerolemia (Mayes, 1990).

Lipid and lipoprotein abnormalities are common in the diabetic population as insulin deficiency and insulin resistance affect key enzymes and pathways in lipid metabolism (Orchard, 1990). In addition, post transcriptional modification of the apoprotein components of plasma lipoproteins by glycosylation may interfere with the binding of lipoproteins to important cell surface receptors and alter their metabolism (Tan and Betteridge, 1991).

In Type 1 (IDDM) diabetic subjects in good glycaemic control, lipid levels are similar to normal controls, whereas those with poor control usually have elevated levels. Hence well-controlled Type 1 diabetics have normal level of triglyceride, cholesterol, Low-density lipoproteins (LDL) cholesterol and Very low-density lipoproteins (VLDL) triglyceride. High-density lipoproteins (HDL) levels, while low in untreated or poorly controlled Type 1 diabetic patients, are similar to appropriate non-diabetic control

subjects or even raised in patients established on insulin therapy (Tan and Betteridge, 1991).

Patients with Type 2 (NIDDM) diabetes are much more likely to have an abnormal lipid profile. The most common abnormality is hypertriglyceridaemia. VLDL triglyceride can be 50-100% higher than control subjects. There is no consistent pattern with regard to concentration of total plasma cholesterol and LDL cholesterol. HDL concentration has been found in many studies to be reduced in Type 2 diabetic patients. VLDL clearance may be reduced in untreated or poorly controlled Type 1 diabetic patients as the enzyme lipoprotein lipase is dependent on adequate insulin concentration for full activity. This situation is reversed with insulin therapy and VLDL kinetics in patients on optimum therapy tend to be normal.

In Type 2 diabetic patients, there is general agreement that VLDL production is increased but the mechanism is not known. It is not clear whether hyperinsulinaemia associated with insulin resistance directly stimulates VLDL production, or relative insulin deficiency at the cellular level secondary to insulin resistance leads to overproduction of VLDL. In addition to quantitative changes in VLDL, qualitative

changes in the lipid and apoprotein composition also occur. There is an increase in the triglyceride content of VLDL with a corresponding increase in particle size. Alteration of apoprotein content with an increased ratio of apoprotein C-III to C-II has been described and this may be of importance as apoprotein C-III is an inhibitor of lipoprotein lipase while apoprotein C-II is an activator of the enzyme. LDL levels are generally raised in poorly controlled patients but return to levels similar to control subjects when normoglycaemia is achieved. LDL levels are more likely to be elevated in Type 2 diabetic patients (Tan and Betteridge, 1991).

In vitro and in vivo experiments have shown that insulin increases LDL receptor activity. A further factor which may alter LDL receptor binding is glycosylation of the lysine residues of LDL apoprotein B. LDL isolated from diabetic patients has been shown to have up to 5% lysine residues glycosylated, three to four-fold higher than control subjects. Whether this will significantly affect binding still remains to be proved. The enrichment of LDL with triglyceride seen in diabetic patients may also cause decreased binding and uptake by cells (Tan and Betteridge, 1991).

In Type 1 diabetic patients, plasma HDL concentrations, particularly the HDL fraction which is most closely associated with protection against vascular disease, are normal or raised. In Type 2 diabetic patients, HDL tends to be low. One explanation is that the hydrolysis of triglyceride-rich lipoproteins is reduced in Type 2 diabetic patients with a consequent reduction in transfer of surface components to the HDL fraction. Other factors are also involved and an inverse relationship, independent of VLDL, has been shown between plasma insulin and C-peptide levels and HDL in Type 2 diabetic patients. Activity of the liver enzyme hepatic lipase which is important in HDL metabolism appears to be elevated in these patients. Several qualitative changes in HDL have been described, particularly enrichment with triglyceride, depletion of apoprotein A and an increased ratio of cholesterol to protein. The significance of these changes needs further clarification (Tan and Betteridge, 1991).

Insulin Biosynthesis

Insulin is synthesized as a precursor molecule, proinsulin, which is converted within the pancreatic beta cell to insulin (Rubenstein et al., 1972; Horuitz et al., 1975). Insulin synthesis within the beta cell

cytoplasm first involves the formation of pre-proinsulin under the influence of messanger-RNA. Ribosomes transfer RNA amino acids to make the A, B and C chains of proinsulin (Figure 11). The chains, linked in the order A-C-B are formed close to the microsomal membrane. The pre-hormone sequence is cleared away so that folded proinsulin with its A, B and C chains can be identified in the cytoplasmic reticulum. Secretion granules containing the A and B chains of insulin separated from the connecting peptide appear in the region of the Golgi zones of the beta cell cytoplasm, ultimately to be secreted from the cell by emiocytosis (Bloom, 1980).

Structure and properties of Proinsulin and Insulin

Proinsulin consists of a single polypeptide chain ranging in length from 78 (dog) to 86 (human, pig, horse, rat) aminoacid residues. The variations in length in the mammalian proteins occur only in the connecting polypeptide portion which links the carboxy terminus of the insulin B to the amino terminus of the insulin A chain (Figure 12). The known mammalian proinsulins have pairs of basic residues at either end of the connecting peptide which link the connecting polypeptide to the insulin chains. These residues are excised during the conversion of proinsulin to insulin,

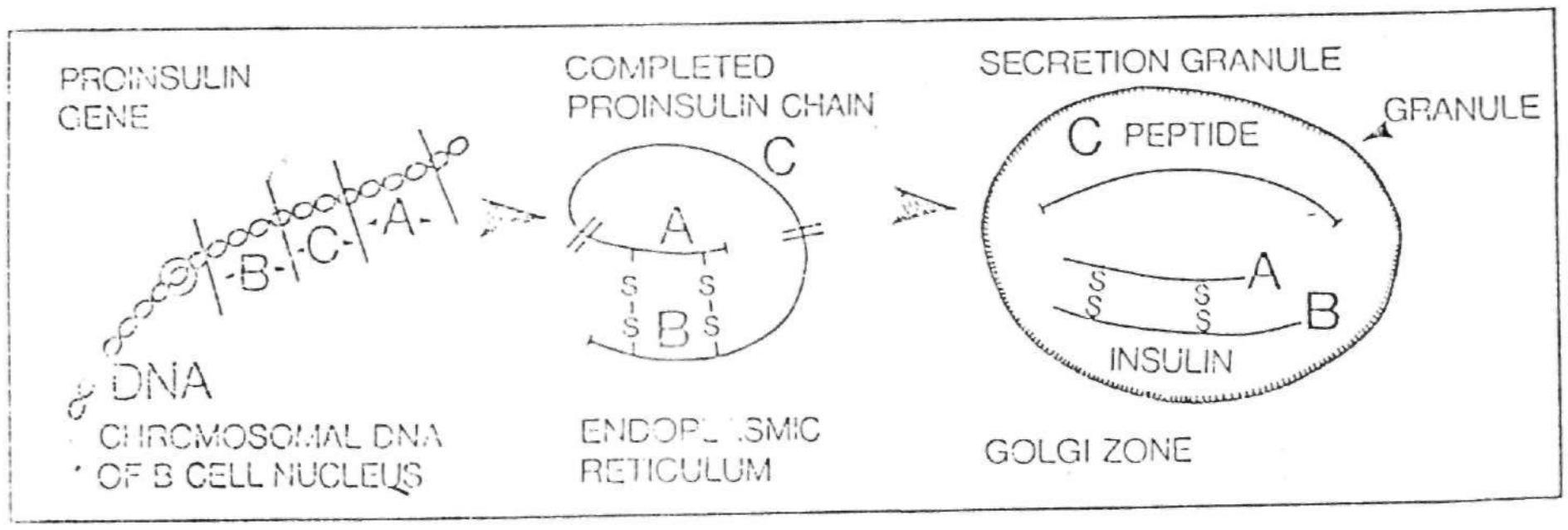


Fig. 11. Insulin Synthesis within the beta cell cytoplasm (from Bloom, 1980).

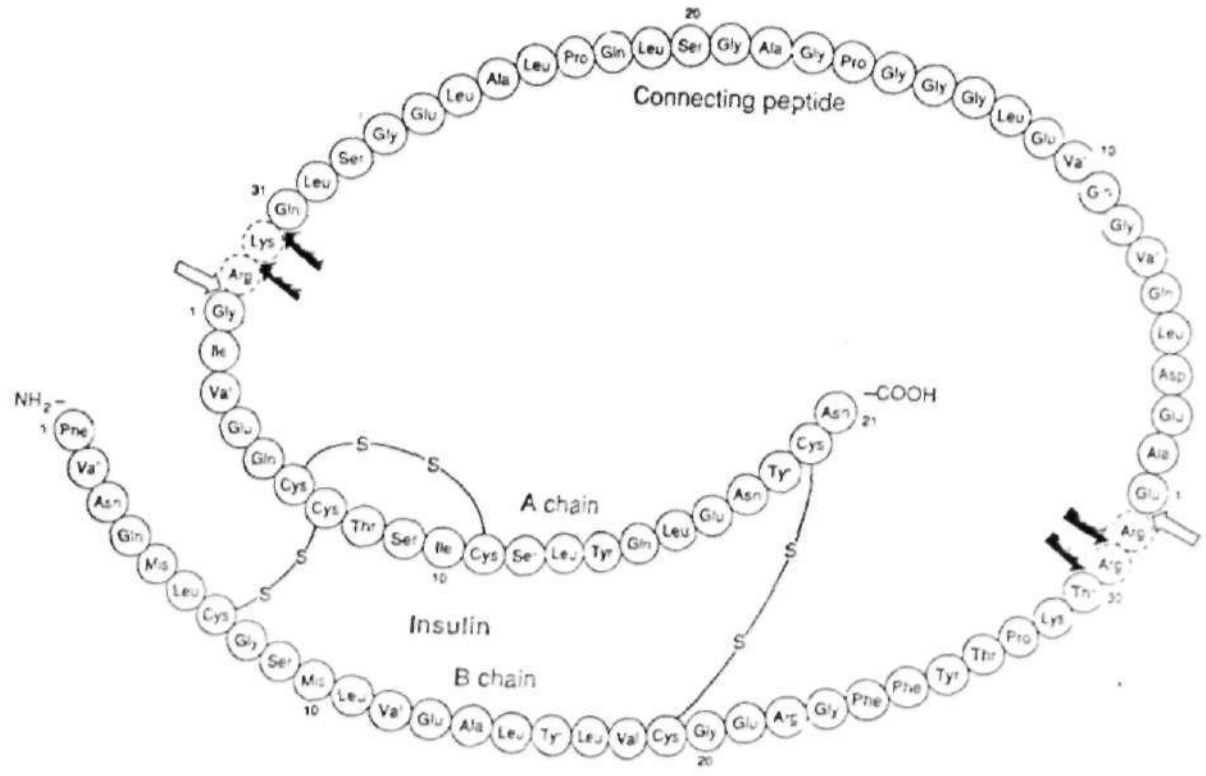


Fig. 12. Structure of human prinsulin. Insulin and C-peptide molecules connected at 2 sites by dipeptide links (from Granner, 1990).

giving rise to native insulin plus the remainder of the connecting polypeptide segment lacking amino - or carboxyterminal basic residues. This peptide is designated connecting-peptide (Rubenstein et al., 1975).

Insulin is a polypeptide consisting of 2 chains, A and B, linked by 2 interchain disulfide bridges that connect A7 to B7 and A20 to B19. A third intra-chain disulfide bridge connects residues 6 and 11 of the A chain. The location of these 3 disulfide bridges is invariant, and the A and B chains have 21 and 30 amino acids respectively, in most species (Granner, 1990).

Despite its considerably larger size, proinsulin is closely similar to insulin in many properties, including solubility, isoelectric point, self associative properties and reactivity with insulin antisera. The connecting peptide (C-peptide) is folded over a portion of the surface of the insulin molecule, but it does not completely mask the "active site", since intact proinsulin exhibits 3-5 percent biological activity of insulin in several systems in vitro (Gliemann and Sorensen, 1970).

Plasma C-peptide as an indicator of insulin secretion

The plasma C-peptide concentration has been interpreted as a semiquantitative measure of insulin secretion in a variety of clinical situations (Polonsky and Rubenstein, 1984). The most important assumptions on which these methods depend are as follows:

1. C-peptide and insulin are secreted on an equimolar basis from the pancreas. Polonsky et al. (1983), performed in vivo studies in dogs and the C-peptide and insulin concentrations were measured directly in the portal vein. Under basal conditions, as well as after stimulation by intravenous glucose, this ratio varied between 1.1 and 1.6 which is very close to the theoretical value of 1.0. Similarly, in the portal vein of man during periods of stimulation of insulin secretion, the C-peptide: insulin molar ratio approximates 1.0 (Horwitz, et al., 1975).

2. The hepatic extraction of C-peptide is negligible under physiologic conditions. Although there is a great deal of experimental evidence that is consistent with a negligible hepatic extraction of C-peptide, most of these studies are not conclusive. In human subjects, Horwitz et al. (1975), noted a portal to peripheral C-peptide ratio of 1.4 in the fasting state,

and this value increased to 2.7 two minutes after the start of a rapid intravenous glucose infusion. Because hepatic vein C-peptide concentrations were not measured, it was not possible to calculate the hepatic C-peptide extraction accurately in the study. However, since hepatic vein C-peptide concentrations are intermediate between those in the portal vein and periphery, the observed ratios are consistent with, but do not prove, negligible hepatic C-peptide extraction.

3. The metabolic clearance rate (MCR) of C-peptide is constant under physiologic conditions and over a wide range of plasma concentrations. An important prerequisite for the use of C-peptide as an indicator of insulin secretion is the demonstration that its metabolic clearance rate is constant under different physiological circumstances and over the range of plasma concentrations usually encountered in these situations. The MCR of C-peptide has been studied under basal conditions in laboratory animals and man. Constant infusion studies performed in the rat using C-peptide isolated from bovine pancreas indicated that its metabolic clearance rate (4.6 $\pm$ 0.2 ml/min) was independent of its plasma concentration over a range of 1ng/ml to more than 15ng/ml (Katz and Rubenstein, 1973). Similarly, the metabolic clearance rate of infused dog C-peptide (11.5 $\pm$ 0.8ml/kg/min) was

constant at concentrations encountered under normal physiologic cirmumstances (Polonsky et al., 1983). Because of lack of availability of sufficient quantities of human C-peptide this question has not been adequately evaluated in man. Faber et al. (1978), were able to study the MCR of synthetic human C-peptide in normal control and type 1 diabetic subjects by analyzing the exponential decay curves after single-dose injections. The C-peptide MCR was indistinquishable in control and type 1 diabetic subjects. In a single subject, the C-peptide MCR measured by the constant-infusion technique was comparable with that obtained after bolus injection, and was found to be independent of its plasma concentration. It should be stressed that the lack of additional data on C-peptide kinetics in human subjects has forced investigators to use the mean result from the above experiments for all calculations in which the insulin secretion rate is derived from peripheral C-peptide levels. The limitations of this situation are: i. all the experiments reported by Faber et al. (1978), were performed under fasting conditions and their application to other situations, such as after meal ingestion, in obesity and cirrhosis is currently un-known.

ii. The use of a single value for the C-peptide MCR does not permit investigators to assess the potential variability in this parameter in different subjects.

iii. The resolution of plasma C-peptide decay curves into more than one exponential as was carried out in the study using the single-injection technique (Faber et al., 1978), is a difficult and at times arbitrary procedure; particularly when the plasma concentrations are low. The difference between results obtained with this technique and those derived from other methods of calculating the C-peptide MCR (such as the constant-infusion technique) are yet to be determined. Untill it is possible for each investigator to study the MCR of C-peptide in individual subjects and use these data for the calculation of C-peptide secretion rates, the validity of using the results of Faber et al. (1978) for all studies will not be known.

Metabolic consequences of lack of Insulin

In diabetes mellitus, because of an inadequate insulin secretion, or an inadequate tissue response to insulin, there is deficiency of insulin actions. Transport of glucose into muscle and adipose tissue is restricted, and blood glucose therefore rises. In addition, the breakdown of neutral fat to free fatty

acids and glycerol is increased and there is a rise in the fatty acid content of the blood. The increased catabolism of fatty acids by the liver results in a greater production of ketone bodies which diffuse out from the liver and pass to the muscles for further oxidation. As the diabetic state worsens the ketone body levels increases and, as the capacity for their oxidation is exceeded, ketosis become progressively more severe. Less amino acids are taken up by tissues and protein breakdown is greatly increased. At the same time the enzymes responsible for gluconeogenesis are activated by the absence of insulin with a consequent increase in the production of glucose, mainly from the liver at the expense of protein. The lack of insulin depresses protein and fat synthesis and, when prolonged, leads to an increase in their breakdown. These changes can be reversed by insulin, although some, such as the reversal of gluconeogenesis, may take many hours (Oakley et al., 1975).

Since all tissues require insulin (bound to the receptor site) for glucose metabolism and normal cellular function, all steps leading to and including this binding are logical sites for genetic errors that could result in diabetes. This permits the speculation as to the location of the primary defects. Among these defects might be an inability of the pancreas to

synthesize, store, and release appropriate amounts of insulin in response to need; errors in the transport of insulin to the target tissues; incompetent of insulin receptor sites that interfere with the normal binding of insulin; a deficiency in glucose tolerance factor that facilitates insulin binding to the receptor site and excess levels of other hormones that interfere or compete with insulin for the binding site (Berdanier, 1976).

Other hormones and the control of blood sugar

Besides insulin there are other hormones which significantly influence the control of blood sugar. The most important of these are growth hormone, glucagon, adrenaline, ACTH and adrenal steroids.

Growth hormone: Growth hormone regulates metabolism in the growth period and probably also in states of carbohydrate deprivation, such as fasting. Growth hormone also increases the breakdown of fat, thereby sparing oxidation of carbohydrate and increasing blood glucose.

Glucagon: Glucagon, produced in the A cells of the islets of Langerhans, opposes the action of insulin and raises blood glucose. It does this by increasing glycogen breakdown (by raising levels of cyclic-AMP in

the liver) and also by increasing gluconeogenesis. Glucagon acts by activating phosphorylase. Glucose-1-phosphate is readily converted to glucose-6-phosphate which is readily hydrolysed to give free glucose.

Adrenaline: Like glucagon, adrenaline raises blood sugar. By raising the level of cyclic-AMP in liver the activity of liver phosphorylase is increased and glycogen is broken down to glucose. Another important effect of adrenaline is to inhibit the secretion of insulin. This effect may be especially important in producing the hyperglycaemia of adrenaline-secreting tumours.

Adrenocorticotropic hormone (ACTH) and Adrenal steroids

Adrenal steroids, particularly those with an oxygen atom at position 11 on the steroid ring, may raise blood sugar and increase glycogen experimentally. The same effect is produced indirectly by ACTH produced by the pituitary. In man, the diabetogenic effects of adrenal steroids such as cortisone are well known. Cushing's disease, in which steroids are produced in excess, and steroid therapy quite commonly lead to mild diabetes and, conversely, in Addison's disease, in which there is steroid deficiency, there is increased insulin sensitivity. Adrenal steroids therefore have a

significant influence on carbohydrate metabolism. Their principle mode of action is to accelerate gluconeogenesis from tissue protein by increasing enzyme activity in the liver. This effect of steroids is important in starvation and in diabetic ketosis. Adrenal steroids may also limit glucose uptake by cells (Oakley et al., 1975).

2.5 Food and drug interactions

Food can be described as what one takes into the body system, which when digested nourishes the body to maintain life and sustain or promote growth while drug is a chemical compound of synthetic, natural or biological origin which interacts with animal cells and the resultant action may be to cure or to reduce ill effects in the human or animal body. Unless advised to the contrary, patients frequently take drugs with meals, the meal time acting as a suitable reminder. Evidence has shown that the presence of food in the gastrointestinal tract (GI tract), and in particular the stomach, can have a marked yet unpredictable effect on drug absorption (Welling, 1977; Melander, 1978; Toothaker and Welling, 1980).

The extent to which food may alter circulating levels of an orally dosed compound is frequently far

greater than that which may occur due to changing a drug brand or formulation, so that the enormous expense involved in producing a particular drug formulation may be wasted if due attention is not paid to this easily controllable and yet often ignored factor (Welling and Tse, 1982).

Food components may interact directly with drugs in a variety of ways, these generally leading to decreased drug availability. Examples are chelation of drug by polyvalent metal ions (Dearborn et al., 1957), or complex formation with proteins (Kohn, 1961). Food can also act as a mechanical barrier, preventing drug access to the mucosal surface of the intestine.

The formulation in which a drug is administered is likely to have a profound effect on the outcome of a particular food-drug interaction. Solutions and suspensions are generally less susceptible to the influence of food than other dosage forms because of their greater mobility within the gastrointestinal tract and the relative ease with which they pass from the stomach into the small intestine. On the other hand, enteric coated tablets are particularly susceptible to food interactions, as the presence of food may cause the intact dosage form to remain in the stomach for several hours after dosing.

Interactions between food components and orally administered drugs may have threee possible outcomes. Drug absorption may be decreased, not affected or increased, and all of these have been observed (Welling and Tse, 1982). Food has been shown to reduce the bioavailability of the non-esterified penicillins (Cronk et al., 1960), ampicillin (Jordan et al., 1981) and amoxycillin (Welling, 1977). Similarly the absorption of many of the cephalosporins is either delayed or reduced by food (McCracken et al., 1978).

Most studies carried out on erythromycin base in plain tablets or capsules have reported that food delays and/or reduces drug bioavailability (Josselyn and Sylvester, 1952; Smith et al., 1953). The majority of the studies with coated tablets reported that absorption is either delayed or unaffected by food (Smith et al., 1953; and Bachtol et al., 1979). Others however, have reported reduced absorption of erythromycin base from enteric coated tablets in non-fasting individuals (Maytyla et al., 1978). The absorption of erythromycin base from capsules containing enteric coated pellets was delayed, but not reduced, when they were taken immediately following a standard breakfast (Rutland et al., 1979).

Food intake reduces the bioavailability of rifampicin (Acocella, 1978; Polasa and Krishnaswamy, 1983) and isoniazid (Melander et al., 1976a). Mannisto et al (1982) have shown that the AUC for ketoconazole is significantly reduced by a high carbohydrate low fat meal. Food delayed but did not reduce the extent of metronidazole absorption in healthy subjects and patients (Melander et al., 1977a). Absorption of the antifungal agent griseofulvin was increased by fatty meals (Crounse, 1961; Kabasakalian et al., 1970), but appears to be unaffected by high protein or high carbohydrate meals (Crounse, 1961). Co-administration with food increases the bioavailability of nitrofurantoin by up to 400% (Bates et al., 1974; Rosenberg and Bates, 1976). This effect is maximal for those formulations of the drug with the poorest dissolution characteristics, suggesting that the effect is at least in part due to better dissolution resulting from delayed gastric emptying (Rosenberg and Bates, 1976). Nigerian fatty meal significantly increased the extent of absorption of nitrofurantoin, the fatty meal gave rise to 40% increase in the bioavailability of the drug (Ogunbona and Oluwatodimu, 1986).

In contrast to these observations concerning nitrofurantoin, the bioavailability of the newer quinolone antibiotics (e.g. ciprofloxacin) is not

greatly perturbed by food (Neuman, 1988).

Mebendazole, when given to fasting healthy subjects, achieved plasma concentrations below 18nmole /L; when the same dose was given to the same subjects with fatty food, the peak plasma concentrations were 91, 112 and 142nmole/ L and AUC was similarly increased (Munst et al., 1980). Flubendazole, a p-fluoro derivative of mebendazole, when given with a fatty meal, like mebendazole gave a higher plasma concentrations (Michiels et al., 1982).

One study reported that food had no effect on pivampicillin absorption (Foltz et al., 1971), but others have demonstrated a distinct reduction in circulating antibiotic levels due to food (Bergan, 1972; Magni and Sjovall, 1972). In a study in which pivampicillin was administered to healthy volunteers, mean peak serum antibiotic levels were reduced from 4.5 to 3.3mg/L by the presence of food and the area under the serum level curve was reduced from 15.3 to 8.0mg /L (Fernandez et al., 1973).

Two studies have shown that the absorption of cephalexin may be significantly reduced when ingested following a meal. Peak serum levels of antibiotic activity were reduced to 12mg/L following single 500mg doses in non-fasted individuals compared to 18mg/L in

fasted individuals and urinary recovery was also reduced from 78-100% to 50-70% of the dose (Mayers et al., 1969). Cephalexin absorption was also reduced by concomitant administration of milk in children (McCracken et al., 1978).

Reduced bioavailability of tetracyclines due to chelation with heavy metal ions, and binding to macromolecules is well documented (Neuvonen and Turaka, 1974; Schumacher, 1975 and Braybrooks et al., 1975). While the absorption of all the oral tetracyclines is reduced to a similar extent by ion salts (Neuvonen et al., 1970), doxycycline absorption is affected less than the other tetracyclines by milk and dairy products (Neuvonen et al., 1971). Doxycycline absorption is also influenced by solid diets than tetracycline or demethylchlortetracycline (Rosenblatt et al., 1967 and Welling et al., 1977a).

Harvengt et al (1973) compared the absorption of cephradine and cephalexin after oral doses to six fasted subjects and then to the same subjects 30 min after breakfast. Serum levels of both compounds were depressed at 30 min after the non-fasting dose, but peak drug levels, times of peak levels, and also the total urinary recovery of antibiotic activity were all unaffected. In a subsequent study by Mischler et al.

(1974), cephradine absorption was shown to be markedly delayed when it was administered immediately after a meal.

While the availability of most sulphonamides is delayed by food (Melander et al., 1976b), the availability of sulphamethoxydiazine is increased (Kaumeier, 1979; Kaumeier et al., 1979). Food caused a delay in absorption of sulphamethoxydiazine, and peak drug levels in plasma were not reached until 10h after dosing, compared to 7h in fasting individuals. However, peak plasma concentrations were higher, and the area under the plasma curve was almost doubled. High fat meals appear to have a greater effect on sulphamethoxydiazine availability than high carbohydrate or high protein meals.

High carbohydrate diet greatly prolonged the half-life of theophylline in children, whereas high protein diet markedly decreased the half-life (Feldman et al., 1980). Heimann et al (1982) reported that the rate of theophylline absorption was significantly decreased when the drug was given with food; mean maximum serum concentrations were reached after 4.7h instead of 1.6h under fasting conditions. However, the amount of the drug absorbed did not differ between the

two groups. Food did not influence the absorption of theophylline from Theolin Retard. The bioavailability was complete (93% after 30h) both with food and without food, and no difference was found in the time to peak of the plasma concentration curve (7h). The absorption characteristics did not change with intake of breakfast (Sips et al., 1984).

Melander and Wahlin-Boll (1978) have shown that the extent of absorption of dicoumarol is significantly increased by food. Melander et al. (1977b) reported an enhancement of canrenone bioavailability from spirono-lactone by food. The results of their study indicates that more canrenone enters the general circulation when spironolactone is taken together with food than when the drug is taken in the fasting state.

The intestinal absorption of propranolol, metoprolol and labetalol is virtually complete (Winstanley and Orme, 1989), but administration of the drugs to non-fasted subjects significantly increases their bioavailability (Melander et al., 1977c; Daneshmend and Roberts, 1982). Food has been shown to increase the bioavailability of propafenone in healthy volunteers (Axelson et al., 1978). A high-protein meal has been reported to have no effect on verapamil bioavailability (Woodcock et al., 1986). The

bioavailability of the hydrophilic drug atenol has been reported to be reduced by food (Melander et al., 1979).

The anticholinergic effects of propantheline are inhibited when given with food (Ekenved et al., 1977). Kennedy and Wade (1982) reported that food delayed the absorption of phenytoin but there was no difference in the total bioavailability as determined by the area under the plasma concentration-time curve. The effect of food on the pharmacodynamics of tolmesoxide had also been studied. Food caused a significant reduction in the peak plasma concentration and a significant increase in the time to reach peak plasma concentration of the drug (Elliott et al., 1982).

Food has been shown to decrease the absorption of paracetamol but area under the blood concentration time curve did not differ significantly (McGilveray and Mattock, 1972). In another study, paracetamol is not affected by fat or protein meals but carbohydrate - rich meals reduced the rate of absorption of the drug (Clement, 1978).

Woo and Greebiatt (1980), demonstrated that ingestion of quinidine with a standard breakfast affects the rate but not the extent of quinidine absorption from the gastrointestinal tract. Conney et al. (1976) reported that a diet containing charcoal-

broiled beef markedly decreased the plasma levels of phenacetin without appreciably influencing the plasma concentrations of phenacetin's metabolite N-acetyl-P-aminophenol, or the plasma half-life of phenacetin.

Concomitant administration of food was found to have little effect on the bioavailability of glibenclamide (Sartor et al., 1980a). However, food intake significantly delayed the absorption of glipizide both in diabetic and in normal subjects (Wahlin-Boll et al., 1980). Antal et al (1982), observed a difference in peak serum concentration of tolbutamide or the time of its occurrence and the rate of absorption in diabetic patients. They however observed a statistically significant decrease in the extent of the drug absorption when taken with meal.

Melander et al. (1977d), demonstrated that food enhances the bioavailability of hydralizine 2-to 3-fold both when non coated and coated tablets are used. Asprin absorption is influenced by food ingestion, the peak concentration of unchanged aspirin was 40 to 50% reduced by food and the rate of absorption was also decreased by food (Koch et al., 1978). Food has been shown to reduce the inhibitory effects on both gastric and salivary secretion of the anticholinergic drug propantheline (Gibaldi and Grundhofer, 1975). This

drug should be given between meals if its maximum antisecretory effects are required. Alcohol bioavailability is reduced when taken with food but the rate of absorption is not affected, suggesting that alcohol interacts with food in the gut lumen (Welling et al., 1977b).

A high protein diet reduces the plasma levels of Levodopa used in treating parkinson's disease (Sweet and McDowell, 1974). On average, a diet containing less than 10g protein daily almost doubles the plasma concentration. This effect may be explained in two ways. Firstly, by delaying gastric emptying, the meal causes levodopa to be retained in the stomach and degraded. Secondly, digestion librates amino acids from the protein which compete with levodopa for active uptake in the small intestine which is its major site of absorption. Beta-adrenergic blocking agents, hydralazine and methoxypsoralen also show increased absorption when given with food (Toothaker and Welling, 1980). Food changed the distribution of diazepam (Naranjo et al., 1980). When fats are eaten following an intravenous injection of diazepam the plasma concentration of unbound (and thus active) diazepam rises, probably because diazepam is displaced from binding to plasma and tissue protein by fatty acids (Naranjo et al., 1980).

2.6 Objective and Scope of the Study

Oral administration of drugs is convenient and linking drug doses to daily routines such as meal times can improve compliance (Haynes et al., 1979). However, interindividual variation in drug response, particularly following oral administration, has long been a problem. Since this variation can result in therapeutic failure or drug toxicity (Routledge, 1988). In the past, variation in the composition, strength or formulation of the drug has often been responsible for such problems; Nowadays, atleast in the 'developed world' such formulation problems are rare, even so dose-response relationships still vary from patient to patient (Winstanley and Orme, 1989). While such variation may result from pharmacodynamic factors, pharmacokinetic factors are also important (Winstanley and Orme, 1989).

Many factors which influence pharmacokinetic/bioavaialability data can be changed by food, both 'acutely', if a drug is taken with a meal and 'chronically', where regularly consumed food items influence drug disposition. The nature of these interactions is complicated and is a function of the type and size of the meal, the chemical and physical form of the drug, the dosage

form and the time relationship between eating and drug administration. It should be noted that as well as changing the pharmacokinetic of some drugs, food can alter their pharmacodynamic effects (Winstanley and Orme, 1989).

The effect of food intake on the absorption of drugs has received much attention in recent years. Published reviews of this topic have demonstrated that drug food interactions may result in reduced, delayed or increased absorption, or there may be no effect at all (Toothaker and Welling, 1980). The clinical significance of these interactions depends both on the type of drug and the extent of change in absorption. Since a specific drug-food interactions may be a result of physiological changes in gastrointestinal tract, or physico-chemical interactions with the drug molecule itself, it is difficult to make general rules regarding potential interactions. Therefore, it is best to evaluate a potential interaction on an individual drug basis, in a group of subjects in a population at risk (Antal et al., 1982).

The best diet for diebetic patients has been a controversial issue for many years and still has not been resolved satisfactorily (Grundy, 1991). There has been, and continues to be disagreement among workers in the field of diabetes as to the relative merits of a high-carbohydrate

versus a low-carbohydrate diet in the treatment of diabetics (Fajans, 1972).

The discovery of insulin in 1921 made it possible to liberalize carbohydrate diets intake. In 1923 Geyelin began to prescribe high-carbohydrate diets to diabetic patients treated with insulin. In 1935, after reviewing 10 years of his experience with the high-carbohydrate, low-fat diet, he concluded that the increased effectiveness of insulin that consistently followed the institution of a high-carbohydrate diet was "chiefly dependent on the degree to which fat is curtailed" (Goodhart and Shills, 1978).

Raising carbohydrate intake from 40 to 60 percent had been reported to have no deleterious effect in diabetic patients (Ernest et al., 1965). The role of carbohydrate intake in the control of the plasma lipid levels was noted by Stone and Connor (1965). They increased the carbohydrate intake from 40 to 64 percent, together with other manipulations of the diet, and noted improvement in the hyperglycaemia, hypertriglyceridemia and hypercholesterolemia in their patients. Their goal was to keep their patients 5 to 10 pounds under their average body weights. They suggested that even less fat be included in the diet than previous observers had advocated and they believed that the restriction of dietary fat would help lower the incidence of cardiovascular - renal disease. They also observed that

"potential diabetes can be activated and mild diabetes can be made severe by rigid restriction of carbohydrate. However, Burns-Cox (1969) reported that a high intake of refined carbohydrate results in an increased incidence of atherosclerosis in diabetics, but this has been seriously challenged.

Brunzell et al. (1971), demonstrated an improved glucose tolerance with high carbohydrate feedings in mild diabetics by maintaining the subjects with mild diabetes on basal (45 percent) and high (85 percent) carbohydrate diets in order to evaluate the effect of increased dietary carbohydrate in diabetes mellitus.

Weinsier et al (1974) studied the effect of high and low carbohydrate diets on diabetes control, insulin secretion and blood lipids. Despite an increased carbohydrate load from 40 to 60 percent (with fat decreased from 45 to 25 percent), insulin secretory capacity was maintained, serum cholesterol was not altered by the change in dietary fat and the high-carbohydrate intake did not increase the mean level of serum triglycerides.

Simpson et al. (1979), demonstrated an improved glucose tolerance in Type 1 and Type 2 diabetic patients by raising the patients carbohydrate intake from 40 to 60 percent. Their results were more impressive in Type 1 than in Type 2

diabetes. In 1979 the Food and Nutrition Committee of the American Diabetes Association published dietary recommendations advocating that the carbohydrate intake of Type 1 (insulin-dependent) diabetic patients be increased to 50-60 percent of total calories.

Anderson and Ward (1979), demonstrated that an increased carbohydrate intake, provided it is in the form of unprocessed carbohydrate, did not worsen the diabetes or result in the elevated triglyceride levels. They maintained diabetic patients receiving low doses of insulin on 35 percent and 70 percent carbohydrate diets and observed lower triglyceride levels in the patients.

Simpson et al. (1981), demonstrated that a high carbohydrate diet improved all aspects of diabetes control and reported that, the restriction of carbohydrate intake has no short-or long-term advantage for the diabetic and does often contributes to poor compliance and failure of dietary therapy. Thus, the prevailing view is that diabetic patients, should eat a low-fat, high-carbohydrate diet and where possible carbohydrate be taken in the form of complex carbohydrate (restricting the intake of simple sugar), primarily to reduce the risk for coronary heart disease, a major killer of NIDDM patients. This view represents the position of several dietary recommending bodies, including, the Special Report Committee of Canadian Diabetes

Association (1981), the British Diabetes Association (1982) and the American Diabetes Association (1986).

The Tufts University Diet and Nutrition Letter (1987), in their article captioned "Sweet News for People with Diabetes" reported that the American Diabetes Association and the American Dietetic Association are putting more emphasis on starch foods. Their new dietary list recommends 60 percent of calories as carbohydrates. The old list advised people to eat closer to 40 percent.

The most important fact that emerges from clinical and epidemiologic studies is the deleterious effects of obesity on diabetes. The fairest vascular complications are found when the habitual dietary intake results in leaness, regardless of the proportion of carbohydrate or fat in the diet (Jenkins et al., 1982).

The present study is intended to evaluate the influence of three varieties of Nigerian meals (cutting across the dietary habits of the three major tribes that is: Hausa, Yoruba and Igbo) on the bioavailability and pharmacokinetic profiles of chlorpropamide tablets following oral administration to both healthy and freshly diagnosed type II diabetic subjects. The aim of the work is to provide some information on the beneficial or otherwise, the effect of the traditional dietary habits among the three major tribes in the management of diabetic patients. Although the three

different types of meals contain essentially carbohydrate, however their methods of preparation differ. On comparative basis it was envisaged that the study will be able to recommend a more appropriate meal for diabetic patients.

Each healthy subject will undergo two treatments:

i. administration of a single dose 250mg *chlorpropamide* tablet *in fasting* state.

ii. administration of a single dose 250mg chlorpropamide tablet concomitantly with a meal.

Each patient will undergo three treatments:

i. Administration of meal only

ii. Administration of a single dose 250mg chlorpropamide tablet 30min before the meal (since the patients cannot be fasted for ethical reason)

iii. administration of a single dose 250mg chlorpropamide tablet concomitantly with a meal.

Thus, every subject will serve as his or her own control. Chlorpropamide and glucose levels will be measured. The batch of the chlorpropamide tablets to be used in this study will be pre-analyzed to assess the quality of the tablets and the meal preparations will be analyzed for their components.

The study will employ a quantitative U.V. spectrophotometric technique for the quality control of the batch of chlorpropamide tablets.

HPLC technique will be used for the determination of chlorpropamide plasma levels.

Colorimetric method will be used for plasma glucose analysis and standard methods of analysis will be employed for the determination of moisture, ash, carbohydrate, fat and protein contents of the standardized meal preparation.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Drugs

Chlorpropamide (Diabinese$^{(R)}$) tablets manufactured by PFIZER PRODUCTS LTD., Plot 16, Akanni Doherty Layout, Oregun, Lagos. Nigeria. Lot. No: 02417006.
Manufacturing Date: 11-90
Expires: 11-93

Chlorpropamide Standard powder supplied by PFIZER INTERNATIONAL, New York, U.S.A.

Tolbutamide standard powder supplied by Boehringer Mannheim GMBH 6800 Mannheim 31, West Germany.

3.1.2 Equipment

Model 206 High performance liquid chromatography (HPLC) system comprising:

- M450 solvent delivery system
- M441 UV/Vis Detector
- M U6K Septumless Injector
- M 510 Pump

- Radial PAK UBONDAPAK C*18* Column and a Z module (WATERS ASSOCIATES CO. LTD., MILLIPORE HOUSE, 78882 St. Quentin en. Yvelines, Cedex, France

SP8 - 100 ultraviolet spectrophotometer (PYE - UNICAM LTD, York Street, Cambridge, England CB1 2PX).

ERWEKA dissolution apparatus and accessories. (Erweka Appatebau G. M. B. H. 6056, Heusenstamm Kr. Offenbuch-Main).

KENT pH Meter Model 7060 with a combination pH Electrode Model EL 887 (Electronic, Instruments, LTd., Chertsey, Surrey, England).

Mettler Balance Model AE 240 (Mettler Instruments LTD., Kingsmead House. Abbey Barn Road. High Wycombe Buckinghamshire).

MAINS STUART Auto Vortex Mixer (Gallemkamp, England.

Centrifuge Machine. ECONOSPIN. Model 120010 (SORVAIL$^{(R)}$ Instruments, Republic of Germany. Biotechnology System Division, U.S.A.)

Hanson Scale (Hanson Industries Ltd., Cleveragh Estate, Sligo, Ireland).

Autostill ^{TM} Distilling machine. (Jencons Scientific LTD, CherryCourt Way Industrial Estate, Stranbridge Road, Leighton Buzzard).

3.1.3 Reagents

Hydrochloric acid - Analar grade (BDH chemicals Ltd., Poole, England).

Sodium Hydroxide pellets - (BDH chemicals Ltd., Poole, England).

Sodium Nitrite - May and Baker Ltd., Dagenham, England).

Methanol - Analar grade (BDH Chemicals Ltd., Poole, England).

Acetone - Analar grade (May and Baker Ltd., Dagenham, England).

Sulphuric Acid - Analar grade (BDH Chemicals Ltd., Poole, England).

Sodium Carbonate analydrous (G.P.R., Hopkin & William, Chadwell Health, Essex, England.

Dichloromethane - Analar grade (BDH Chemicals Ltd., Poole, England).

Acetic acid - Analar grade (BDH Chemicals Ltd., Poole, England).

Diethylether - Analar grade (BDH Chemicals Ltd., Poole, England).

Perchloric Acid - Analar grade (BDH Chemicals Ltd., Poole, England).

Glucose powder - Analar grade (BDH Chemicals Ltd., Poole, England).

Phenol - Analar grade (BDH Chemicals Ltd., Poole, England).

Sodium chloride - Analar grade (BDH Chemicals Ltd., Poole England).

Sodium acetate anhydrous (G.P.R., Hopkin & William, Chadwell, Health, Essex, England).

4-Amino phenazone - Analar grade (BDH Chemicals Ltd., Poole, England).

Benzoic acid - Analar grade (BDH Chemicals Ltd., Poole, England).

3.2 METHODS

3.2.1 Quality Control of Chlorpropamide tablets

3.2.1a Identification

A quantity of the powdered tablets equivalent to 1g of chlorpropamide was extracted with five quantities each of 4ml of acetone, filtered and evaporated carefully to dryness on a water-bath.

The residue was used to carryout the following tests:

i. 0.1g of the residue was boiled with 8ml of a 50 percent w/w solution of sulphuric acid under a reflux condenser for 30 minutes, cooled and filtered, the filtrate was reserved. The melting point of the precipitate was taken.

ii. The filtrate reserved above was made alkaline with 5M sodium hydroxide and heated.

iii. 0.1g of the residue was heated with 1g of anhydrous sodium carbonate at a dull red heat for ten minutes, and cooled. The residue was extracted with water and filtered. The filtrate was acidified with 2M nitric acid and silver nitrate solution was added.

iv. U.V. spectrum of the sample was run together with that of the reference sample supplied by PFIZER INTERNATIONAL.

3.2.1b Chemical Assay

Twenty chlorpropamide tablets were accurately weighed and the average weight determined. They were powdered and a quantity of the powder equivalent to 0.25g of chlorpropamide was weighed and added to 40ml of methanol in a 50ml volumetric flask, and shaken for 20 minutes. Sufficient methanol was added to produce 50ml, mixed and filtered. 5ml of the filtrate was diluted to 100ml with 0.1M hydrochloric acid and mixed. 10ml of the resulting solution was diluted to 250ml with 0.1M Hcl acid and mixed, and the absorbance of the resulting solution measured at the maximum at 232nm. The percentage content of chlorpropamide was calculated taking 598 as the value of A (1%, 1cm) at 232 nm (B.P. 1988).

Formula

$$\frac{\text{Absorbance}}{\text{Weight of the drug in gm} \times A\ (1\%,1cm)} \times \text{Dilution factor}$$

$$A\ (1\ \text{percent, 1cm}) = \frac{A}{cd}$$

Where A (1 percent, 1cm) is the absorbance of a 1cm layer of a 1% w/v solution (1g in 100ml) of the absorbing solute. It

is also called the specific absorbance; c is the concentration of the absorbing solute expressed as % w/v and d is the thickness of the absorbing layer in centimeter.

3.2.1c Dissolution Rate Tests

One litre of physiological buffer solution, pH 6.8 as the dissolution medium was introduced into the vessel and immersed in a constant temperature water bath maintained at $37\pm0.5^{o}C$. One chlorpropamide tablet was placed in a dry basket in an assembled dissolution apparatus, and the basket was lowered into position. The dissolution apparatus was operated and the basket containing the chlorpropamide tablet was rotated at 100 revolutions per minute. 10ml of the sample was withdrawn at 45 minutes from a point half-way between the surface of the dissolution medium and the top of the rotating basket, not less than 10mm from the wall of the vessel. The sample was filtered at $37^{o}C$ and the amount of active ingredient present in the sample was determined by measuring the absorbance of the diluted sample at 230nm. The total content of chlorpropamide in the medium was calculated by taking 469 as the value of A (1%, 1cm) at the maximum at 230nm. Complete operation was repeated four times (B.P., 1988).

Preparation of buffer

6.80g of potassium dihydrogen orthophosphate was accurately weighed and dissolved in 100ml of water, sufficient distilled water was added to produce 1000ml. It was warmed to 37°C and the pH adjusted to pH 6.8, with IM sodium hydroxide (B.P., 1988).

3.2.2 Food Samples Analysis

3.2.2a Determination of the Moisture Content

A clean petri dish was dried in an oven at 80°C for about 30 minutes, cooled in a dessicator and weighed. 5.00g of the ground meal sample was placed in the dish and accurately weighed again. The sample in the container was then dried in an oven at (50 to 60°C) for 5 hours. The sample was immediately transferred to a dessicator after drying to cool. It was quickly weighed after cooling. The procedure was repeated by drying for 3 hours for each subsequent weighing till constant weight was obtained. Duplicate determination of each sample was made and the average determined (Pearson, 1973).

The moisture content of the sample was calculated using the following relation:

$$\text{Moisture (\%)} = \frac{\text{Loss in weight due to drying}}{\text{Weight of sample taken}} \times 100$$

3.2.2b Determination of the ash content

A porcelain crucible with lid was ignited in a Bunsen burner flame and transferred to a dessicator to cool, and weighed.

5g of the ground meal sample was weighed into the crucible and weighed again. The crucible containing the sample was heated gently on a Bunsen burner in a fume cupboard until smoking ceased, and then transferred into a muffle furnace heated at 550 - 570^{o}C untill it turned white. The crucible was taken out, covered immediately and transferrred to a dessicator to cool and it was weighed (Pearson, 1973).

The ash content of the sample was calculated by using the relation:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

3.2.2c Determination of fat (lipid) content

A soxhlet extractor was fitted to a reflux condenser and a 500ml round bottom flask. 5g of the dried meal sample was weighed into a fat-free extraction thimble which was

previously dried in an oven and weighed. It was plugged with cotton wool and re-weighed again. The thimble was placed in the extractor and diethylether was added until it siphoned over, more ether was added until the barrel of the extractor was half full.

The condenser was replaced, and placed on a heating mantle. The solvent was allowed to boil gently and left to siphon over for 12 hours. Finally, the ether was left to siphon over until the barrel of the extractor was just empty. The condenser was detached, and the thimble was removed. The thimble was placed in a fat-free clean beaker and transferred into an oven to dry at $50^{o}C$ to a constant weight (Pearson, 1973).

The weight of the extracted lipid is given by either the weight of lipid in the flask after extraction or the weight from the thimble as (weight of thimble containing the sample before extraction) - (weight of thimble and sample after extraction).

The content of fat in the sample was calculated by the following relation:

$$\text{Lipid}^{(f)}\ \% = \frac{\text{Weight loss of sample (extracted fat)}}{\text{Weight of sample}} \times \frac{100}{1}$$

3.2.2d Determination of the total carbohydrate content

0.03g of the dried food sample was weighed and transferred to 100ml beaker.
10ml water was added and the content of the beaker was stirred thoroughly. 13ml of 52% perchloric acid was added and the mixture was stirred with a glass rod for 30 minutes. The rod was washed down with 20ml of water and the content of the beaker transferred into a 250ml volumetric flask made to volume with distilled water.

The contents of the flask were mixed, filtered and 1ml filtrate was taken in a test tube in duplicate.
1ml distilled water was taken in another set of test tubes as blank. 5ml of anthrone reagent was added to each tube and mixed.
The tubes were placed in boiling water for 12 minutes and cooled at room temperature. The absorbance was determined in 1-cm cell at the wavelength of 630nm against the reagent blank of distilled water. The sample was assayed by reference to a standard calibration curve (Joslyn, 1970). The standard solutions were treated in the same manner and the carbohydrate content was calculated by the formula:

$$\frac{\text{Concentration of sample} \times \text{Dilution factor}}{\text{Weight of Sample}} \times \frac{100}{1}$$

Calibration curve

50mg of standard glucose powder was accurately weighed, and dissolved in 100ml of 0.1M perchloric acid in a 100-ml volumetric flask. Thus, the standard solution contained 500Mcg/ml. .05, 0.1, 0.2 and 0.3 ml of the standard solution were transferred into series of 10-ml volumetric flasks; enough distilled water was added to volume. Solutions of various concentrations (0.025 - 0.15Mg) were obtained. 1ml standard solutions were taken in a set of test tubes and treated in the same manner as sample and blank. The absorbance was measured at 630nm against the reagent blank. Graph of absorbance against concentration was plotted.

3.2.2e Determination of protein content by estimation of Nitrogen using Kjeldahl method

0.25g of the dried sample was weighed into a micro Kjeldahl digestion flask.

0.8g of catalyst mixture and 2ml of nitrogen free concentrated sulphuric acid were added and stirred to mix. The flask which was fitted with a loose pear-stopper in an inclined position was heated in a fume cupboard. The heat was applied gently first, but when the initial frothing subsided it was increased gradually untill the liquid boiled at a moderate rate. The flask was swirled from time to time in order to wash down any chaired material adhering to the

flask. The heating proceeded for 1h after the liquid had become clear. The flask was left to cool. The content of the flask was distilled using the Markham semi-micro Kjeldahl distillation apparatus (figure 13).

Distillation procedure: The content of the Kjeldahl flask was transferred quantitatively to the reaction vessel through the funnel. The flask and funnel were rinsed with two successive 5-ml volumes of water. 15ml of 40% sodium hydroxide solution was added through the funnel and the mixture was allowed to distill into 20 ml of 0.1M Hcl containing 2 drops of phenolphthalein indicator in a conical flask. The content of the conical flask was titrated with 0.1M NaoH (Pearson, 1973).

Nitrogen content of proteins varies from 15-18% with an average value of 16%. Therefore, the crude protein value was estimated by the relation. Nitrogen x 6.25 (factor), and the protein content of the sample was given by the relation:

$$\frac{\text{g protein}}{\text{g sample}} \times \frac{100}{1}$$

Preparation of catalyst mixture:

200g of sodium sulphate was mixed with 8g of hydrated copper sulphate and 1.5g of selenium dioxide.

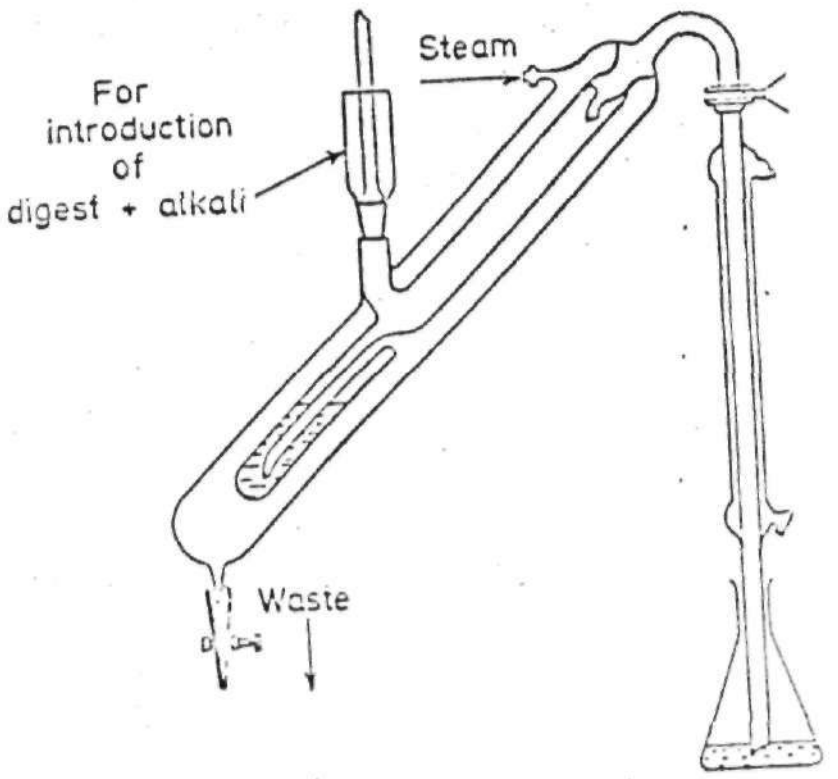


Fig. 13. Markham semi-micro Kjeldahl distillation apparatus (Pearson, 1973).

3.2.3 Meal-Chlorpropamide Interaction Studies In Healthy Human Subjects

3.2.3a Subjects

Normal healthy adult human subjects (16 males and 2 females) volunteered for the study. Their age ranged from 28 to 35 years, mean ±S.D 33.50 (± 3.25) height ranged from 1.50 to 1.75m, mean ±S.D 1.70 (± 0.08) and their weight range was 55 to 70kg, mean ±S.D 62.33 (± 5.76). Fifteen males completed the study. There were three groups of meals and five subjects were used for each group. Every subject served as his own control.

3.2.3b Groups of Meals

1. Cooked Maize flour "Tuwon masara" and "Miyan kuka" (*Adansonia digita Linn*) soup.
2. Cooked Cassava flour "Gari" and vegetable (*Amaranthus tricolor Linn*) soup.
3. Cooked Yam flour "Amala" and okra (*Hibiscus esculentus*) soup.

350g meal consisting of 25g meat was dished out to each healthy and patient volunteers.

3.2.3c Study Design

Each subject received the following two treatments at two weeks intervals, using a two way cross over design: (i) one 250mg chlorpropamide tablet administered in a fasting state; (ii) one 250mg chlorpropamide tablet administered orally immediately after a meal of the subjects' group. 150ml water was taken with each treatment.

The protocol of the study was explained to each of the subjects and an informed consent was obtained. No drugs including alcohol was allowed one month before the study and each subject fasted overnight before taking part on the following day. On the day of each test, prior to oral ingestion of the 250mg chlorpropamide tablet each subject attended the trial room and was allowed 5-min relaxation on a couch. A cannula was placed in an antecubital vein and 5ml of blood taken in a heparinized tube predose.

For the first treatment, the tablet was swallowed on an empty stomach at 08.00 a.m. and the subject fasted for the next 4 hours after drug administration. The subject was then given cooked "jollof" beans (350gm) before further blood samplings. A 5ml blank saliva sample was also collected after stimulation with a rubber band which was chewed for 2 min. Further saliva samples were collected from six subjects whose saliva pH varied within a narrow

range (7.00 - 7.40), and were able to produce 5ml in 2 min.

Further samplings were at times 0.5, 1, 2, 4, 6, 8, 12, 36, 48, 72, 144 and 168 hours. The subject undergoing the second treatment took the meal of his or her group together with 250mg dose of chlorpropamide tablet. Only the blood samples were collected for this treatment. No food or other drink was allowed during the four hours of the study. Blood was collected in heparinized tubes and plasma was obtained by centrifuging at 2000g for 10 minutes. Using pasteur pipette, the plasma was transferred into bijoux bottle, stoppered and stored alongside the saliva samples which were collected in plain plastic containers in a deep freezer at -20°C until analyzed.

3.2.4 Meal-chlorpropamide Interaction Studies in Diabetic Patients

3.2.4a Subjects

Eighteen type II diabetic patients (16 males and 2 females) freshly diagnosed participated in the study. The diagnostic criteria were based on the National Diabetes Data Group's recommendation in 1979. Their age ranged from 32-55 years, mean ±S.D 45.86(± 8.83), height ranged from 1.55 to 1.82m, mean ±S.D 1.67(± 0.06) and their weight range was 50 to 72 kg, mean ±S.D 64.86(± 8.30). Six subjects were used for each group of the three groups of meals. The meals were

as described for the healthy subjects study.

3.2.4b Study Design

Each patient received the following three treatments at 2 weeks intervals, using a three way cross over design: (i) a standardized meal only; (ii) a standardized meal given 30 min after the administration of one chlorpropamide 250mg tablet orally and (iii) a standardized meal given together with 250mg chlorpropamide tablet administered orally.

2ml blood sample was drawn for the first treatment (meal only) while 5ml blood sample was drawn for other treatments. The sampling times were 0, 0.5, 1, 2,4, 6, 8 hours, for the first treatment (only meal) and further samplings for the other two treatments were at times 24, 48, 72, 144 and 168 hours. Other procedures were as described for the healthy subjects.

3.2.5 Drug Level Analysis

3.2.5a Extraction Procedure

The extraction profile of chlorpropamide and Tolbutamide (I.S) were studied in hexane, toluene, diethylether, chloroform and dichloromethane.
Blank plasma sample (0.5ml) was spiked with 50mcg/ml chlorpropamide, 100mcg/ml tolbutamide (I.S) and 1M Hcl (0.5ml). 5ml of each solvent was added and mixed on an automixer for

10 seconds, centrifuged at 2000g for 3 min and the aqueous layer was aspirated. The aliquot layer was removed and evaporated to dryness in a stream of nitrogen at 40^{o}C. The dried residue was dissolved in 50UL of methanol and 10UL injected on to the column.

The percentage extraction recoveries of chlorpropamide was calculated using the following relation

$$\frac{\text{Peak Height Ratio of The Extracted Chlorpropamide}}{\text{Peak Height Ratio of The standard chlorpropamide.}} \times \frac{100}{1}$$

The above extraction procedure was repeated with 3.00ml of the most suitable solvent (dichloromethane) using different concentrations of chlorpropamide and maintaining the same concentration of tolbutamide (I.S).

3.2.5b Optimization studies

The ratio of the components of the solvent system methanol:acetic acid was varied and the effect of pH of the mobile phase on the suitable ratio of the solvent system was studied to optimize the system.

3.2.5c Preparation of standard solutions and standard curves

Stock solutions of chlorpropamide and tolbutamide (1mg/ml) were prepared by dissolving 50mg of the reference

sample in 1ml of 0.1M sodium hydroxide and diluting to 50ML with water. These solutions were found to be stable for at least 5 months when stored at $4^{o}C$. Working solutions of appropriate concentration were prepared every week by dilution of the stock solutions. Plasma calibration curve was prepared by spiking blank plasma sample 0.5ml with 1, 5, 10, 20, 30, and 40 mcg/ml chlorpropamide standard and 100mcg/ml tolbutamide as internal standard. The saliva calibration curve was also prepared by spiking blank (1ml) saliva samples with 0.2, 0.4, 0.6, 0.8, 2, 3 and 4 mcg/ml chlorpropamide standard and 10mcg/ml tolbutamide as internal standard. The samples were prepared as described under extraction procedure using 3ml of dichloromethane as the extraction solvent.

3.2.5d Precision

The precision of the assay was determined by analyzing drug-free human plasma/saliva spiked with known amounts of chlorpropamide. The coefficient of variation (C.V.) at three different concentrations of plasma and saliva for five samples at each level were studied (Within-assay and day-to-day assay).

3.2.6 Plasma glucose analysis

3.2.6a Principle

Glucose oxidase is a specific enzyme which promotes the oxidation of glucose to acid with the production of an equivalent amount of hydrogen peroxide. In the presence of a second enzyme - peroxidase, oxygen from peroxide can be transferred to a suitable acceptor with the production of a coloured complex compound. The intensity of which is proportional to the concentration of glucose in sample (Martinek, 1964).

$$\text{Glucose} \xrightarrow[\text{oxidase}]{\text{Glucose}} \text{Gluconolactone} \xrightarrow[O_2]{H_2O} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Oxygenacceptor} \xrightarrow{\text{Peroxidase}} \text{colour complex} + H_2O$$

Fig. 14 Summary of the enzymic methods for glucose estimation.

3.2.6b Reagents and Preparation

1. Phenol Reagent: 21.25mmol/L phenol in 0.154M sodium chloride solution (1.94g colourless phenol crystals in 1 litre of 0.9% sodium chloride).

2. Enzyme of colour reagent:
15ml Fercozyme (glucose oxidase), 15mg peroxidase, 300mg of 4-amino phenazone were mixed and made up to volume with sodium acetate/acetic acid buffer (12.88g of sodium acetate

anhydrous dissolved in 200ml of distilled water, 320ul of glacial acetic acid was added) in 1000 - ml volumetric flask. Solution was stable for 8 weeks at $4^{o}C$.

3. Stock glucose solution:

18.02g of glucose dissolved in 1-litre of distilled water saturated with benzoic acid.

4. Standard glucose working solution:

1.0ml of stock glucose solution diluted to 10ml with distilled water saturated with benzoic acid.

3.2.6c Procedure

Three tubes were labelled as test, standard and blank. The phenol reagent, plasma, standard glucose, distilled water and colour reagent were mixed as shown in the table below.

Reagent	Test Ml	Standard Ml	Blank Ml
Phenol Reagent	2.5	2.5	2.5
Plasma	0.05	-	-
Standard (10Mmol/L)	-	0.05	-
Distilled Water	-	-	0.05
Colour Reagent	2.5	2.5	2.5

The tubes were incubated at 37°C for 20 minutes and cooled at room temperature for 45min. The absorbance was measured at 520nm using the reagent blank. The amount of glucose in the sample was calculated using the following formula:

$$\text{Glucose Mmol/L} = \frac{\text{Absorbance of Test-Blank}}{\text{Absorbance of standard-Blank}} \times \frac{10}{1}$$

3.2.7 Data Analyses

After the single oral dose of chlorpropamide, the pharmacokinetic parameters were calculated using a one compartment open-model itinerated computer programme KINEMP[1]

Results were expressed as mean ± S.D. They were analysed for statistical significance by students t-test for paired data and the differences among meals were compared by analysis of variance (ANOVA), the significant points were further analysed using the least square difference (LSD). Healthy and patient volunteers' results were compared using students t-test for independent data. $P < 0.05$ being considered significant in all cases.

CHAPTER FOUR

RESULTS

4.1 Quality Control of Chlorpropamide Tablets:

The melting point of the recrystallized product of the extracted chlorpropamide was 142.5^{0}C. The filtrate obtained when treated with 5M NaoH solution produced an ammoniacal odour. The extracted drug heated with anhydrous sodium carbonate and treated with $AgNO_3$ produced white precipitate. The solution of the sample obtained in the assay exhibited an absorption maximum at 232nm (Figure 15).

The chlorpropamide tablets and standard powder contained 98.66 and 100.5 percent of the active ingredients respectively. Thus, the sample and the standard powder complied with the B.P. (1988) specification of drug content (92.5 - 107.5)%. The drug content values and the percentage composition are shown in Table 1a.

The percentages of the active ingredient released in solution during the dissolution test are shown in table 1b. The mean amount released was 78.97$\pm$0.23%. The results complied with the offical B.P. (1988) requirement of not less than 70% in 45min.

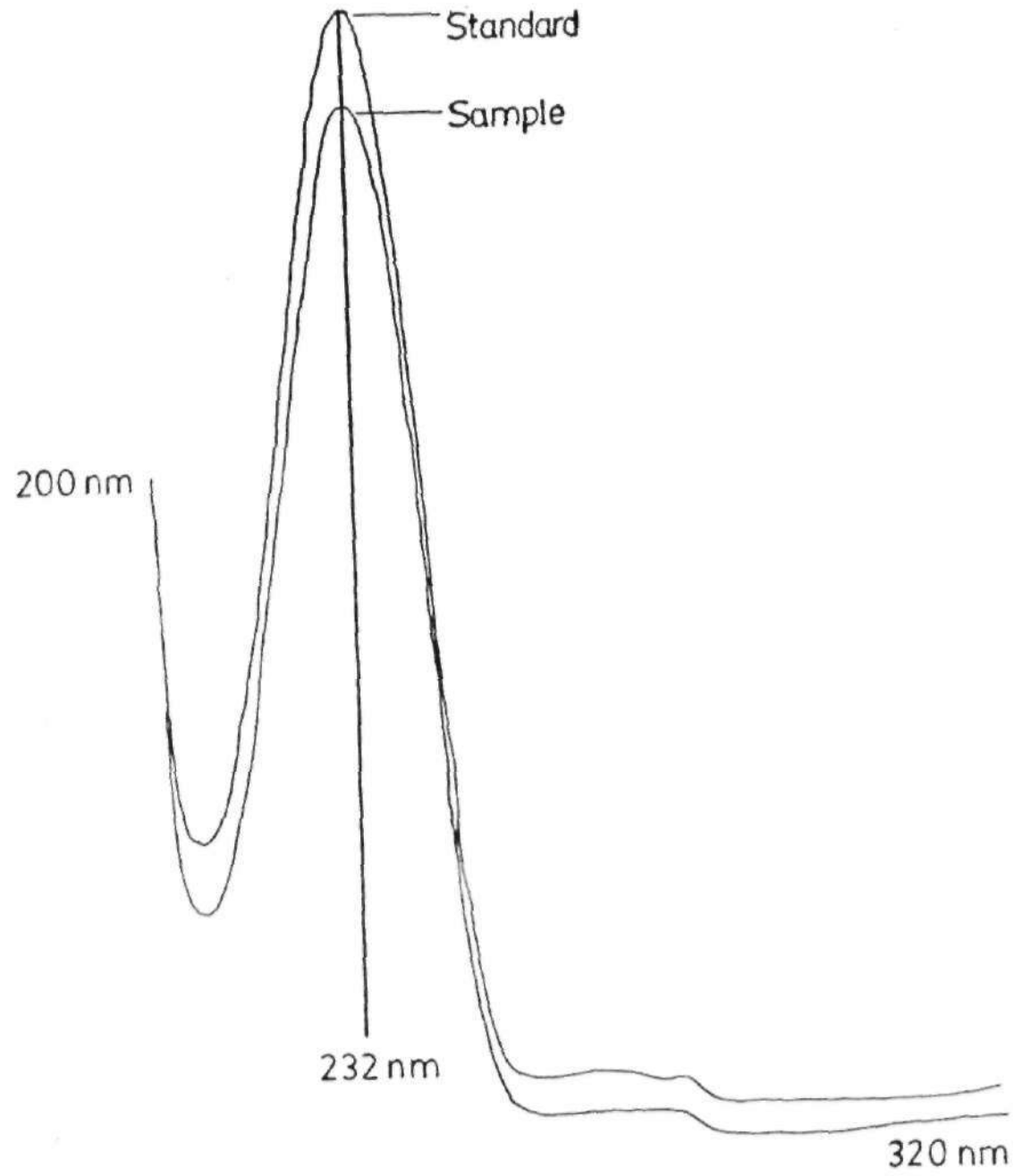


Fig. 15. Absorption spectra for Chlorpropamide standard powder and tablet.

TABLE 1a: QUANTITATIVE DETERMINATION OF CHLORPROPAMIDE TABLETS AND STANDARD POWDER USING B.P. (1988) METHOD

CHLORPROPAMIDE	DRUG CONTENT	% ± S.D.
Tablets	246.65	98.66±0.003
Standard Powder	251.25	100.5±.001

Table 1b: DISSOLUTION OF CHLORPROPAMIDE TABLETS IN PHOSPHATE BUFFER pH 6.8 MAINTAINED AT 37±0.5^{0}C USING USP XVIII ROTATING BASKET METHOD WITH A STIRRING RATE OF 100 r.p.m.

No. of tablets	Percentage Dissolved
1	78.67
2	78.93
3	79.10
4	79.19
Mean ±S.D.	78.97±0.23

S.D. = Standard Deviation

4.2 Food Samples Analysis:

The mean percentage composition of the meals with their energy values are shown in Table 2. The calibration curve for carbohydrate content determination is shown in Figure 16. It can be seen that the 3 meals (B, C and D) used in this study have low protein and high carbohydrate contents.

4.3 Drug Levels Analysis:

Extraction from Biological Samples:- Out of all the solvent media tried for the extraction of chlorpropamide and tolbutamide (I.S.) from plasma and saliva, dichloromethane was found to be most suitable at pH of about 3. The percentages of chlorpropamide recovery from plasma and saliva are shown in Table 3.

Solvent system: The effects of varying the ratio of methanol and acetic acid (0.2%) on the resolution of chlorpropamide and tolbutamide are shown in Table 4a, Figure 17a and Table 4b, Figure 17b respectively. The influence of pH of the mobile phase on chlorpropamide and tolbutamide resolution is shown in Table 4c, Figure 17C. There was no separation at pH below 4.5. Resolution of chlorpropamide

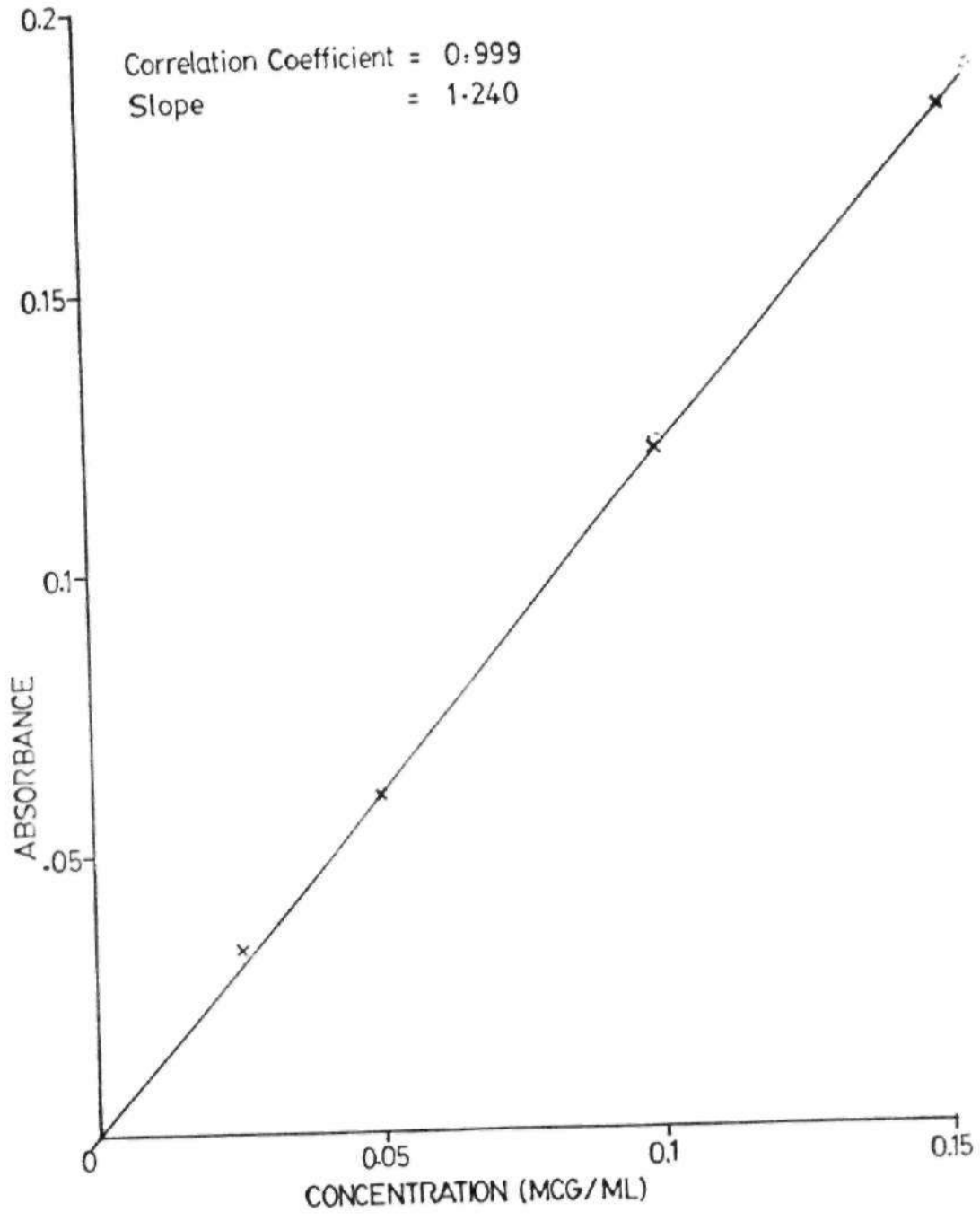


Fig. 16. Calibration curve for glucose in distilled water using colorimetric method.

Table 2: MEAN PROXIMATE PERCENTAGE COMPOSITION OF THE MEALS WITH ENERGY VALUES

Sample Code	Energy Value Cal.	KJ	Percentage Moisture	Percentage Composition of Dried Sample ±S.D. Ash	Fat	Protein	Carbohydrate
A	446.40	1867.73	61.80	5.25±0.39	15.20±0.5	17.90±0.20	59.50±1.00
B	428.46	1792.67	76.70	2.00±0.14	10.50±0.30	2.99±0.89	80.50±0.20
C	447.30	1871.50	75.00	3.50±0.20	14.70±0.14	2.75±0.42	76.00±0.50
D	417.00	1744.73	79.60	3.80±0.28	8.00±0.50	1.75±0.35	84.50±1.06

A = Jollof Beans

B = Cooked maize flour and "aiyan kuka" meal

C = Cooked cassava flour and okra soup meal

D = Cooked yam flour and vegetable soup meal.

S.D. = Standard Deviation

Table 3: PERCENTAGE EXTRACTION RECOVERIES OF CHLORPROPAMIDE AT DIFFERENT CONCENTRATIONS FROM PLASMA AND SALIVA

Concentration (mcg/ml)	% Recoveries Plasma	Saliva
0.20	95.60	98.60
0.50	97.40	98.52
2	96.00	97.52
10	95.60	96.50
20	94.32	96.00
30	93.56	95.32
Mean	95.41	97.07
±S.D.	1.34	1.35

S.D. = Standard Deviation

Table 4a: EFFECT OF METHANOL ON THE RESOLUTION (R) OF CHLORPROPAMIDE AND TOLBUTAMIDE USING SOLVENT SYSTEM METHANOL 0.2% ACETIC ACID WITH U-BONDA - PAK C18 RADIAL PAK CATRIDGE (WATERS ASSOCIATE)

Solvent Percentage Methanol	0.2% Acetic Acid	Resolution R
20	80	3.93
40	60	1.34
50	50	1.94
60	40	1.77
80	20	1.42

Table 4b: EFFECT OF 0.2% ACETIC ACID ON THE RESOLUTION (R) OF CHLORPROPAMIDE AND TOLBUTAMIDE USING SOLVENT SYSTEM METHANOL 0.2% ACETIC ACID WITH U-BONDA- PAK C18 RADIAL PAK CATRIDGE (WATERS ASSOCIATE)

Solvent Percentage 0.2% Acetic Acid	Methanol	Resolution R
20	80	1.42
40	60	1.77
50	50	1.94
60	40	1.34
80	20	3.93

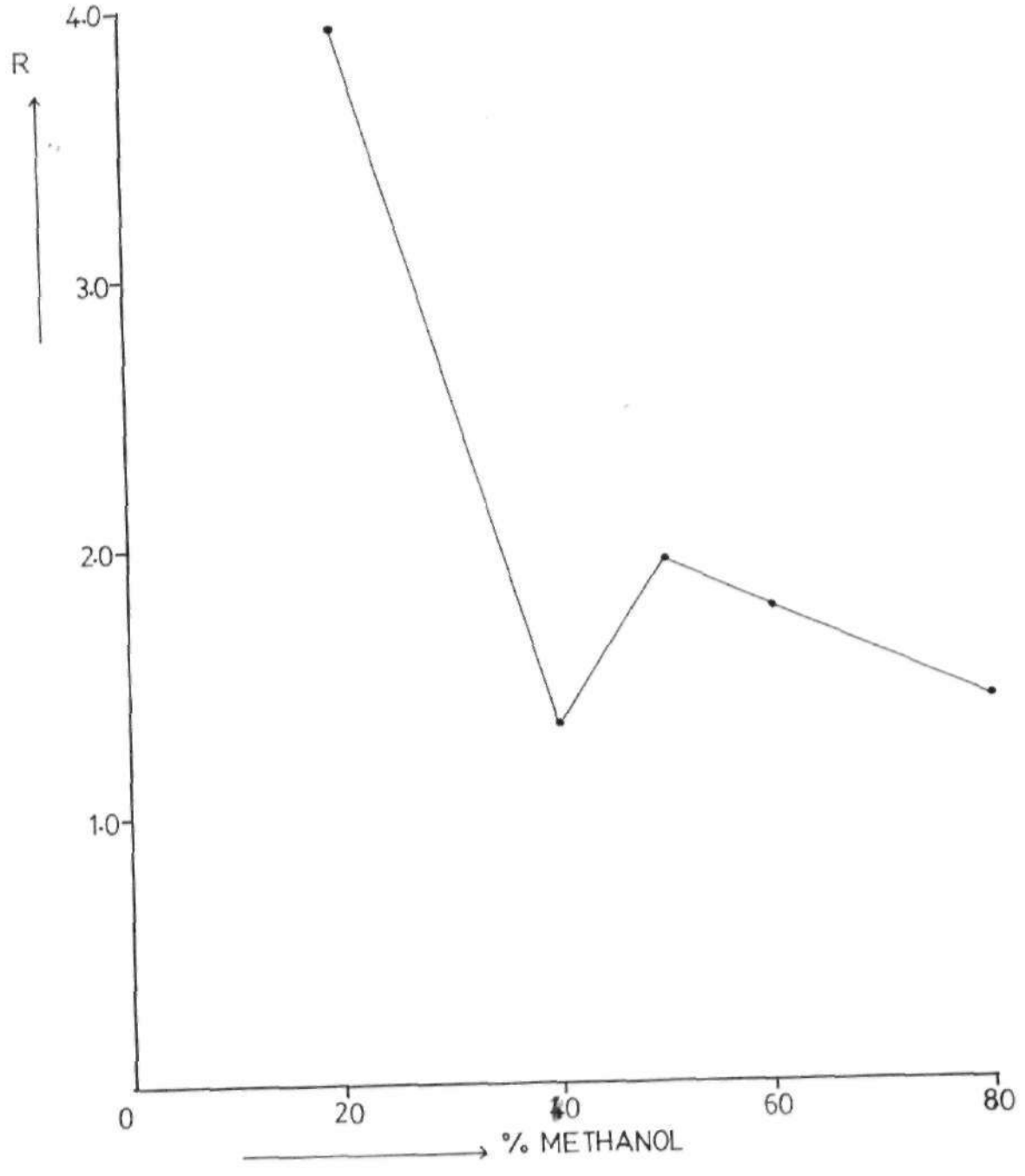


Fig. 17a. *Effect of methanol on the resolution (R) of Chlorpropamide and tolbutamide using solvent system methanol: 0.2% acetic acid with μ-Bonda-Pak C_{18} Radial Pak Catridge (Waters Associate).*

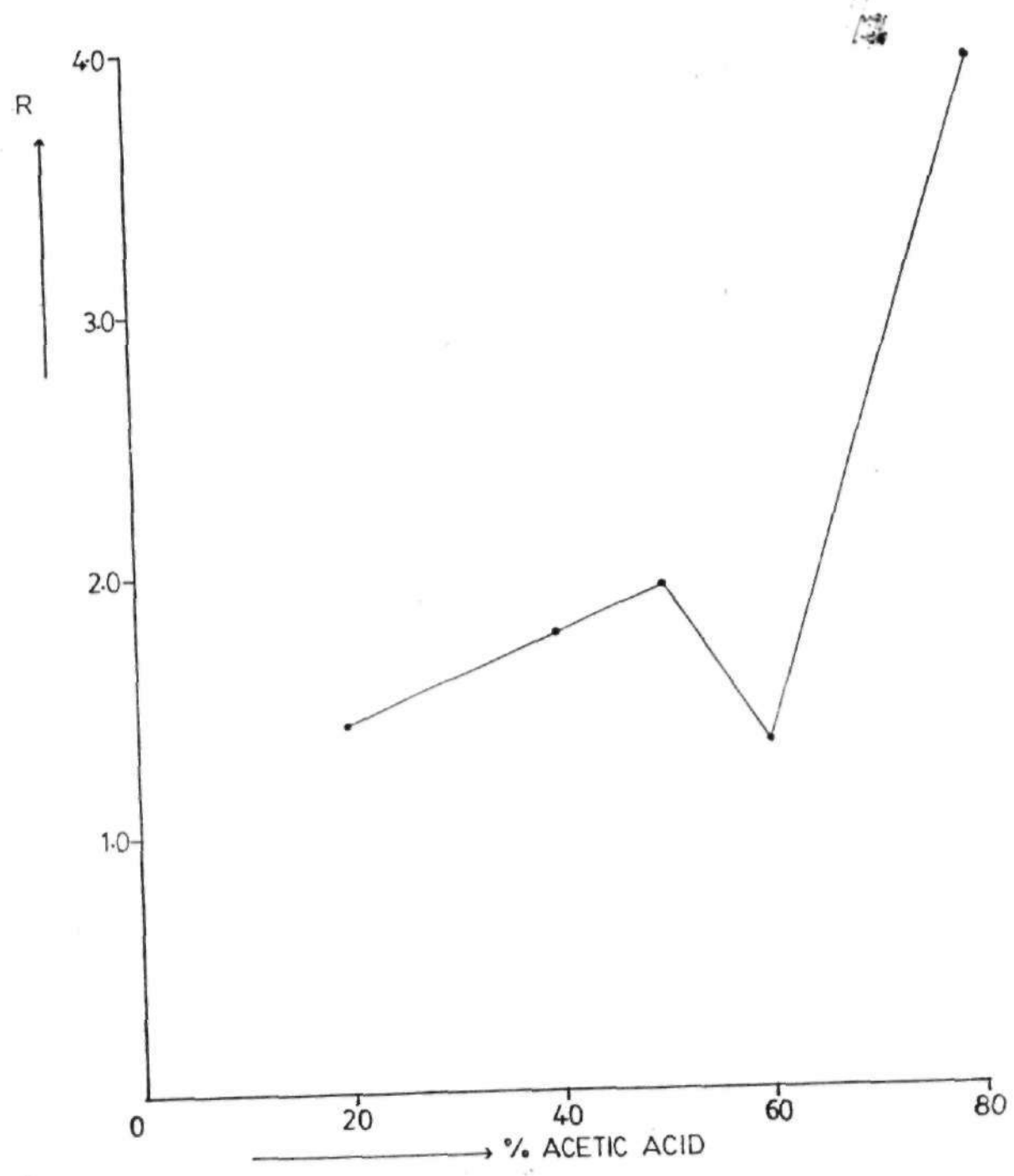


Fig.17b. Effect of 0.2% acetic acid on the resolution (R) of Chlorpropamide and tolbutamide using solvent system methanol: 0.2% acetic acid with μ-Bonda-Pak C_{18} radial Pak Catridge (Waters Associate).

Table 4c: EFFECT OF pH ON THE RESOLUTION (R) OF CHLORPROPAMIDE AND TOLBUTAMIDE (INTERNAL STANDARD) USING SOLVENT SYSTEM METHANOL 0.2% ACETIC ACID (3:2) WITH U-BONDA-PAK C18 RADIAL PAK CATRIDGE (WATERS ASSOCIATE)

pH of Mobile Phase	Resolution (R)
4.50	0.63
5.00	0.90
5.50	1.13
6.0	1.33
6.50	1.55
6.70	1.60
7.00	1.22

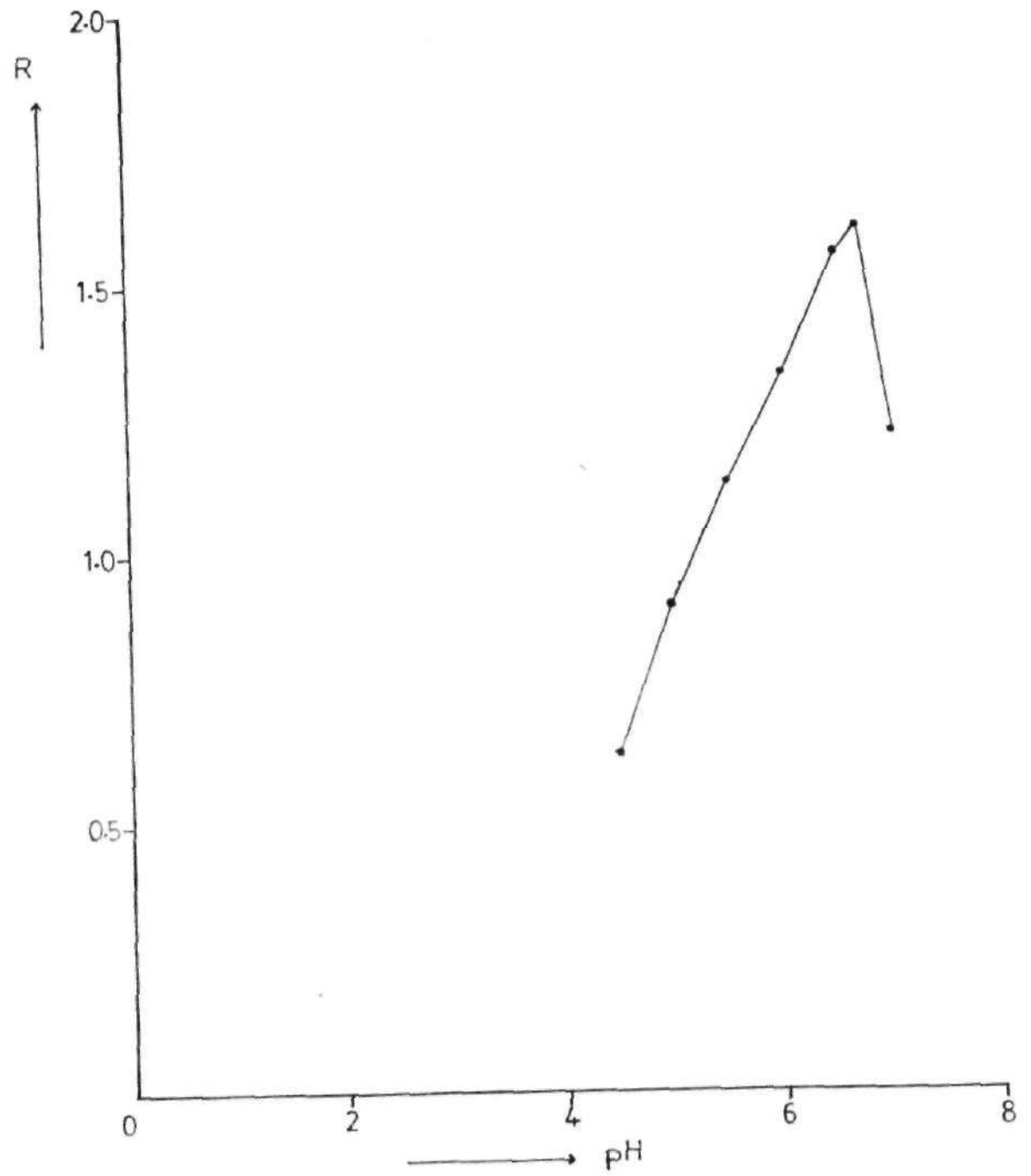


Fig. 17c. Effect of PH on the resolution (R) of Chlorpropamide and internal standard (tolbutamide) using solvent system methanol: 0.2% acetic acid (3:2) with μ-Bonda-Pak C_{18} radial Pak catridge (Waters Associate).

and tolbutamide (I.S) increased with the increase in pH up to pH 6.7. However at pH above 6.7 there was a decrease in the resolution. The retention times of chlorpropamide and tolbutamide using the solvent system methanol and 0.2% acetic acid (3:2) at pH about 6.5 were 8 and 11 min respectively. The results of within and day-to-day assay studies are shown in Table 5. Typical chromatograms of an extract of blank plasma and blank saliva containing the internal standard are shown in Figures 18a and 18b respectively.

Table 5: WITHIN-DAY AND DAY-TO-DAY PRECISION OF PLASMA/ SALIVA CHLORPROPAMIDE DETERMINATION

Biological fluid	Concentration (mcg/ml)	Coefficient of variation %	n
Within-run			
Plasma	1.0	3.0	5
	10	2.0	5
	30	1.2	5
Saliva	0.2	5.3	6
	0.5	3.5	6
	1.0	2.8	6
Day-to-day			
Plasma	1.0	4.3	5
	10	3.5	5
	30	1.6	5
Saliva	0.2	6.3	6
	0.5	4.2	6
	1.0	3.0	6

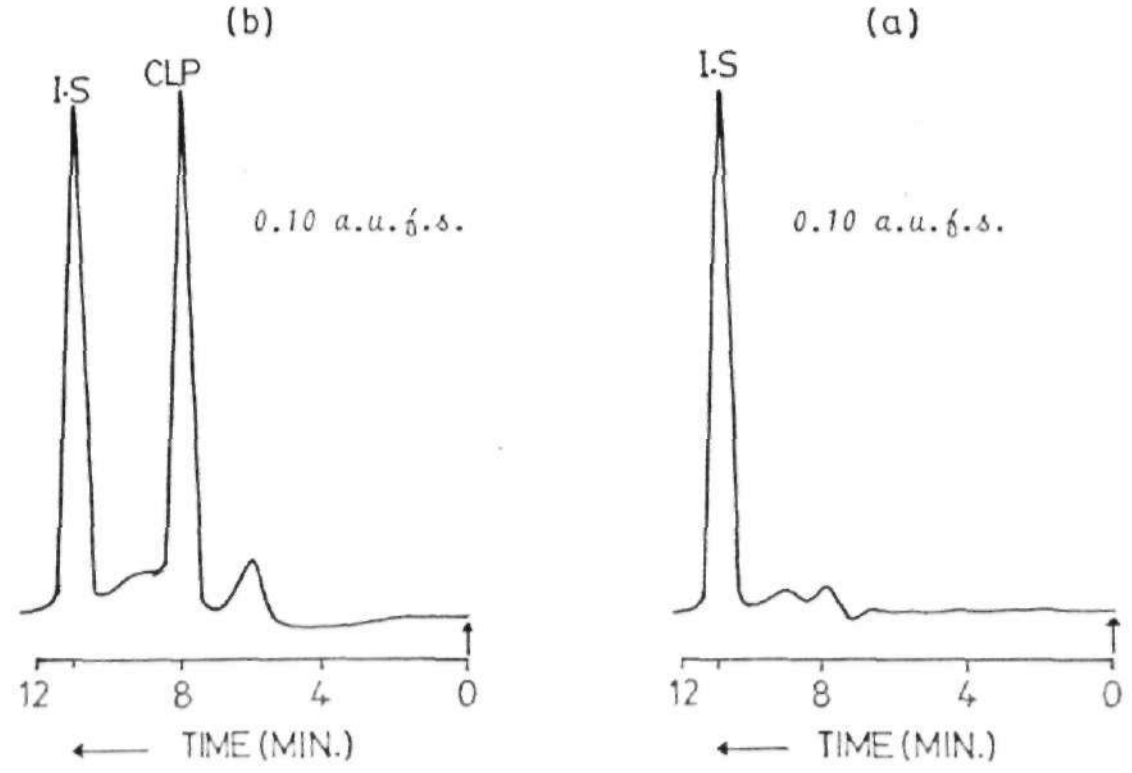


Fig. 18a. High-Performance Liquid Chromatograms of (a) an extract of blank plasma containing the internal standard (I.S), and (b) an extract of test plasma sample obtained from a volunteer 6h following an oral administration of 250mg Chlorpropamide (ClP) tablet.

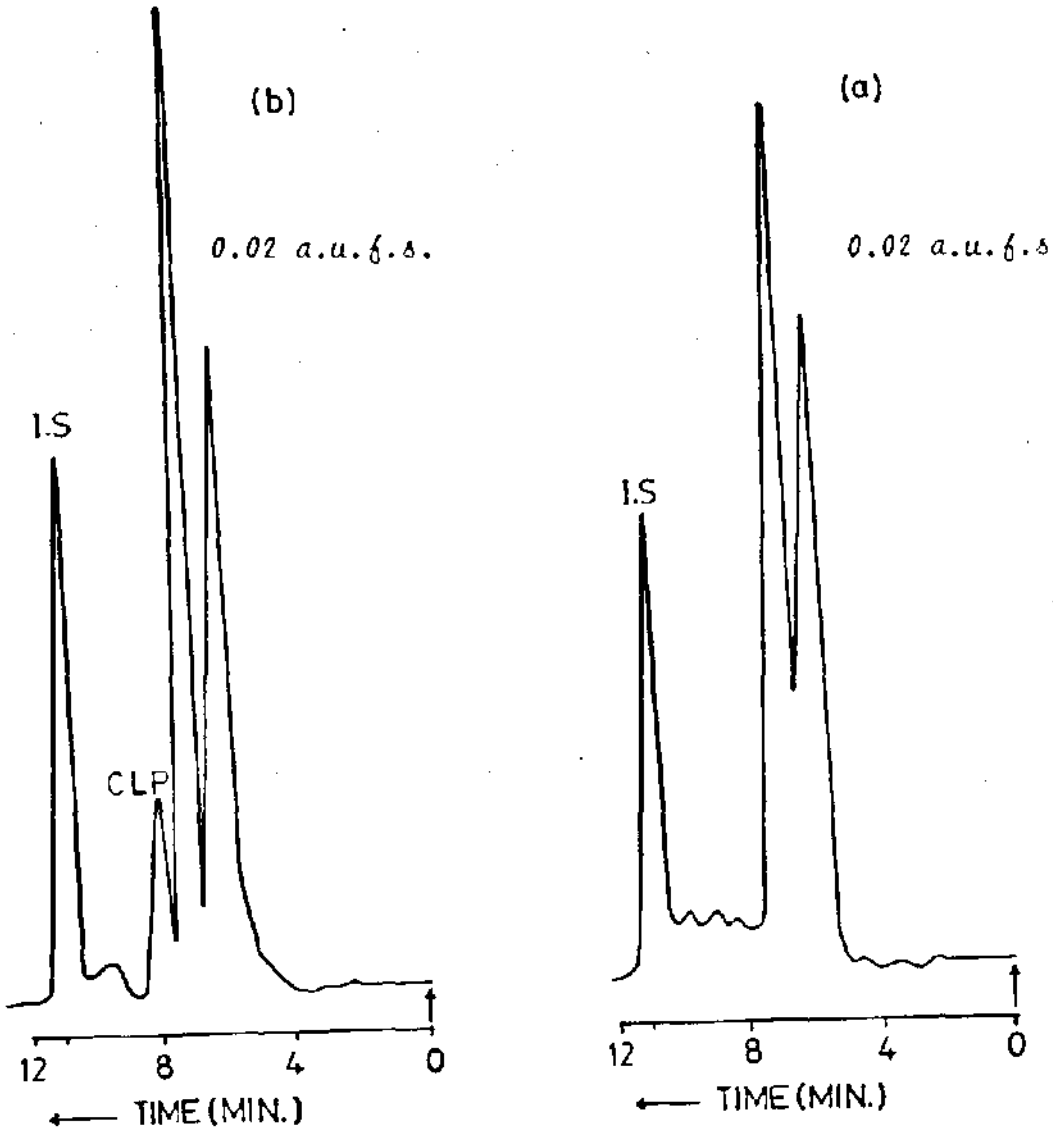


Fig.18b. High-Performance Liquid Chromatograms of an extract of blank saliva containing the internal standard (I.S), and (b) an extract of test saliva sample obtained from a volunteer 6h following an oral administration of 250mg Chlorpropamide (CLP) tablet.

4.4 Cooked Maize Flour Meal (B) Chlorpropamide Interactions Studies In Healthy Volunteers And Diabetic Patients

Figure 19 shows the calibration curve obtained in plasma for chlorpropamide. Following oral administration of a single 250mg chlorpropamide tablet to healthy and patient volunteers as described under the materials and methods, the mean maximum plasma concentrations (Cmax) ranged from 26.34±0.94 to 31.33±3.33 mcg/ml. The mean time to peak plasma concentration (tmax) ranged from 4.33±2.33 to 6.40±1.67h. The absorption half-life (t1/2x) ranged from 0.60±0.21 to 1.65±1.14h and the elimination half-life (t1/2B) ranged from 31.46±8.69 to 34.70±13.05h.

The mean chlorpropamide pharmacokinetic parameters obtained for healthy subjects are shown in table 6a while the mean parameters obtained for patients are shown in Table 6b. The similarities between the parameters are obvious. The differences in the derived parameters compared for both healthy subjects (fasting state versus meal treatment) and patients (30min. before meal and concomitant administration of drug with meal) were however not statistically significant. The total area under the plasma concentration-time curve (AuCo-oo) for the healthy subjects in fasting state when compared with that obtained when the drug was administered concomitantly with meal did not differ

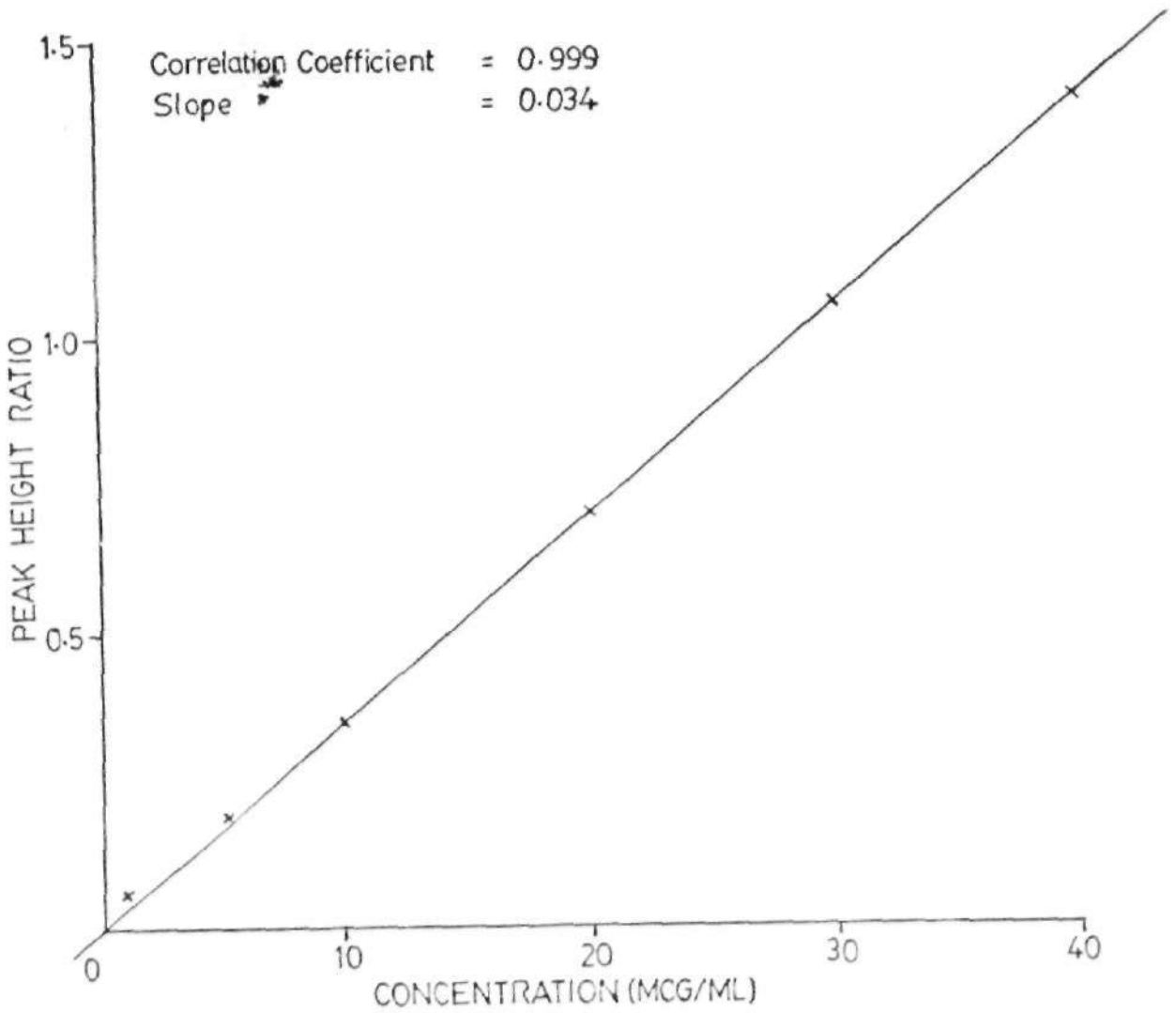


Fig. 19. Calibration curve for Chlorpropamide in plasma using HPLC.

Table 6a: MEAN (±S.D.) PHARMACOKINETIC PARAMETERS DERIVED FROM PLASMA IN 5 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET IN FASTING STATE (A) AND WITH COOKED MAIZE FLOUR MEAL (B)

Mean Parameter ±S.D.	A	B	Paired sample T-Test Results
Lag Time (h)	0.39±0.09	0.53±0.05	P<0.02
Absorption half-life. t1/2 x (h)	0.60±0.21	1.65±1.14	P<0.09
Absorption rate constant. ka (h^{-1})	1.33±0.69	0.53±0.23	P<0.06
Maximum concentration. Cmax mcg/ml	26.34±0.94	26.68±0.35	P<0.36
Time to peak concentration tmax (h)	5.20±1.09	6.40±1.67	P<0.20
Area Under the Curve (AUC) AUCo-168h (mcg/h/ml)	1472.03±398.49	1399.32±378.02	P<0.25
AUCo-o (mcg/h/ml)	1576.89±481.59	1483.72±420.29	P<0.11
Relative bio-availability. FR	0.62±0.19	0.58±0.16	P<0.67
Volume of distribution Vd (L)	7.77±0.83	7.53±0.80	P<0.68
Clearance CL (L/h)	0.17±0.06	0.18±0.06	P<0.70
Elimination half life. t1/2 B (h)	34.70±13.05	31.46±8.69	P<0.28
Elimination rate constant. ke (h^{-1})	0.02±0.01	0.02±0.004	P<0.62

Table 6b: MEAN (±S.D.) PHARMACOKINETIC PARAMETERS DERIVED FROM PLASMA IN 6 DIABETIC PATIENTS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET 30 MIN. BEFORE (A) AND WITH COOKED MAIZE FLOUR MEAL (B)

Mean Parameter ±S.D.)	A	B	Paired sample T-Test Results
Lag Time (h)	0.28±0.13	0.36±0.12	P<0.29
Absorption half-life. t1/2 x (h)	0.81±0.63	1.12±0.58	P<0.17
Absorption rate constant. ka (h^{-1})	1.26±0.72	0.75±0.35	P<0.15
Maximum concentration. Cmax mcg/ml	31.06±3.73	31.33±3.33	P<0.59
Time to peak concentration tmax (h)	4.33±2.33	5.66±1,50	P<0.17
Area Under the Curve (AUC) AUCo-168h (mcg/h/ml)	744.55±395.71	1758.98±467.49	P<0.88
AUCo-o (mcg/h/ml)	1812.28±423.51	1831.26±512.75	P<0.86
Relative bio-availability. FR	0.63±0.15	0.63±0.15	P<0.74
Volume of distribution Vd (L)	6.85±0.77	6.67±0.66	P<0.48
Clearance CL (L/h)	0.14±0.03	0.14±0.03	P<0.81
Elimination half life. t1/2 B (h)	33.96±6.89	33.79±9.25	P<0.17
Elimination rate constant. ke (h^{-1})	0.02±0.001	0.02±0.008	P<0.36

significantly (P<0.11); although the mean value was 5.91% higher in the fasting state. However, the mean AUCo-oo obtained for patients was 10% higher when chlorpropamide was ingested concomitantly with the meal compared with the drug ingestion 30min. before the meal (P<0.86).

The mean pharmacokinetic parameters obtained for healthy subjects and diabetic patients are compared in Table 6c. Significantly different mean values were observed for lag time (tlag), Cmax and volume of distribution (vd) in diabetic patients compared to healthy subjects. No significant difference was observed in the other absorption and the elimination parameters (P>0.05).

The mean plasma chlorpropamide concentration for healthy and patient volunteers are shown in Table 7a, Figure 20a and Table 7b, Figure 20b respectively. Higher, but non statistically significant chlorpropamide levels were detected for 4h when the drug was administered to healthy subjects in fasting state relative to when the drug was concomitantly administered with the meal. Chlorpropamide concentrations were however significantly higher for 2h in patients when the drug was taken 30min before the meal compared with concomitant administration (0.5h, P<0.045; 1h, P<0.008 and 2h, P<0.016). The differences observed in the concentrations in healthy and patient volunteers at other sampling points were however not statistically significant.

Table 6c: MEAN (±S.D.) PHARMACOKINETIC PARAMETERS OBTAINED IN HEALTHY VOLUNTEERS AND DIABETIC PATIENTS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET WITH COOKED MAIZE FLOUR MEAL (B)

Mean Parameter ± S. D.	Healthy Volunteers A	Diabetic Patients B	Paired sample T-Test Results
Lag Time (h)	0.53±0.05	0.36±0.12	P<0.05
Absorption half-life. t1/2 x (h)	1.65±1.14	1.12±0.58	P<0.47
Absorption rate constant. ka (h^{-1})	0.53±0.23	0.75±0.35	P<0.43
Maximum concentration. Cmax mcg/ml	26.68±0.35	31.33±3.33	P<0.013
Time to peak concentration tmax (h)	6.40±1.67	5.66±1.50	P<0.69
Area Under the Curve (AUC) AUCo-168h (mcg/h/ml)	1399.32±378.02	1758.98±467.49	P<0.27
AUCo-o (mcg/h/ml)	1483.72±420.49	1831.26±512.75	P<0.34
Relative bio-availability. FR	0.58±0.16	0.63±0.15	P<0.84
Vd (L)	7.53±0.80	6.67±0.66	P<0.05
Clearance CL (L/h)	0.18±0.06	0.14±0.03	P<0.46
Elimination half life. t1/2 B (h)	31.46±8.69	33.79±9.25	P<0.87
Elimination rate constant. ke (h^{-1})	0.02±0.014	0.02±0.008	P<0.67

Table 7a: MEAN (±S.D.) PLASMA CONCENTRATIONS IN 5 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET IN FASTING STATE (A) AND WITH COOKED MAIZE FLOUR MEAL (B)

Time (h)	Concentration (mcg/ml) ±S.D. A	B
0.5	4.62±3.91	6.83±9.81
1	14.84±5.30	10.83±7.92
2	23.22±2.67	18.37±4.31
4	26.08±0.57	23.95±2.49
6	25.46±0.82	24.85±2.31
8	24.42±1.17	24.36±2.16
12	22.32±1.87	22.49±1.72
36	13.40±4.02	13.08±1.76
48	10.46±4.03	10.05±2.10
72	6.58±3.54	6.03±2.18
144	1.86±1.36	1.46±1.12
168	1.25±1.13	0.94±0.83

S.D. = Standard Deviation

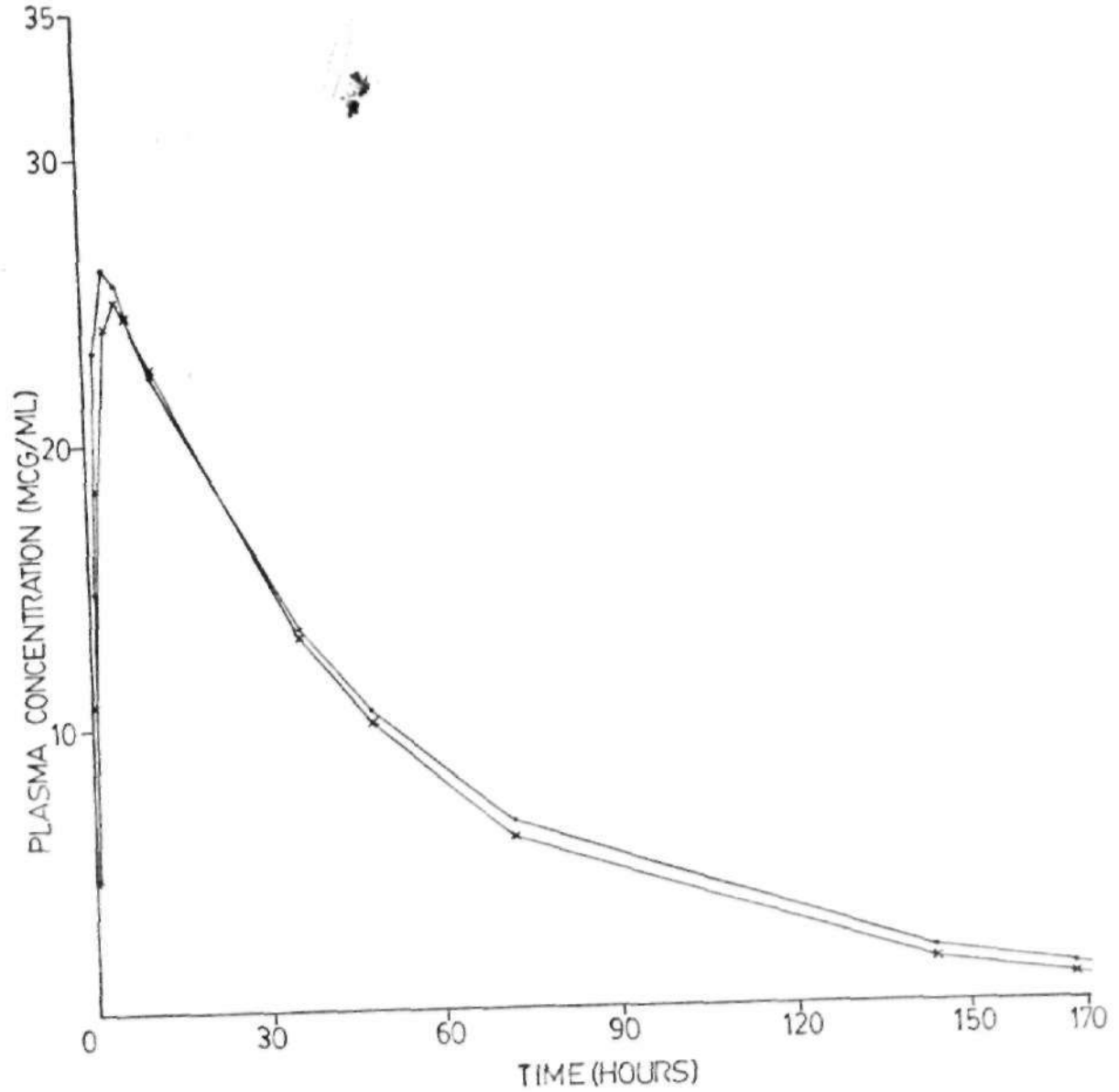
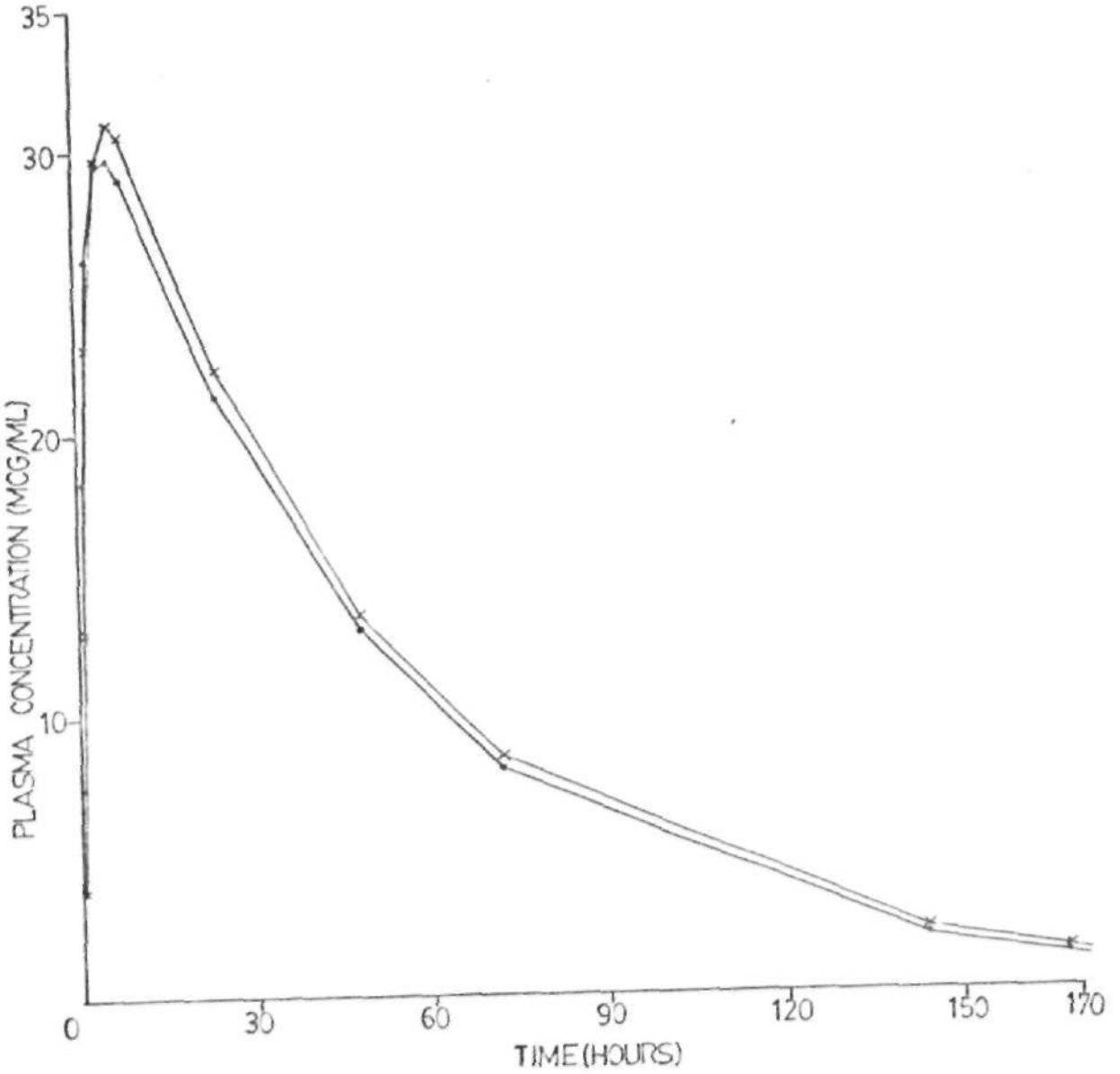


Fig. 20a. Mean plasma concentration - time curves in healthy volunteers following oral administration of 250mg Chlorpropamide tablet in fasting state (●——●) and with cooked maize flour meal (x——x).

Table 7b: MEAN (±S.D.) PLASMA CONCENTRATIONS IN 6 DIABETIC PATIENTS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET 30 MIN. BEFORE (A) AND WITH COOKED MAIZE FLOUR MEAL (B)

Time (h)	Concentration (mcg/ml) ±S.D. A	B
0.5	7.71±5.64	3.67±3.00
1	18.77±6.18	12.45±5.98
2	28.44±7.29	22.45±6.30
4	29.89±3.56	29.30±3.36
6	29.91±3.80	30.64±2.53
8	29.19±4.30	30.35±3.09
24	21.28±4.53	22.18±4.66
48	12.98±3.73	13.54±4.56
72	7.97±2.93	8.44±3.76
144	1.91±1.17	2.13±1.48
168	1.20±0.83	1.43±1.07

S. D. = Standard Deviation



20b. *Mean Plasma concentration - time curves in diabetic patients following oral administration of 250mg Chlorpropamide tablet 30 min. before (●——●) and with cooked maize flour meal (x——x).*

The results of the effects of each mode of treatment on the plasma glucose levels in healthy and patient volunteers are shown in Table 8a, Figure 21a and Table 8b, Figure 21b respectively. Following administration of a single 250mg chlorpropamide tablet to healthy volunteers (in fasting state and concomitantly with meal) the glucose levels decreased consistently and fell below the preprandial values. Significant decrease in the plasma glucose values were however observed in the fasting state compared with when the drug was administered concomitantly with meal at 0.5h, ($P<0.05$); 1h, ($P<0.04$) and at 8h ($P<0.01$). In the case of patients, the glucose levels increased for the 3 treatments reaching a mean maximum in 2h in each case. Thereafter, the glucose levels decreased and fell below the preprandial levels in the two cases where drug was taken. This occurred in the 4th hour when chlorpropamide was administered 30min before meal, in the 6th hour when the drug was administered concomitantly with the meal. Plasma glucose level had not returned to the preprandial value at the 8th hour when meal alone was taken.

The respective mean postprandial plasma glucose values were significantly lowered at each point when chlorpropamide was taken 30min. before meal ($P<0.02 - P<0.001$) and for 6h when the drug was administered concomitantly with meal ($P<0.04 - P<0.003$) as compared to when meal alone was

Table 8a: MEAN (±S.D.) PLASMA GLUCOSE CONCENTRATIONS IN 5 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET IN FASTING STATE (A) AND WITH COOKED MAIZE FLOUR MEAL (B)

Time (h)	Plasma Glucose Mmol/L (±S.D.)	
	A	B
0	4.67±0.25	4.80±0.13
0.5	4.47±0.32	4.75±0.12
1	4.31±0.31	4.50±0.19
2	4.12±0.28	4.30±0.11
4	3.92±0.29	4.12±0.13
6	4.01±0.20	4.08±0.07
8	4.14±0.05	4.06±0.06

Table 8b: MEAN (±S.D.) PLASMA GLUCOSE CONCENTRATIONS IN 6 DIABETIC PATIENTS GIVEN COOKED MAIZE FLOUR (M) AND FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET 30MIN BEFORE (A) AND WITH THE MEAL (B)

Time (h)	Plasma Glucose Mmol/L (±S.D.)		
	M	A	B
0	17.68±3.23	17.72±3.59	17.91±3.64
0.5	21.25±4.09	18.66±4.31	19.29±4.24
1	22.39±3.12	19.61±4.16	20.57±4.06
2	22.94±3.87	19.88±4.87	20.84±4.94
4	21.10±3.68	17.39±4.31	18.58±4.92
6	18.87±4.28	16.31±4.43	17.02±4.64
8	18.48±3.80	15.55±4.64	16.18±5.06

S. D. = Standard Deviation

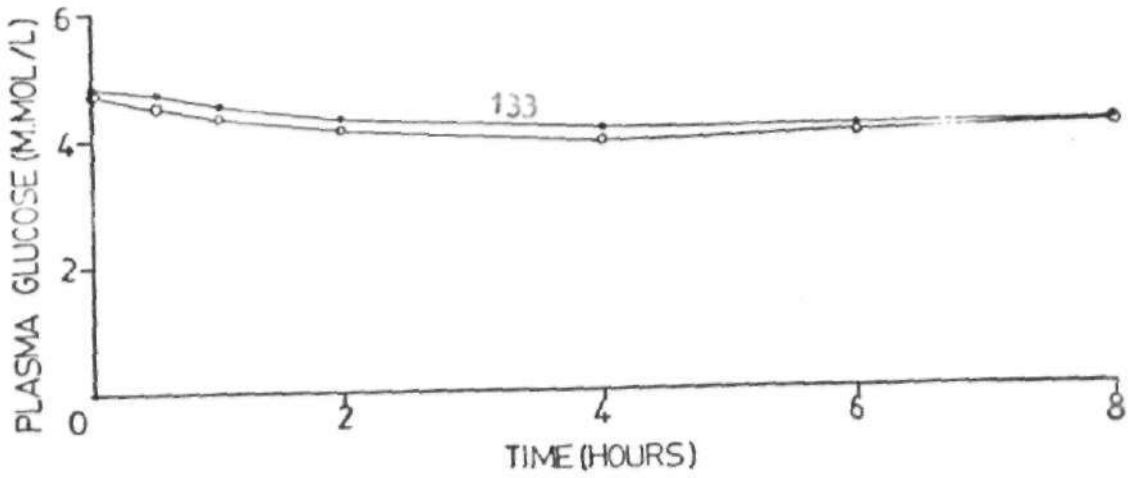


Fig. 21a. *Mean plasma glucose concentration - time curves in healthy volunteers following oral administration of 250mg Chlorpropamide tablet in fasting state (o——o) and with cooked maize flour meal (•——•).*

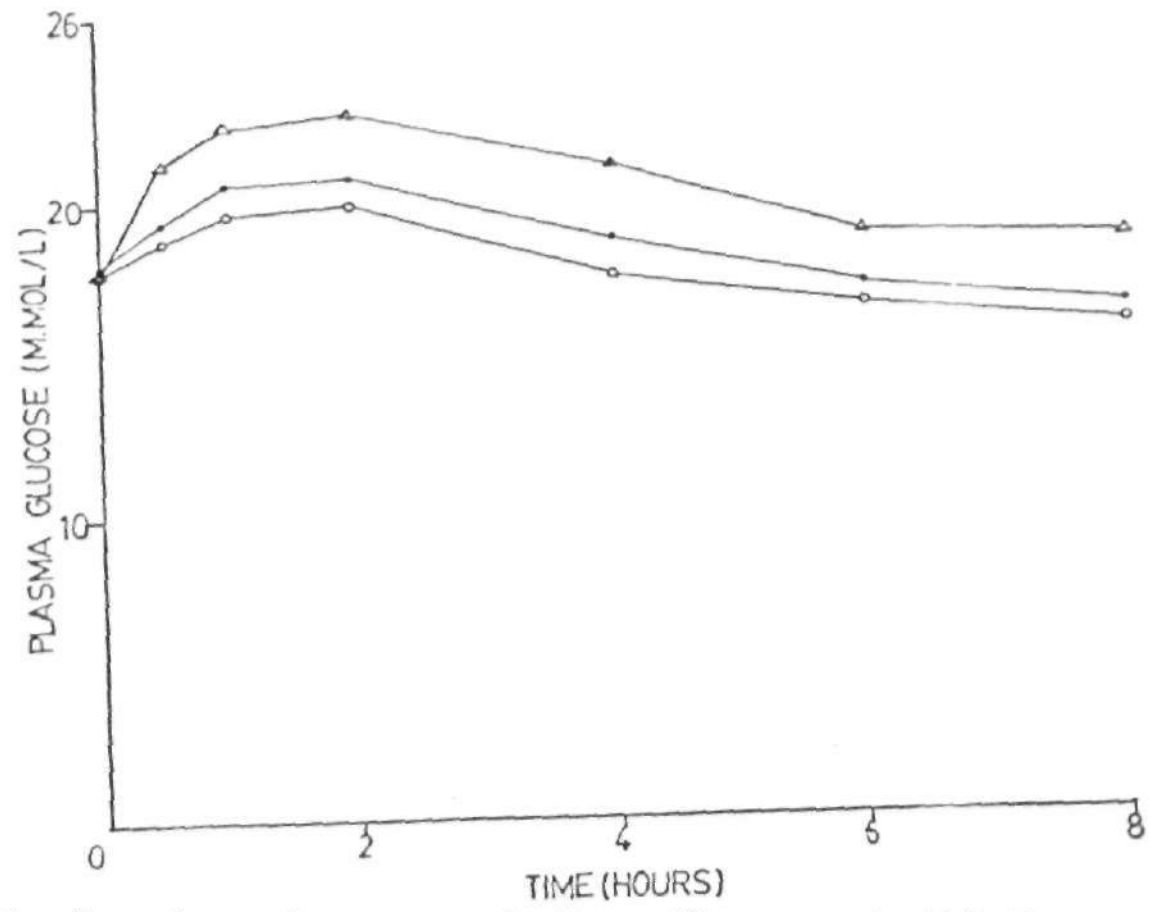


Fig. 21b. *Mean plasma glucose concentration - time curves in diabetic patients given cooked maize flour (▸——◂) and following oral administration of 250mg Chlorpropamide tablet 30 min. before (o——o) and with the meal (•——•).*

administered. The differences detected in the lowered postprandial glucose levels when the drug was administered 30 min. prior to meal compared with concomitant administration of drug and meal were statistically significant at 0.5h (P<0.001), 1h (P<0.02), 4h (P<0.02) and 6h (P<0.004).

4.5 Cooked Cassava Flour Meal (C) Chlorpropamide Interactions Studies In Healthy Volunteers And Diabetic Patients

Following oral administration of a single 250mg chlorpropamide tablet to healthy and patient volunteers as described under the materials and methods, the mean Cmax ranged from 31.06±2.10 to 23.06±1.72 mcg/ml and the mean tmax ranged from 2.16 ±0.98 to 11.20±1.78h. The mean t1/2x ranged from 0.33±0.07 to 4.23±2.75h and the mean t1/2B ranged from 33.67±13.86 to 41.28±9.78h.

The mean chlorpropamide pharmacokinetic parameters obtained for healthy subjects and diabetic patients are shown in Table 9a and 9b respectively. Concomitant administration of drug with meal increased the lag time, tmax and t1/2x while the ka was reduced relative to when the drug was administered in fasting state.

Table 9a: MEAN (±S.D.) PHARMACOKINETIC PARAMETERS DERIVED FROM PLASMA IN 5 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET IN FASTING STATE (A) WITH COOKED CASSAVA FLOUR MEAL (C)

Mean Parameter ± S. D.	A	C	Paired sample T-Test Results
Lag Time (h)	0.24±0.09	0.50±0.15	P<0.004
Absorption half-life. t1/2 x (h)	0.52±0.17	4.23±2.57	P<0.02
Absorption rate constant ka (h^{-1})	1.44±0.48	0.24±0.19	P<0.002
Maximum concentration. Cmax mcg/ml	31.690±2.29	31.06±2.10	P<0.13
Time to peak concentration tmax (h)	3.60±0.89	11.20±1.78	P<0.001
Area Under the Curve (AUC) AUCo-168h (mcg/h/ml)	1977.40±308.43	1907.33±283.39	P<0.40
AUCo-oo (mcg/h/ml)	2116.53±378.41	2032.86±291.31	P<0.22
Relative bioavailability. FR	0.83±0.15	0.80±0.11	P<0.21
Vd (L)	7.02±1.10	5.79±2.13	P<0.11
Clearance CL (L/h)	0.12±0.02	0.13±0.02	P<0.09
Elimination half life. t1/2 B (h)	41.28±9.78	33.67±13.86	P<0.14
Elimination rate constant ke (h^{-1})	0.02±0.006	0.02±0.011	P<0.3

Table 9b: MEAN (±S.D.) PHARMACOKINETIC PARAMETERS DERIVED FROM PLASMA IN 6 DIABETIC PATIENTS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET 30MIN. BEFORE (A) AND WITH COOKED CASSAVA FLOUR MEAL (C)

Mean Parameter ± S. D.	A	C	Paired sample T-Test Results
Lag Time (h)	0.23±0.13	0.27±0.10	P<0.43
Absorption half-life. t1/2 x (h)	0.33±0.07	1.50±0.53	P<0.002
Absorption rate constant. ka (h^{-1})	2.12±0.46	0.53±0.23	P<0.0003
Maximum concentration. Cmax mcg/ml	32.58±2.95	33.06±1.72	P<0.72
Time to peak concentration tmax (h)	2.16±0.98	7.33±1.03	P<0.0002
Area Under the Curve (AUC) AUCo-168h (mcg/h/ml)	1768.09±316.34	2177.31±349.17	P<0.12
AUCo-oo (mcg/h/ml)	1844.32±340.30	2273.29±338.41	P<0.14
Relative bioavailability. FR	0.64±0.12	0.79±0.13	P<0.13
Vd (L)	6.67±0.61	6.47±0.64	P<0.34
Clearance CL (L/h)	0.14±0.02	0.11±0.02	P<0.12
Elimination half life. t1/2 B (h)	33.94±5.66	40.40±5.53	P<0.18
Elimination rate constant. ke (h^{-1})	0.02±0.003	0.017±0.002	P<0.20

S. D. = Standard Deviation

Following chlorpropamide administration to healthy subjects in fasting state or concomitantly with meal, the mean AUCo-oo did not differ significantly (P<0.22). However, it was 4.11% higher in the fasting state. In the case of patients, there was a 23.25% reduction in the mean AUCo-oo when the drug was administered 30min. before meal compared to when the drug was concomitantly administered with meal (P<0.14).

The mean pharmacokinetic parameters obtained for healthy and patient volunteers are compared in Table 9c. Significant reductions were observed for lagtime, ka,t1/2 x and tmax in patients relative to those of the healthy subjects. No significant difference was observed for other parameters in healthy and patients volunteers (P>0.05).

The *mean plasma chlorpropamide concentrations* are shown in Table 10a, Figure 22a and Table 10b, Figure 22b for healthy and patient volunteers respectively. In healthy subjects, chlorpropamide concentrations were significantly higher in fasting state relative to when the drug was concomitantly administered with meal (P<0.02 - P<0.001). In the case of the patients, significantly higher chlorpropamide concentrations were observed for 2h when the drug was administered 30min. before meal compared with concomitant administration (P<0.02 - P<0.001).

Table 9c: MEAN (±S.D.) PHARMACOKINETIC PARAMETERS OBTAINED IN HEALTHY VOLUNTEERS AND DIABETIC PATIENTS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET WITH COOKED CASSAVA FLOUR MEAL (C)

Mean Parameter ± S. D.	Healthy Volunteers	Diabetic Patients	Independent Sample T-Test Results
Lag Time (h)	0.50±0.15	0.27±0.10	P<0.016
Absorption half-life. t1/2 x (h)	4.23±2.57	1.50±0.53	P<0.04
Absorption rate constant. ka (h^{-1})	0.24±0.19	0.53±0.23	P<0.052
Maximum concentration. Cmax mcg/ml	31.06±2.10	33.06±1.72	P<0.19
Time to peak concentration tmax (h)	11.20±1.78	7.33±1.03	P<0.0027
Area Under the Curve (AUC) AUCo-168h (mcg/h/ml)	1907.33±283.43	2177.31±349.17	P<0.27
AUCo-oo (mcg/h/ml)	2032.86±291.31	2273.29±338.41	P<0.37
Relative bio-availability. FR	0.80±0.11	0.79±0.13	P<0.87
Vd (L)	5.79±2.15	6.47±0.64	P<0.43
Clearance CL (L/h)	0.13±0.02	0.11±0.02	P<0.30
Elimination half life. t1/2 B (h)	33.67±13.86	40.40±5.53	P<0.31
Elimination rate constant. ke (h^{-1})	0.02±0.11	0.017±0.002	P<0.20

S.D = Standard Deviation

Table 10a: MEAN (±S.D.) PLASMA CONCENTRATIONS IN 5 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET IN FASTING STATE (A) AND WITH COOKED CASSAVA FLOUR MEAL (C)

Time (h)	Concentration (mcg/ml) ±S.D.	
	A	C
0.5	12.21±7.32	2.21±2.31
1	22.30±5.10	5.35±2.96
2	29.68±2.47	11.40±3.28
4	31.61±2.57	19.39±2.51
6	30.85±2.55	24.17±2.10
8	29.81±2.37	27.06±3.11
12	27.71±2.08	29.61±5.21
36	18.27±2.43	21.30±3.97
48	14.87±2.68	16.21±2.72
72	9.92±2.73	9.40±2.83
144	3.09±1.62	2.28±1.65
168	2.12±1.26	1.50±1.19

S. D. = Standard Deviation

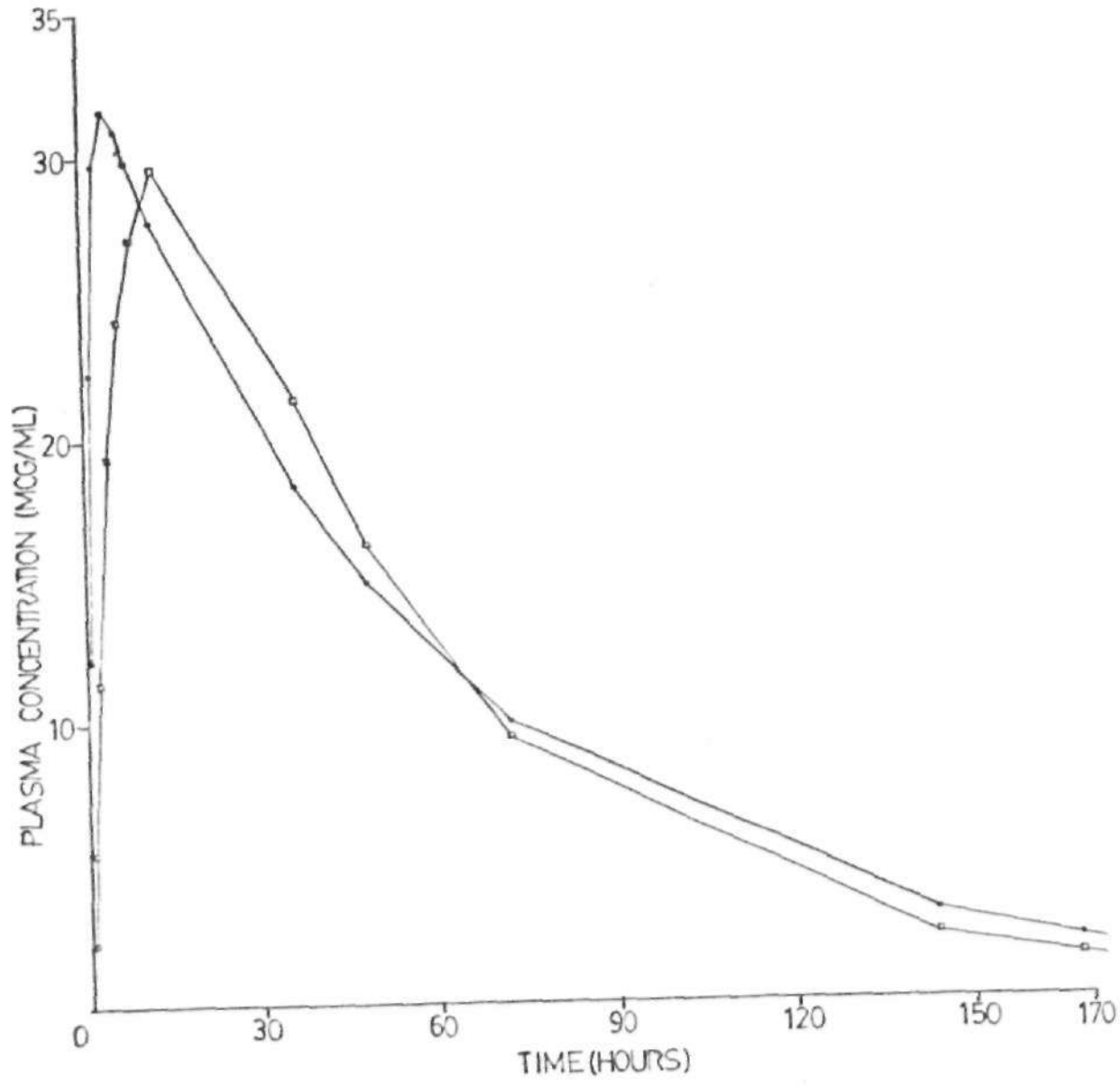


Fig. 22a. Mean plasma concentration - time curves in healthy volunteers following oral administration of 250mg Chlorpropamide tablet in fasting state (●——●) and with cooked cassava flour mean (▫——▫).

Table 10b: MEAN ±S.D.) PLASMA CONCENTRATIONS IN 6 DIABETIC PATIENTS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET 30 MIN BEFORE (A) AND WITH COOKED CASSAVA FLOUR MEAL (C)

Time (h)	Concentration (mcg/ml) ±S.D.	
	A	C
0.5	15.85±8.10	4.13±2.10
1	27.24±1.91	11.42±3.41
2	32.38±2.91	20.92±3.75
4	32.01±3.51	29.46±1.34
6	30.73±3.48	32.20±0.95
8	29.49±3.44	32.76±2.12
24	21.19±3.23	26.00±3.16
48	12.97±2.88	17.20±2.85
72	7.98±2.40	11.40±2.47
144	1.91±1.06	3.37±1.27
168	1.20±0.77	2.26±0.97

S. D. = Standard Deviation

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F-tg. 22b. Mean *PZci&ma conco.yitA.aXA.on - time. CUAVCA in diabetic patients ^oVCo\^inQ OAOX adminiAtAation o& 250mg ChloApAopamidc tabluX 30 Min. bz^oAo.* (*———•) *and with cooked* ca44aua *ilouA meat [a*———a).

The results of the effects of each mode of treatment on the plasma glucose levels are shown in Table 11a, Figure 23a and in Figure 23b, Table 11b, for healthy and patient volunteers respectively. Following administration of a single 250mg chlorpropamide tablet to healthy subjects in fasting state, the glucose levels decreased and fell below the preprandial levels. However, when the drug was administered to the subjects concomitantly with meal, the glucose level increased for 1h and fell below the preprandial value between the 2nd and 4th hour. The glucose levels when the drug was administered in fasting state were however significantly different for 4 h compared with concomitant administration of drug and meal ($P<0.03$ – $P<0.01$). In the case of patients, plasma glucose levels increased for each treatment, reaching a mean maximum in 2 h when meal alone was ingested and when the drug was concomitantly administered with the meal. The corresponding time to reach mean maximum plasma glucose level when the drug was given 30min. before the drug was 4h. Thereafter, the values decreased and fell below the preprandial levels in the 6th hour when the drug was administered 30min. before meal and 8th hour when it was administered concomitantly with meal. The glucose level had not returned to the preprandial level in the 8th hour when the meal alone was ingested.

Table 11a: MEAN (±S.D) PLASMA GLUCOSE CONCENTRATIONS IN 5 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET IN FASTING STATE (A) AND WITH COOKED CASSAVA FLOUR MEAL (C)

Time h	Plasma Glucose M.Mol/L (±S.D.) A	C
0	4.65±0.14	4.44±0.22
0.5	4.42±0.14	4.64±0.21
1	4.24±0.14	4.79±0.24
2	4.08±0.10	4.54±0.25
4	3.95±0.12	4.36±0.25
6	4.01±0.09	4.22±0.20
8	4.03±0.05	4.23±0.21

Table 11b: MEAN (±S.D) PLASMA GLUCOSE CONCENTRATIONS IN 6 DIABETIC PATIENTS GIVEN COOKED CASSAVA FLOUR (N) AND FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET 30 MIN. BEFORE (A) AND WITH THE MEAL (C)

Time h	Plasma Glucose M.Mol/L (±S.D.) N	A	C
0	17.34±2.07	17.12±2.08	17.09±2.02
0.5	23.51±2.97	17.38±0.98	19.25±1.83
1	25.13±2.29	18.74+1.79	20.95±2.77
2	25.54±2.94	19.05±1.83	21.14±3.38
4	23.01±3.74	19.10±3.50	19.44±3.34
6	19.88±3.23	16.78±1.82	17.38±2.04
8	17.78±1.55	16.12±1.63	16.02±1.42

S. D. = Standard Deviation

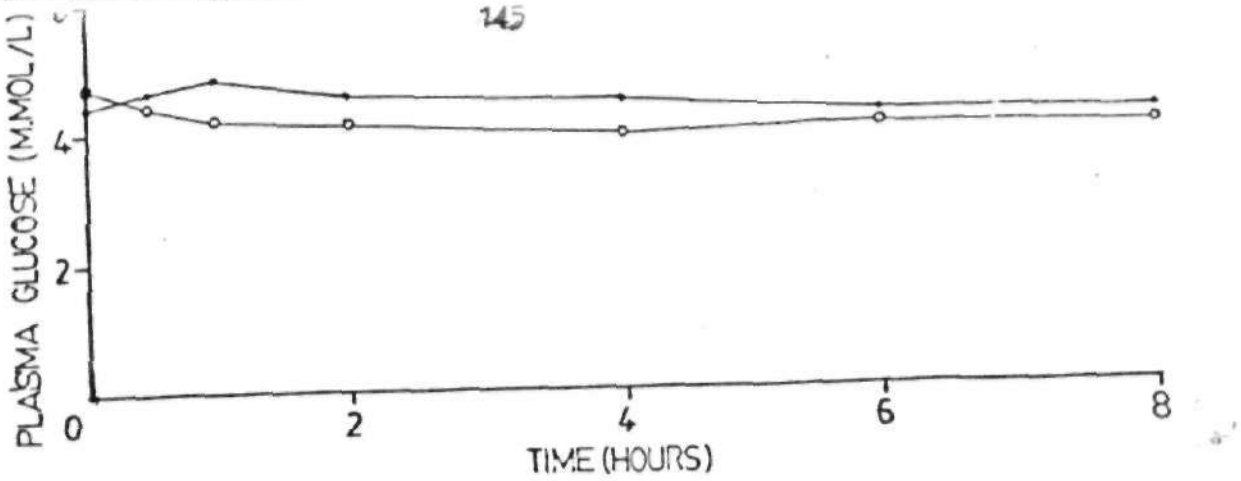


Fig. 23a. Mean plasma glucose concentration - time curves in healthy volunteers following oral administration of 250mg Chlorpropamide tablet in fasting state (o——o) and with cooked cassava flour meal (•——•).

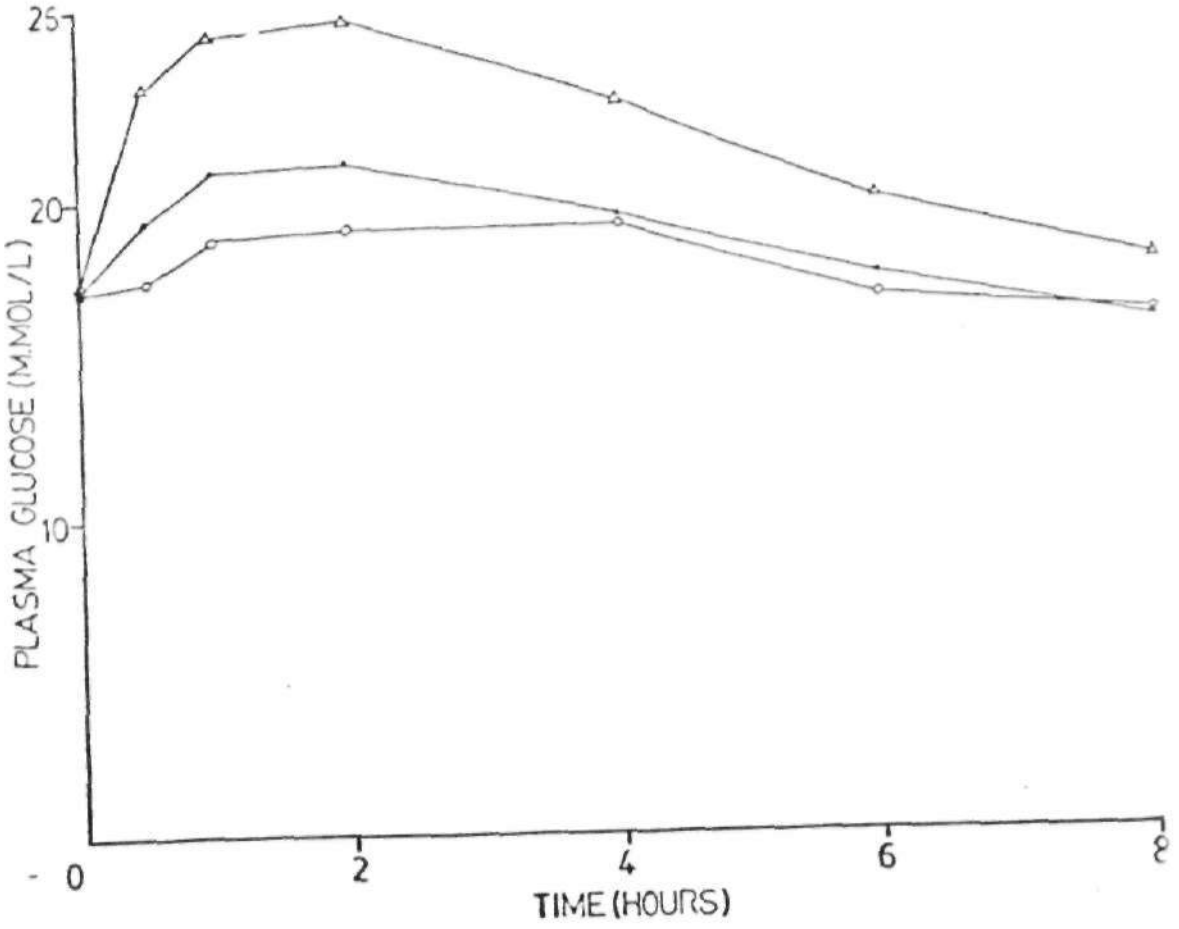


Fig. 23b. Mean plasma glucose concentration - time curves in diabetic patients given cooked cassava flour (◁——◁) and following oral administration of 250mg Chlorpropamide tablet 30 min. before (o——o) and with the meal (•——•).

The mean postprandial glucose levels were significantly lowered when drug was administered 30min. before meal ($P<0.03$ - $P<0.001$) and when the drug was concomitantly administered with meal ($P<0.02$ - $P<0.001$) than the glucose levels when meal alone was ingested. However, the postprandial plasma glucose levels were comparatively lowered when the drug was administered 30min. before meal than the levels when the drug was concomitantly administered with meal (0.5h, $P<0.017$; 1h, $P<0.021$ and 2h $P<0.03$).

4.6 Cooked Yam Flour Meal (D) Chlorpropamide Interactions Studies In Healthy Volunteers And Diabetic Patients:

Following oral administration of a single 250mg chlorpropamide tablet to healthy and patient volunteers as described under the materials and methods, the mean Cmax ranged from 28.51±1.57 to 30.76±2.37 mcg/ml and the mean tmax ranged from 1.60±0.54 to 4.80±1.09h. The mean t1/2 x ranged from 0.26±0.21 to 0.87±0.34h and t1/2 B ranged from 35.71±6.43 to 40.43±4.40h. The mean pharmacokinetic parameters obtained for healthy volunteers and diabetic patients are showin in Table 12a and 12b respectively.

Table 12a: MEAN (±S.D.) PHARMACOKINETIC PARAMETERS DERIVED FROM PLASMA IN 5 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET IN FASTING STATE (A) AND WITH COOKED YAM FLOUR MEAL (D)

Mean Parameter ± S. D.	A	D	Paired sample T-Test Results
Lag Time (h)	0.23±0.10	0.33±0.15	P<0.009
Absorption half-life. t1/2 x (h)	0.87±0.34	0.26±0.21	P<0.01
Absorption rate constant. ka (h^{-1})	0.88±0.38	8.57±5.98	P<0.04
Maximum concentration. Cmax mcg/ml	29.29±2.16	28.31±1.57	P<0.58
Time to peak concentration tmax (h)	4.80±1.09	1.60±0.54	P<0.001
Area Under the Curve (AUC) AUCo-168h (mcg/h/ml)	1814.83±123.58	1637.31±272.98	P<0.09
AUCo-oo (mcg/h/ml)	1911.19±131.34	1730.84±311.60	P<0.10
Relative bio-availability. FR	0.75±0.05	0.68±0.12	P<0.10
Vd (L)	7.63±0.79	7.44±0.29	P<0.67
Clearance CL (L/h)	0.13±0.008	0.15±0.02	P<0.07
Elimination half life. t1/2 B (h)	40.43±4.40	35.71±6.43	P<0.16
Elimination rate constant. ke (h^{-1})	0.017±0.002	0.019±0.003	P<0.50

S. D. = Standard Deviation

Table 12b: MEAN (±S.D.) PHARMACOKINETIC PARAMETERS DERIVED FROM PLASMA IN 6 DIABETIC PATIENTS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET 30MIN. BEFORE (A) AND WITH COOKED YAM FLOUR MEAL (D)

Mean Parameter ± S. D	A	D	Paired Sample T-Test Results
Lag Time (h)	0.22±0.13	0.37±0.29	P<0.33
Absorption half-life. t1/2 x (h)	0.43±0.43	0.77±1.15	P<0.35
Absorption rate constant. ka (h^{-1})	3.12±2.70	1.82±1.70	P<0.26
Maximum concentration. Cmax mcg/ml	30.76±2.37	30.49±2.93	P<0.87
Time to peak concentration tmax (h)	3.16±1.83	3.66±2.33	P<0.45
Area Under the Curve (AUC) AUCo-168h (mcg/h/ml)	1795.56±290.65	1831.54±293.95	P<0.77
AUCo-oo (mcg/h/ml)	1894.40±323.24	1901.20±304.40	P<0.96
Relative bio-availability. FR	0.66±0.11	0.66±0.10	P<0.89
Vd (L)	7.35±1.14	7.25±1.39	P<0.84
Clearance CL (L/h)	0.13±0.02	0.14±0.02	P<0.94
Elimination half life. t1/2 B (h)	38.54±9.043	38.15±8.86	P<0.91
Elimination rate constant. ke (h^{-1})	0.017±0.003	0.018±0.002	P<0.18

S. D. = Standard Deviation

In healthy volunteers, concomitant administration of chlorpropamide with meal significantly increased the lagtime, reduced the t1/2 x and decreased tmax when compared with the values of the corresponding parameters derived in fasting state. In the case of the patients, concomitant administration of drug with meal did not significantly affect any of the chlorpropamide pharmacokinetic parameters when compared with the data obtained in fasting state (P>0.05).

The mean pharmacokinetic parameter obtained for meal in healthy and patient volunteers are compared in Table 12c. Following oral administration of chlorpropamide concomitantly with meal to healthy and patient volunteers, all the derived pharmacokinetic parameters (except the absorption rate constant, ka) were similar. Meal concurrently administered with chlorpropamide reduced ka in patient compared with healthy subjects (P<0.05).

The mean plasma chlorpropamide concentrations are shown in Table 13a, Figure 24a and in Table 13b, Figure 24b for healthy and patient volunteers respectively. In healthy subjects, significantly higher chlorpropamide levels were observed for 1h when the drug was concomitantly administered with meal to healthy subjects relative to when the drug was administered in fasting state (0.5h, P<0.02 and 1h,

Table 12c: MEAN (±S.D.) PHARMACOKINETIC PARAMETERS OBTAINED IN HEALTHY VOLUNTEERS AND DIABETIC PATIENTS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET WITH COOKED YAM FLOUR MEAL (D)

Mean Parameter ± S. D.	Healthy Volunteers	Diabetic Patients	Independent sample T-Test Results
Lag Time (h)	0.33±0.13	0.37±0.29	P<0.73
Absorption half-life.t1/2 x (h)	0.26±0.21	0.77±1.15	P<0.37
Absorption rate constant. ka (h^{-1})	8.57±5.98	1.82±1.70	P<0.05
Maximum concentration. Cmax mcg/ml	28.51±1.57	30.49±2.93	P<0.39
Time to peak concentration tmax (h)	1.60±0.54	3.66±2.33	P<0.13
Area Under the Curve (AUC) AUCo-168h (mcg/h/ml)	1637.00±272.98	1831.54±293.95	P<0.45
AUCo-oo (mcg/h/ml)	1730.84±311.60	1901.20±304.40	P<0.56
Relative bio-availability. FR	0.68±0.12	0.66±0.10	P<0.64
Vd (L)	7.44±0.29	7.25±1.39	P<0.98
Clearance CL (L/h)	0.15±0.02	0.14±0.02	P<0.54
Elimination half life. t1/2 B (h)	35.71±6.43	38.15±8.86	P<0.63
Elimination rate constant. ke (h^{-1})	0.019±0.003	0.018±0.002	P<0.35

S. D. = Standard Deviation

Table 13a: MEAN (±S.D.) PLASMA CONCENTRATIONS IN 5 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET IN FASTING STATE (A) AND WITH COOKED YAM FLOUR MEAL (D)

Time (h)	Concentration (mcg/ml) ±S.D.	
	A	D
0.5	6.18±1.83	20.10±6.74
1	15.02±3.29	27.54±2.41
2	23.88±3.50	28.18±1.59
4	28.74±2.73	27.30±1.68
6	29.10±2.30	26.23±1.75
8	28.42±2.10	25.232±1.84
12	26.66±1.80	23.32±2.00
36	17.62±1.21	14.59±2.44
48	14.33±1.16	11.56±2.41
72	9.48±1.10	7.29±2.12
144	2.77±0.66	1.89±1.00
168	1.84±0.51	1.22±0.72

S. D. = Standard Deviation

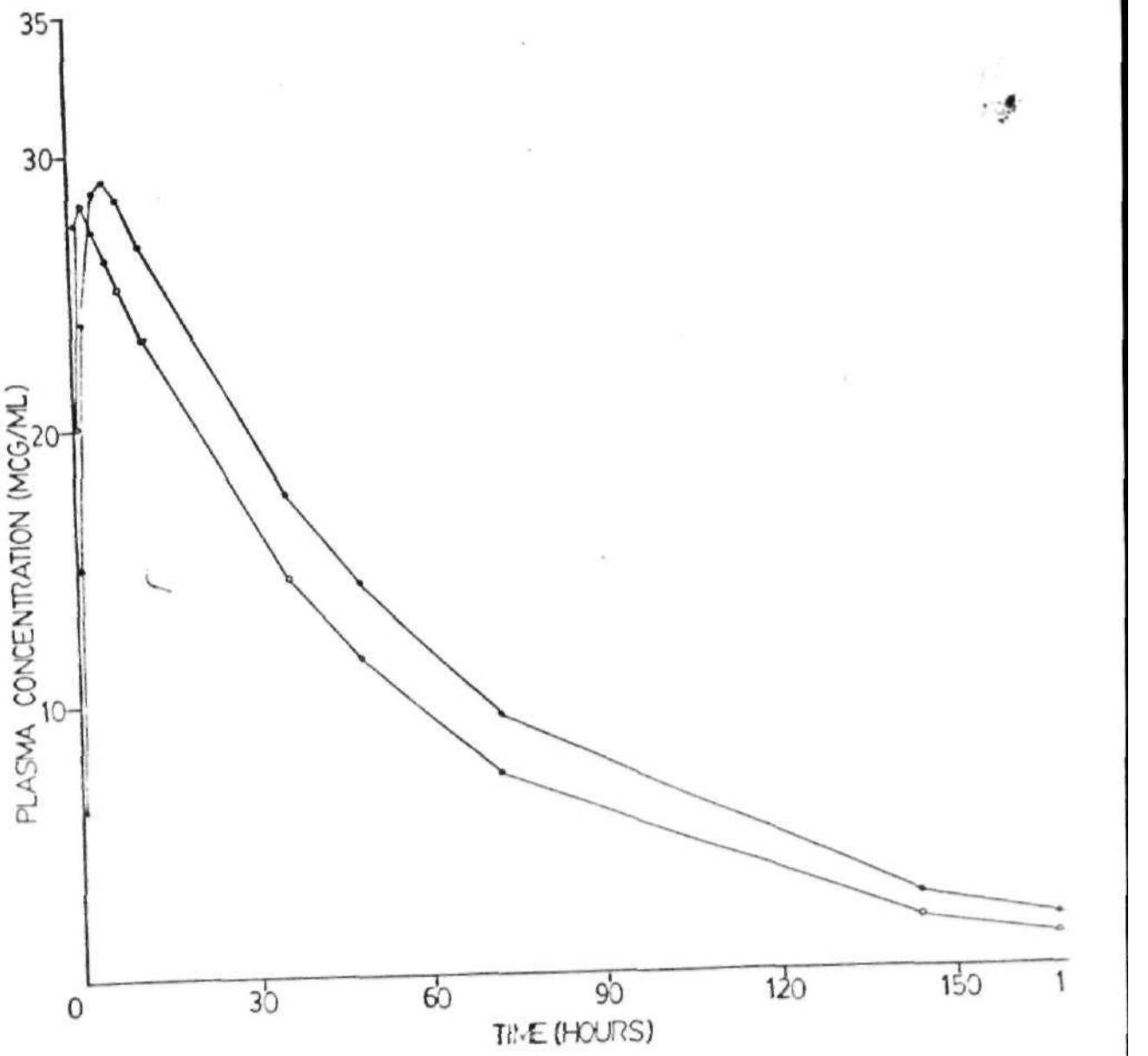


Fig. 24a. *Mean plasma concentration - time curves in healthy volunteers following oral administration of 250mg Chlorpropamide tablet in fasting state (●——●) and with cooked yam flour meal (o——o).*

Table 13b: MEAN (±S.D.) PLASMA CONCENTRATIONS IN 6 DIABETIC PATIENTS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET 30 MIN BEFORE (A) AND WITH COOKED YAM FLOUR MEAL (D)

Time (h)	Concentration (mcg/ml) ±S.D.	
	A	D
0.5	18.54±6.29	16.61±3.55
1	26.44±4.91	23.18±4.52
2	29.22±3.17	27.50±4.06
4	29.89±2.80	29.22±2.73
6	29.17±2.85	29.15±2.87
8	28.20±2.81	28.79±3.38
24	20.95±2.54	22.84±4.29
48	13.48±2.66	15.02±3.88
72	8.74±2.52	9.67±3.04
144	2.50±1.41	3.54±2.91
168	1.67±1.09	1.81±0.98

S. D. = Standard Deviation

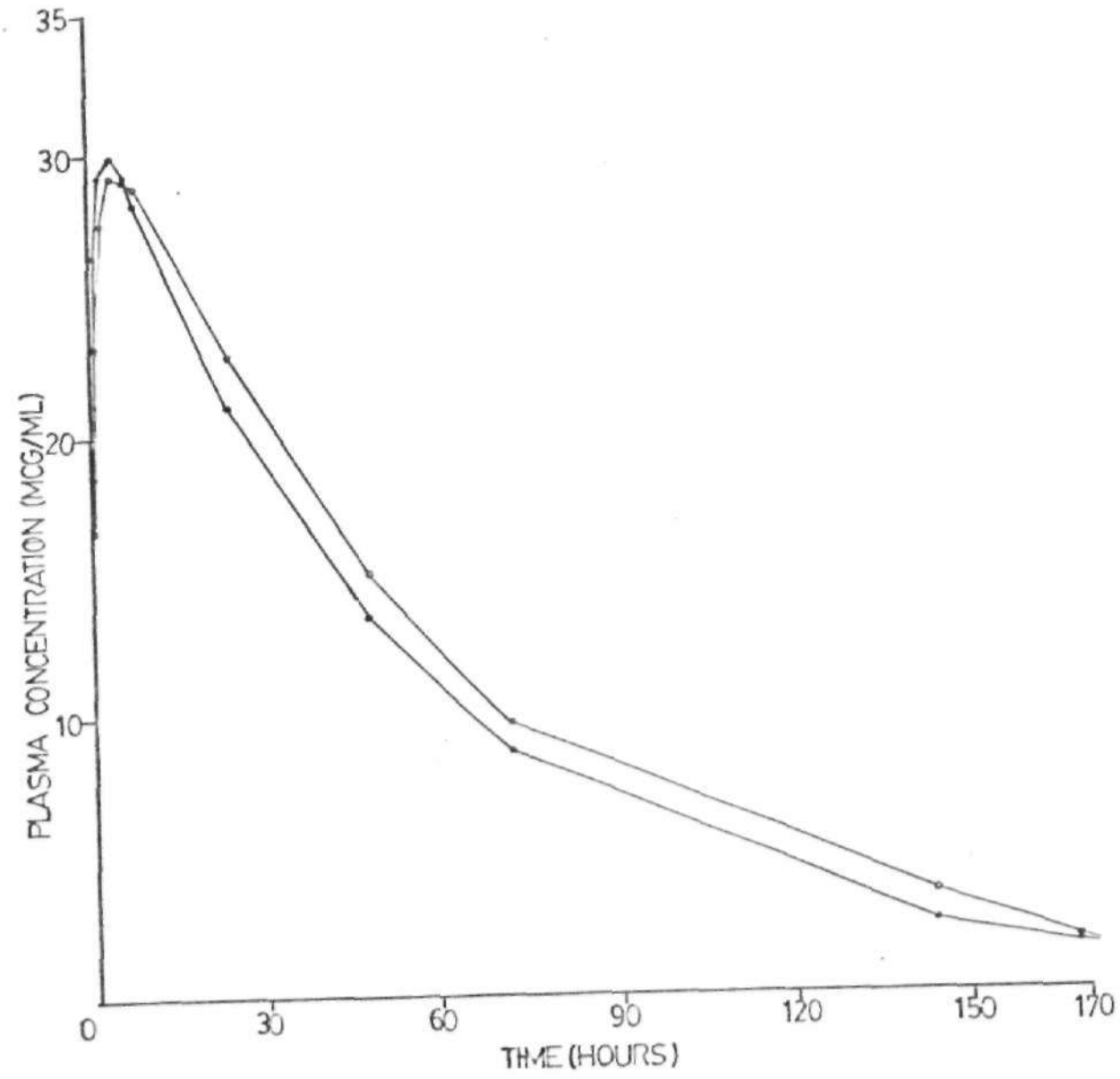


Fig. 24b. Mean plasma concentration - time curves in diabetic patients following oral administration of 250mg Chlorpropamide tablet 30 min. before (●——●) and with cooked yam flour meal (o——o).

P<0.003). In the case of patients, there was no significant difference in chlorpropamide levels when the drug was administered 30min. before meal compared to concomitant administration of drug and meal.

The results of the effects of each mode of treatment on the plasma glucose levels are shown in Table 14a, Figure 25a and in Table 14b, Figure 25b for healthy and patient volunteers respectively. Following oral administration of chlorpropamide to healthy subjects in fasting state or concomitantly with meal, the plasma glucose levels decreased in each case and fell below the preprandial values. No significant difference was observed when the drug was administered in the fasting state compared with concomitant administration of the drug and meal. In the case of the patients, the plasma glucose levels increased for the three treatments reaching a maximum in 2h in each case. However, the glucose levels fell below the preprandial levels in the 6th h in the two cases where drug was taken. In contrast, the plasma glucose level had not fully returned to the preprandial value in the 8th h when meal alone was taken. The respective mean postprandial plasma glucose values were significantly lowered at each point when the drug was administered 30min before meal (P<0.001 - P<0.0002) and when the drug was administered concomitantly with meal (P<0.007 - P<0.0002) as compared to the ingestion of the meal

Table 14a: MEAN (±S.D) PLASMA GLUCOSE CONCENTRATIONS IN 5 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET IN FASTING STATE (A) AND WITH COOKED YAM FLOUR MEAL (D)

Time h	Plasma Glucose M.Mol/L (±S.D.) A	D
0	4.62±0.06	4.72±0.08
0.5	4.59±0.06	4.44±0.07
1	4.38±0.13	4.24±0.04
2	4.24±0.23	4.11±0.15
4	3.98±0.17	4.08±0.40
6	3.85±0.21	3.97±0.38
8	3.64±0.16	3.89±0.34

Table 14b: MEAN (±S.D) PLASMA GLUCOSE CONCENTRATIONS IN 6 DIABETIC PATIENTS GIVEN COOKED YAM FLOUR (O) AND FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET 30 MIN. BEFORE (A) AND WITH THE MEAL (D)

Time h	Plasma Glucose M.Mol/L (±S.D.) O	A	D
0	15.25±1.98	15.08±2.08	15.36±1.86
0.5	17.45±2.63	14.57±2.27	15.93±2.28
1	20.19±2.69	15.80+2.89	18.28±3.09
2	22.31±2.32	16.93±2.65	18.47±3.21
4	21.18±2.76	16.13±2.63	16.46±2.27
6	17.89±1.76	14.49±2.43	14.77±1.82
8	16.18±1.48	13.53±2.16	14.11±1.97

S. D. = Standard Deviation

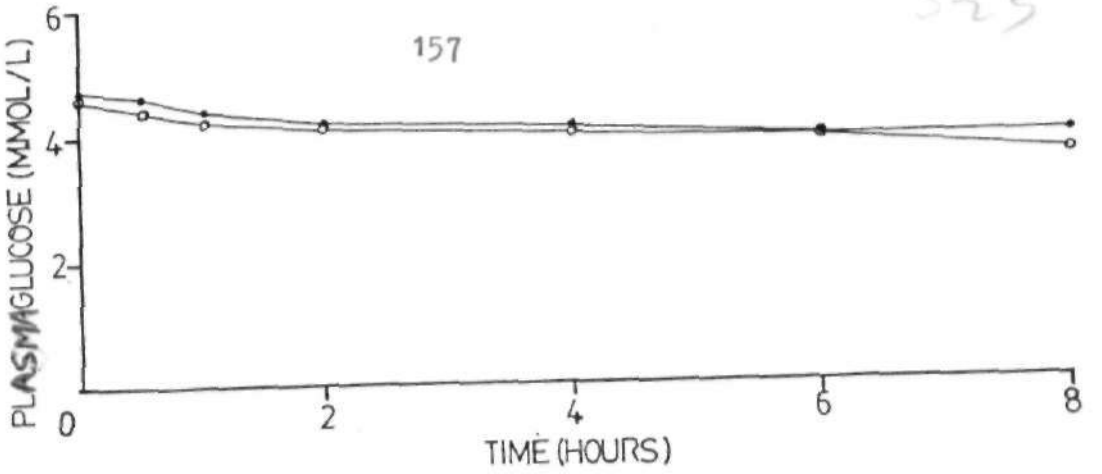


Fig. 25a. Mean plasma glucose concentration - time curves in healthy volunteers following oral administration of 250mg Chlorpropamide tablets in fasting state (o——o) and with cooked yam flour meal (●——●).

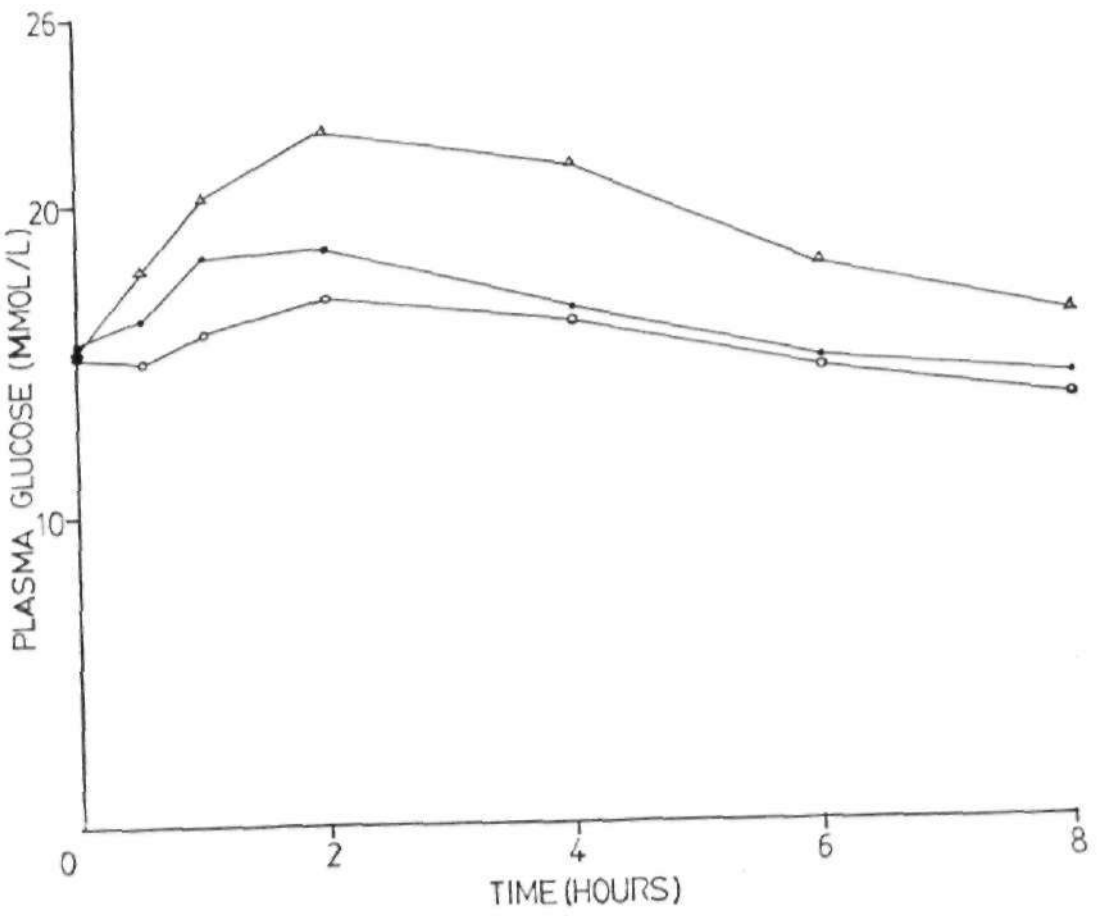


Fig. 25b. Mean plasma glucose concentration - time curves in diabetic patients given cooked yam flour (◁——▷) and following oral administration of 250mg Chlorpropamide tablet 30 min. before (o——o) and with the meal (●——●).

alone. The values were even lowered at 0.5h and 1h (P<0.02 at each sampling point) when the drug was taken 30min. before meal compared with when taken concomitantly with the meal.

4.7 Effects of Cooked Maize (B), Cassava (C) and Yam (D) Flour Meals On Chlorpropamide Pharmacokinetic Parameters

Following oral administration of a single 250mg chlorpropamide tablet to healthy subjects concomitantly with different meals (B, C and D), the t1/2x, ka, Cmax and tmax were significantly affected. The AUC values, relative bioavailability (FR), volume of distribution (Vd) and the parameters of elimination calculated were not significantly affected. The different pharmacokinetic parameters are shown in Table 15 and the plasma concentration time data are graphically represented in Figure 26. Statistically significant differences observed in t1/2 x and Cmax were between meals B versus C and meals C versus D. However, for ka, the statistical significant difference was between meals B versus D and meals C versus D. The statistical significant difference in tmax was among the three treatments compared. That is, drug treatment with meals B versus C, meals B versus D and meals C versus D. In the case of diabetic patients, ingestion of meals B, C or D alone resulted in significant differences in the 0.5h and 1h postprandial

Table 15: MEAN (±S.D.) PHARMACOKINETIC PARAMETERS OBTAINED IN 5 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET WITH COOKED MAIZE (B) CASSAVA (C) AND YAM (D) FLOUR MEALS

Mean Parameter ± S. D.	B	C	D	ANOVA (Among treatments	LSD Value
Lag Time (h)	0.53±0.05	0.50±0.15	0.33±0.15	NS	NA
Absorption half-life. t1/2 x (h)	1.65±1.14	4.23±2.57	0.26±0.21	P<0.01	2.25
Absorption rate constant. ka (h^{-1})	0.53±0.23	0.24±0.19	8.37±5.98	P<0.01	4.47
Maximum concentration. Cmax mcg/ml	26.68±0.35	31.06±2.10	28.51±1.57	P<0.01	2.11
Time to peak concentration tmax (h)	6.40±1.67	11.20±1.78	1.60±0.54	P<0.01	1.99
Area Under the Curve (AUC) *AUCo-168h* (mcg/h/ml)	1399.32±378.02	1907.33±283.43	1637.00±272.98	NS	NA
AUCo-oo (mcg/h/ml)	1483.72±420.29	2032.86±291.31	1730.84±311.60	NS	NA
Relative bioavailability. FR	0.58±0.16	0.80±0.11	0.68±0.12	NS	NA
Vd (L)	7.53±0.80	5.79±2.15	7.44±0.29	NS	NA
Clearance CL (L/h)	0.18±0.06	0.13±0.02	0.15±0.02	NS	NA
Elimination half life. t1/2 B (h)	31.46±8.69	33.67±13.86	35.71±6.43	NS	NA
Elimination rate constant. ke (h^{-1})	0.02±0.004	0.02±0.011	0.019±0.003	NS	NA

S.D.= Standard Deviation
ANOVA = Analysis of Variancwe for Complete Cross-over design
L.S.D. = Least Square Difference
NS = Not significant At 95% Confidence Level (P>0.05)
NA = Not applicable

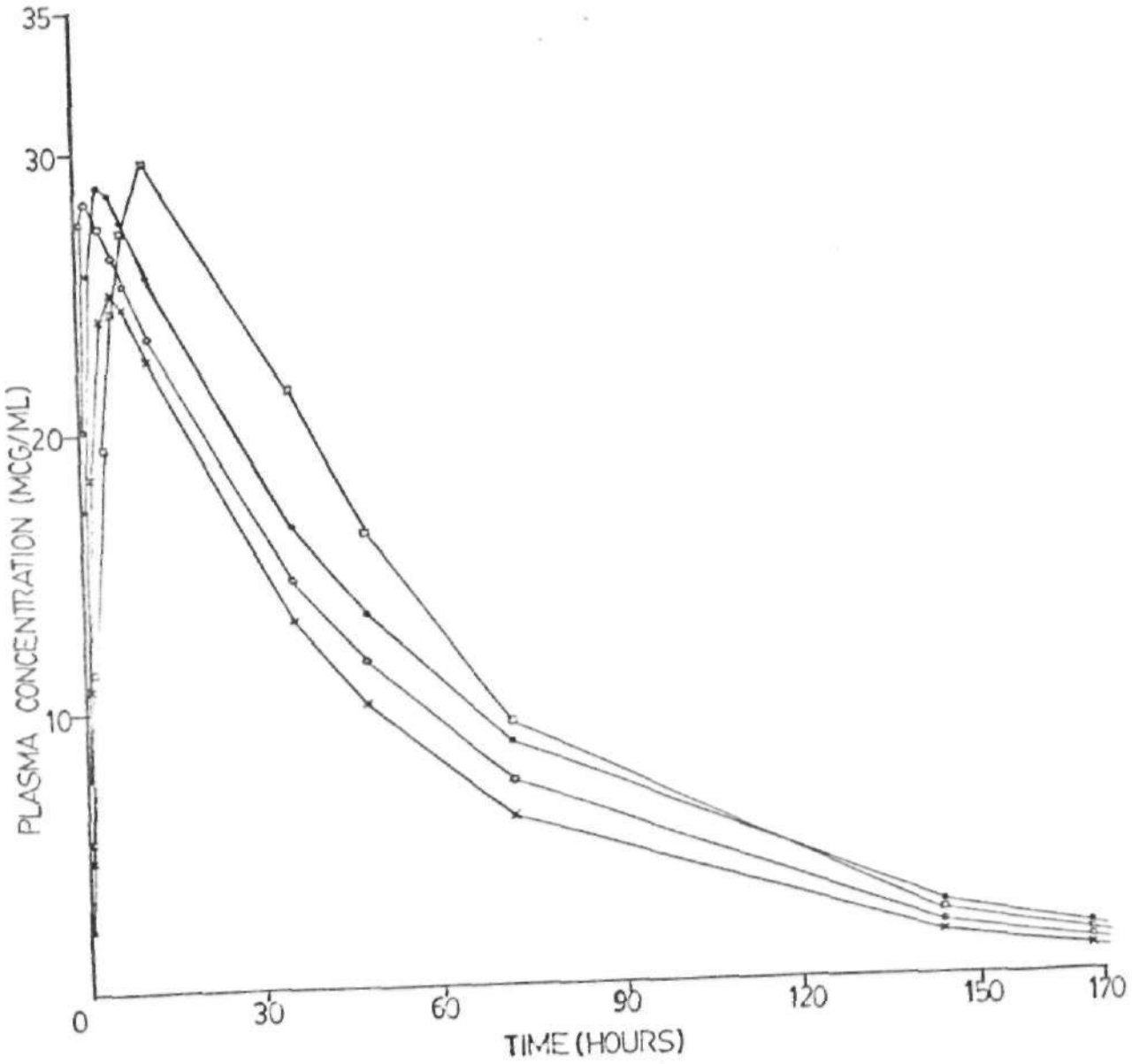


Fig. 26 *Comparative Mean Plasma concentration - time curves in healthy volunteers following oral administration of 250mg Chlorpropamide tablet in fasting state (●——●) with cooked maize (x——x), cooked cassava (□——□) and cooked yam (o——o) flour meals.*

plasma glucose levels. The corresponding plasma glucose levels are shown in Table 16 and graphically represented in Figure 27. Statistically significant differences observed at the 2 sampling points were between meals C and D.

Following oral administration of a single 250mg chlorpropamide tablet 30min. before meals, B, C or D, the differences in the derived pharmacokinetics of the drug did not reach level of significance in diabetic patients. The mean pharmacokinetic parameters are shown in Table 17a and the plasma concentration-time curves are shown in Figure 28a. The plasma glucose levels were also not significantly affected by the meal at any sampling point (Table 17b, Figure 28b). Concomitant administration of 250mg chlorpropamide tablet with different meals B, C, and D resulted in similar pharmacokinetic parameters. However, the tmax was signicantly affected by the meals. The mean pharmacokinetic parameters are shown in Table 18a and the plasma concentration-time data are graphically represented in Figure 29a. The statistically significant difference observed in tmax was between meals C and D. No significant difference was detected in the plasma glucose levels for patients at any sampling point (Table 18b and Figure 29b).

Table 16: MEAN (±S.D) PLASMA GLUCOSE CONCENTRATION IN 6 DIABETIC PATIENTS FOLLOWING INGESTION OF COOKED MAIZE (M) CASSAVA (N) AND YAM (O) FLOUR MEALS

Time h	Plasma Glucose M.Mol/L (±S.D.) M	N	O	ANOVA (Among treatment	LSD Value
0	17.68±3.23	17.37±2.07	13.25±1.98	NS	NA
0.5	21.25±4.09	23.31±2.97	17.88±2.85	P<0.05	4.04
1	22.39±4.09	25.13±2.29	20.19±2.69	P<0.05	3.35
2	22.94±3.87	25.54±2.94	22.26±2.37	NS	NA
4	21.10±3.68	23.01±3.74	21.18±2.78	NS	NA
6	18.87±4.28	19.88±3.23	17.89±1.76	NS	NA
8	18.48±3.00	17.78±1.55	16.18±1.48	NS	NA

S. D. = Standard Deviation
ANOVA = Analysis Of Variance For Complete Cross-Over Design
N.S. = Not Significant At 95% Confidence Level (P>0.05)
N.A. = Not Applicable

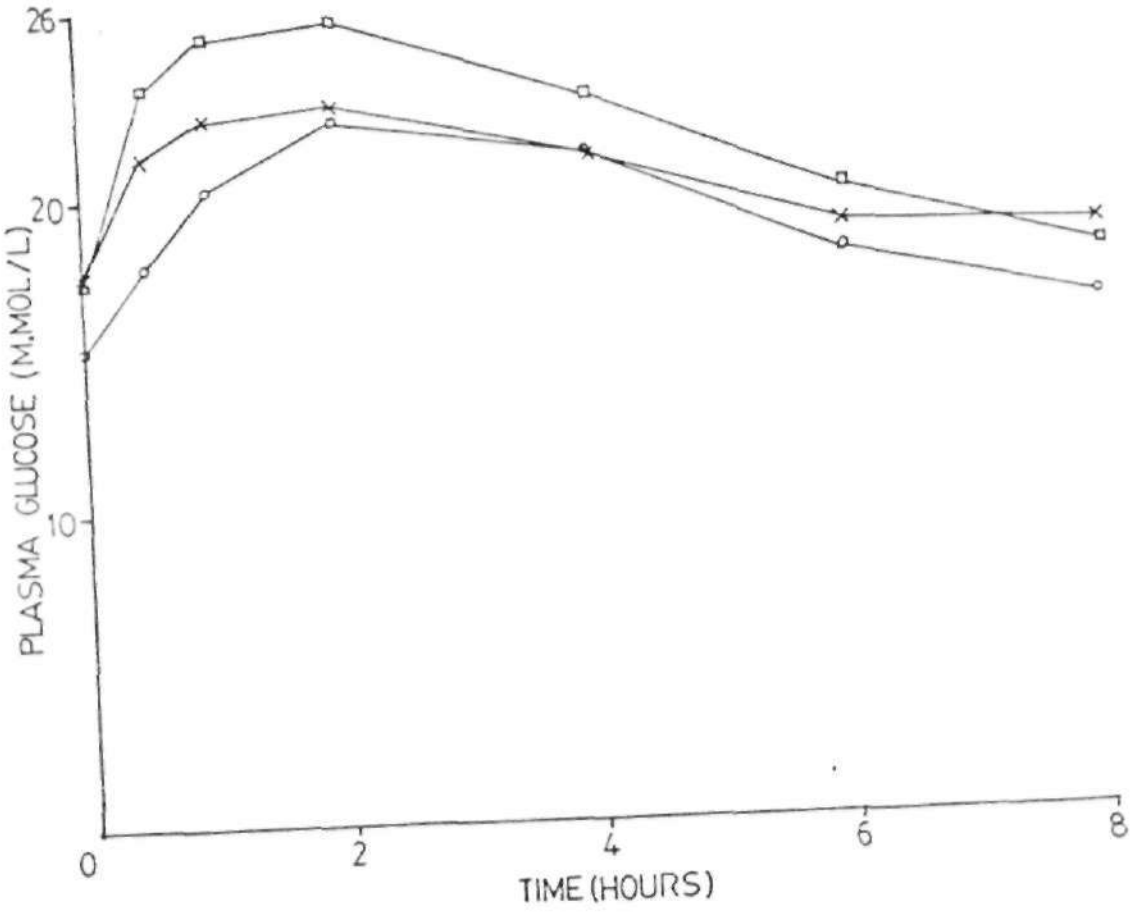


Fig. 27. Comparative mean plasma glucose concentration - time curves in diabetic patients following administration of cooked maize (x——x) cooked cassava (□——□) and cooked yam (o——o) flour meals alone.

Table 17a: MEAN (±S.D) PHARMACOKINETIC PARAMETERS OBTAINED IN 6 DIABETIC PATIENTS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET FOLLWED 30MIN. LATER WITH COOKED MAIZE (B) CASSAVA (C) AND YAM (D) FLOUR MEALS

Mean Parameter ± S. D.	B	C	D	ANOVA (Among Treatments)
Lag Time (h)	0.28±0.13	0.23±0.13	0.22±0.13	NS
Absorption half-life. t1/2 x (h)	0.81±0.63	0.33±0.07	0.43±0.43	NS
Absorption rate constant. ka (h^{-1})	1.26±0.72	2.12±0.46	3.12±2.70	NS
Maximum concentration. Cmax mcg/ml	31.06±3.73	32.58±2.95	30.76±2.37	NS
Time to peak concentration tmax (h)	4.33±2.33	2.16±0.98	3.16±1.83	NS
Area Under the Curve (AUC) AUCo-168h (mcg/h/ml)	1744.55±395.71	1768.09±316.34	1795.56±290.65	NS
AUCo-oo (mcg/h/ml)	1812.28±423.51	1844.32±340.30	1894.40±323.24	NS
Relative bio-availability. FR	0.63±0.15	0.64±0.12	0.66±0.11	NS
Vd (L)	6.85±0.77	6.67±0.61	7.35±1.14	NS
Clearance CL (L/h)	0.14±0.03	0.14±0.02	0.13±0.02	NS
Elimination half life. t1/2 B (h)	33.96±6.89	33.94±5.66	38.54±9.04	NS
Elimination rate constant. ke (h^{-1})	0.02±0.001	0.02±0.003	0.017±0.003	NS

S. D. = Standard Deviation
ANOVA = *Analysis Of Variance For Complete Cross-Over Design*
N.S. = Not Significant At 95% Confidence Level (P>0.05).

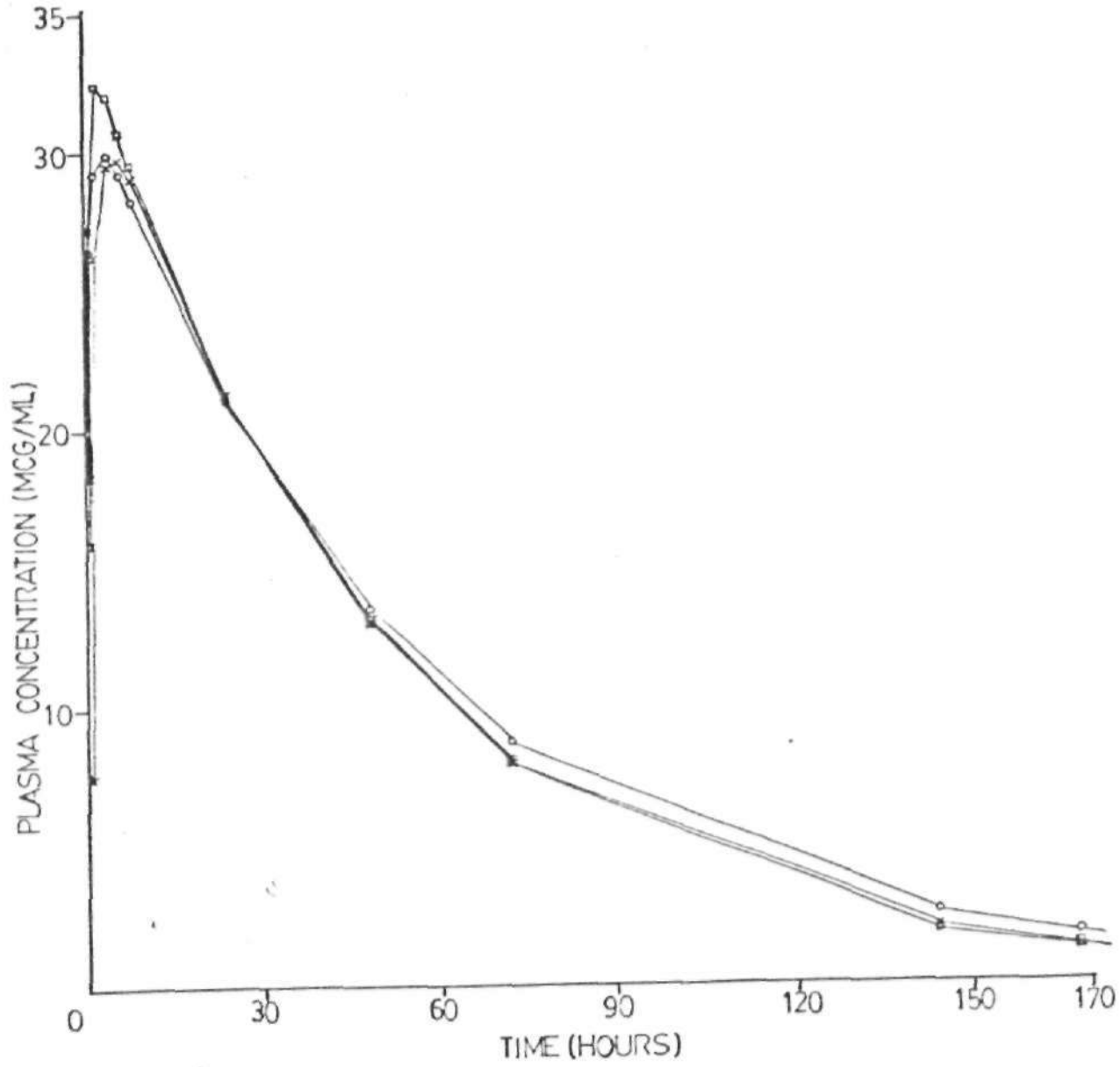


Fig. 28a. *Comparative mean plasma concentration - time curves in diabetic patients following oral administration of 250mg Chlorpropamide tablet followed 30 min later with cooked maize (x——x) cooked cassava (□——□) and cooked yam (o——o) flour meals.*

Table 17b: MEAN (±S.D) PLASMA GLUCOSE CONCENTRATION IN 6 DIABETIC PATIENTS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET FOLLOWED 30MIN. LATER WITH COOKED MAIZE (M) CASSAVA (N) AND YAM (O) FLOUR MEALS

Time h	Plasma Glucose M.Mol/L (±S.D.) M	N	O	ANOVA (Among treatment
0	17.72±3.59	17.12±2.08	15.08±2.08	NS
0.5	18.66±4.31	17.38±0.98	14.91±2.64	NS
1	19.61±4.16	18.74±1.79	15.80±2.89	NS
2	19.88±4.87	19.05±1.83	16.93±2.65	NS
4	17.39±4.31	19.10±3.50	16.13±2.63	NS
6	16.31±4.43	16.78±1.82	14.49±2.43	NS
8	15.55±4.64	16.12±1.63	13.53±2.16	NS

S. D. = Standard Deviation
ANOVA = Analysis Of Variance For Complete Cross-Over Design
N. S. = Not Significant At 95% Confidence Level (P>0.05).

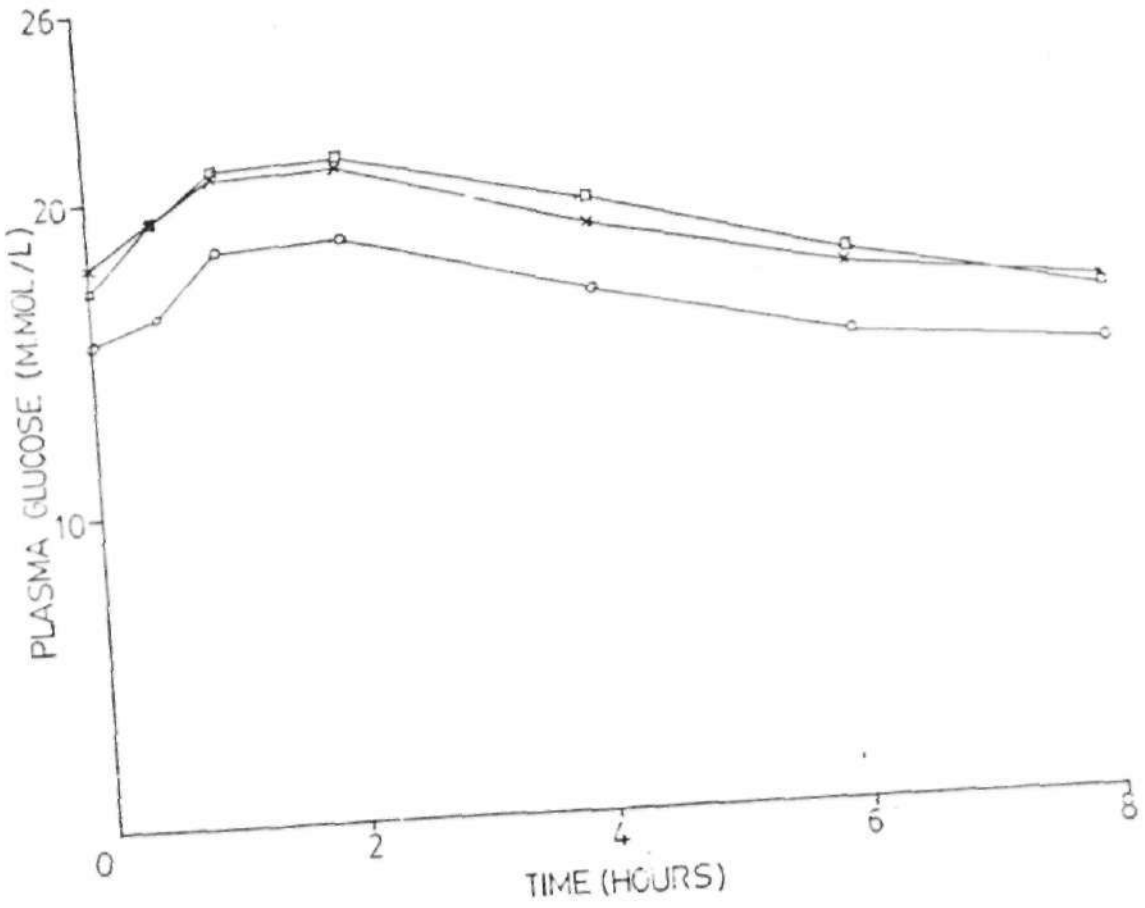


Fig. 28 (b). Comparative mean plasma glucose concentration - time curves in diabetic patients following oral administration of 250mg Chlorpropamide tablet followed 30 min. later with cooked maize (x——x) cooked cassava (□——□) and cooked yam (o——o) flour meals.

Table 18a: MEAN (±S.D) PHARMACOKINETIC PARAMETERS OBTAINED IN 6 DIABETIC PATIENTS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMDE TABLET WITH COOKED MAIZE (B) CASSAVA (C) AND YAM (D) FLOUR MEALS

Mean ± S. D. Parameter	B	C	D	ANOVA (Among treatments)	LSD Value
Lag Time (h)	0.36±0.12	0.27±0.10	0.37±0.29	NS	NA
Absorption half-life. t1/2 x (h)	1.12±0.58	1.50±0.53	0.77±1.15	NS	NA
Absorption rate constant. ka (h^{-1})	0.75±0.35	0.53±0.23	1.82±1.70	NS	NA
Maximum concentration. Cmax mcg/ml	31.33±3.33	33.06±1.72	30.49±2.93	NA	NA
Time to peak concentration tmax (h)	5.66±1.50	7.33±1.03	3.66±2.33	P<0.01	2.10
Area Under the Curve (AUC) AUCo-168h (mcg/h/ml)	1758.98±467.49	2177.31±349.17	1831.54±293.95	NS	NA
AUCo-op (mcg/h/ml)	1831.26±512.75	2273.29±338.41	1901.20±304.40	NS	NA
Relative bio-availability. FR	0.63±0.15	0.79±0.13	0.66±0.10	NS	NA
Vd (L)	6.67±0.66	6.47±0.64	7.256±1.39	NS	NA
Clearance CL (L/h)	0.14±0.03	0.11±0.02	0.14±0.02	NS	NA
Elimination half life. t1/2 B (h)	33.79±9.25	40.40±5.53	38.15±8.86	NS	NA
Elimination rate constant. ke (h^{-1})	0.02±0.008	0.017±0.002	0.018±0.002	NS	NA

S. D. = Standard Deviation
ANOVA = Analysis of Variance For Complete Cross-Over Design
N.S. = Not Significant At 95% Confidence Level
N.A. = Not Applicable
L.S. = Least Square Difference

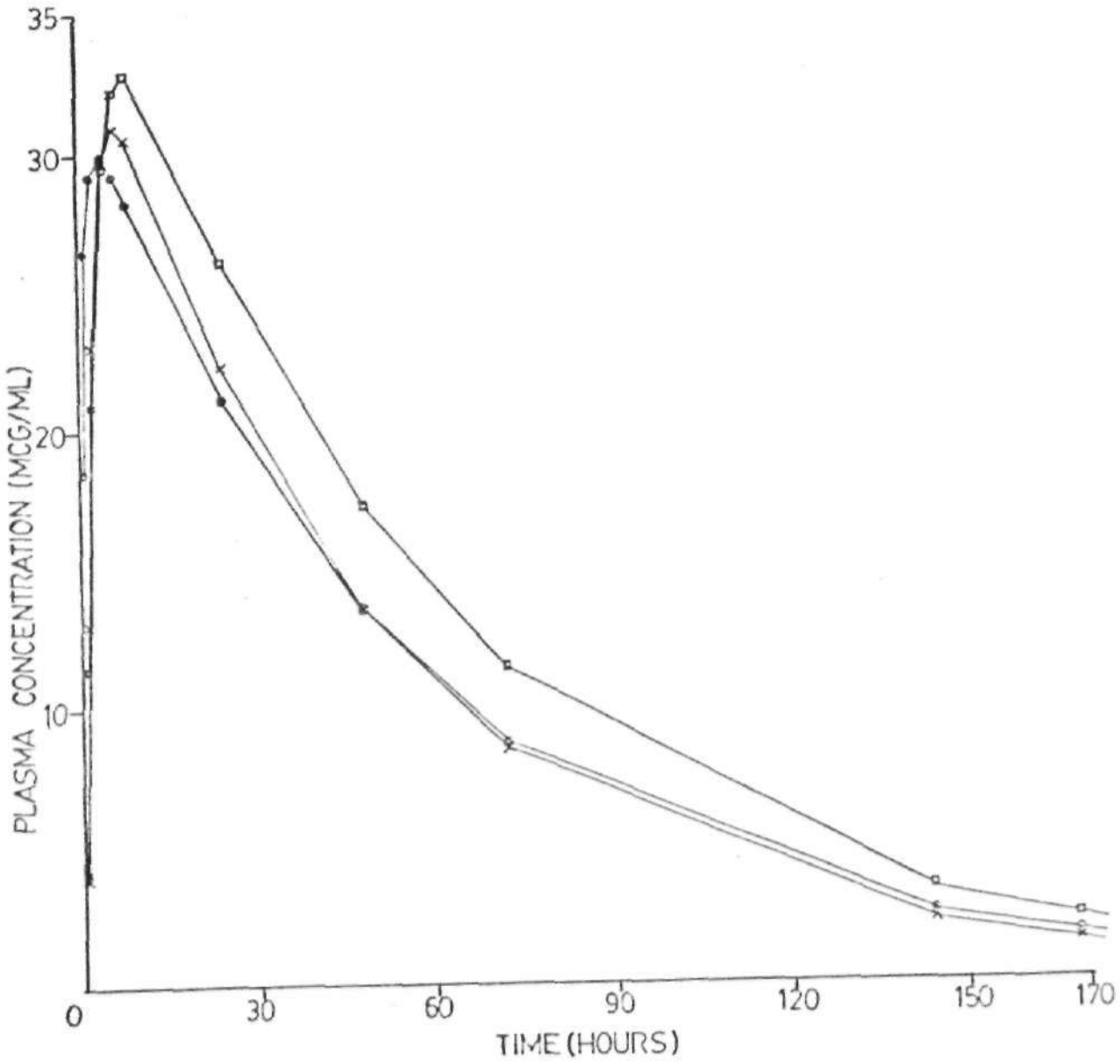


Fig. 29a *Comparative mean plasma concentration - time curves in diabetic patients following oral administration of 250mg 'Chlorpropamide tablet with cooked maize (x——x) cooked cassava (□——□) and cooked yam (o——o) flour meals.*

Table 18b: MEAN (±S.D) PLASMA GLUCOSE CONCENTRATIONS IN 6 DIABETIC PATIENTS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET WITH COOKED MAIZE (B) CASSAVA (C) AND YAM (D) FLOUR MEALS

Time (h)	Plasma Glucose M.Mol/L (±S.D.)			ANOVA (Among treatments)
	B	C	D	
0	17.91±3.64	17.09±2.02	15.36±1.83	NS
0.5	19.29±4.24	19.25±1.83	16.30±2.47	NS
1	20.57±4.06	20.95±2.77	18.28±3.09	NS
2	20.84±4.94	21.14±3.88	18.47±3.21	NS
4	18.58±4.92	19.44±3.34	16.46±2.27	NS
6	17.02±4.64	17.38±2.04	14.77±1.82	NS
8	16.18±5.06	16.02±1.42	14.11±1.97	NS

S. D. = Standard Deviation
ANOVA = Analysis Of Variance For Complete Cross-Over Design
N.S. = Not Significant At 95% Confidence Level (P>0.05).

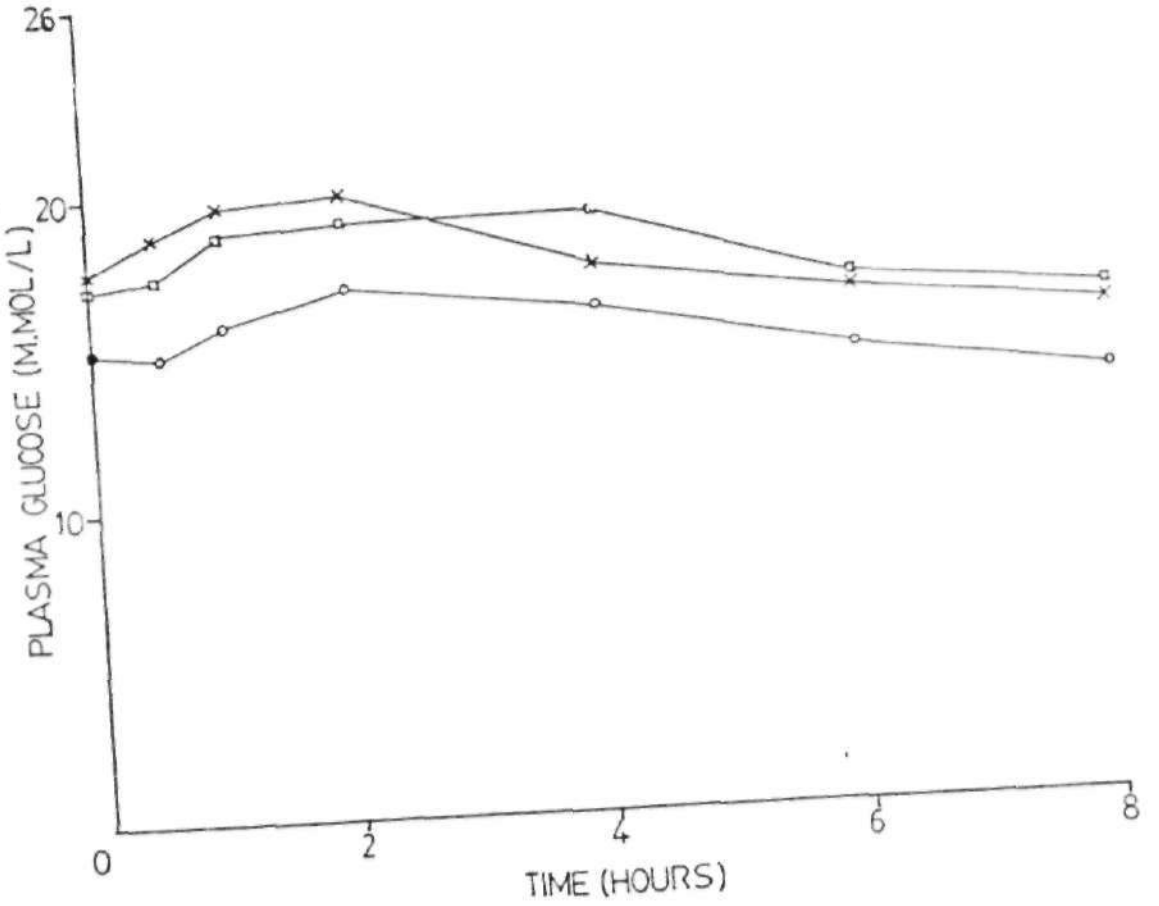


Fig.29 (b) Comparative mean plasma glucose concentration - time curves in diabetic patients following oral administration of 250mg Chlorpropamide tablet with cooked maize (x——x) cooked cassava (□——□) and cooked yam (o——o) flour meals.

Tables 19a, b and c show the hypoglycaemic responses to chlorpropamide in both healthy volunteers and diabetic patients following the administration of the drug in fasting state and with cooked maize, cassava and yam flour meals respectively. Comparison of these responses with plasma chlorpropamide levels in healthy volunteers shows that there was a relationship between the hypoglycaemic responses and plasma chlorpropamide levels attained after administration of the drug. Chlorpropamide administered in fasting state gave a mean correlation, r = 0.87$\pm$ 0.10 (range 0.75 - 0.94) while administration of chlorpropamide concomitantly with a meal gave a mean correlation, r = 0.76 $\pm$ 0.18 (range 0.55 - 0.91). However in the case of patients, there was no simple direct relationship between the plasma chlorpropamide levels and the hypoglycaemic responses. Chlorpropamide administered in fasting state gave a mean correlation, r = 0.37 $\pm$ 0.06 (range 0.32 - 0.44) while the concomitant administration of the drug with a meal gave a correlation, r = 0.41 $\pm$ 0.25) (range 0.12 - 0.56).

4.8 Salivary Excretion of Chlorpropamide:

Figure 30a shows the calibration curve obtained in saliva for chlorpropamide. Following oral administration of a single 250mg chlorpropamide tablet to healthy subjects in fasting state, plasma Cmax ranged from 26.09 to

Table 19a: MEAN HYPOGLYCAEMIC RESPONSES IN HEALTHY VOLUNTEERS AND DIABETIC PATIENTS FOLLOWING ORAL ADMINISTRATION OF 250 MG CHLORPROPAMIDE TABLET IN FASTING STATE (A) AND WITH COOKED MAIZE FLOUR MEAL (B)

Time (h)	Hypoglycaemic Responses Mean Percentage (%) Change In Basal Glucose Healthy Volunteers A	Healthy Volunteers B	Diabetic Patients A	Diabetic Patients B
0.5	4.28	1.05	5.30	7.70
1	7.71	6.25	10.66	14.85
2	11.78	10.42	12.18	16.35
4	16.06	14.17	1.87	3.74
6	14.14	15.00	8.02	4.97
8	11.35	15.42	12.25	9.66

Table 19b: MEAN HYPOGLYCAEMIC RESPONSES IN HEALTHY VOLUNTEERS AND DIABETIC PATIENTS FOLLOWING ORAL ADMINISTRATION OF 250 MG CHLORPROPAMIDE TABLET IN FASTING STATE (A) AND WITH COOKED CASSAVA FLOUR MEAL (C)

Time (h)	Hypoglycaemic Responses Mean Percentage (%) Change In Basal Glucose Healthy Volunteers A	Healthy Volunteers C	Diabetic Patients A	Diabetic Patients C
0.5	4.95	4.50	1.51	12.63
1	8.82	7.88	9.46	12.58
2	12.26	2.25	11.27	23.69
4	15.06	1.81	11.56	13.75
6	13.77	4.96	1.99	1.69
8	13.34	4.73	5.85	6.27

Table 19c: MEAN HYPOGLYCAEMIC RESPONSES IN HEALTHY VOLUNTEERS AND DIABETIC PATIENTS FOLLOWING ORAL ADMINISTRATION OF 250 MG CHLORPROPAMIDE TABLET IN FASTING STATE (A) AND WITH COOKED YAM FLOUR MEAL (D)

Time (h)	Hypoglycaemic Responses Mean Percentage (%) Change In Basal Glucose			
	Healthy Volunteers		Diabetic Patients	
	A	D	A	D
0.5	0.65	5.94	3.39	3.71
1	5.20	10.17	4.77	19.01
2	8.23	12.93	12.26	20.24
4	13.85	13.56	6.96	7.16
6	16.67	15.89	3.92	3.85
8	21.22	17.59	10.28	8.14

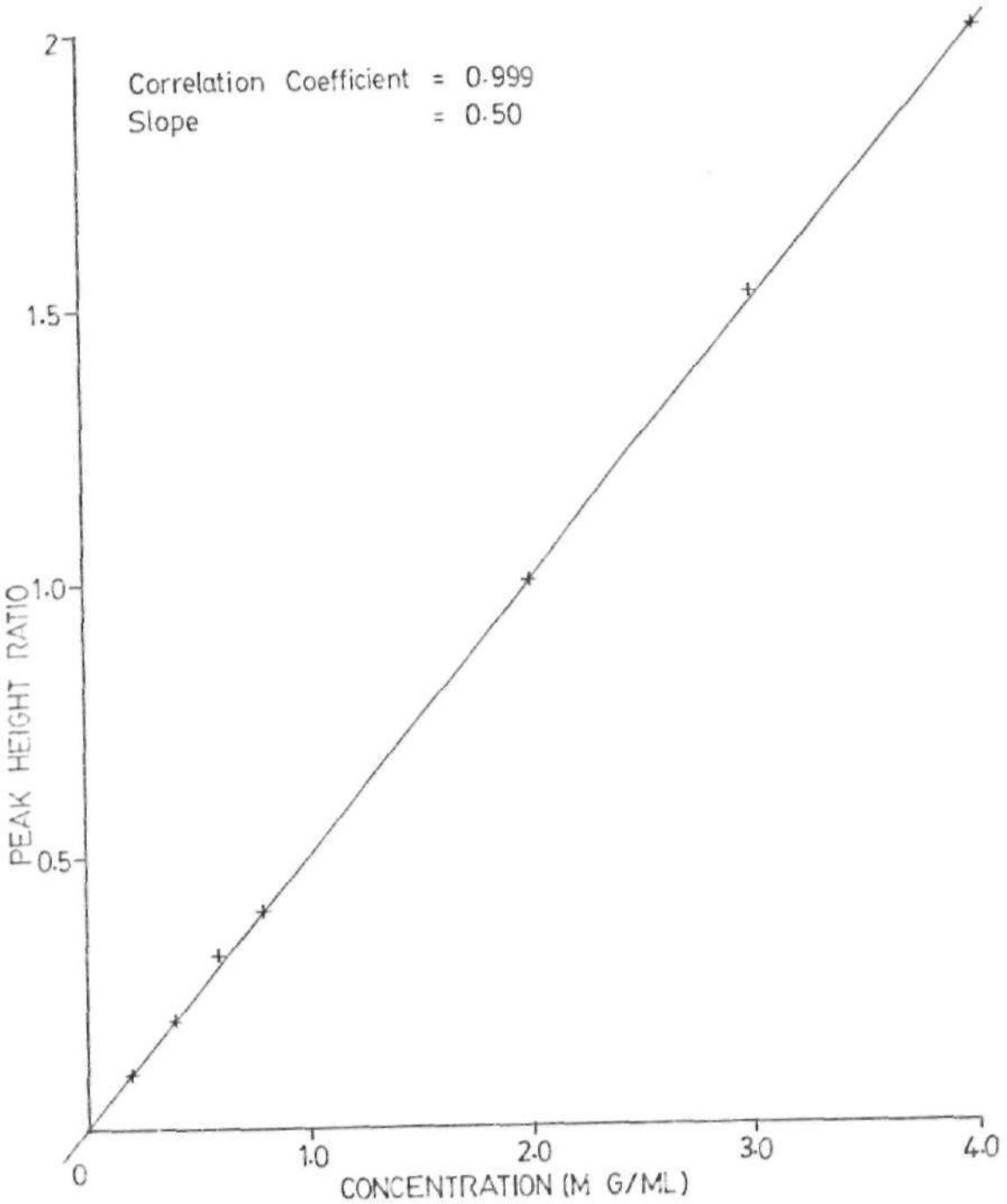


Fig. 30a Calibration curve for Chlorpropamide in saliva using HPLC.

33.06±2.71mcg/ml (mean, 30.06±2.71mcg/ml). The salivary Cmax ranged from 1.25-2.04 mcg/ml (mean, 1.46±0.30mcg/ml). The plasma tmax ranged from 2-6h (mean, 4.33±1.50h) and the salivary tmax ranged from 4-6h (mean, 4.66±1.30h). The mean pharmacokinetic parameters obtained for chlorpropamide in plasma and saliva are shown in Table 20. The derived plasma pharmacokinetics, except t1/2x, ka and tmax differed significantly from those of saliva. The mean plasma and saliva chlorpropamide concentration data are shown in Table 21 and are graphically represented in Figure 30b. Consistently higher levels of chlorpropamide in plasma relative to those in saliva were observed. The saliva and plasma (S/P) ratio ranged from 0.009 - 0.05 (mean, 0.03±0.015). There was a correlation (r) of 0.93 between the plasma and saliva levels of chlorpropamide after a single oral dose in fasting state.

Table 20: MEAN (±S.D.) PHARMACOKINETIC PARAMETERS DERIVED FROM PLASMA AND SALIVA IN 6 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET IN FASTING STATE

Mean Parameter ± S. D	Biological Fluid Plasma	Saliva	Paired sample T-Test Results
Lag Time (h)	0.27±0.05	0.19±0.08	P<0.01
Absorption half-life. t1/2 x (h)	0.67±0.24	1.07±0.35	P<0.08
Absorption rate constant. ka (h^{-1})	1.16±0.53	0.71±0.29	P<0.16
Maximum concentration. Cmax mcg/ml	30.06±2.71	1.46±0.31	P<0.00
Time to peak concentration tmax (h)	4.33±1.50	4.66±1.03	P<0.61
Elimination half life. t1/2 B (h)	42.39±4.51	21.44±5.37	P<0.0003
Elimination rate constant. ke (h^{-1})	0.02±0.001	0.03±0.01	P<0.017
Clearance CL (L/h)	0.13±0.02	4.76±.001	P<0.0002
Vd (L)	7.66±0.69	154.88±76.02	P<0.005
Area Under the Curve (AUC) AUCo-168h (mcg/h/ml)	1892.49±227.09	53.43±12.41	P<0.00
AUCo-oo (mcg/h/ml)	2006.63±271.99	54.17±12.54	P<0.00

S. D. = Standard Deviation

Table 21: MEAN (±S.D.) SIMULTANEOUS PLASMA AND SALIVA CONCENTRATIONS IN 6 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 250MG CHLORPROPAMIDE TABLET IN FASTING STATE

Time (h)	Concentration (mcg/ml) ±S.D.	
	Plasma	Saliva
0.5	8.28±6.64	0.31±0.09
1	17.62±6.25	0.67±0.08
2	26.07±3.85	1.07±0.09
4	30.06±2.71	1.46±0.30
6	29.53±2.44	1.36±0.23
8	28.77±2.38	1.19±0.23
12	26.89±2.19	0.96±0.28
36	18.13±1.66	0.47±0.09
48	14.90±1.54	0.29±0.07
72	10.05±1.36	0.13±0.05
144	3.12±0.80	0.03±0.02
168	2.11±0.63	0.002±0.002

S. D. = Standard Deviation

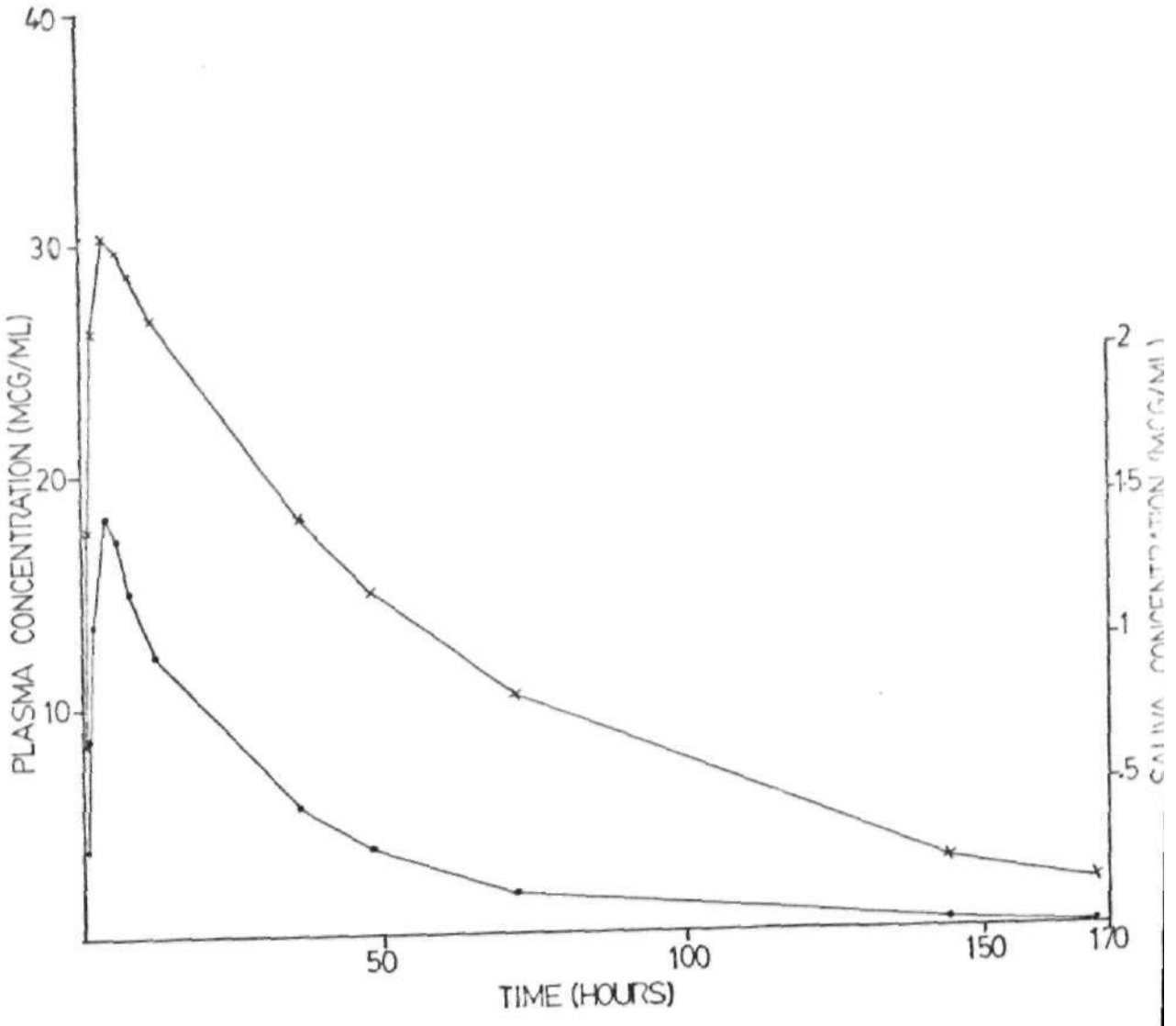


Fig. 30 b. Mean plasma (x——x) and saliva (•——•) concentration - time curves in healthy subjects following oral administration of 250mg Chlorpropamide tablet in fasting state.

CHAPTER FIVE

DISCUSSIONS

5.1 Quality Control of Chlorpropamide Tablets:

The melting point of the recrystallized tablet product which was 142.5°C is close to the B.P. value of 143°C. The ammoniacal odour produced by the filtrate of the recrystallized product, and the white precipitate produced by the filtrate on adding NaOH are in agreement with the B.P. (1988) specifications. The 232nm absorption maximum exhibited by the solution of the sample also agreed with the B.P. (1988) specification. Thus, the tablets complied with the identification tests described for chlorpropamide in the B.P. (1988). The percentage of the active ingredient in the tablets (98.66%) and in the standard powder (100.5%) indicated that the batch of chlorpropamide tablets and the standard powder used in this study complied with the official B.P. (1988) specification of drug content (92.5 - 107.5)%. The average amount of the active ingredients in solution (78.97±6.23%) indicated that the batch of chlorpropamide tablets used in this study meets the official B.P. (1988) requirement for dissolution rate test (not less than 70% in 45 minutes).

5.2 Drug Levels Analysis:

Dichloromethane was used as the extraction solvent because other media when tried either extracted chlorpropamide or tolbutamide badly and yielded interfering peaks from their residue or gave lower percentage recovery compared with dichloromethane. Plasma and saliva, acidified with HCl and extracted with dichloromethane, however abolished these problems (Figures 18a and 18b). Although it seems as if there was no linear effect of varying the ratio of the solvent system on the resolution factor (R) of chlorpropamide and tolbutamide, it can be inferred that increase in the amount of methanol (Figure 17a) decreased R whereas increase in the amount of acetic acid increased R (Figure 17b). Considering the peak height produced for chlorpropamide and tolbutamide with the different ratios, 60% methanol and 40% acetic acid (0.2%) at a pH of 6.5 to 6.7 (Figure 17c) was considered to be optimal. The method involved a less cumbersome extraction procedure than those previously reported (Hill and Crechiolo, 1978. Wahlin-Boll and Melander, 1979 and Hartvig et al., 1980). The retention time (tR) of chlorpropamide and tolbutamide (8 and 11 min respectively), the single extraction step using a small volume of plasma (0.5ml) with about 15min total analytical time per sample are appropriate for a routine assay procedure. The assay method is reliable and reproducible

with a coefficient of variation (CV) of <7%, for both day-to-day and within-day assay.

5.3 Food Samples Analysis:

The three types of meals (B, C and D) contained high carbohydrate but their energy values differ due to the different physico-chemical properties of the meals.

5.4 Cooked Maize Flour Meal (B) Chlorpropamide Interactions Studies In Healthy Volunteers And Diabetic Patients:

The non-significant differences observed in chlorpropamide pharmacokinetic parameters when the drug was administered in fasting state and with meal are in agreement with the results obtained following concomitant administration of chlorpropamide with a meal in healthy subjects (Sartor et al., 1980b) and of glibenclamide with a meal in diabetic patients (Sartor et al., 1980a). Lack of effect of food on sulfaisodimidine pharmacokinetics in adults was reported by Melander et al. (1977) and on amoxycillin pharmacokinetics by Eshelman and Spyker (1978). Lag time and Vd were reduced whilst Cmax was increased in patients relative to that of healthy volunteers. Arrigoni et al. (1987) reported significant differences in the

pharmacokinetics of chlorpropamide for healthy and patient volunteers with a higher Vd for patients which is not in agreement with the finding of this study. However they envisaged that, there could be error in their values of Vd and t1/2 β due to 24 h sampling time used. An increase in Vd could indicate an increase in free fraction of chlorpropamide.

In healthy subjects, the plasma levels of chlorpropamide were higher when the drug was administered in fasting state compared with when it was taken concomitantly with meal. In agreement with this finding, little increase in chlorpropamide absorption had been reported in healthy subjects when the drug was administered with food (Sartor et al., 1980b). In contrast, no difference was observed in tolbutamide absorption with food (Sartor et al., 1980b; Samanta et al., 1984). In the case of patients, chlorpropamide absorption was significantly delayed for 2h when the drug was administered concomitantly with meal compared with when it was taken 30min before the meal. However, the postprandial glucose levels were significantly lowered at each sampling point under the latter compared with the former condition. Wahlin-Boll et al. (1980) reported a similar delay in absorption when glipizide was administered concomitantly with food and, the glucose values when the drug was taken 30min before food were significantly

lowered in diabetic patients. In addition, Sartor et al. (1982) and Jeng et al. (1986) obtained similar results for glibenclamide in diabetic patients. Delay in drug absorption following concomitant administration of drug with meal presumably reflects the meal-induced delay in gastric emptying. The presence of meal in the stomach changed gastric motility to a typical postpranial pattern, during which gastric secretion and residence time are increased (Winstanley and Orme, 1989). According to Sartor et al. (1978), the increased postprandial plasma glucose levels obtained in the patients when the drug was administered 30min before meal may be due to a stronger action of the drug on insulin release or a more optimal timing between induction of insulin release and the blood glucose increment evoked by the meal.

5.5 Cooked Cassava Flour Meal (C) Chlorpropamide Interactions Studies In Healthy Volunteers And Diabetic Patients:

Following concomitant administration of a single 250mg chlorpropamide tablet with meal to healthy and diabetic subjects, the lag time, tmax and t1/2x significantly increased whilst the ka was reduced compared with when the drug was administered in fasting state. Several factors could explain the observed significant alteration in the

parameters. The presence of the meal in the stomach might have acted as a physical barrier between the intestinal mucosa and lumina contents and thus reduced the rate of drug absorption (Jaffe et al., 1971). Also, in view of the greater efficiency of drug absorption from the small intestine, any factor which increases the residence time of a drug in the stomach will in general, reduce the absorption rate of the drug and t1/2x will be increased. Gastric emptying is reduced by high bulk diet while administration of drug in fasting state with large volume of fluid may result in a shortened residence time of the drug resulting in a more rapid rate of absorption (Sampson, 1987).

A more realistic view, which has been expressed generally is that "dietary effects on drug absorption are quite variable and frequently depend on the components of the meal and the physico-chemical properties of the drug (Jaffe et al., 1971; Toothaker and Welling, 1980; Rogers and Chir, 1982 and Winstanley and Orme, 1989).

Concomitant administration of chlorpropamide with meal (c) reduced the mean AUCo -oo in healthy subjects and increased the AUCo-oo in patients as compared to the administration of the drug in the fasting state. The difference in the AUC can be explained by considering the fact that, tmax is dependent on ka while AUC is

proportional to the amount or extent of drug absorption. The greater the total amount of drug absorbed, the greater is the bioavailability (Abdel-Monem and Henkel, 1978). Absorption may be rapid (large ka) with a low bioavailability as in the case of healthy subjects or conversely, the absorption may be slow (small ka) with a high bioavailability. There is of course, a limit to how slow absorption may be without reducing the bioavailability. This is determined by how long the drug remains in the gastrointestinal tract (GItract) (Tozer, 1979 and Svensson et al., 1983). Following concomitant administration of chlorpropamide and meal (C), the pharmacokinetic parameters derived in healthy subjects when compared with those derived in diabetic patients, indicated significant differences in the lagtime, tmax, t1/2x and ka. This finding agrees with the results of Wahlin-Boll et al. (1980) and Arrigoni et al. (1987).

Significant reduction in plasma glucose levels was observed in healthy and patient volunteers following administration of a single 250mg chlorpropamide tablet in the fasting state compared with concomitant administration of the drug with meal. This finding might be due to the significantly increased plasma levels of chlorpropamide detected in the fasting state for both healthy and patient volunteers compared to when the drug was administered

concomitantly with meal. The degree of hypoglycaemic effect produced by chlorpropamide in healthy subjects is related to the plasma concentration of the drug (West and Johnson, 1960). A more optimal timing between induction of insulin release and the blood glucose increment evoked by the meal could also explain the reduction in plasma glucose levels detected in the patients (Sartor et al., 1978).

5.6 Cooked Yam Flour Meals (D) Chlorpropamide Interactions Studies In Healthy Volunteers And Diabetic Patients:

Following concomitant administration of a single 250mg chlorpropamide and meal (D) to healthy subjects, the lagtime and ka increased while the t1/2x and the tmax were reduced when compared with the corresponding values observed when the drug was administered in the fasting state. According to Roe (1985), with a full stomach the delivery of the drug to the absorptive site in the small intestine is delayed, the drug in a solid dosage form occupies the stomach for a longer period resulting in a longer lagtime and rapid rate of absorption. Also, the physical nature of this meal with the increased fluid volume could have improved dissolution prior to absorption (Rogers and Chir, 1982), resulting in a shortened residence time of the drug and rapid rate of absorption. The increase in the ka following administration of the drug in the fasting state resulted in decreased AUCo-oo (not significant) when the drug was administered

concurrently with meal (Abdel-Monem and Henkel, 1978). In case of patients, the differences observed in the pharmacokinetic parameters when the drug was administered in fasting state compared with concomitant administration of the drug with meal were not statistically significant and the ka was decreased by the meal. Also, comparison of the pharmacokinetic parameters obtained in healthy volunteers with those of patients indicated significant decrease in ka for the patients. This could be due to the effect of age on the GItract, most of the diabetic patients that participated in the study involving this meal had their age within the upper limit of the used age ranged. According to Sklar (1978), the GI tract is the most common site of distress in the aged.

Following the concomitant administration of a single 250mg chlorpropamide tablet and meal to healthy subjects, the plasma levels of the drug increased significantly at 0.5h and 1h but the reduction in glucose levels did not reach a level of statistical significance at any point and no postprandial rise in glucose with meal was observed. In the case of the patients, the differences in plasma chlorpropamide levels when the drug was administered 30min. prior to the meal compared with when the drug was concurrently administered with meal, did not reach a level of statistical significance at any point, but, the

postprandial plasma glucose levels were significantly lowered under the former condition at 0.5h and 1h. According to Campbell (1980), there appears to be a 'threshold' chlorpropamide concentration which must be attained before activity occurs; an 'All-or-nothing' response would then be observed: when chlorpropamide levels are below a certain limit, almost no effect on blood glucose is measured, whilst levels above this 'threshold' concentration produce an activity unrelated to the drug concentrations in the blood. Alternatively, the findings might represent a direct effect of chlorpropamide on the hepatic output of glucose, and/or some other extrapancreatic effect (Marshall et al., 1970; Lebovitz and Feinglos, 1978 and Almer et al., 1982).

5.7 Effects Of Cooked Maize (B), Cassava (C) and Yam (D) Flour Meals On Chlorpropamide Pharmacokinetic Parameters

The statistical analyses of the results of the different meal treatments (concomitant administration of 250mg chlorpropamide with cooked maize, cassava and yam flour meals) indicated significant differences in t1/2x, ka, Cmax and tmax obtained in healthy subjects (Table 15). The significant difference in tmax was found in all the meal treatments. However, it can be inferred that the tmax was significantly increased by cassava flour meal compared with

the maize or yam flour meal. Also, the Cmax was significantly increased by cassava flour meal compared with the treatment with either maize or yam flour meal. The significant increase observed in t1/2x when the results of the three meal treatments were compared was between the treatment with cassava flour meal compared with the treatment with maize or yam flour meal. Also, the ka was significantly reduced by cassava flour meal compared with the yam flour meal. However, the reduction in the ka by cassava flour meal did not reach a level of significance when compared with that of maize flour meal. These observations further confirmed the upredictable effect of meals on drug absorption. Drug-food interactions may result in reduced, delayed or increased absorption, or there may be no effect at all (Toothaker and Welling, 1980). Considering the fact that tmax is dependent on ka, the significant reduction in ka by cassava flour meal increased the tmax and resulted in increased t1/2x.

Statistical analyses of the results of plasma glucose levels obtained in patients following the meal treatments without chlorpropamide revealed significant differences in the 0.5h and 1h mean postprandial values (Table 16, Figure 27). The reduction in the plasma glucose values was observed to be by yam flour meal as compared with cassava flour meal. Dietary carbohydrate has been repeatedly

demonsatrated to improve diabetes control especially in patients with non-insulin-dependent diabetes mellitus (Brunzell et al., 1971; Weinsier et al., 1974; Spannhake, 1976; Anderson and Ward, 1979 and Simpson et al., 1981). Enhancement of insulin secretion or increased sensitivity of peripheral tissue to insulin (Grundy, 1991); suppression of glucagon and augmentation of insulin secretion by exposure to glucose (Spannhake, 1976) might be the mechanisms for such an action. It has also been reported that the glycaemic response of starchy food differs (Jenkins et al., 1982). Thus, it is possible that the glycaemic index of yam flour meal was higher than that of cassava flour meal. The difference in the carbohydrate content of yam flour meal (84.5%) when compared with that of cassava flour meal (76%) might also be responsible for the improved glucose tolerance observed with yam flour when compared with cassava flour meal.

Following the administration of chlorpropamide to diabetic patients 30min prior to the ingestion of the meals (maize, cassava and yam flour), the differences observed in the drug pharmacokinetic parameters did not reach a level of significance (Table 17a). However, the tmax was different in patients following concomitant administration of the drug and each meal to patients (Table 18a). The difference in tmax was statistically significant in patients taking

cassava and yam flour meals. The postprandial plasma glucose levels were significantly reduced when each meal was taken with 250mg chlorpropamide than when given alone in patients. In addition to this finding, the early postprandial increment was reduced and there was a subsequent decrease to values below preprandial levels. In view of the potent stimulatory effect of chlorpropamide and other sulphonylureas on B-cell, it will be logical to postulate that the single oral dose of chlorpropamide administered in fasting state or concomitantly with each of these meals could be responsible for these effects. It is also worth mentioning that the high carbohydrate meals used in this study might have contributed to the marked reduction in the glucose levels. The mechanism for such an action might be either an enhancement of insulin secretion or increased sensitivity of peripheral tissue to insulin by the carbohydrate content of the meal (Anderson and Ward, 1979; Simpson et al., 1981; Garg et al., 1988 and Grundy, 1991). This point would have been confirmed if it were possible to measure insulin and C-peptide levels. However, the therapeutic end-point of oral hypoglycaemic drug is reduction of glucose levels which were measured.

The sulphonylureas act by causing the release of insulin from the islet cells of the pancreas. It is not therefore suprising that there was a relationship between the hypoglycaemic action and plasma chloropropamide

concentration in the healthy volunteers. However, the choice of oral hypoglycaemic agent in Type II diabetics is often based on empirical grounds rather than upon any certainty of the presence of residual islet cells. Thus the patient population studied may not be suitable for a sylphonylurea therapy. In any case the derangement in nutrient metabolism that characterized diabetes mellitus might have masked any direct relationship between the hypoglycaemic responses and the drug levels. Moreover some authours have shown that for most sulphonylureas, the blood levels are a poor guide to their hypoglycaemic responses (Balant, 1981 and Ferner, 1988).

Considering the individual variations in the calculated individual pharmacokinetic parameters (see appendices 7a-9b), there were differences as well as similarities. For example the Ke and AUC were similar within subjects but, Ka and tmax were different. Inter-individual and intra-individual variability in drug responsiveness can result from genetic differences in rates of drug metabolism. However, the parameters derived for each subject were within the range of previously reported values (Taylor, 1972 and Sartor et al., 1980b).

5.8 Salivary Excretion of Chlorpropamide:

Comparison of chlorpropamide pharmacokinetic parameters obtained from saliva with those obtained from plasma of healthy subjects (Table 19) shows that the calculated sali-

vary chlorpropamide pharmacokinetics (except the t1/2x, Ka and tmax) were significantly different from those of plasma. The occurrence of tmax at about the same time in saliva and in plasma suggests that equilbrium is reached rapidly between them, in line with Posti's hypothesis that saliva should be regarded as an integral part of the central compartment (Posti, 1982) rather than a 'deep' pharmacokinetic compartment as suggested by Galeazzi et al. (1976). The similar values of Ka which resulted in similar t1/2x can be explained by assuming that the rate of the drug absorption was proportional to its concentration at the absorption site which has nothing to do with different biological fluids. In contrast, elimination parameters are related to bioavailability and Cmax of the drug in either blood or saliva. Therefore the significant differences observed in the bioavailability and elimination of the drug in plasma compared with saliva agreed with the fact that chlorpropamide is highly bound to plasma protein (Crooks and Brown, 1974), and it also explains the mean saliva/plasma (S/P) ratio of less than unity (0.009 to 0.05) obtained at each sampling point in this study.

According to Crooks and Brown (1974), about 96% of chlorpropamide is bound to plasma protein. Hence at any point in time, only about 4% of the plasma drug concentration will be free to traverse the lipoidal membrane of the salivary gland. It has been asserted that saliva pH

is the major variable determining the concentration of ionizable drugs in saliva. Also, the appearance in saliva of acidic drugs with pka under 7 is profoundly affected by minor alteration in salivary flow rate and thus the pH (Mucklow et al., 1978). Since chlorpropamide has pka 4.8 (Mucklow et al., 1978), its salivary excretion would be expected to be affected by the pH of saliva. However, a correlation (r = 0.93) was observed to exist between saliva and plasma levels of chlorpropamide. This is a reflection of the advantage of collection of stimulated saliva which has a narrow pH range around the value of 7.0 whereas the pH of unstimulated saliva showed a large variability (Gilbadi and Prescott, 1983). However, a poor correlation (r = 0.54) of S/P chlorpropamide was observed by Mucklow et al. (1978). The authors collected single sample of venous blood and saliva from diabetic patients on regular chlorpropamide therapy ranging from 100-500mg daily, and measured the drug concentration using GLC method. They however envisaged erros in their study due to non standardization of salivary flow rate and pH. The use of a more sensitive technique (HPLC) in this study may also contribute to the observed improved correlation of S/P ratio of chlorpropamide.

CONCLUSIONS

The batch of chlorpropamide tablets and standard powder used in this study satisfied the official B. P. (1988) requirements. It has been demonstrated that certain specific dietary components for example, cassava and yam flour meals can significantly alter the absorption pattern of orally administered chlorpropamide tablet, while maize flour meal resulted in only little difference in the drug absorption. These findings further confirm the unpredictable effect of meal components on absorption of drug and the fact that no general rule can be formulated concerning food-drug interactions.

The results showed that administration of 250mg chlorpropamide tablet prior to a standard high carbohydrate meal or concomitantly with the meal results in lowered postprandial glucose levels compared with the ingestion of the meal alone. The results further showed that the most pronounced hypoglycaemic effect was obtained when chlorpropamide was administered 30min before meal, as evidenced by lowered plasma glucose levels. Thus, the results suggest that in the long term therapy, pre-prandial treatment is generally more effective than with food dosing due to a more optimal timing between induction of insulin

release and the blood glucose increment evoked by the meal. The principal objective in the treatment of a patient with diabetes mellitus is to permit him to lead a satisfactory life in good health in his normal environment. The treatment program should be designed to correct defects in metabolism, preserve pancreatic functions, prevent chronic diabetic complications and promote psychosocial adjustment (Goodhart and Shils, 1980). There is no doubt of the beneficial influence of a high-carbohydrate diet on the pancreas. Starvation or a normal caloric diet in which carbohydrate is 'restricted, produces an impairment of the insulin production in response to a glucose challenge and may result in an abnormal glucose tolerance test. Preparing the patient with 300gm of glucose for 3 days prior to the test may then result in a normal test. This is a clinical example of the beta cell reserve which is present even in diabetic patients. Such patients, when presented with sufficient carbohydrate for a period of time, are able to increase the amount of insulin produced following a subsequent carbohydrate challenge (Goodhart and Shils, 1980). In view of the present results and the decision of several dietary recommending bodies, including, the Special Report Committee of Canadian Diabetes Association (1981), the British Diabetic Association (1982) and the American Diabetes Association (1986), these results suggest that

diabetic patients should be allowed to take starch meals (complex carbohydrate) such as cereals, yam product meals, and rice. Such meals may be taken 30min. after the drug administration to achieve a critical plasma concentration of the drug before entrance of food into the gastrointestinal tract.

It has been demonstrated that pharmacokinetic values of chlorpropamide cannot be fully extrapolated from a healthy population to a diabetic population. This may be due to pathological changes that occur in patients with diabetes. The results also demonstrated that there was a relationship between the hypoglycaemic responses and chlorpropamide levels attained after administration of the drug to healthy subjects whereas there was no direct relationship between chlorpropamide levels and the hypoglycaemic responses in patients. The derangement in nutrient metabolism that characterized diabetes mellitus might have masked any direct relationship between the hypoglycaemic responses and the drug levels in the patients. Considering individual variations the absorption rate as reflected by the time to reach peak plasma concentration differed markedly between individuals, whereas the elimination rates varied only slightly. Inter-individual and intra-individual variability in drug responsiveness can result from genetic variations of rates of drug metabolism.

Significantly lowered chlorpropamide concentrations were detected in saliva compared with plasma, and the saliva/plasma ratio was less than unity at each sampling point. Plasma/saliva chlorpropamide time to maximum concentration (tmax) and absorption half-life (t1/2x) were similar while the maximum concentration (Cmax) and elimination half-life (t1/2B) were different. This might not be unexpected since chlorpropamide is extensively bound to plasma proteins.

The sulfonylureas are a class of drugs characterized by marked intersubject variations in blood levels and therapeutic effectiveness (Melander, 1978). Because of this variability and the integral role of diet in the control of diabetes, the uptake of these drugs must be examined when administered not only in fasting state but with different varieties of meals consumed by different population groups for meaningful understanding of the influence of these meals on the absorption characteristics of the drugs. In addition, C-peptide and insulin levels measurement together with blood glucose are also recommended for further studies. This will make it easy to relate sulphonylurea blood levels to hypoglycaemic effect since food intake is a major component of this relationship. The pharmacokinetics of most drugs are usually studied in fasting healthy subjects whereas, drugs are administered to patients with a meal. It is recommended that pharmacokinetic studies should be carried

out both in healthy subjects (under fasting and non-fasting conditions) and in patients using standardized test meals. This type of information is slowly becoming available, and it is likely to take sometime before health practitioners have sufficient information regarding food-drug interactions to optimize drug dosage in order to obtain maximum therapeutic effect.

Salivary concentrations of chlorpropamide could have two uses; its specific identification and estimation in the saliva of patients suffering from chlorpropamide overdose may assist the clinician to decide further treatment; and the measurement of the biological or systemic availability of chlorpropamide formulations could be achieved without the discomfort, possible hazard and necessary attendance of medical staff required for repeated venepunctures. There is however the need for more study on salivary excretion of chlorpropamide with a view to develop more sensitive technique for measuring the drug in saliva.

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APPENDICES

Appendix 1a

Chemical Assay of Chlorpropamide Tablets and Chlorpropamide Standard Powder.

Chlorpropamide	Sample	Standard
Absorbance (232nm)	0.590	0.601

Appendix 1b

Dissolution Test Of Chlorpropamide Tablets In Phosphate Buffer pH 6.8 Mainteained at 37 ± 0.5^{o}C, Using USP XVIII Rotating Basket Method. Absorbance Measured at 230nm

Time (Min.)	No. of Tablet			
45	I	II	III	IV
Absorbance	0.922	0.925	0.927	0.928

Appendix 2a

Plasma Concentrations Of Chlorpropamide In 5 Healthy Volunteers Following Oral Administration Of 250mg Chlorpropamide Tablet in Fasting State (A) And With Cooked Maize Flour Meal (B)

	concentration (mcg/ml									
Volunteers	I		II		III		IV		V	
Time (h)	A	B	A	B	A	B	A	B	A	B
0.5	4.00	0.61	3.11	0.33	1.39	0.20	3.19	22.54	11.42	10.05
1	15.23	6.63	12.41	6.07	10.65	8.56	12.05	24.99	23.86	7.90
2	23.40	15.09	21.77	13.89	21.95	20.28	21.23	24.68	27.79	17.92
4	25.18	23.27	26.40	20.25	26.15	26.79	25.99	23.87	26.71	25.61
6	24.45	25.81	26.50	21.75	25.00	26.71	26.09	23.08	25.30	26.91
8	23.58	26.09	25.91	21.76	23.23	25.36	25.41	22.32	23.96	26.31
12	21.92	24.55	24.54	20.77	19.87	22.33	23.81	20.88	21.49	23.95
36	14.14	14.00	17.92	14.62	7.74	10.20	16.01	13.96	11.19	12.64
48	11.35	10.53	14.92	12.26	4.83	6.89	13.12	11.42	8.08	9.17
72	7.32	5.96	10.71	8.62	1.88	3.14	8.82	7.64	4.21	4.83
144	1.96	1.08	3.96	2.99	0.11	0.29	2.68	2.28	0.59	0.70
168	1.26	0.61	2.84	2.10	0.04	0.13	1.89	1.53	0.30	0.37

Appendix 2b

Plasma Concentrations Of Chlorpropamide (mcg/ml) in 6 Diabetic Patients Following Oral Administration Of 250mg Chlorpropamide Tablet 30Min Before (A) And With Cooked Maize Flour Meal (B)

	Concentration (mcg/ml)											
Patients	I		II		III		IV		V		VI	
Time (h)	A	B	A	B	A	B	A	B	A	B	A	B
0.5	3.53	0.66	7.19	4.41	4.51	3.08	10.20	7.84	2.97	0.11	17.82	5.94
1	20.42	8.60	20.18	11.11	14.25	11.44	25.78	22.19	8.88	5.32	23.13	16.34
2	28.18	18.97	26.21	19.73	25.15	21.74	35.24	33.27	17.85	15.31	28.01	25.72
4	28.00	27.39	25.97	26.70	31.77	29.45	36.02	35.77	28.10	27.24	29.53	29.26
6	26.64	28.83	24.78	28.10	32.34	30.90	34.48	34.38	32.54	32.84	28.68	28.79
8	25.33	27.94	23.63	27.73	31.64	30.63	32.93	32.77	34.09	35.16	27.53	27.88
24	16.93	15.78	16.16	19.96	24.47	24.10	22.74	22.15	27.80	29.80	19.59	21.21
48	9.25	6.45	9.14	11.92	16.57	16.14	13.05	12.31	18.15	19.79	11.76	14.07
72	5.05	2.64	5.17	7.12	11.22	11.60	7.49	6.84	11.85	13.14	7.06	9.33
144	0.82	0.18	0.94	1.52	3.48	3.85	1.42	1.17	3.29	3.84	1.53	2.72
168	0.45	0.04	0.52	0.90	2.35	2.67	0.81	0.65	2.15	2.55	0.92	1.8

Appendix 3a

Plasma Concentration Of Chlorpropamide In 5 Healthy Volunteers Following Oral Administration Of 250mg Chlorpropamide Tablet In Fasting State (A) And With Cooked Cassava Flour Meal (C).

	Concentration (mcg/ml)									
Volunteers	I		II		III		IV		V	
Time (h)	A	C	A	C	A	C	A	C	A	C
0.5	5.44	0.48	10.94	4.67	6.50	0.12	21.18	4.78	9.01	1.00
1	18.93	2.37	26.03	8.24	16.73	2.11	29.05	7.97	20.76	6.07
2	30.77	7.54	31.20	14.15	26.97	8.11	32.36	13.34	27.14	13.89
4	34.67	16.01	32.15	22.19	31.79	17.65	31.95	20.88	27.51	20.25
6	33.57	22.37	31.42	26.81	31.55	24.51	31.08	25.42	26.63	21.75
8	31.99	27.03	30.60	29.29	30.54	29.25	30.22	28.00	25.74	21.76
12	28.94	32.51	29.01	30.78	28.36	34.18	28.22	29.82	24.04	20.77
36	15.82	25.31	21.06	22.47	18.09	22.20	20.42	21.92	15.98	14.62
48	11.70	17.95	17.94	18.49	14.45	14.46	17.76	17.91	13.03	12.26
72	6.40	8.28	13.02	12.52	9.55	5.86	12.33	11.94	8.66	8.62
144	1.04	0.72	4.98	3.89	2.39	0.30	4.50	3.54	2.54	2.99
168	0.57	0.31	3.61	2.63	1.52	0.11	3.21	2.35	1.69	2.10

Appendix 3b

Plasma Concentrations Of Chlorpropamide In 6 Diabetic Patients Following Oral Administration of 250mg Chlorpropamide Tablet 30min Before (A) And With Cooked Cassava Flour Meal (C).

Patients	Concentration mcg/ml											
	I		II		III		IV		V		VI	
Time (h)	A	C	A	C	A	C	A	C	A	C	A	C
0.5	11.46	6.43	29.22	2.37	12.58	2.59	9.27	5.50	22.46	6.13	10.12	1.77
1	26.69	15.86	29.29	8.34	28.76	9.35	24.22	15.03	28.35	11.95	26.10	8.03
2	31.33	25.99	28.77	17.54	36.19	19.13	30.76	25.14	31.44	20.34	35.64	17.44
4	30.53	31.52	27.49	28.43	36.18	29.24	30.51	30.63	31.04	28.95	36.32	28.03
6	29.38	31.53	26.26	33.48	35.07	32.89	29.34	30.81	29.54	32.06	34.83	32.48
8	28.26	30.51	25.09	35.86	33.97	33.73	28.21	30.04	28.08	32.81	33.33	33.97
24	20.76	22.00	17.41	30.86	26.31	25.99	20.58	23.14	18.71	26.77	23.41	27.30
48	13.06	13.43	10.07	21.79	17.95	16.31	12.82	15.60	10.17	18.56	13.78	17.54
72	8.22	8.19	5.81	15.38	12.24	10.23	7.98	10.52	5.53	12.87	8.11	11.26
144	2.05	1.86	1.12	5.41	3.88	2.52	1.93	3.23	0.88	4.28	1.65	2.98
168	1.29	1.14	0.65	3.81	2.64	1.58	1.20	2.17	0.48	2.97	6.97	1.91

Appendix 4a

Plasma Concentrations Of Chlorpropamide (mcg/ml) In 5 Healthy Volunteers Following Oral Administration Of 250mg Chlorpropamide Tablet In Fasting State (A) And With Cooked Yam Flour Meal (B)

Volunteers	Concentration (mcg/ml)									
	I		II		III		IV		V	
Time (h)	A	B	A	B	A	B	A	B	A	B
0.5	5.92	22.58	5.11	22.13	9.25	10.07	4.48	28.25	6.17	17.89
1	11.13	27.61	15.77	27.00	19.85	28.10	12.94	30.89	15.44	24.14
2	18.20	29.23	26.40	26.46	26.60	27.46	22.89	30.41	25.31	27.37
4	24.60	28.45	31.39	25.40	27.53	26.15	29.62	29.47	30.39	26.96
6	26.45	27.50	31.20	24.39	26.75	24.89	30.53	28.56	30.57	25.85
8	26.36	26.57	30.23	23.41	25.91	23.70	30.04	27.68	29.57	24.76
12	25.23	24.81	28.13	21.58	24.32	21.48	28.36	25.99	27.30	22.72
36	17.09	16.45	18.18	13.22	16.62	11.92	19.50	17.82	16.72	13.56
48	14.04	13.39	14.62	10.35	13.74	8.87	16.17	14.75	13.08	10.48
72	9.48	8.87	9.44	6.34	9.39	4.92	11.12	10.11	8.01	6.25
144	2.92	2.58	2.55	1.44	2.99	0.84	3.61	3.28	1.80	1.33
168	1.97	1.71	1.64	0.89	2.04	0.46	2.48	2.23	1.10	0.79

Appendix 4b

Plasma Concentrations Of Chlorpropamide (mcg/ml) In 6 Diabetic Patients Following Oral Administration Of 250mg Chlorpropamide Tablet 30Min Before (A) And With Cooked Yam Flour Meal (B)

	Concentration (mcg/ml)											
Patients	I		II		III		IV		V		VI	
Time (h)	A	B	A	B	A	B	A	B	A	B	A	B
0.5	26.41	21.46	20.58	19.64	10.19	15.57	23.22	17.09	12.19	11.84	18.71	14.09
1	29.83	25.71	26.19	27.64	27.19	20.99	31.98	24.99	17.61	15.08	25.98	24.67
2	29.51	27.47	30.01	27.95	26.56	26.67	32.47	29.89	24.48	20.45	32.32	32.63
4	28.73	26.92	30.30	27.21	23.46	29.47	31.02	29.72	29.84	27.73	34.03	34.29
6	27.97	26.47	29.36	26.48	24.43	29.14	29.61	28.16	30.83	31.83	32.87	33.24
8	27.23	25.25	28.37	25.77	23.43	28.23	28.26	26.61	30.49	33.91	31.44	32.83
24	21.96	19.53	21.56	20.74	16.76	21.16	19.49	16.89	24.19	29.34	21.78	23.73
48	15.90	13.28	14.29	14.98	10.14	13.63	11.16	8.53	16.87	18.38	12.55	15.13
72	11.52	9.03	9.47	10.82	6.14	8.81	6.39	4.31	11.76	11.42	7.24	9.66
144	4.37	2.04	2.75	4.07	13.36	2.37	1.19	0.55	3.99	2.74	1.38	2.50
168	3.17	0.93	1.1	2.94	0.82	1.53	0.60	0.28	2.78	1.71	0.79	1.60

Appendix 5a

Pharmacokinetic Parameters Of Chlorpropamide In Plasma Of Individual Healthy Volunteers Following The Ingestion of 250mg Tablet In Fasting State (A) And With Cooked Maize Flour Meal (B)

	Volunteer Number									
	I		II		III		IV		V	
Parameter	A	B	A	B	A	B	A	B	A	B
Auco-168 (mcg/ml/h)	1503.37	1362.44	1948.02	1944.53	910.01	883.07	1711.84	1460.05	1286.92	1346.51
Auco-oo (mcg/ml/h)	1588.43	1444.90	2177.23	2042.41	916.58	885.52	1856.09	1649.48	1346.13	1396.32
Clearance (L/h)	0.16	0.17	0.12	0.12	0.27	0.28	0.14	0.15	0.18	0.18
Vd (L)	8.63	7.31	8.30	6.96	6.89	8.64	8.19	8.05	6.87	6.70
Cmax (mcg/ml)	25.18	26.09	26.50	26.66	26.15	26.79	26.09	26.99	27.79	26.91
tmax (h)	4.00	8.00	6.00	8.00	4.00	4.00	6.00	6.00	6.00	6.00
Ke (h^{-1})	0.018	0.023	0.014	0.017	0.039	0.03	0.016	0.016	0.027	0.020
t1/2E (h)	38.08	29.30	50.10	39.43	17.51	21.21	42.18	41.41	25.64	25.95
Ka (h^{-1})	1.37	0.45	0.92	0.18	0.97	0.78	0.87	0.66	2.52	0.59
t1/2a (h)	0.50	1.54	0.74	3.66	0.71	0.80	0.78	1.05	0.27	1.16
t lag (h)	0.38	0.43	0.37	0.57	0.55	0.58	0.36	0.57	0.30	0.50
FR	0.62	0.57	0.86	0.80	0.36	0.35	0.73	0.65	0.53	0.55

Pharmacokinetic Parameters Of Chlorpropamide In Plasma Of Individual Diabetic Patients Following The Ingestion Of 250mg Tablet 30Min Before (A) And With Cooked Maize Flour Meal (B)

	Volunteer Number											
	I		II		III		IV		V		VI	
Parameters	A	B	A	B	A	B	A	B	A	B	A	B
AUCo-168h (ncg/ml/h)	1335.36	1039.42	1307.60	1582.08	2151.60	2069.40	1799.33	1607.23	2238.43	2403.68	1634.93	1852.1
AUCo-oo (ncg/ml/h)	1377.14	1043.45	1354.09	1648.23	2255.17	2155.52	1856.39	1648.08	2344.75	2344.04	1686.16	1948.2
Clearance (L/h)	0.18	0.24	0.18	0.15	0.11	0.12	0.13	0.15	0.11	0.10	0.15	0.1
V (L)	7.22	6.45	7.79	7.06	7.26	7.13	5.82	6.20	5.99	5.75	6.97	7.4
Cmax(ncg/ml)	28.18	28.82	26.21	28.10	32.34	30.91	26.02	35.77	34.09	33.16	29.23	29.2
tmax (h)	2.00	6.00	2.00	6.00	6.00	6.00	4.00	4.00	8.00	8.00	4.00	4.0
Ke (h^{-1})	0.02	0.04	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.0
t1/2 B (h)	27.57	18.65	29.25	32.28	45.43	42.63	29.96	28.31	38.99	40.53	32.57	40.1
Ka (h^{-1})	2.03	0.53	2.01	0.56	0.63	0.77	1.61	1.27	0.34	0.32	0.98	1.0
t1/2 x (h)	0.34	1.30	0.34	1.23	1.09	0.90	0.43	0.54	1.98	2.14	0.70	0.4
t lag (h)	0.43	0.46	0.35	0.23	0.34	0.32	0.30	0.32	0.27	0.38	0.03	0.3
FR	0.47	0.36	0.47	0.57	0.78	0.75	0.65	0.57	0.82	0.89	0.59	0.4

Appendix 6a

Pharmacokinetic Parameters Of Chlorpropamide In Plasma Of Individual Healthy Volunteers Following The Ingestion of 250mg Tablet In Fasting State (A) And With Cooked Cassava Flour Meal (C)

	Volunteer Number									
	I		II		III		IV		V	
Parameters	A	C	A	C	A	C	A	C	A	C
AUCo-168h (ncg/ml/h)	1760.05	1835.94	2326.36	2247.49	1853.78	1706.69	2286.66	2154.22	1668.21	1592.33
AUCo-oo (ncg/ml/h)	1858.26	1961.07	2542.94	2373.36	1930.67	1778.96	2506.84	2302.98	1748.05	1749.92
Clearance (L/h)	0.13	0.13	0.10	0.11	0.15	0.15	0.10	0.11	0.14	0.14
Vd (L)	5.35	3.72	7.36	6.46	6.91	3.59	7.08	6.45	8.42	8.73
(max(ncg/ml/)	34.67	32.51	32.15	30.78	31.79	32.18	32.18	32.36	29.82	27.51
tmax (h)	4	12	4	12	4	12	2	12	4	8
Ke (h^{-1})	0.03	0.03	0.01	0.02	0.02	0.04	0.01	0.02	0.02	0.01
t1/2 B (h)	27.55	20.24	51.89	42.54	37.00	17.22	49.23	41.18	40.37	47.21
Ka (h^{-1})	1.09	0.89	1.35	0.23	0.91	0.19	2.09	0.21	1.79	0.58
t1/2 a (h)	0.63	7.29	0.51	2.96	0.75	4.53	0.33	3.24	0.38	1.17
t 3aq (h)	0.35	0.58	0.11	0.25	0.27	0.67	0.21	0.54	0.29	0.50
FR	0.73	0.77	1.00	0.93	0.76	0.70	0.99	0.91	0.69	0.69

Appendix 6b

Pharmacokinetic Parameters Of Chlorpropamide In Plasma Of Individual Diabetic Patients Following The Ingestion Of 250mg Tablet 30Min Before (A) And With Cooked Cassava Flour Meal (C)

	Volunteer Number											
	I		II		III		IV		V		VI	
Parameters	A	C	A	C	A	C	A	C	A	C	A	C
AUCo-168h mcg/ml/h)	1745.58	1691.65	1477.85	2735.83	2348.63	2089.12	1665.49	1996.19	1520.53	2306.63	1850.48	2244.48
AUCo-oo (mcg/ml/h)	1807.79	1717.89	1561.93	2873.30	2480.32	2154.07	1706.05	2088.78	1584.45	2447.32	1925.23	2358.22
Clearance (L/h)	0.14	0.15	0.16	0.09	0.10	0.12	0.15	0.12	0.16	0.10	0.13	0.11
V (L)	7.17	7.01	7.01	5.98	6.33	6.03	7.43	7.29	6.23	6.69	5.88	5.74
Cmax (mcg/ml/h)	31.33	31.53	29.29	35.55	36.19	33.72	30.76	30.81	31.64	32.81	36.32	33.97
t max (h)	2.00	6.00	1.00	8.00	2.00	8.00	2.00	6.00	2.00	8.00	4.00	8.00
Ke (h^{-1})	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02
t 1/2 B (h)	35.93	33.68	30.34	47.63	43.52	36.00	35.13	42.21	27.34	45.40	31.39	37.53
Ka (h^{-1})	2.64	0.81	2.68	0.33	2.05	0.41	2.11	0.84	1.60	0.42	1.66	0.35
t 1/2 x (h)	0.26	0.85	0.25	2.08	0.33	1.67	0.32	0.83	0.43	1.65	0.41	1.92
t lag (h)	0.33	0.25	0.09	0.32	0.30	0.33	0.34	0.28	0.02	0.07	0.31	0.37
FR	0.63	0.60	0.54	1.00	0.86	0.75	0.59	0.73	0.55	0.85	0.67	0.82

Appendix 7a

Pharmacokinetic Parameters Of Chlorpropamide In Plasma Of Individual Healthy Volunteers Following The Ingestion Of 250mg Tablet In Fasting State (A) And With Cooked Yam Flour Meal (B)

	Volunteer Number									
	I		II		III		IV		V	
Parameters	A	B	A	B	A	B	A	B	A	A
AUCo-168h (ncg/ml/h)	1782.32	1847.04	1761.14	1459.88	1712.49	1370.71	2029.34	2003.38	1788.97	1504.85
AUCo-o (ncg/ml/h)	1940.84	1969.44	1817.43	1541.85	1803.29	1429.12	2125.46	2150.95	1868.93	1562.84
Clearance (L/h)	0.12	0.13	0.14	0.16	0.14	0.17	0.12	0.12	0.13	0.16
Vd(L)	7.88	7.39	7.51	7.92	8.74	7.09	7.53	7.39	6.52	7.41
Cmax (ncg/ml)	26.45	29.23	31.39	27.00	27.53	28.10	30.53	30.89	30.59	27.37
tmax (h)	6.00	2.00	4.00	1.00	4.00	1.00	6.00	2.00	4.00	2.00
Ke (h^{-1})	0.016	0.017	0.018	0.02	0.015	0.02	0.02	0.02	0.02	0.02
t 1/2 B (h)	42.41	40.39	37.87	33.86	43.69	28.11	44.40	44.07	33.78	32.18
Ka (h^{-1})	0.50	2.38	0.92	14.98	1.52	12.35	0.70	12.31	0.89	1.77
t 1/2 x (h)	1.38	0.29	0.74	0.05	0.44	0.06	0.98	0.56	0.83	0.38
t lag (h)	0.05	0.07	0.32	0.46	0.25	0.38	0.29	0.41	0.25	0.35
FR	0.76	0.77	0.72	0.61	0.71	0.56	0.84	0.85	0.74	0.61

Appendix 7b

Pharmacokinetic Parameters Of Chlorpropamide In Plasma Of Individual Diabetic Patients Following The Ingestion Of 250mg Tablet 30Min Before (A) And With Cooked Yam Flour Meal (B)

	Volunteer Number											
	I		II		III		IV		V		VI	
Parameters	A	B	A	B	A	B	A	B	A	B	A	B
AUCo-168h (mcg/ml/h)	2043.30	1697.36	1812.42	1941.18	1329.79	1748.46	1622.76	1357.95	2130.90	2153.09	1834.21	2091.22
AUCo-∞ mcg/ml/h)	2199.89	1772.10	1927.65	2050.32	1387.09	1838.46	1691.63	1387.87	2254.09	2213.79	1906.08	2144.63
Clearance (L/h)	0.11	0.14	0.13	0.12	0.18	0.14	0.15	0.18	0.11	0.11	0.13	0.12
Vd (L)	8.47	8.79	7.55	8.99	8.61	7.46	6.36	6.34	7.39	5.71	5.71	6.22
Cmax (mcg/ml)	29.83	27.47	30.39	27.95	27.10	29.47	32.47	29.89	30.83	33.91	34.03	34.29
t max (h)	1.00	2.00	4.00	2.00	2.00	4.00	2.00	2.00	6.00	8.00	4.00	4.00
Ke (h^{-1})	0.013	0.016	0.017	0.014	0.012	0.018	0.02	0.02	0.02	0.02	0.02	0.02
t 1/2 β (h)	51.67	43.21	40.33	51.10	33.09	38.03	29.83	24.39	46.15	35.05	30.19	37.10
Ka (h^{-1})	7.72	2.03	1.38	5.08	3.80	0.82	4.21	1.51	0.59	0.22	1.06	1.31
t 1/2 a (h)	0.89	0.34	0.50	0.14	0.95	.08	0.16	.46	1.17	3.10	0.65	0.53
t lag (h)	0.19	0.22	0.24	0.27	0.46	0.46	0.07	0.22	0.26	0.93	0.10	0.13
FR	0.77	0.62	0.67	0.71	0.48	0.64	0.59	0.48	0.78	0.77	0.66	0.77

Appendix 8a

Plasma Glucose Levels (M.Mol/L) In 5 Healthy Volunteers Following Oral Administration Of 250mg Chlorpropamide Tablet In Fasting State (A) And With Cooked Maize Flour Meal (B)

	Plasma Glucose (M.Mol/L)									
Volunteers	I		II		III		IV		V	
Time (h)	A	B	A	B	A	B	A	B	A	A
0	4.62	4.76	4.46	4.62	4.68	4.80	4.50	4.85	5.11	5.00
0.5	4.42	4.82	4.20	4.60	4.50	4.77	4.23	4.65	5.00	4.90
1	4.29	4.48	4.00	4.25	4.40	4.65	4.10	4.40	4.80	4.75
2	4.00	4.28	3.90	4.12	4.10	4.40	4.00	4.30	4.62	4.42
4	3.82	4.00	3.62	3.98	3.90	4.20	3.85	4.12	4.41	4.31
6	4.00	4.00	3.68	4.00	4.20	4.10	4.00	4.12	4.16	4.18
8	4.06	4.00	4.12	4.00	4.18	4.08	4.15	4.10	4.20	4.16

Appendix

Individual Plasma Glucose Levels (M.Mol/L) In 6 Diabetic Patients Given Cooked Maize Flour (M) And Following Oral Administration Of 250mg Chlorpropamide Tablet 30Min Before (A) And With The Meal (B)

	Plasma Glucose (M.Mol/L)																	
Patients																		
Time (h)	M	A	B	M	A	B	M	A	B	M	A	B	M	A	B	M	A	B
0	22.80	23.60	24.00	18.70	18.90	19.00	18.90	18.70	18.70	17.00	16.80	17.10	15.11	15.01	15.20	13.60	13.32	13.5
0.5	26.50	25.30	25.80	21.10	19.00	19.80	25.30	21.50	22.00	19.30	17.00	17.30	19.71	16.20	17.23	13.40	13.00	13.6
1	27.10	25.80	26.77	22.20	19.50	20.20	22.90	21.80	22.20	22.60	20.00	20.70	22.32	17.10	19.42	17.25	13.80	14.2
2	30.20	28.40	29.80	21.50	20.50	20.98	22.20	21.50	21.68	22.65	17.80	18.20	22.60	16.52	19.21	18.50	14.61	15.2
4	28.20	24.50	27.10	20.90	18.80	20.32	20.90	19.20	19.60	29.70	13.25	14.80	17.62	15.00	16.10	19.30	13.63	13.8
6	26.50	23.60	24.80	20.00	18.30	19.20	18.90	17.85	18.20	18.00	12.75	13.50	15.46	12.52	13.44	14.41	12.85	13.1
8	25.30	22.52	24.00	19.50	18.00	19.00	18.50	18.00	18.00	17.00	11.00	10.50	14.50	12.30	13.40	13.60	11.52	12.2

Appendix 9a

Plasma Glucose Level (M.Mol/L) In 5 Healthy Volunteers Following Oral Administration Of 250mg Chlorpropamide Tablet In Fasting State (A) And With Cooked Cassava Flour Meal (C)

	Plasma Glucose (M.Mol/L)									
Volunteers	I		II		III		IV		V	
Time (h)	A	C	A	C	A	C	A	C	A	C
0	4.73	4.63	4.85	4.70	4.63	4.22	4.52	4.40	4.54	4.23
0.5	4.56	4.86	4.54	4.85	4.40	4.38	4.23	4.62	4.35	4.48
1	4.42	5.00	4.32	3.01	4.28	4.47	4.12	4.83	4.08	4.62
2	4.20	4.75	4.12	4.82	4.12	4.20	4.01	4.52	3.95	4.43
4	4.02	4.65	4.00	4.61	4.04	4.12	3.95	4.20	3.75	4.22
6	4.04	4.32	4.52	4.08	4.02	4.00	4.00	4.12	3.85	4.11
8	4.04	4.34	4.06	4.53	4.09	4.02	4.02	4.13	3.95	4.12

Appendix 9b

Plasma Glucose Level (M.Mol/L) In 6 Diabetic Patients Given Cooked Cassava Four (N) And Following Oral Administration Of 250Mg Chlorpropamide Tablet 30Min Before (A) And With The Meal (C)

	Plasma Glucose (M.Mol/L)																	
Patients	I			II			III			IV			V			VI		
Time (h)	N	A	C	N	A	C	N	A	C	N	A	C	N	A	C	N	A	C
0	19.60	19.40	19.35	16.60	16.25	16.40	19.80	19.60	19.40	16.02	15.80	15.80	17.52	17.30	17.30	14.50	14.35	14.30
0.5	20.70	18.51	19.80	24.50	17.50	20.90	27.50	18.30	21.71	19.50	16.50	17.12	25.35	17.50	18.00	23.52	16.00	18.00
1	27.52	20.20	25.40	26.85	19.50	22.00	23.25	21.10	22.00	21.65	17.32	17.85	26.52	17.80	20.00	25.00	16.50	18.50
2	30.40	22.40	27.80	24.20	19.00	20.70	22.54	19.60	20.80	23.00	18.00	18.52	27.00	17.30	19.00	26.10	18.00	20.00
4	27.80	26.12	23.81	18.20	17.50	16.90	20.20	19.00	19.50	21.00	17.50	16.72	25.23	17.00	18.20	25.62	17.50	19.52
6	25.80	20.20	20.82	16.52	16.50	15.12	19.50	17.00	18.21	17.50	13.50	15.60	16.50	17.56	16.50	20.00	13.00	17.00
8	20.00	19.00	17.80	16.00	16.00	15.00	19.00	16.00	17.00	16.50	13.02	15.00	18.22	17.00	17.00	14.00	14.20	14.30

Appendix 10a

Plasma Glucose Levels (M. Mol/L) In 5 Healthy Volunteers Following Oral Administration of 250mg Chlorpropamide Tablet In Fasting State (A) And With Cooked Yam Flour Meal (B)

Volunteers	Plasma Glucose (M.Mol/L)									
	I		II		III		IV		V	
Time (h)	A	B	A	B	A	B	A	B	A	B
0	4.65	4.86	4.65	4.75	4.60	4.68	4.67	4.62	4.52	4.72
0.5	4.56	4.45	4.50	4.48	4.65	4.45	4.66	4.52	4.62	4.32
1	4.37	4.25	4.22	4.26	4.32	4.20	4.57	4.31	4.45	4.20
2	4.46	4.35	3.85	3.95	4.22	4.02	4.32	4.12	4.36	4.12
4	4.20	3.80	3.72	3.55	4.08	4.32	3.98	4.55	3.95	4.22
6	4.12	4.22	3.52	3.30	3.95	4.15	3.85	4.20	3.85	4.00
8	3.92	3.98	3.52	3.32	3.55	4.12	3.68	4.18	3.55	3.85

Appendix 10b

Individual Plasma Glucose Level (M.Mol/L) In 6 Diabetic Patients Given Cooked Yam Flour (0) And Following Oral Administration Of 250Mg Chlorpropamide Tablet 30 Min Before (A) And With The Meal (B)

Patients	Plasma Glucose (M.Mol/L)																	
	I			II			III			IV			V			VI		
Time (h)	0	A	B	0	A	B	0	A	B	0	A	B	0	A	B	0	A	B
0	15.20	15.10	15.30	12.20	12.00	12.90	14.52	14.00	14.40	14.80	14.60	14.42	17.80	17.60	17.80	17.00	17.20	17.25
0.5	18.70	14.42	16.20	14.70	11.35	13.50	16.00	13.30	14.80	15.80	14.20	14.60	17.50	16.00	16.22	22.00	18.00	20.00
1	22.20	15.25	20.00	18.20	12.25	16.30	18.25	14.20	15.50	17.00	14.60	15.00	22.00	18.52	20.10	23.52	20.00	22.58
2	19.80	15.62	17.80	21.32	14.28	18.87	20.50	16.52	15.20	22.25	15.00	15.50	24.00	19.00	19.50	26.00	21.20	24.00
4	18.84	14.00	16.41	18.65	15.00	15.35	19.30	15.00	14.20	21.40	14.00	14.50	23.50	18.50	18.50	25.41	20.32	19.85
6	15.80	12.61	15.20	16.42	13.22	13.00	18.82	13.50	13.80	16.80	12.50	13.00	20.00	17.00	16.12	19.50	18.12	17.50
8	15.40	12.20	14.00	14.00	11.50	12.20	17.00	13.00	13.00	15.40	12.00	12.00	17.50	16.00	16.00	17.80	16.50	17.00

Appendix II

Simultaneous Plasma/Salivary Concentrations Of Chlorpropamide (mcg/ml) In 6 Healthy Volunteers Following Oral Administration Of 250mg Chlorpropamide Tablet In Fasting State

	Concentration (mcg/ml)											
Volunteers	I		II		III		IV		V		VI	
Time (h)	Plasma	Saliva	Plasma	Saliva	Plasma	Saliva	Plasma	Saliva	Plasma	Saliva	Plasma	Saliva
0.5	6.50	0.29	9.25	0.25	21.18	0.45	3.19	0.19	4.48	0.29	5.11	0.41
1	16.73	0.65	19.85	0.68	29.03	0.62	12.05	0.65	12.94	0.63	15.77	0.84
2	26.97	1.11	26.66	0.89	32.36	1.13	21.23	1.07	22.89	1.08	26.40	1.18
4	31.99	1.48	27.93	1.25	33.24	2.04	25.99	1.21	29.82	1.45	31.39	1.25
6	31.55	1.53	26.75	1.15	31.08	1.68	26.09	1.17	30.53	1.51	31.25	1.17
8	30.54	1.47	25.91	0.85	30.22	1.18	25.41	1.11	30.04	1.46	30.23	1.08
12	28.36	1.26	24.32	0.71	28.22	0.59	23.81	1.00	28.36	1.31	28.13	0.91
36	18.09	0.48	16.62	0.53	20.42	0.49	16.01	0.53	19.50	0.58	18.18	0.31
48	14.49	0.27	13.74	0.24	17.26	0.29	13.12	0.39	16.17	6.39	14.62	0.21
72	9.22	0.09	9.39	0.14	12.33	0.11	8.82	0.20	11.12	0.18	9.44	0.07
144	2.39	0.06	2.99	0.03	4.50	0.05	2.68	.015	3.61	0.02	2.33	0.004
168	1.52	0.002	2.04	.001	3.51	0.001	1.80	.001	2.48	0.007	1.64	0.001

Appendix 12

Pharmacokinetic Parameters Of Chlorpropamide In Plasma And Saliva Of Individual Volunteers Following The Ingestion Of 250mg Tablet In Fasting State

a. Plasma

Parameters	Volunteer Number					
AUCo-168h (mcg/ml/h)	1853.78	1712.49	2286.6	1711.84	2029.34	17.61.14
AUCo- (mcg/ml/h)	1930.67	1803.29	2506.84	1856.09	2125.46	1817.43
Clearance (L/h)	0.13	0.14	0.10	0.14	0.12	0.14
Vd (L)	6.91	8.74	7.08	8.19	7.53	7.51
Cmax (mcg/ml)	31.99	27.93	33.24	25.99	29.82	31.39
tmax (h)	4.00	4.00	2.00	6.00	6.00	4.00
Ke (h^{-1})	0.018	0.015	.014	0.016	0.015	0.018
t1/2 B (h)	37.90	43.69	49.23	42.18	44.90	37.87
Ka (h^{-1})	0.09	1.52	2.09	0.87	0.78	0.90
t1/2 a (h)	0.75	0.44	0.33	0.78	0.98	0.74
t lag (h)	0.27	0.25	0.21	0.36	0.29	0.32

b. Saliva

AUCo-168 (mcg/ml/h)	57.34	34.67	68.81	54.91	61.62	43.68
AUCo-o (mcg/ml/h)	58.34	35.52	69.62	55.67	62.22	43.48
Clearance (L/h)	4.27	7.04	3.59	4.49	4.02	5.15
Vd (L)	99.78	299.52	97.22	171.10	120.80	140.90
CMax (mcg/ml)	1.53	1.25	2.04	1.21	1.51	1.25
tmax (h)	6.00	4.00	4.00	4.00	6.00	4.00
Ke (h^{-1})	0.04	0.02	0.04	0.03	0.03	0.04
t1/2 B (h)	16.19	29.49	18.76	26.40	20.83	16.98
Ka (h^{-1})	0.51	0.51	0.59	1.05	0.53	1.13
t1/2 a (h)	1.35	1.38	1.16	0.65	1.31	0.61
t lag (h)	0.16	0.10	0.20	0.34	0.16	0.19

Appendix 12

Pharmacokinetic Parameters Of Chlorpropamide In Plasma And Saliva Of Individual Volunteers Following The Ingestion Of 250mg Tablet In Fasting State

a. Plasma

Parameters	Volunteer Number					
AUCo-168h (ncg/ml/h)	1853.78	1712.49	2286.6	1711.84	2029.34	17.61.14
AUCo- (ncg/ml/h)	1930.67	1803.29	2506.84	1856.09	2125.46	1817.43
Clearance (L/h)	0.13	0.14	0.10	0.14	0.12	0.14
Vd (L)	6.91	8.74	7.08	8.19	7.53	7.51
Cmax (ncg/ml)	31.99	27.93	33.24	25.99	29.82	31.39
tmax (h)	4.00	4.00	2.00	6.00	6.00	4.00
Ke (h^{-1})	0.018	0.015	.014	0.016	0.015	0.018
t1/2 B (h)	37.90	43.69	49.23	42.18	44.40	37.87
Ka (h^{-1})	0.09	1.52	2.09	0.87	0.70	0.90
t1/2 a (h)	0.75	0.44	0.33	0.78	0.98	0.74
t lag (h)	0.27	0.25	0.21	0.36	0.29	0.32

b. Saliva

AUCo-168 (ncg/ml/h)	57.14	34.67	68.81	54.91	61.62	43.48
AUCo-∞ (ncg/ml/h)	58.54	35.52	69.62	55.67	62.22	43.48
Clearance (L/h)	4.27	7.04	3.59	4.49	4.02	5.15
Vd (L)	99.78	299.52	97.22	171.10	120.80	140.90
CMax (ncg/ml)	1.53	1.25	2.04	1.21	1.51	1.25
tmax (h)	6.00	4.00	4.00	4.00	6.00	4.00
Ke (h^{-1})	0.04	0.02	0.04	0.03	0.03	0.04
t1/2 B (h)	16.39	29.49	18.76	26.40	20.83	16.98
Ka (h^{-1})	0.51	0.51	0.59	1.05	0.53	1.13
t1/2 b (h)	1.35	1.38	1.16	0.65	1.31	0.61
t lag (h)	0.16	0.10	0.20	0.34	0.16	0.19

Appendix 12

Pharmacokinetic Parameters Of Chlorpropamide In Plasma And Saliva Of Individual Volunteers Following The Ingestion Of 250mg Tablet In Fasting State

a. Plasma

Parameters	Volunteer Number					
AUCo-168h (ncg/ml/h)	1853.78	1712.49	2286.6	1711.84	2029.34	17.61.14
AUCo- (ncg/ml/h)	1930.67	1803.29	2506.84	1856.09	2125.46	1817.43
Clearance (L/h)	0.13	0.14	0.10	0.14	0.12	0.14
Vd (L)	6.91	8.74	7.08	8.19	7.53	7.51
Cmax (ncg/ml)	31.99	27.93	33.24	25.99	29.82	31.39
Tmax (h)	4.00	4.00	2.00	6.00	6.00	4.00
Ke (h^{-1})	0.018	0.015	.014	0.016	0.015	0.018
t1/2 B (h)	37.00	43.69	49.23	42.18	44.40	37.87
Ka (h^{-1})	0.09	1.52	2.09	0.87	0.70	0.90
t1/2 a (h)	0.75	0.44	0.33	0.78	0.98	0.74
t lag (h)	0.27	0.25	0.21	0.36	0.29	0.32

b. Saliva

AUCo-168 (ncg/ml/h)	57.14	34.67	68.81	54.91	61.62	43.48
AUCo-∞ (ncg/ml/h)	58.54	35.52	69.62	55.67	62.22	43.48
Clearance (L/h)	4.27	7.04	3.59	4.49	4.02	5.15
Vd (L)	99.78	299.52	97.22	171.10	120.80	140.90
CMax (ncg/ml)	1.53	1.25	2.04	1.21	1.51	1.25
Tmax (h)	6.00	4.00	4.00	4.00	6.00	4.00
Ke (h^{-1})	0.04	0.02	0.04	0.03	0.03	0.04
t1/2 B (h)	16.19	29.49	18.76	26.40	20.83	16.98
Ka (h^{-1})	0.51	0.51	0.59	1.05	0.53	1.13
t1/2 a (h)	1.35	1.38	1.16	0.65	1.31	0.61
t lag (h)	0.16	0.10	0.20	0.34	0.16	0.19

Appendix 12

Pharmacokinetic Parameters Of Chlorpropamide In Plasma And Saliva Of Individual Volunteers Following The Ingestion Of 250mg Tablet In Fasting State

a. Plasma

Parameters	Volunteer Number					
AUCo-168h (ncg/ml/h)	1853.78	1712.49	2286.6	1711.84	2029.34	17.61.14
AUCo- (ncg/ml/h)	1930.67	1803.29	2506.84	1856.99	2125.46	1817.43
Clearance (L/h)	0.13	0.14	0.10	0.14	0.12	0.14
Vd (L)	6.91	8.74	7.08	8.19	7.53	7.51
Cmax (ncg/ml)	31.99	27.93	33.24	25.99	29.82	31.39
tmax (h)	4.00	4.00	2.00	6.00	6.00	4.00
Ke (h^{-1})	0.018	0.015	.014	0.016	0.015	0.018
t1/2 B (h)	37.00	43.69	49.23	42.18	44.40	37.87
Ka (h^{-1})	0.09	1.52	2.09	0.87	0.70	0.90
t1/2 a (h)	0.75	0.44	0.33	0.78	0.98	0.74
t lag (h)	0.27	0.25	0.21	0.36	0.29	0.32

b. Saliva

AUCo-168 (ncg/ml/h)	57.14	34.67	68.81	54.91	61.62	43.48
AUCo-o (ncg/ml/h)	58.54	35.52	69.62	55.67	62.22	43.48
Clearance (L/h)	4.27	7.04	3.59	4.49	4.02	5.15
Vd (L)	99.78	299.52	97.22	171.10	120.80	140.90
CMax (ncg/ml)	1.53	1.25	2.04	1.21	1.51	1.25
tmax (h)	6.00	4.00	4.00	4.00	6.00	4.00
Ke (h^{-1})	0.04	0.02	0.04	0.03	0.03	0.04
t1/2 B (h)	16.19	29.49	18.76	26.40	20.83	16.98
Ka (h^{-1})	0.51	0.51	0.59	1.05	0.53	1.13
t1/2 h (h)	1.35	1.38	1.16	0.65	1.31	0.61
t lag (h)	0.16	0.10	0.20	0.34	0.16	0.19