INFLUENCE OF AMPICILLIN/CLOXACILLIN ON THE PHARMACOKINETICS OF CHLORPROPAMIDE IN TYPE II DIABETES MELLITUS PATIENTS

BY

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN PHARMACEUTICAL CHEMISTRY

DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY , FACULTY OF PHARMACEUTICAL SCIENCES,
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ZARIA, NIGERIA

1999

DECLARATION

I hereby declare that the work reported in this thesis was carried out by me under the supervision of DR.(Mrs.) M. T. Bakare - Odunola and Dr. Garba Magaji both of the Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, A.B.U Zaria. This work has not been presented in any previous application for higher degree. The work of other investigators are acknowledged and referred to accordingly.

Isa'ac Jmali 13/10/99

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CERTIFICATION

This thesis entitled THE INFLUENCE OF AMPICLOX (AMPICILLIN/CLOXACILLIN) ON THE PHARMACOKINETICS OF CHLORPROPAMIDE IN TYPE II DIABETES MELLITUS PATIENTS by ENEMALI SHAIBU ISAAC meets the regulations governing the award of the Degree of MASTER OF SCIENCE of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and Literary Presentation.

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DEDICATION

TO

The memory of the one, I called, Iya,

who nurtured my dreams,

And

The only Sister I call, Mercy,

who for our tomorrow, gave her today

believing that shattered and delayed

dreams can be redesigned and realized.

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Since 'He exist before all things and in Him all things are held together as One' (Col.1.17b),. I Want to thank the Almighty God of the heavens for the opportunity and the enablement in bringing this study to fruition.

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For my entire family, I want to say thank you for your patience; and how can I forget you sister Mercy, for living up our dreams, Amos 3:3 is always there for us.
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ABSTRACT

Infection is very common in diabetics and its treatment should be prompt. This study is to examine the influence of ampiclox (Ampicillin/Cloxacillin) Capsules on the pharmacokinetics of chlorpropamide tablets in Type II diabetic patients. Seven type II diabetic patients with age ranging from 35-70 yrs, weight ranging from 55-70 kg and height from 1.65 - 1.78m participated in the study. Each patient received the following treatments at two weeks interval:

250mg Chlorpropamide tablet alone and one 250mg Chloropromede table with one 500mg Ampiclox capsule.

Blood samples were taken at intervals for 168hours. Plasma Samples were analysed by sp8 - 100 spectrophotometer. The plasma peak concentration (C_{max}), the amount of drug absorbed (AUC) and the elimination half - life ($t½_{el}$) were all significantly ($P<0.05$) increased following the second treatment with the lag-time. The statistical analysis was carried out using student t- test for paired data.

The elimination rate constant (K_{et}), the clearance time CI and the apparent volume of distribution (Vd) were significantly ($P<0.05$) reduced following the second treatment.

Ampiclox might have competitively displaced chlorpropamide from the biding site thus giving rise to higher bioavailability and C_{max} in the second treatment compared with treatment. The effect of higher bioavaibility and C_{max} followinj-the second treatment can be observed by the significant ($P<0.05$) reduction in the plasma glucose level following the second treatment compared with the first. Co-administration of ampiclox with, the long acting chlorpropamide may also affect the renal tubular secretion of the later drug thus slowing down its tubular secretion which can lead to reduced Clearance rate. Thus, the second treatment resulted in increased bioavailability of chlorpropamide with longer half - life of elimination.

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ABBREVIATIONS

B.P	=	British Pharmacopoeia
°C	=	Degree Centigrade (Celsius)
g	=	Gram
µg	=	Microgram
mg	=	Milligram
ml	=	Milliliter
Min	=	Minute
hr	=	Hour
HCl	=	Hydrochloric Acid
NaOH	=	Sodium Hydroxide
H.P.L.C	=	High Performance liquid Chromatography
USP	=	United States Pharmacopoeia
HP	=	Hydrogen - ion Concentration
U.V	=	Ultraviolet

CHAPTER ONE

INTRODUCTION

1.10 PHARMACOKINETICS

The overall biological response of an organism to a drug depends on two major underlying phenomena: One, termed the pharmacodynamic response, is the action of the drug at specialised and specified sites in the body to give a pharmacological response. The other pharmacokinetic, is the action of the organism on the drug to impede its penetration or absorption, to dilute, distribute, transform and excrete the active principles, and thus diminish its availability at the site of action. The elucidation of the parameters which describes these procedures which modify the time-course of the active drug in the body is a function of pharmacokinetics (Paxton, 1981).

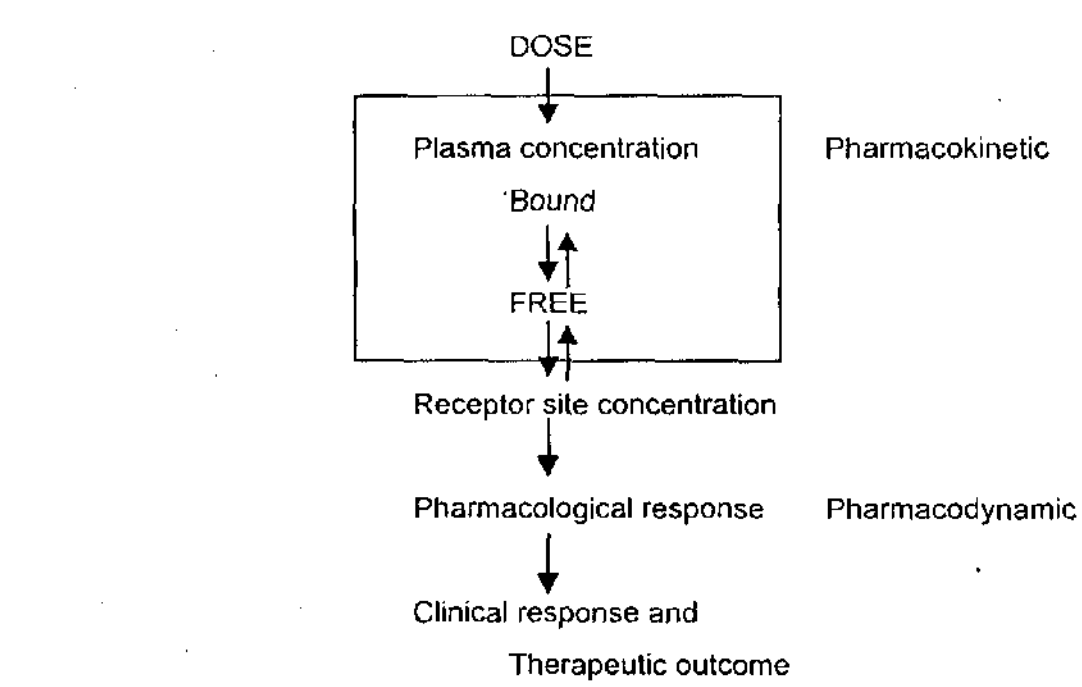


Figure 1.1 Showing schematic representation of main factors and terms involved in drug dosage and therapeutic outcome (Paxton,1981).

Pharmacokinetics is concerned with obtaining the appropriate amount of drug at the receptor site for the appropriate length of time for a particular patient, thus obtaining maximum therapeutic benefit with minimum side effects (Wagner,1967).

Pharmacokinetics deals with the change of drug concentration in the drug product and changes of concentration of drug and or its metabolites in the human or animal body following administration. That is, the change of drug concentration in the different body fluids and tissues in the dynamic system of liberation, absorption, distribution, body storage, building, metabolism and excretion (Shar,1993).

Pharmacokinetic knowledge provides the followings:

- Estimation of rates of absorption, metabolism and urinary excretion.
- Bioavailability- estimation of relative extent of absorption and relative rates of absorption following administration of two or more products or formulations.
- Prediction of blood levels after multiple doses from blood levels measured after single doses.
- Optimising dosage regimens of specific drugs
- Determining quantitative effects of drugs in patients.
- Aid in determining effect of plasma protein binding of drugs on drug distribution.
- Aid in elucidating drug interactions.

When applied to therapy, clinical pharmacokinetics are used for selecting and adjusting drug dosage schedules and to facilitate the interpretation of measured serum concentration of drugs (Tooms, 1993).

The knowledge of pharmacokinetics of drugs in the same class allows a choice that would be most beneficial to the patient. Looking at the multitude of new dihydropyridine - derived calcium antagonists makes it imperative to evaluate the pharmacokinetics of these agents in relation to disease management (Faulknor et al,1986).

Also the relevance of pharmacokinetic knowledge has recently been demonstrated in an attempt to make the best choice of dosage form of the same drug. Pharmacokinetic study involving several formulations of aspirin: Soluble aspirin, mouth-dispersible aspirin, plain aspirin and enteric-coated aspirin granules was carried out.

The result revealed rapid absorption of aspirin from the soluble formulation compared with that from plain aspirin or enteric -coated aspirin granules. The pharmacokinetic data in this study clearly demonstrate the rapid availability of aspirin from a soluble formulation, suggesting that the formulation of choice for use in acute myocardial infarction should be soluble aspirin. Based on this study, it was recently recommended that patients judged to be at high risk of myocardial infarction should carry such product in their pockets for emergency use(Muir et al,1997).

Blood or plasma is a practical and the most convenient site of pharmacokinetic measurement and it is the most logical one for determining drug in the body. Blood receives the drug from the site of administration after its absorption and carries the drug to all the tissues (Paxton,1981). The transfer of a drug and the various steps involved in the distribution, and elimination of the drug in the body are as follows:

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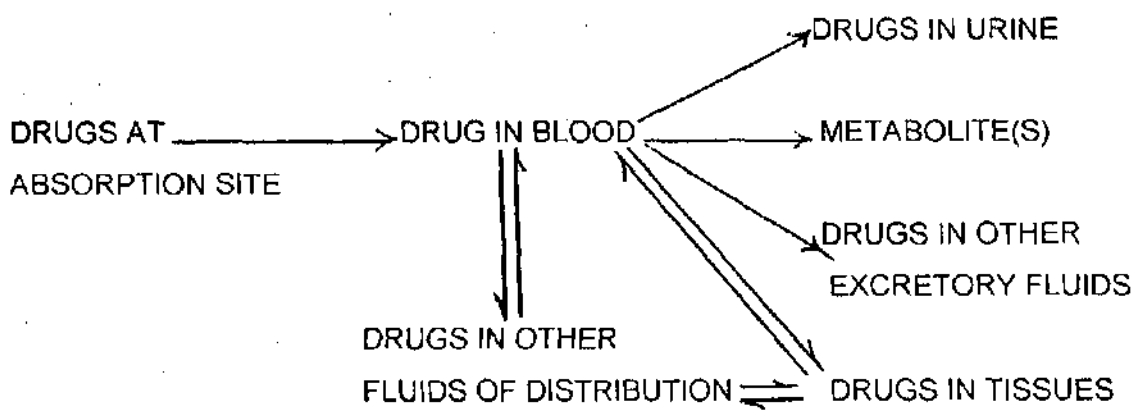


Figure. 1.2 Showing steps involved in the distribution and elimination of drug in the body. (Tozer,1979).

Pharmacokinetic study based on the data obtained following intraveneous administration of drug in which the entire dose administered is placed directly into the blood stream, will give parameters that are unaffected by all the potential rate-limiting factors associated with drug absorption following other routes. It has been established that the intensity of the pharmacokinetic effects of many drugs is related to their concentration and or to the concentration of their metabolite(s) in plasma (Gibaldi and Perrier,1975).

A model in Pharmacokinetics is a hypothetical structure which can be used to characterise with reproducibility the behavior and the fate of a drug in biological systems when given by a certain route of administration and in a particular dosage form.

Reasonable complex models to describe the pharmacoketics of certain drugs have been developed. In such models, biological event(s) are descbribed in mathematical terms, and the body is represented as a system of compartments with no apparent physiologic or anatomic reality.

A compartment is an entity which can be described by a definite volume and a concentration of drug contained in the volume. These compartments are classified into one, two or multi compartment models (Gibaldi and Perrier, 1975).

Before any equations may be derived from these models, some basic assumptions have to be made. Firstly, drug enters the system only via the central compartment. Secondly, reversible transfer occurs between a central and peripheral compartments, and thirdly, the exit of drugs from all compartments in the system is described by first-order elimination rate constant which has units of reciprocal time like min^{-1} or hr^{-1} (Paxton,1981). All these assumptions are relatively valid for most drugs. The majority of drugs enters the body via the blood stream, and are eliminated from the blood stream by the kidney.

There are a few exceptions which are primarily eliminated by biotransformation, and if given in high doses results in saturation of the metabolic enzyme system. The elimination kinetics then becomes zero order which means that, rate constant are independent of drug concentration, (Wagner,1967).

1.1.1 SINGLE OR ONE COMPARTMENT MODEL

This is the simplest model which depicts the body as a single homogeneous unit in which the drug entering the body distributes instantly between the blood and body fluid or tissues (that is, the exchange of drug between the plasma and tissue proceeds rapidly compared with the rate of elimination). The whole body is then considered mathematically as a single compartment. This model is useful for the pharmacokinetic analysis of salivary and urinary excretion data for drugs which are rapidly distributed between plasma and other body fluids and tissues upon entry into the systemic circulation (Paxton,1981).

The assumption that the body behaves as a one - compartment model does not mean that the drug concentration in all body tissues at any given time are the same. However, a one compartment model does assume that any changes that occur in the plasma quantitatively reflects changes occurring in tissue drug-levels. Assuming instantaneous distribution after an i.v injection into this model according to Figure1.4, the concentration (C_o) in the plasma immediately after injection is equal to the dose (D) divided by the volume of this compartment (V_{dc}).

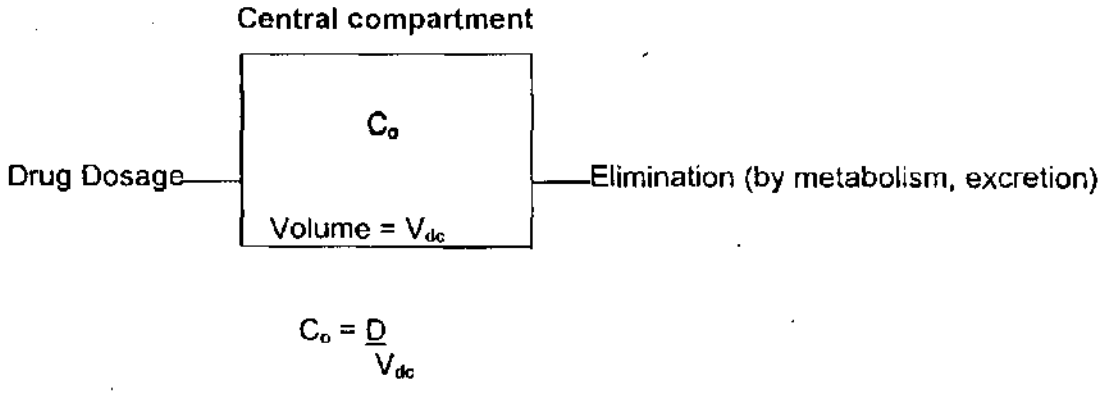


Figure 1.3 Showing a single compartment model, rapid i.v injection of drug into a single compartment (Paxton,1981).

This apparent volume of distribution (V_{dc}) of the drug is not a true volume, but that volume into which all the drug in the body would appear to be distributed to achieve a concentration the same as that in the plasma (Shar,1993). Elimination follows a first order kinetics after instantaneous distribution which means that a constant fraction of drug is eliminated per unit time. Thus, a plot of plasma drug concentration against time, with each unit representing the time for one half of the drug to be eliminated result in an exponential curve as shown in Figure 1.5

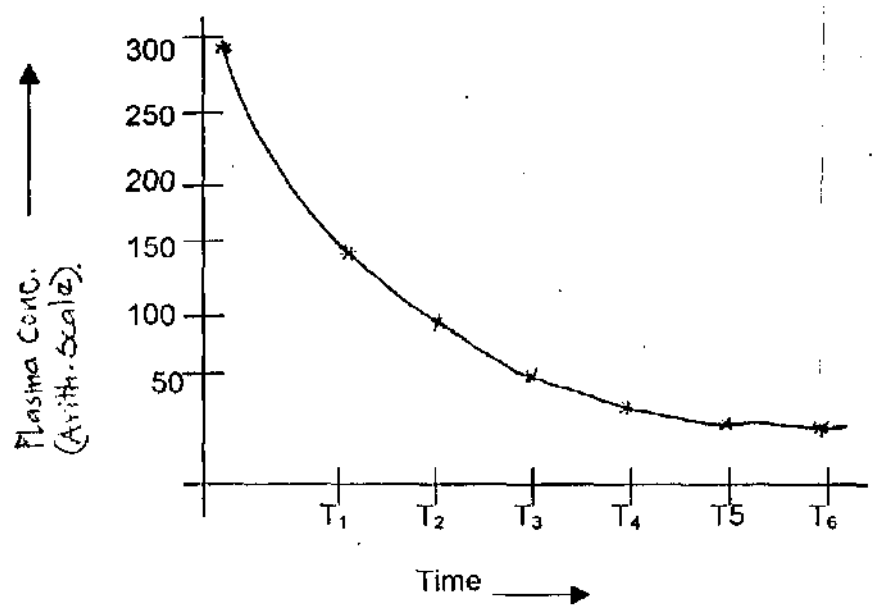


Figure 1.4 Showing plasma concentration Vs time curve (Wagner,1967).

Replotting the above curve on a logarithmic scale results in a straight line curve as in figure 1.5.

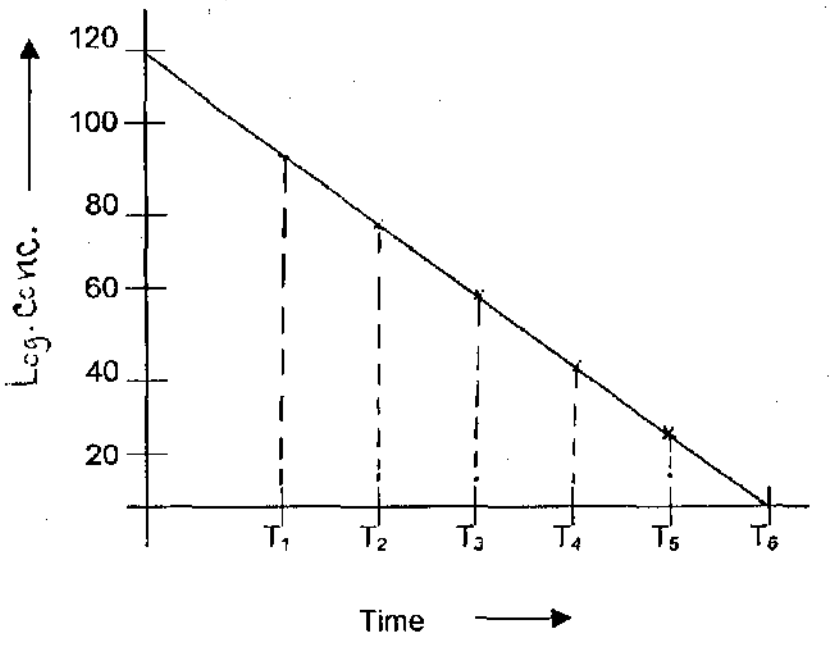


Figure 1.5 Showing plasma log.conc.-time curve for one compartment model (Wagner,1967).

Extrapolation of the plot in figure 1.6 to time zero gives the theoretical initial concentration (C_o). Accepting first order kinetics between drug concentration and time, the decline in drug concentration may be expressed mathematically:

$\frac{d_c}{d_t} = \beta_{ct}$, where

$_{ct}$ is the concentration and β is the rate constant of elimination.

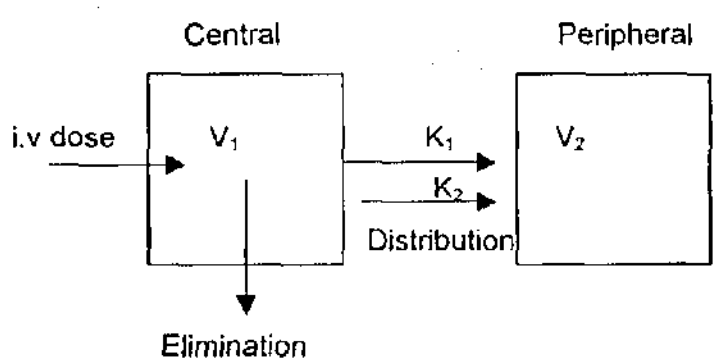
By integration and conversion to log base 10,

$$\log_{ct} = \log C_o - \frac{\beta_t}{2.303}$$

(Paxton,1982)

1.1.2 TWO COMPARTMENT MODEL

The plasma concentration - time curve of a drug is often not a straight line on a semi-log plot (Paxton,1981).If distribution of the drug is however so slow that it can not be disregarded, a model must then be considered which contained a central compartment and at least one other peripheral compartment. Figure 1.6 illustrate this compartment model.



K_1 and K_2 are transfer rate constants between the two compartments.

Figure 1.6 Showing two compartment model (Paxton,1981).

Drugs distribute within a few minutes through this compartment and equilibrium between plasma and tissue is rapidly established. The peripheral compartments are then formed by less perfused tissues like skin, muscle, or adipose tissue in which drugs enter more slowly. The combined effect of two compartments give rise to a biphasic curve on i.v injection with two distinct linear portions when drawn on a semi-log, Scale as shown in figure 1.8.

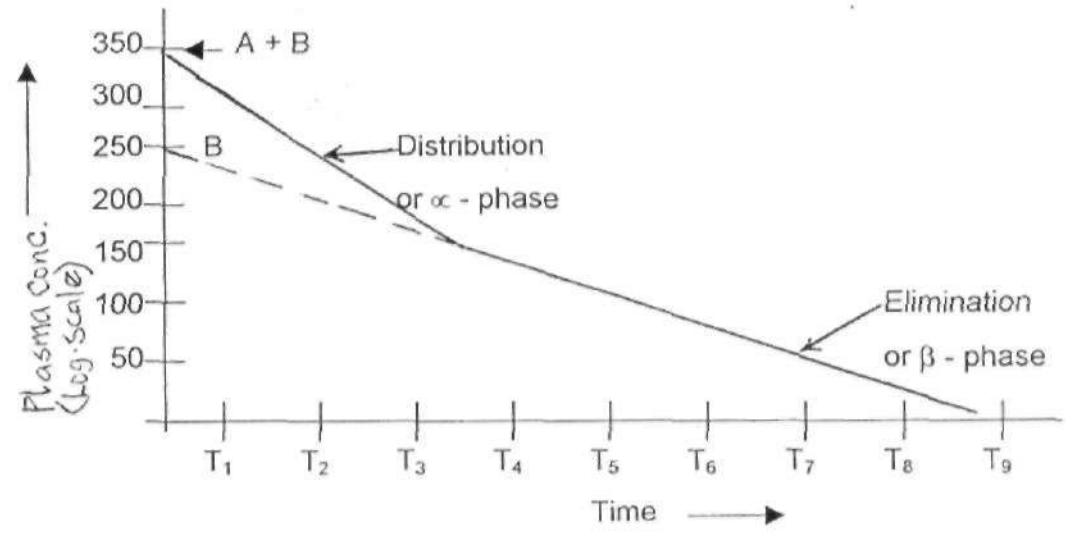


Figure 1.7 Showing plasma log-conc Vs time curve for a two compartment model. (Paxton,1981).

Although drug distribution is slow, it is usually much faster than elimination. Thus, the initial rapid fall in concentration (the α-phase) mainly represents the relatively rapid process of drug distribution from central to peripheral compartment. Once distribution is complete, the curve enters the relatively slow (β-phase) or elimination phase during which drug disappearance is determined mainly by irreversible elimination from the central compartment (Gibaldi and Perrier,1975).

The two compartment model may be expanded to contain additional compartment which can be described mathematically as the sum of as many individual exponential function as there are relevant compartments (Multiple Compartment Models). In reality, a maximum of three compartments is allowed in assay techniques, (Tozer, 1979).

1.1.3 THREE COMPARTMENT MODEL

This is a design or a modified model for oral dosing in which an additional compartment is incorporated to represent the volume (V_a) from which absorption occurs at a first - order rate, (Paxton,1982).

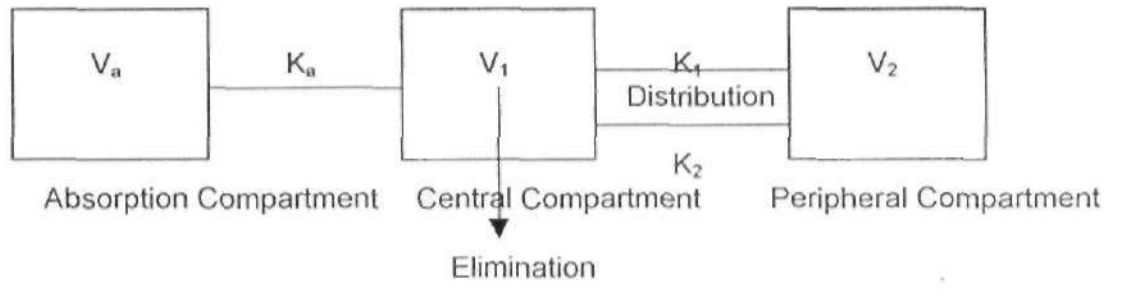


Figure 1.8.Showing a three compartment model (Paxton,1982).

K_a = absorption constant

k_1/k_2 = transfer rate constant between compartments.

A typical concentration time curve for a single oral dose is shown in figure1.10. Similar plots are obtained for other administration routes, such as the intramuscular or subcutaneous and involves a preliminary absorption phase (Paxton,1981)

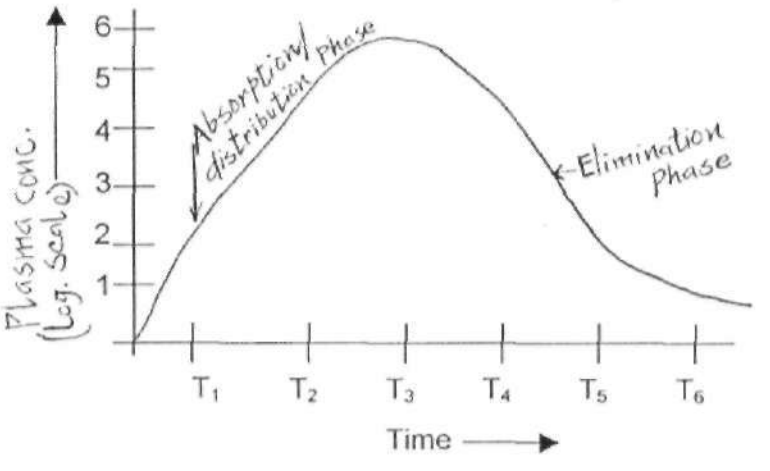


Figure 1.9 Showing a typical concentration Vs time plot after oral dose (Paxton,1981).

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1.2 ABSORPTION

It is the process by which a drug proceeds from the site of application to the site of measurement within the body (Kobayashi et al,1993). There are several sites at which drugs are commonly administered. These sites may be classified as either extravascular or intravascular. Intravascular administration refers to the placement of a drug directly into the blood, either intra-arterial or intravenously. Extravascular modes of administration includes the oral, buccal, sublingual, intramuscular, subcutaneous and rectal routes. To enter the blood, drug administered extravascularly must be absorbed but no absorption step is required when drug is administered intravascularly (Paxton,1981).

1.3 DISTRIBUTION

After introduction into the systemic circulation, a drug is subjected to the multiple processes of distribution, governed by its lipid solubility, its pk_a, the pH of body fluids, the extent of protein and tissue binding, and differences in regional blood flow. Organs such as the liver, heart, and endocrine glands are richly perfused with blood, and rapid equilibration takes place between plasma and tissue. In contrast, in tissues such as muscle and adipose without an extensive blood supply, equilibration takes much longer (Mc Namara et al,1979).

Although distribution is less variable than metabolism, which is greatly influenced by genetic factors, it is still considered to be an important source of variability of drug effect, for example, changes in body water either in development or in disease condition can affect the distribution of certain compounds, (Paxton,1982).

1.4 ELIMINATION

The term elimination is used to express the removal of drug activity from the body. Drug effects are usually terminated by two processes of elimination, metabolism or excretion (Mc Namara et al,1979). Metabolism or drug biotransformation chiefly occurs in the liver, but can also occur in plasma, gastrointestinal tract, kidney and lungs. The biochemical mechanism are complex, but common to all is an increase in the water solubility or polarity of the Metabolic product as compared with the parent drug. This means there is a progressive decrease in lipid solubility which makes metabolites less able to enter cells and more readily excreted in the urine. Metabolism usually leads to deactivation and loss of pharmacological activity. However, an increase number of exceptions to this rule are becoming apparent (Vesta and Wool,1980). The other process of elimination is excretion of the unchanged drug (and metabolite). This takes place mainly in the urine, although biliary excretion and from there into feasces may be important for some drugs (Paxton,1982). Other minor routes of excretion include exhalation of some anaesthetics by the lungs (Rowland,1978).

1.5 PHARMACOKINETIC PARAMETERS

Some of the most useful pharmacokinetic parameters obtained following drug administration are half-life, Area under curve of blood level versus time, systemic and total clearance, apparent volume of distribution and rate constants (Shar,1993).

Half-life ($t_{1/2}$) of elimination is the time taken for the post - absorption plasma concentration of a drug to be reduced by 50% of its original value ((Rowland,1978). It can be determined for both one and two compartment models from the terminal data of the plasma logarithm concentration time plot. It is calculated by dividing 0.693 (ln2) by

the calculated slope of each exponential phase of mulltiphasic blood level versus time curve:

$$t\frac{1}{2} = \frac{\ln 2}{K} = \frac{0.693}{K}$$

(Paxton,1982)

Although t½ is a poor index of drug elimination processes and dosage requirements of individual patients, it is a useful parameter in the determination of suitable dosage intervals and the time required to attain the steady - state during constant intraveneous or chronic oral therapy (Paxton,1982).

Area under curve is an integral of blood level over time from zero to infinity ($o \rightarrow \infty$) and it is a measure of quantity of drug absorbed (bioavailable) in the body (Paxton,1981). The methods of calculating area under curve are discussed by various authors. The methods include planimetry, cut and weigh method, trapezoidal rule and the use of a computer. Many authors have also used the area analysis to a pharmacokinetic pathway and obtain the rate constants associated with the proposed pharmacokinetic model (Wagner, 1967). The area under curve parameter is important for calculating important pharmacokinetic parameters such as relative oral bioavailability, plasma clearance and apparent volume of distribution. It is represented by the equation:

$$AUC = C_o.d_t$$

(Paxton,1982)

Systemic or plasma clearance is the volume of plasma from which a drug is totally and irreversibly removed per unit time, and it is a direct index of drug elimination. (Shar,1993).

Total plasma clearance (Cl_s) of a drug, which results from all processes of elimination (Such as hepatic metabolism, renal and feacal excretion and exhalation by the lung), is the sum total of individual clearances of the drug by the various organs and tissues and can be expressed by two types of relationship. First, one can divide the absorbed dose by the Area under curve , as the latter value basically represent the total bioavailable drug subsequently eliminated, (Paxton '82).

$$Cl_s = \frac{FD}{AUC}$$

(Paxton, 1982).

F is the bioavailble fraction and D is the dose administered.

Secondly, the product of the elimination constant (β) and the apparent volume of distribution (V_d) equals to the clearance (Paxton, 1982).

Clearance has dimensions of volume per unit time and expresses the efficacy with which a drug is eliminated, and is made up of the sum of all the clearances due to individual organs. If only one organ is involved such as the kidney, the plasma clearance is equal to renal clearance. For any particular organ, the physiological determinants of drug clearance are organ blood flow, the ability of the organ to extract the drug from the blood and the extent of binding of the drug to plasma proteins (Mc Namara, Levy and Gibaldi,1979).

Apparent volume of distribution (V_d) is defined as that volume in which the amount of drug in the body would need to be uniformly distributed to produce the absorbed plasma concentration (Paxton,1981).

The volume of distribution is inversely proportional to blood level which means that, the larger the volume of distribution, the more extensively the drug distributes from the blood stream into the various tissues, organs, and binding sites of the body. The concept of volume of distribution is used to relate the plasma concentration of a drug to the dose administered as the body is obviously not homogeneous, even if it can be treated as such in a mathematical model (Rowland,1978).

Drug concentrations in the liver, kidney, muscle fat and other tissues will therefore differ from one another as well as from the plasma concentration . The apparent volume of distribution does not relate to a real physiological or anatomical space but provides an estimate of the extent of the drugs distribution through the body fluid compartments and its uptake by tissues.

A large volume of distribution value implies wide distribution or extensive tissue uptake or both (Paxton,1981). After oral administration, volume of distribution can be determined from the following relationship:

$$V_d = \frac{F.D}{\beta . AUC}$$

where,
F is the fraction of dose absorbed
D is the dose
β is the elimination rate constant
AUC is the area under curve.
(Paxton,1981).

1.6 BIOAVAILABILITY

Some drug formulation are superior to others in their ability to give up their contained drug, which will have an effect on the amount that ultimately reaches the systemic

circulation (Muir et al,1997). Bioavailability is the term used to indicate measurement of the relative amount of an administered dose that reaches the systemic circulation. The absolute bioavailability of an oral dose (or by any other route) can not be determined unless an intraveneous study with the same dose in the same subject is performed (paxton,1982). It is worthwhile noting here that a low oral bioavailability may not necessarily indicate poor absorption. Loss of drug to various degrees may occur due to rapid uptake and metabolism in the liver, as the drug is being transferred by the portal system from its absorption site in the gastrointestinal tract to the general circulation (Paxton, 1981). This so-called "first-pass elimination" during the first circulation through (Wagner '67) the organ is observed for a number of drugs in common use, such as propranolol and alprenolol. Many other drugs such as morphine and lignocaine have such extensive first-pass elimination that they are rarely given orally. According to this understanding of bioavailability, the absorption of an intravenously administered drug is rapid and complete (Wagner, 1967).

The therapeutic action of a drug is dependent on an adequate concentration of the drug reaching the site of action and this concentrations is dependent on the passage of the drug across biological membranes. The rate and extent of absorption of a drug by the body is influenced by a number of biological and physiological factors but it may be affected also by the rate at which the drug dissolves in the body fluid at or before the site of absorption, (Rowland, 1978). Frequently, this dissolution is the rate-limiting factor in the absorption process.

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Effective dispersal of the drug particles in the body fluid and its solubility may both affect the rate of dissolution. These factors may be dependent on the physiochemical

characteristics of the drug itself and on other materials and manufacturing processes used in the preparation of the dosage form (Rowland,1978).

Bioavailability or bioequivalence studies are usually based on measurement of the active drug moiety or and its metabolite(s) in biological fluids as a function of time. In vivo bioavailability testing in humans usually dictates that the biological fluid sample be limited to blood, urine and saliva (Tooms,1993).

The dosage form is administered to healthy subjects who are usually in the fasted state (≅ 10 hours) and who are not allowed to take food for some hours (usually 4 hours) following administration. Blood samples are then collected before and after drug administration at a frequency sufficient to permit an estimate of peak plasma or serum concentration of the active ingredient and the total area under the plasma level versus time curve (Balant, 1980). In most studies where oral dosage forms are compared, the sampling time after each drug administration will be identical (Paxton, 1982).

In figure 1.10 typical plasma concentration versus time curve obtained after oral administration of a dosage form can be seen.

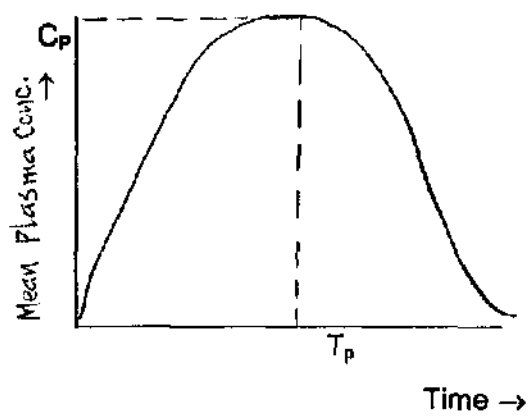


Figure 1.10 showing plasma concentration-time curve (Paxton, 1981).

The shape of the curve in figure 1.10 is dependent on a number of factors. Elimination of the drug begins as soon as it appears in the blood and absorption, distribution and elimination (metabolism and excretion) will all modify the shape of the curve. There are three important parameters that can be derived from this curve, namely the peak-plasma concentration (C_{max}), the time of the peak-plasma concentration (T_{max}) and the area under the plasma level versus time curve (AUC) (Tooms, 1993). The first two parameters, C_{max} and T_{max} are related to and used as a measure of the rate of availability from the dosage form. The extent of absorption from the dosage form is related to the (Henkel 1978) third parameter, AUC. On bioequivalence study, the objective is to determine whether the tested dosage form is equivalent to the reference form. Alternatively, one might demonstrate that no statistically significant differences exist between the plasma levels derived from each drug product at each sampling time (Abdel-monem and Henkel, 1978). The determination of "absolute" bioavailability of a drug involve a comparison of AUC obtained following oral and intraveneous administration is often not possible, and in such cases, 'relative" bioavailability is determined by comparing the Area under curves 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 (Paxton, 1981). A number of circumstances usually arise thereby making the determination of the availability of a drug following administration of a single dose difficult (Paxton, 1981). The plasma level of some drugs maybe 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 period of time. Thus, it is sometime

desirable to determine availability based on steady-state plasma drug levels following multiple dosing (Paxton, 1981).

For the estimation of bioavailability, the area under curve determination is required. The most commonly employed methods for calculating AUC are the cut and weigh method, planimetry, trapezoidal rule and by computer operations. (Bakare, 1992)

Important areas of application of bioavailability include: determination of drug-drug interactions; determination of the effect of food on drug dispersion following ingestion (that is, drug-food interaction); and determination of those formulation factors that alter the bioavailability of an active ingredient in a drug product or products (Bakare, 1992).

1.6.1 FACTORS INFLUENCING DRUG BIOAVAILABILITY

The most widely used oral dosage form is the compressed tablet which often present bioavailability problem due to the reduction in drug surface area in the formulation together with the tableting methodologies, excipients and coating that are used (Rowland, 1978).

Absorption may also be affected by similar dosage forms from different manufacturers and also by different batches of a dosage form from a single source. Most common conventional oral dosage forms in decreasing order of relative dissolution and absorption rates are solutions, suspensions, capsules, tablets, coated tablets, and sustained-release preparations (Muir et al, 1993).

Dosage form related factors which can produce profound differences in bioavailability include formulation and manufacturing variables such as particle sizes, the chemical form and solubility of the drug; the type and quality of excipients used and the compaction pressure (Shar, 1997). Other factors include crystal form, salt, complex and pH of the active Ingridients (Rowland, 1978).

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 amorphous forms) relatively common, being exhibited by at least one-third of all organic compounds. Apart from the polymorphic forms, they can also occur in non-crystalline or amorphous forms. The energy required for a molecule of drug to escape from the crystal in which it is located is very much greater than that required to escape from an amorphous powder. Thus, the amorphous form of a compound is always more soluble than a corresponding crystalline form and may exhibit correspondingly, different therapeutic properties. This observation have been revealed in in-viitro studies with novobiocin and chloramphenicol (Benet and Sheiner, 1985).

Some drugs are capable of forming solvent-addition compounds or solvates, and this may affect their bioavailability. The solubility and rate of solution of a solvate may significantly differ from that of non-solvated form of the drug such as caffeine, theophyline and glutethimide (Bello, 1998).

Drugs may interact reversibly to form complexes with substances occuring in the body or with other drugs or with pharmacologically inactive components of the

pharmaceutical dosage form. Such drug complexes may differ in physicochemical properties from the pure drug, for example, its ability to permeate biological membranes may become altered. Such complexes are thus pharmacologically inactive since dissociation prior to drug absorption usually fails to occur (Bello, 1998).

Surfactants are substances frequently used to increase solubility or act as emulsifiers, 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 the formulation. It was reported that these agents may change the absorptive surface itself in the small intestine, and that in other cases, the agents promote micelle formation, thus (Sheiner 1985) enabling lipid soluble substances to disperse in an aqeous medium (Benet and Sheiner, 1985).

Pharmaceutical factors are therefore extremely important in the control of drug availability. In addition to the drug substance, there are excipients, disintegrating agents, binders, diluents, lubricants and dyes, all of which may contribute to the performance of the dosage form as a drug delivery system. The active drug substance may comprise less than 10% of the total tablet weight under conditions of fast disintegration, the dissolution rate of the solid dosage form will limit the appearance of drug in the blood (Benet and Sheiner, 1985).

The Noyes-Whitney equation which characterises the dissolution rate of a drug in solid dosage form can be written as follows:

$$\frac{d_w}{d_t} = KS(C_s - C)$$

where

$\frac{d_w}{d_t}$ represents the dissolution rate; K is the rate constant; s represent the surface area of dissolving solid; C is the concentration of drug in dissolution medium and C_s represent the concentration of a saturated drug solution.

The dissolution rate may therefore be controlled by manipulating the surface area of the drug particles or solubility (Noyes and Whitney, 1897).

Another important factor influencing bioavailability of drug is the influence of food and specific dietary components. Hayes and Borzelleca in 1985 extensively reviewed the influence of food and specific dietary components on drug absorption. 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 (Bakare, 1992). The presence of food has been shown to decrease, delay, increase or not to affect drug absorption. The following factors have been found to be responsible for the observed effects. That is, the type of drug and dosage form, the nature and size of meals, fluid intake, the condition of the subject and the time sequence between dieting and drug administration (Bakare, 1992).

Though drug bioavailability studies are routinely carried out in healthy human subjects, drugs are administered in practice to patients who may be suffering a variety of illness. Diseases affecting the liver, cardiovascular system and gastrointestinal tract may alter circulating drug or metabolite level after oral dosing. Surgical removal of part of GIT

reduces the absorption of most compounds, suggesting that patients who have undergone such procedure may need to be monitored carefully when taking drugs whose absorption efficiency is critical (Paxton, 1981).

Drug-drug interaction could also affect bioavailability. Drug interaction could be direct as in the case of tetracycline chelation by polyvalent met al ions or indirect as in the case of increased acetaminophen absorption through the stimulating effect of metochlorpromide on the stomach emptying rate (Ivan, 1994).

Volume of co-administered fluid may have a considerable effect on drug absorption. Drug absorption might be expected to occur more readily from concentrated solutions than dilute solutions; nevertheless, the reverse appears to be the case. The pharmacological activity of sodium phenobarbitone and also the circulating levels of salicylate, were greater from dilute than from concentrated solutions (Rowland, 1978).

Age, pregnancy, stress, disease state, cigarette smoking and exposure to chemicals such as insecticides also affect drug disposition. Gender is also a significant variable in some animals but appear to be less in humans (Vesta and Wood, 1980).

1.6.2 Difference in Drug Bioavailability and their Clinical Implications

The change in clinical response which may accompany altered drug bioavailability is a function of both the drug's mode of action (Pharmacokinetics) and also the extent of bioavailability variance (Balant, 1981). However, several reports have documented actual changes in clinical response which have been associated with changes in bioavailability of some drugs. Most of the drugs have well defined blood or tissue level

for therapeutic effect, step dose response relationships or narrow therapeutic indices or have suitable absorption or elimination mechanisms (Paxton, 1981).

It is appropriate to divide bioavailability changes into three major groups, whether the changes in bioavailability of drug is caused by drug interaction, pathological condition and product substitution and any other factor (Rowland, 1978).

In general term, change in bioavailability if sufficiently large will be capable of causing a clinical change for all drugs (Tooms, 1993). Nevertheless, small change in bioavailability are unlikely to be of clinical significance if their level in the body far exceeds the minimum required for activity (Fincher, 1968).

Group of drugs such as anticoagulants, antihypertensives, cardiac glycosides, anticonvulsants, antineoplastic agents, and inorganic ions like lithium belong to the class where likely change in their bioavailability will have a measurable clinical implications (Tooms, 1993).

Describing the effect of formulation changes in hydrochlorothiazide, Tannenbaum et al, in 1968 revealed a marked clinical change to be associated with impaired bioavailability of this drug. Shaldon et al (1963) revealed similar clinical change in chlorothiazide and Spironolactone while Eadie et al and Tyret et al working independently in 1968 and 1970 respectively revealed similar result with phenytoin (Bello, 1998).

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CHAPTER TWO

LITERATURE REVIEW

2.10 DIABETIC MELLITUS

Diabetic mellitus is a chronic disease caused by the deficiency or diminished effectiveness (decreased responsiveness) to insulin, which results in the underutilization of glucose. The consequence of this is the persistent elevation of plasma glucose level or hyperglycaemia which is usually demonstrated by a glucose Tolerance Test (GTT) (Danowski, 1964). Although the cause of diabetes mellitus is not known, the following conditions have been implicated: Heridity, pancreatic disorder/disease (pancreatitis, trauma, etc), drugs (corticosteroids and thiazide groups), and stress conditions. Diabetic mellitus is a metabolic disease with eventual manifestation of glycosuria. The deficiency in insulin in diabetes mellitus could be an absolute lack of insulin as in patients who developed the disorder early in life (juvenile or growth onset), or a relative deficiency as found in diabetics who developed the disease later in life (adult onset or maturity onset) (Ellenberg and Riftin, 1970).

Insulin is synthesized and stored in granules in the β -islet cells of the pancrease. Daily secretion amounts to 30 - 40 units, which is about 25% of total pancreatic insulin content (Williams, 1974). Insulin is a polypeptide with two peptide chains (A - chain, 21 amino acids and B - chain, 30 amino acids) linked by two disulphide bridges, first isolated by Banting and Best, (Pfeiffer, 1969). Its action includes the reduction of blood sugar due to increased glucose uptake in the peripheral tissue (which convest it into glucose or fat), and reduction of hepatic output of glucose (diminished breakdown of glycogen and diminished gluconeogenesis) (Williams, 1974). When the blood glucose

level falls below the renal threshold (10mmol/l or 180mg\100ml) glycosuria ceases in diabetes, as does the osmotic diuresis of water and electrolytes.

Polyuria with dehydration and excessive thirst are thus alleviated. If the blood - glucose falls much below normal levels, appetite is stimulated. Also at metabolic level, in addition to enabling glucose to pass across cell membranes, the transist of amino acids and potassium into the cell is enhanced. Insulin regulates carbohydrate utilization and energy production; and enhances protein synthesis (Bondy and Felig, 1971).

The absolute or relative deficiency of insulin results in the inability of the body to carryout its normal physiological function which would have been possible in the presence of insulin resulting in the disorder. Since the level of glucose in the blood varies only within narrow limits, it rarely rises above 120 mg/100ml or falls below 60mg/100ml. A decrease in blood glucose below a level of about 30mg/100ml results into serious disturbance of mental function which may lead to coma. There is usually a mechanism by which the level of glucose is maintained within the normal range both in fasting and after a Carbohydrate meal (Oakley et al, 1975).

Diabetes mellitus is classified into type I (Insulin Dependent Diabetes Mellitus, IDDM) and type II (Non - Insulin Dependent Diabetes Mellitus, NIDDM). Diabetes mellitus should be distinguished from Diabetes insipidus which is caused by renal reabsorption of water (Danowski, 1964).

Diabetes Mellitus is characterized with Polyuria, nocturia, decreased visual accuity, frequent thirst, dry mouth, Vulvular pruritis in women, severe skin infection, impotence, darkspot due to skin necrosis. Gangrene of the feet and other extremities, coronary artery disease, retinal diseases and parathesia due to neuropathy are complications of

diabetes mellitus. Others include Urinary tract Infection due to favourable growth media, pulmonary or respiratory diseases due to depressed body immune system, And skin Infections with staphylococcus organisms as a result of skin abrassion, (Bloom, 1980).

In large population, clinical investigations include detecting sugar in urine by the use of clinistix. Investigation also involves the assessment of blood sugar level of patients; and levels above 7m mol/l is a strong indication of the disease condition. Glucose Tolerance Test can also be carried out (Williams,1974). Management plan for diabetic patients involves: diet alone, diet and oral hypoglycaemic agents, regular exercise, education of patients and insulin therapy (Elienberg and Riftin, 1970).

2.1.1 INCIDENCE OF DIABETES MELLITUS

According to World Health Organisation and the American Diabetes Association's criteria, an adult is considered diabetic if the fasting blood sugar measured in various plasma exceeds 8 mmol/l on more than one occasion; or if on two occasions, the concentration of glucose in various plasma exceeds 11 mmol/l 30, 60, 90 minutes after ingesting about 75g of glucose and remains above this levels two hours after ingestion.

Diagnosis of the disease in children follows the same pattern and if the child manifests the symptoms of the disease. In pregnancy, a diagnosis of diabetes is made through the manifestations of the symptoms of diabetes mellitus and if the concentration of glucose in venous plasma is in excess on at least two occasions when measured 1,2 and 3 hours after the ingestion of 100g of glucose, and the concentration should not exceed 11mmol/l, 1 hour after taking the glucose, or 9 mmol/l, 3 hours afterwards, (BDA, 1982).

Diabetes mellitus is one of the major cause of death in Europe and the United States (ADA, 1986).From various studies, 5% of the general population of the USA will eventually develop the disease. The prevalence is close in similar other countries. However, the incidence of juvenile diabetes mellitus is unusual in Africa and in the East, especially in Japan. About 30,000,000 people of the world population have been reported to be afflicted with diabetes mellitus (BDA, 1982). In Zaria and environs, about 1 (one) out of 50 cases in medicine are diabetic cases from the reported cases at the Ahmadu Bello University Teaching Hospital, Zaria. The prevalence is high among the aged both in the cities and in the rural areas. The rate of hospital visit among the affected group of the rural dwellers is quite low as they often resort to traditional medication and could only visit the hospital at the worsening of their condition. The demand for constant checkups often makes some patients to even abandon the therapy altogether among this group (Fajans 1972).

2.1.2 BIOCHEMICAL BASIS OF DIABETES

After a meal containing carbohydrates, blood sugar is expected to rise due principally to the breakdown of starch to glucose (Oakley et al, 1975). Absorbed glucose is then carried in the portal vein to the liver where it is converted to and stored as the polysaccharide, glycogen. It is in this way, that glucose from excess dietary carbohydrate is synthesised into glycogen.

In times of carbohydrate requirement, glycogen is conversely broken down to glucose and thus released into the blood stream. Figure. 2.1 shows the synthesis and breakdown of glycogen.

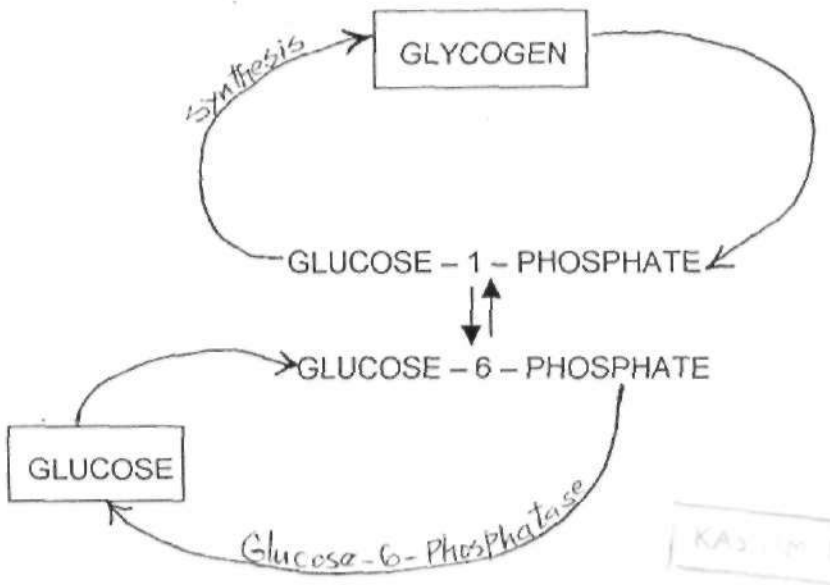


Figure 2.1 synthesis and breakdown of glycogen (Oakley et al, 1975).

The first step is the phosphorylation of glucose to glucose - 6 - phosphate before being converted to glucose - 1 - phosphate which adds glucose to the end of an existing glycogen molecule, thus increasing the length of the chain (Oakley, et al, 1975).

The pathway involved in glycogen breakdown involves the activities of enzymes: phosphorylase, which produces glucose - 1 - phosphate from glycogen and glucose - 6 - phosphates which hydrolyses glucose - 6 - phosphate to free glucose. The activity of both enzymes are under the influence of two hormones, glucagon and adrenaline. These hormones activates phosphorylase, thereby depleting liver glycogen with the consequence of raising blood glucose. The action of these hormones is regulated by cyclic - Amp intervention (Stryer, 1998).

Proteins like complex carbohydrates are similarly split into amino acids. Certain amino acids can be metabolized to glucose. This process is active in starvation leading to severe loss of body protein all in an attempt to maintain the blood sugar level. The most important change after about 3 days of fasting or starvation is that large amounts of acetoacetate, 3-hydroxybutyrate and acetone are formed in the liver and are released into the blood, from where they are carried to the brain (Oakley et al,1975). Figure 2.2 explains this relationship.

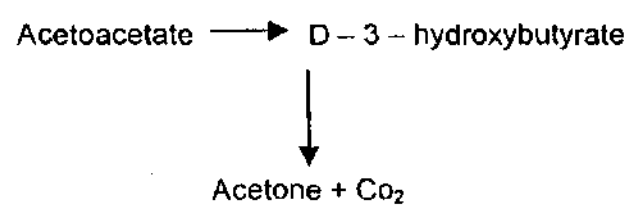


Figure 2.2 Relationship of ketone bodies (Oakley et al,1975).

2.1.2.1 Glycolysis

Glycolysis involves the sequence of reactions which converts glucose into pyruvate with the production of ATP. That means, glycolysis is the metabolism of glucose with pyruvic acid as the end product; except in oxygen deficiency where it can be reduced to lactic acid (stryer,1988). This pathway is shown in Figure 2.3.

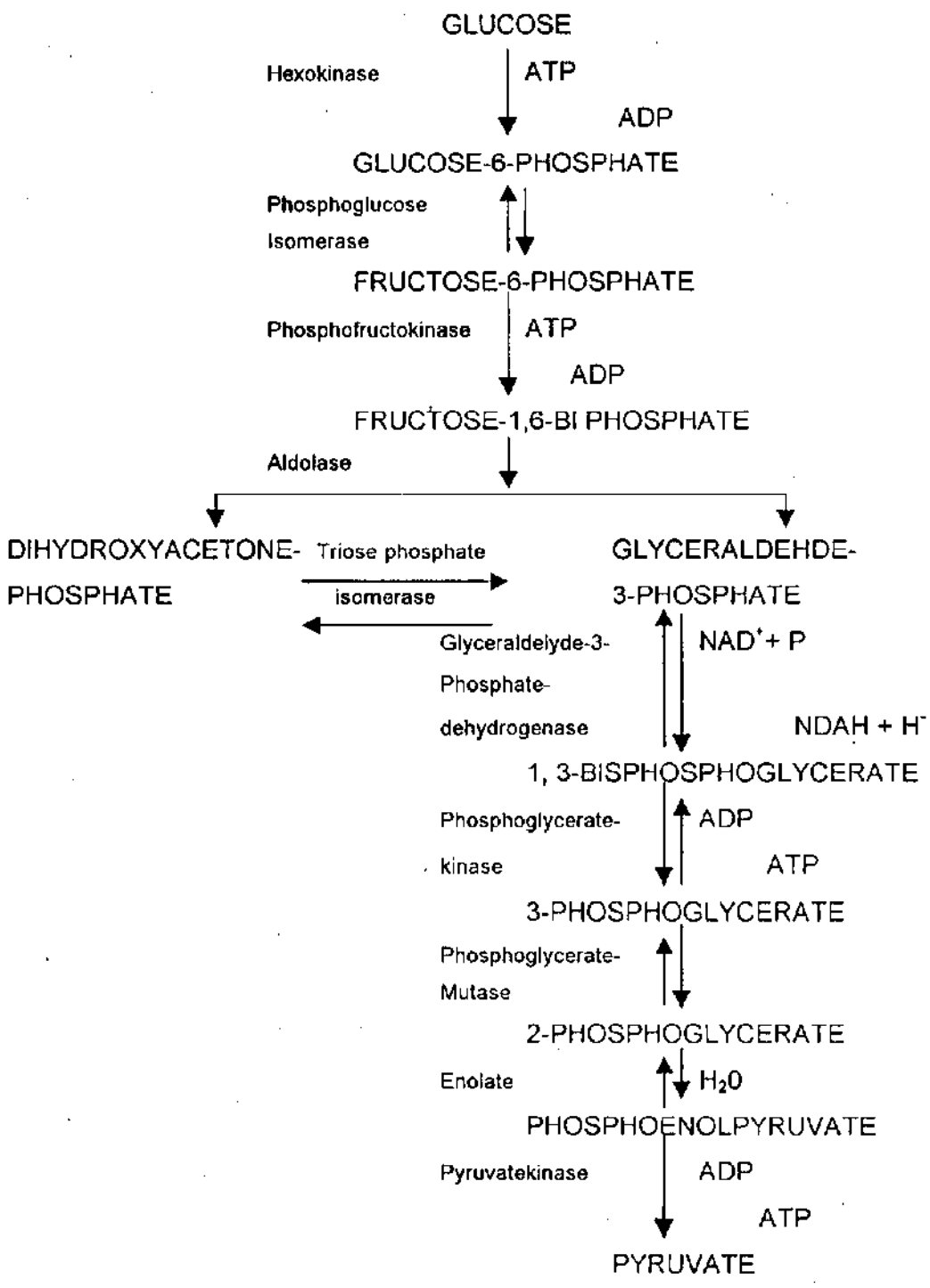


Figure 2.3 The glycolytic pathway (Stryer,1988)

2.1.2.2 GLUCONEOGENESIS

The Synthesis of glucose from non-carbohydrate precursors is known as gluconeogenesis. This process maintain blood sugar in periods of fasting or food deprivation or during intense exercise. Gluconeogenesis is accelerated in diabetes where breakdown of protein contributes substantially to the maintenance of blood sugar (Williams, 1974).

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In this pathway, pyruvate is converted into glucose. Thus, non - carbohydrate precursors of glucose enters the gluconeogenic pathway as pyruvate, oxaloacetate, and dihydroxyacetone phosphate.

Lactate from active skeletal muscle in citric acid cycle, Amino acids from diet and from skeletal muscle protein breakdown, and glycerol from hydrolysis of triacylglycerols in fat cells all constitute non - carbohydrate procussors (Stryer,1988). The major site of gluconeogenesis is the liver and the enzymes are located in cytosol. Other sites of gluconeogenesis include the cortex of the kidney, the brain, skeletal muscle or the heart muscle (Garg et al, 1988).

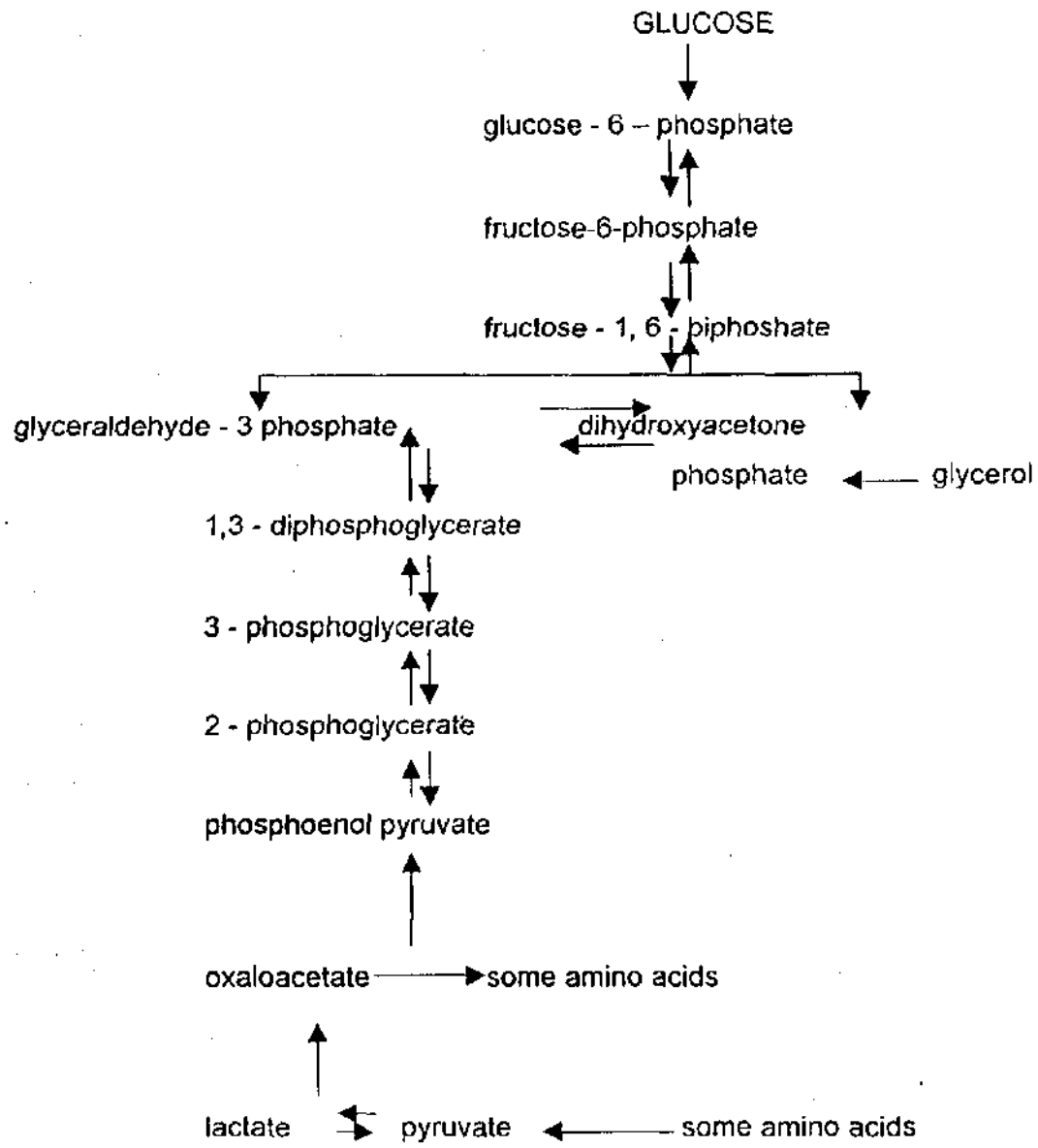


Figure 2.4 Gluconeogenic pathway (Stryer,1988)

2.1.2.3 HYPERLIPIDAEMIA IN DIABETES MELLITUS

2.1.2.4

Lipids from the intestines and from the liver in form of very low density lipoproteins (VLDL) are usually transported to most tissues for oxidation and to adipose tissue for storage. Lipoproteins are means of these mobilizations. Abnormalities of lipid metabolism is bound to occur at the sites of production or utilization of lipoproteins (Garg et al,1988).

In diabetes mellitus, excessive mobilisation of free fatty acids from adipose tissue occurs due to insulin deficiency resulting in underutilization of VLDL leading to hypertriacylglycerolemia. Since insulin deficiency and insulin resistance affect key enzymes and pathways in lipid metabolism, it is expected that lipid and lipoprotein abnormalities would be common in diabetic population (William,1974). The most common abnormality is hypertriglyceridaemia in type II diabetes. VLDL triglycerides can be 50 - 100% higher than control subjects. High density Lipoproteins(HDL) concentration has been found in many studies to be reduced in type II diabetic patients.

Although the mechanism is unknown, there is general consensus that VLDL production is increased in type II diabetic patients. It is possible that hyperinsulinaemia associated with insulin resistance directly stimulates VLDL production, or that insulin deficiency at the cellular level secondary to insulin resistance leads to overproduction of VLDL (Bondy and Felig,1971).

Qualitative changes in lipid and apoprotein composition also occur in diabetes mellitus. Increase in triglyceride content of VLDL with corresponding increase in particle size have been observed (Oakley et al, 1975).

Low density lipoprotein (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 II diabetic patients (Stryer,1988).

Figure 2.5 shows the overall complication of diabetes.

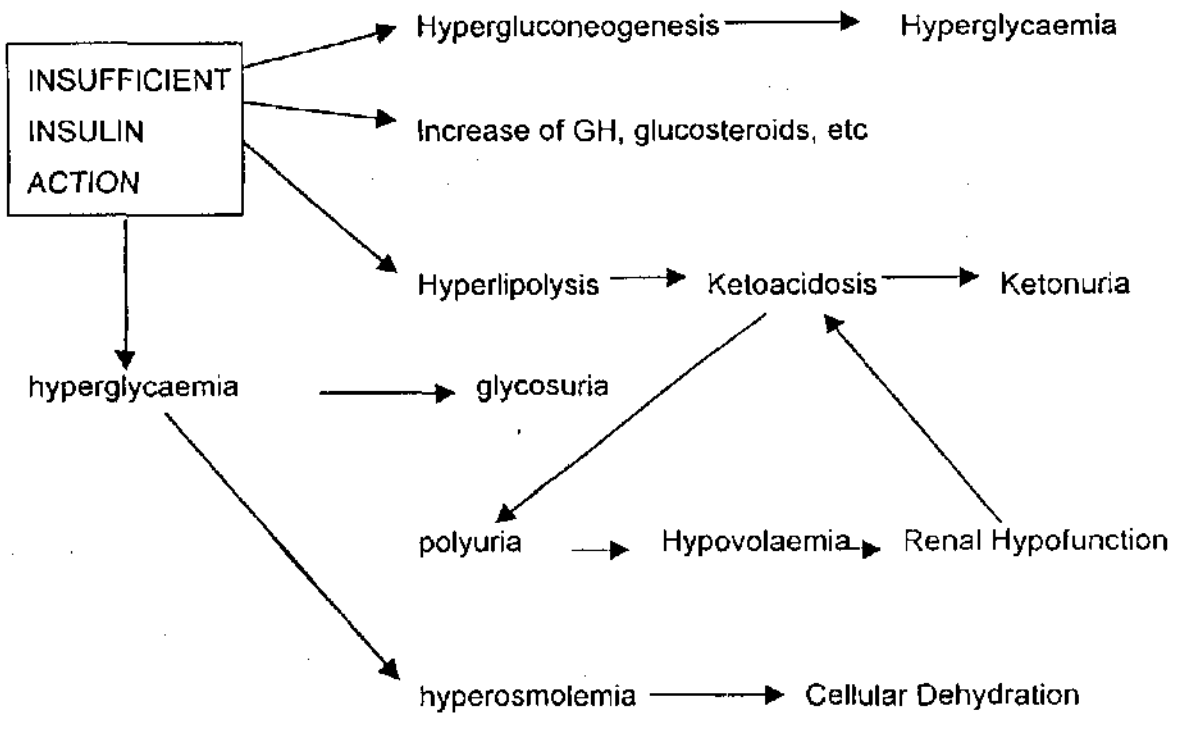


Figure 2.5 overall complication of Diabetes (Williams,1974).

With marked insufficiency in insulin action, there are increased gluconeogenesis, ketogenesis, glycogenolysis, hyperglycaemia, hyperosmolaemia, excessive loss of water and electrolytes, acidosis, and many additional sequelae (Williams,1974).

2.1.3 TREATMENT OF DIABETES MELLITUS AND HYPOGLYCAEMIC AGENTS

Good control of diabetes always involves diet, and some patients need insulin or oral hypoglycaemic in addition (Krall,1971). The aim of treatment is to keep the blood sugar level within the normal range throughout the 24 hours, to avoid ketosis and infections, and the long - term complications. It is pertinent to note that each patient must be assessed individually (Fajans, 1972).

Oral hypoglycaemics should only be used initially when there is no significant ketonuria. Oral drugs are useless if no insulin is present and are most useful in maturity - onset cases. Careful trial is the only sure way of deciding who can be maintained on oral therapy rather than on insulin, (Stowers et al, 1959).

Oral hypoglycaemic drugs are of two kinds, sulphonamide derivatives (sulphonylureas) and quanidine derivatives (biquanides), and are used by 30% of all diabetics, (Balant, 1981). Following the observation in 1918 that guanidine had a potent hypoglycaemic effect, quanidines were tried in diabetes in 1926, but were abandoned a few years later for fear of hepatic toxicity, (Fajans,1972).

In 1930, it was noted that sulphonamides could cause hypoglycaemia, and in 1942, severe hypoglycaemia was found in patients with typhoid fever during a therapeutic trial of a sulphonamide derivative (Jackson,1969). Examples of biquanides in current use include metformin (glucophage), phenformin (meltrol), and Buformin (Butformin). They reduce absorption of carbohydrates from the gut and increase the utilization of glucose in peripheral tissues provided insulin is present, and they reduce hepatic gluconeogenesis (Burgen and Mitchell, 1985).

Examples of sulphonylureas in current use include chlorpropamide (Diabenese), Glibenclamide (Daonil), Tolbutamide (Orinase), and Tolazamide (Tolinase). They act primarily by stimulating the β-islet cells of the pancrease to release stored insulin. They are therefore ineffective in totally insulin- deficient patients (Williams, 1974).

2.2 DRUGS EVALUATED IN THE STUDY

The drugs evaluated in this study include chlorpropamide (antidiabetic sulphonylurea), an *oral hypoglycaemic agent and Ampicillin/cloxacillin (antibiotic, Penicillins), an* antibacterial agent.

2.2.10 HISTORY AND CHEMISTRY OF CHLORPROPAMIDE

Drug management of Diabetes demanded an oral hypoglycaemic agent due to the limitation of insulin therapy. Such limitation is the fact that insulin can only be administered parenterally. Apart from the inconvenience, compliance to medication was generally poor. The curiosity for an oral hypoglycaemic agent and the attendant challenge led to the observation that certain sulphonamide derivatives possesses hypoglycaemic action (Fajans,1972). Two guanidine derivatives synthalin A and B were introduced into the therapy of diabetes in 1920's though less toxic than insulin, their use was discontinued in 1930's for fear of renal and hepatic toxicity. Thus, Arylsulphonylurea became the drug of choice with the following general formular (Williams,1974).

Ar ..... So_2 - NHCONH – R

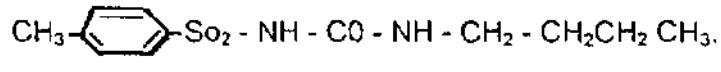
(arylgroup) (urea moiety) (alkylgroup)

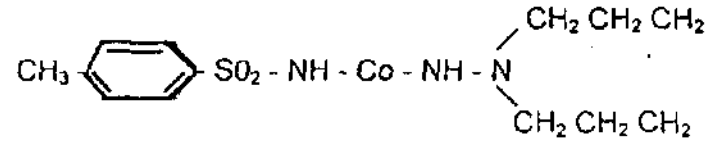
(Williams,1974).

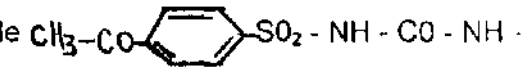
Chemistry

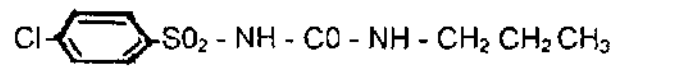
Variations on the arylgroup gave rise to classical agents in this class. Methylgroup on the arylmoiety gave rise to Tolbutamide. Tolbutamide is an effective hypoglycaemic agent, but it was found to be rapidly metabolised by oxidation of the ρ - methylgroup to a Carboxyl function and excreted (Jackson,1969). Replacement of the methylgroup by a halogen, chlorine gave a novel compound with greater potency and along duration of

action. The series in Figure 2.6 represents the various modifications. The 3 - propylderivative was found to be most effective with a generic name of chlorpropamide. The lipophilic properties of the compounds are derived from the alkyl group (Williams, 1974). The hologen (chlorine) substituent in chlorpropamide structure is responsible for its long duration of activity. T1/2 of chlorpropamide is as long as 35 hours with a pka of 7.8. Modification of the Urea moiety led to loss of activity while variation of the aryl group showed that chains of 3 or 4 carbon atoms gave optimum activity. Later developments included the introduction of acetohexamide (Ajibola, 1989) .

Tolbutamide (Oninase) 

Acetohexamide (Dymelor) 

Tolazamide (Tolinase) 

Chlorpropamide (Diabenese) 

1 – [(p - chlorophenyl) sulphonyl] - 3 - propylurea.

Figure 2.6 showing chemical structures of some available sulphonylureas (Williams, 1974).

2.2.1.1 ABSORPTION, METABOLISM AND EXCRETION OF CHLORPROPAMIDE

Although the sulphonylureas probably interact with the β-cells by a common mechanism, there are major differences in their degradation, rates of absorption, and methods of excretion (Balant,1980). As an oral agent, chlorpropamide's absorption from the alimentary tract is good and with a pka of 7.8, the rate of association and dissociation is slow at a physiologic pH of 7.4. (Balant, 1980).

In general, the compounds with short action are metabolised in the liver to inactive forms which are then excreted in the urine. The longer acting compounds are metabolised poorly, if at all, by the liver, and are excreted unchanged in the urine. They or their metabolites may induce hypoglycaemia. In the case of a completely non - metabolised drug, binding to plasma protein plays an important role in the duration of action (Stowers et al,1959).

Chlorpropamide, the longest acting compound of the group, is very poorly metabolised. It is tightly bound to protein and is therefore active for a long period. It is dependent upon renal excretion for the termination of its activity, and is therefore contraindicated in individuals with significantly reduced renal function (Ellenberg and Riftin,1970).

Using chromatographic techniques, Brothertion et al (1969) identified two metabolites of chlorpropamide in the urine of diabetic patients. These metabolites are p - chlorobenzene sulphonylureas and p - chlorobenzene sulphonamide. Two additional metabolites, 2 - hydroxyl - chlorpropamide and 3 - hydroxyl - chlorpropamide were further identified by Taylor (1972) from the urine of four diabetic patients receiving 250 - 400mg of chlorpropamide daily; however, he reported that unchanged drug accounted (Bello,1998).

2.2.1.2 THERAPEUTIC SIGNIFICANCE OF CHLORPROPAMIDE

The limitation of insulin therapy in diabetes chiefly lies in the fact that it can only be administered parenterally, making therapy inconvenient to a number of patients (Fajans,1972). Where the insulin dose has to be administered by the patients unto themselves, ignorance of dose schedules and inability of self administration is also a major set back in insulin therapy. Thus, the development of sulphonylureas had a tremendous therapeutic significance in the therapy of diabetes (Stowers et al,1959). These agents are orally effective in reducing blood glucose levels and are also safe beside being more acceptable than insulin to many patients. Therapy with these agents is easy with high compliance rate, and the reduction in the rate of hospital visit is also of considerable benefit interms of health economics. These agents are not prescribed for type I diabetes, pregnant women, those with renal diseases or those whose hyperglycaemia can be controlled by diet alone. Patients who can be placed on these agents include those who show evidence of pancreatic reserves, who requires less than 40 units of insulin daily for control. The older the patient at onset of diabetes, the better the chance of satisfactory control with these agents (Oakley et al,1975).

Chlorpropamide which is a prototype of sulphonylurea is readily absorbed from the gastro instestinal tract and it is extensively bound to plasma protein, with half - life of about 36 hours. It is partly metabolised in the liver, and the metabolites and the unchanged drug are excreted in the urine (Aguwa,1986).

In a study to evaluate the use of these agents in the prevention of long term complication of diabetes in 1961 by the University Group Diabetes program (UGDP), it was reported that there were more cardiovascular related death in patients on

treatment than those on placebos (Bello, 1998). The use of these agents thus suffered a set back by this revelation as many patients then on these agents were switched on to diet/insulin treatment, from which better results were obtained. Nevertheless, when diet alone cannot control diabetes and in patients responsive to these agents where the inconvenience of insulin therapy can not be tolerated, orally active sulphonyurea becomes then the best choice to date in the management of diabetes type II (Krall,1971).

2.2.1.3 MECHANISM OF ACTION OF CHLORPROPAMIDE

The mechanism of each sulphonylurea is similar but the strength and duration of action differs considerably (Jackson,1969). The presence of responsive islets is necessary for these compounds to lower blood sugar. Since they can all be shown to increase acutely, the secretion of insulin. It is generally believed that this is the primary mechanism of action (Stowers,1959). The primary effect of the drug is on the fasting glucose level, with little alteration in post glucose increments in long - term treated patients. Thus, over a period of time, fasting blood sugar decreases while insulin out put, which can be related to this fasting blood sugar, remains the same, suggesting that in the basal state, the sulphonylurea itself is now partly responsible for the insulin secreted (Krall,1971).

The nature and mechanism of the β-cell response to these compounds is largely unknown. In general, it is believed that there is no increase in β-cell size or number with the most commonly used compounds (Jackson,1969).

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However, some authors have suggested that the more potent compounds may actually increase the number of β- cells (Jackson,1969). The specific details of the mechanism by which sulphlonylureas increase insulin release is unknown, but studies in vitro suggest that it is quite different from that of glucose or other substrate. In particular, it appears that insulin synthesis, which is definitely stimulated by glucose, is not increased by sulphonylureas (Burgen and Mitchell,1985). From the above discussion, it can be said that Chlorpropamide do however require the presence of insulin containing pancreatic tissue for activity. This has led to the theory that its main action is to cause the release of "bound" or "complexed" insulin both in plasma and in the tissues (Ajibola, 1989)

2.2.1.4 ADVERSE EFFECTS AND TOXICITY OF CHLORPROPAMIDE

Toxic reactions to the sulphonylurreas are similar to those of the sulphonamides from which they are derived (Harris,1971).

All the sulphonylureas can cause toxic and allergic reactions. The most common reactions are seen in blood, skin, gastrointestinal tract, and liver (Tucker, et al, 1972).

In the blood, the most dangerous adverse reaction is agranulocytosis. Fatal marrow aplasia after chlorpropamide and pure aplastic anaemia were also reported (Harris,1971). Skin reactions include *pruritus, rashes, erythemanodosum, erythema* multiforme, Steven - Johnson syndrome, erythrodermia, exfoliative dermatitis, sometimes photosensitization occurs, in some cases combined with coproor uroporphyrinuria. Purpura, with or without thrombocytopemia, can occur (Harris,1971). Rare and often fatal are more generalised hypersensitivity reactions with rashes, fever,

arthralgia, proteinuria, and jaundice. Skin manifestations subsides promptly when stopping the medication. The skin manifestations may sometimes disappear as treatment is continued, but continuation is dangerous in view of the possibility of a more generalised hypersensitivity reaction of toxicodermia.

In the gastrointestinal tract, minor side effects are nausea, vomiting, metallic taste in the mouth, heartburn and dyspepsia, (Williams,1974).

Disturbances in the liver function tests are frequently seen during treatment with most sulphonylureas. The significance is not always clear. Jaundice during treatment may be due to a sulphonylurea, but it is difficult to distinguish it from viral hepatitis. Some sulphonylurea like chlorporpamide in higher dose are hepatotoxic (Pryor,1960).

In the cardiovascular system, angiitis have been described in association with sulphonylureas (Harris,1971).

It is uncertain whether sulphonylureas in therapeutic dosage have caused congenital malformations. It seems however wise at present not to use these drugs during pregnancy, insulin is to be preferred (Burger and Mitchell,1985).

2.2.1.5 DRUG INTERRACTIONS WITH CHLORPROPAMIDE

Certain drugs when co - administered with chlorpropamide has been found to potentiate its action; which means, they are capable of increasing the hypoglycaemic effect of chlorpropamide (Tucker et al, 1972). Such drugs will of course, alter certain pharmacokinetics of chlorpropamide. These include salicylates, probenecid, Allopurinol,

sulphonamides, chloramphenicol, monoamine oxidase inhibitors ($MAOI_S$) and β-adrenoceptor blockers (Daubresse et al, 1976). Latendre et al (1986) reported that when certain drugs are concurrently administered with chlorpropamide, they tends to oppose the hypoglycaemic effect of chlorpropamide. Instead of hypoglycaemic effect, hyperglycaemia are observed in patients in whom such combinations are administered. Such drugs include Rifampicin, Epinephrine, thiazide diuretics, glucagon, corticosteroids, Oral contraceptives and dextrothyroxine.

Tetracycline may increase the hypoglycaemic effect of Tolbutamide by an unestablished mechanism (Pryor,1960).

Smoking may result in increased glucose concentrations due to altered response to insulin administration, because smoking may result in release of endogeneous substances that antagonises the hypoglycaemic effect of insulin (Vesta and wood,1980). Thus, if in the presence of insulin, smokers are unresponsive to therapy, it could follow that diabetic smokers on chorpropamide may need a change of habit.

Probenecid may increase chlorpropamide concentration as it may inhibit the renal tubular secretion of chlorpropamide (Tucker et al, 1972). In six patients with concomitant administration, the half - life of chlorpropamide was found to be increased from 36 hours to 50 hours (Pryor,1960). Thus, probenicid may enhance the effect of chlorpropamide.

Phenylbutazone increase the serum concentration of oral hypoglycaemic drugs and may increase their hypoglycaemic action. Since the drug had been shown to prolong the half - life of the active metabolite of acetohexamide (hydroxyhexamide); the

increased serum concentration of the drugs may be due to inhibition of their metabolism or displacement of the drug from plasma protein binding may be involved in the enhancement of hypoglycaemic effect of these agents (Ivan,1994).

Excessive intake of alcohol lead would to altered glycaemic control, most commonly hypoglycaemia. An antabuse - like reaction may occur in patients taking sulphonylureas (Balant,1981). Alcohol inhibit intrinsic hypoglycaemic activity, although hyperglycaemia has also been noted. Chlorpropamide and to a lesser extent, others, provoke "antabuse reaction" with flushing and headache. Alcohol may also inhibit the antidiuretic effect of chlorpropamide used to treat diabetes insipidus. Thus, there is risk of severe hypoglycaemia especially in fasting patients (Podgainy, et al,1968).

Since "antabuse reactions" may occur following ethanol ingestion in patients receiving sulphonylureas, they should be informed of this possibility when therapy is initiated.

Allopurinol or its metabolite might compete with chlorpropamide for renal tubular secretion leading to increased chlorpropamide effect (Daubresse et al, 1976).

In conclusion, interraction with chlorpropamide could either lead to the potentiation of chlorpropamide effect or to the inhibition of the hypoglycaemic effect of the drug.

Potentiation of chlorpropamide effect could be by any of the mechanisms to be described: competition for renal - tubular secretion whereby, compounds involved competes for secretion. Suppression of chlorpropamide secretion will lead to increased half - life of the drug and hence, the enhancement of its hypoglycaemic activity

(Peaston et al,1968). Examples of compounds capable of such action include salicylates, probenicid, Dicoumarol, Allopurinol to mention but a few.

Interference with protein binding, whereby, certain compounds interfers with the binding of chlorpropamide making more unbound fraction of this drug, available for activity since activity is measured by the proportion of the unbound fraction. Salicylates, Phenylbutazone, Halofenate, and Clofibrate are compounds that can act in this way (Dalgas et al, 1965).

The metabolism and elimination of chlorpropamide can be decreased by the inhibition of hepatic microsomal enzymes (Pryor,1960). More levels of chlorpropamide would therefore be available for pharmacological action by the activity of such drugs. Examples of such drugs include chloramphenicol, Dicoumarol anticoagulants and anabolic steroids.

Decarboxylation of chlorpropamide could be inhibited by agents such as Phenylbutazone and sulphonamides. Such action will increase the hypoglycaemic activity of chlorpropamide (Daubresse et al, 1976).

When chlorpropamide is given concomitantly with compounds highlighted above, patients should be watched more closely for signs of excessive hypoglycaemia and where necessary, adjustment of dosage could be considered (Ivan,1994).

Inhibition of chlorpropamide effect could occur through any of the mechanisms to be described: By glycogenolysis, where drugs capable of glycogenolysis stimulate the

body, bringing about glycogen breakdown. Thus, glycogen are converted into glucose and released into the blood, precipitating hyperglycaemia even in the presence of chlorpropamide therapy. Epinephrine is a classical agent that exhibit such action (Latendre et al, 1986).

Drugs capable of stimulating hepatic microsomal enzymes would increase the activity of such enzymes leading to increased metabolism of chlorpropamide and its eventual elimination and thus decreasing the pharmacological action of chlorpropamide. Rifampicin acting this way decreases the hypoglycaemic activity of chlorpropamide when concurrently administered (Petitpierre et al,1972).

Certain compounds possess intrinsic ability to cause hyperglycaemia when given alone or concurrently with chlorpropamide. They include thiazide diuretics, glucagon, corticosteroids, oral contraceptives and dextrothyroxine (Guigliano, 1981).

2.2.1.6 PREPARATIONS, PRESENTATIONS, DOSAGES AND CLINICAL USE OF CHLORPROPAMIDE.

The choice of sulphonylureas depends on its potency, biological half - life, cost and side effects. In general, the most potent compounds are those that are most slowly metabolised and therefore the most likely to produce unwanted hypoglycaemia (Williams,1974).

In diabetics taking insulin, it is not possible to predict with certainty whether a sulphonylurea can be used satisfactorily as a substitute (Burgen and Mitchell,1985).

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In general, individuals who have had episode of Ketoacidosis or who use more than 40 units of insulin per day are unlikely to be responsive to chlorpropamide. The dosage used is that necessary to maintain blood sugar at the desired level up to the maximum indicated for each compound (Williams,1974).

Generic name	Trade name	Common/g	Range/g	Dose/Day/g	Duration(hrs)
Tolbutamide	Orinase	1.50	0.5-0.3	2-3	6-10
Acetohexamide	Dymelor	0.70	0.25-1.25	1-2	10-16
Tolazamide	Tolinase	0.25	0.1-0.5	1	10-16
Chlorpropamide	Diabenese	0.25	0.1-0.5	1	40-72

Table 2.1 Dosages of common sulphonylureas in use, (Williams, 1974).

Chlorpropmide is presented in 100mg and 250mg tablets. 200mg daily dose will maintain a plasma level of about 10ug/100ml, while minimum and maximum effective concentrations are 100ug and 500ug/100ml respectively. The starting dose is usually 250mg daily (MIMS Africa,1996).

2.2.20 HISTORY AND CHEMISTRY OF AMPICILLIN/CLOXACILLIN

The history of Ampicillin/cloxacillin is as old as the history of the Penicillins since they are mere analogues of the Penicillins (Hills, 1970). Pasteur and Joubert (1877) and Lister (1871) had already described the phenomenon of antibiosis- using the products of one living organism to kill another living organism - and penicillin mould had been used by Gossio (1896) in an unsuccessful attempt to produce an antibacterial substance. In 1928, Alexander Fleming, working in Almroth Wright's department in St. Mary's hospital, Paddington, noted that some colonies of staphylcocci were lysed when the medium was contaminated by a mould, later classified as penicillium notatum. In 1929, Fleming published an account of his work, he described the antibacterial action

of the substance which he named penicillin, mentioned its lack of toxicity for human leucocytes and wounds, and was impressed by the possibility that it could be used to suppress bacterial growth (Batchelor et al, 1959).

Early research workers at the Sir William Dunn school of pathology, including Florey, Chain and Heatley, became engaged in extracting and purifying penicillin from cultures of the mould. By February 1941, enough penicillin was available to treat an Oxford policeman dying of staphylococcal pyaemia; the infection was arrested, the patient rallied, but died when supplies stopped. Clinical trials in America confirmed the previous studies at oxford. The main problem was to obtain sufficient quantity, and because of this, supplies were limited to British and American armed forces until 1946. By this time, supplies became sufficient for civilian use (Eriksen,1947). Today, owing to its high level of safety, the Penicillins are the most widely prescribed antibiotic in the world covering both gram positive and Gram negative cocci (Petersdorf and Turck,1966).

The limitation of the Penicillins however is the fact that they are destroyed by B - lactamase enzyme; they are also acid labile, and resistant strains of bacteria have been found against this class of antibiotic (Hills, 1970). These limitations were the basis for various modifications of the Penicillins. Penicillins are therefore, the derivative of 6-Aminopenicillanic Acid (6 - APA), the key to understanding the chemistry of the Penicillins. Various side chains may then be added to produce the other active compounds or analogues in use today (Ajibola,1989).

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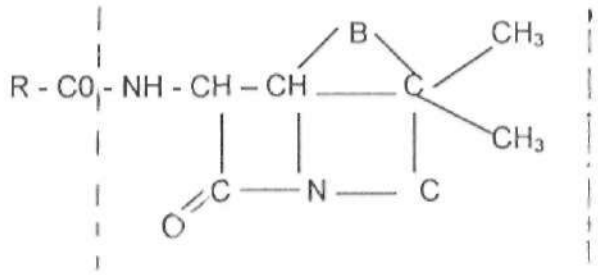


Figure 2.7 – 6 – Aminopenicillanic Acid (6-APA) (Hills, 1970)

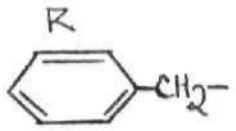
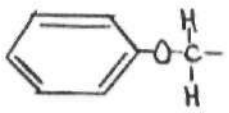
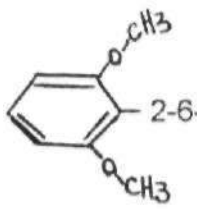
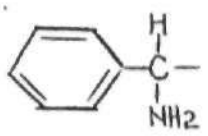
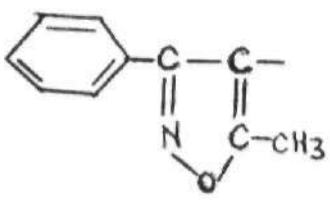
R	Compounds	Remarks
	Benzylpenicillin (Penicillin G):	*The enzyme β- lactamase splits the β-lactam ring producing inactive penicilloic acid, and amidase removes the side chain to produce 6 - APA.*
	α - Phenoxy - methyl (penicillin V):	*This is acid stable and thus orally administered; but still destroyed by β - lactamase.*
	2-6- dimethoxy phenyl penicillin (methicillin):	*It is active against penicillinase – producing staphylococci,acid unstable only given parenterally.*
	Aminobenzyl penicillin (Ampicillin):	*The addition of an α- NH3 group led to this compound with wide spectrum of activity and covers many Gram negative organisms.*
	3 -chlorophenyl - 5 - methyl -4- isoxazolyl penicillin (cloxacillin) :	*an evolution of the penicillins with hindered compounds which are penicillinase - resistant and relatively acid stable.*

Figure 2.8 showing chemical modifications of penicillins (Hills, 1970, Ajibola, 1989).

The aim in Ampicillin/ cloxacillin combination therefore is to confer on Ampicillin the ability to be penicillinase resistant while retaining its broad spectrum capabilities (Hills, 1970).

2.2.2.1 ABSORPTION, METABOLISM AND EXCRETION OF AMPICILLIN/CLOXACILLIN

These penicillins are rapidly absorbed, diffuse easily into body cavities and joint spaces and are very quickly excreted by the renal tubules (Shantz,1985). Very little crosses from the blood into the cerebrospinal fluid (CSF) when the meninges are intact. The level of this drug in the bile is usually several times that of the plasma; if there is obstruction to the bile ducts, no penicillin may reach the bile. Because of very rapid renal excretion, high blood levels of penicillin can only be maintained by large dose at frequent intervals or by reducing the rapidity of renal excretion. A planned pharmacological approach by Beyer led to the discovery of carinamide and later, the more effective probenecid which depresses renal tubular excretion, but does not eliminate glomerular filtration of penicillin (Hills,1970). Excretion of antibiotics other than penicillins, cephalosporins and amino salicylic acid is not affected. Penicillins may be found in inflammatory exudate, (Pines et al, 1970). Another variable in the availability of penicillin at any given site is the proportion bound in plasma and tissues. The bond between penicillins and serum is reversible so that, an equilibrium is established between the protein and free antibiotic on one hand, and the protein - bound drug on the other (Kunin,1966).

Ampicillin is well absorbed when given orally, peak blood levels appear in 1-3 hours and detectable amounts persist for about 6 hours (Petersdorf and Turck,1966). It is

excreted for the most part unchanged in the urine and reaches quite high levels (up to ten times the plasma level) in bile. Cloxacillin also is well absorbed when administered orally; peak levels appear in 2 hours and persist in blood for about 8 hours being higher than oxacillin and intramuscular injection provides double the plasma level of a comparable oral dose (Hills,1970). The t½ of Ampicillin and Cloxacillin is 75 minutes and 30 minutes respectively. While Ampicillin is 20% bound to protein, Cloxacillin is 95% protein bound (Hills, 1970)

2.2.2.2 THERAPEUTIC SIGNIFICANCE OF AMPICILLIN/CLOXACILLIN

Penicillin sensitive organisms are Gram positive, penicillin insensitive organisms are Gram negative, notable exceptions being meningococci and gonococci. The introduction of the reactive group, NH_2 into the penicillin molecule leads to widening of the antibacterial spectrum and the most useful compound to emerge is amino phenylacetamido-penicillanic acid (Ampicillin), (Ajibola, 1989). This compound is comparable to the tatracyclines and the chloramphenicol; it is active against non-penicillinase-producing staphylococci, and most streptococci; also, Pneumococci, meningococci and gonococci, but ampicillin is less active against these cocci than penicillin G. It is highly active against H. influenzae and many strains of E. coli. It is inactive against pseudomonas and some strains of proteus (Hills,1970). It shows in vitro activity against Shigella and Salmonella, but its clinical usefulness in infections due to these organisms has been disappointing. Ampicillin is well absorbed when given by mouth, peak blood levels appear in 1-3 hours and detectable amounts persist for about 6 hours. Ampicillin has several disadvantages : It is destroyed by some penicillinases, resistance to coliform bacteria develop rapidly and hypersensitivity reactions to this drug may be severe (Stewart,1970). Such disadvantages were the

basis for the formulation of Ampicillin/ Cloxacillin combination resulting in a therapeutic advantage over the individual agent (Ajibola). In antibacterial spectrum, cloxacillin resembles penicillin G , covering organisms such as streptococci, pneumococci *meningococci, gonococci and staphylococci. Cloxacillin is less active against those* organisms for which penicillin G might be considered specific, such as pneumococci and haemolytic group A streptococci (Duguid,1946). In chronic pulmonary infections, cloxacilin may not always remove all staphylococci and the drug has little effect on nasal carriers of staphylocci (Eriksen,1947).

2.2.2.3 MECHANISM OF ACTION OF AMPICILLIN/CLOXACILLIN

The possession of mucopeptide by bacterial cells appears to be a unique factor distinguishing it from human cells. It is this difference which confers on penicillin its ability to be selectively toxic for bacterial cells (Park and Strominger,1957).

When Fleming discovered penicillin, he also discovered an enzyme, lysozyme and recognized that lysis resulting in bacteria from the action of lysozyme was caused by the disruption of the bacterial cell wall (Hills, 1970). When the cell wall is impaired or destroyed, *the organism is unable to withstand osmotic action if the surrounding* medium is hypotonic in relation to the interior of the cell. It assumes spherical shape, enlarges and finally ruptures. These changes occurred in both Gram positive and Gram negative bacteria, indeed were most marked in Gram negative organisms which showed most resistance to lysis by penicillin (Hills,1970). These observations on the *morphological changes in bacteria subjected to the action of penicillin focused attention* on the cell wall and interest was then centred on the biochemical and metabolic behaviour of this structure.

From biochemical point of view, Gale and Taylor (1947) showed that penicillin prevented the uptake of glutamic acid in staphylococci but not in Gram negative Coliform bacilli, and in 1949, Park and Johnson found that phosphates accumulated in staphylococci grown in low concentrations of penicillin. It was therefore proposed that *uridine diphospho-acetyl muramyl-pentapeptide, the major accumulated peptide*, was a cell wall precursor, and its accumulation resulted from inhibition of cell wall synthesis by penicillin (Hills, 1970). This hypothesis has been confirmed by isotopic measurements of cell wall synthesis in Gram positive and Gram negative bacteria (Park and Johnson,1949).

Recent studies suggest that the rigidity of the cell wall is at least in part accounted for by cross-linking between the various mucopeptide structures, and it seems probable from recent work that a transpeptidation is inhibited by the action of penicillin (Duguid,1964).

In conclusion, the action of these penicillin analogues may be summarized by the observed physical changes; swelling of the cell, inhibition of cell divisions, and production of spheroplasts. The biochemical factors include the accumulation of nucleotide precursors of both mucopeptide and teichoic acids, and in the breaking of the transpeptide bridge between two glycopeptide chains (Hills,1970).

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2.2.2.4 ADVERSE EFFECTS AND TOXICITY OF AMPICILLIN/CLOXACILLIN

Although all antibiotics have in common, that in low concentrations, they exert a biostatic or biocidal action on microorganisms or certain cell systems, they differ in many microbiological, pharmacokinetic and toxicological aspects (Hills, 1970).

The penicillins have a very low acute toxicity both in humans and in most animals. The lack of intrinsic toxicity of the penicillins to man and animals is explained by their peculiar mode of action. In bacteria, they interfere with the formation of cell walls, which are chemically unrelated to any substances to be found in the human and animal body (Duguid,1964).

The penicillins and their analogues are notorious because of the frequency of hypersensitivity reactions they may provoke (Kasik and Thompson, 1970). In the absence of allergy however, they belong to the safest drugs now available. This also applies to their use during pregnancy and early childhood.

Cloxacillin cause relatively few side reactions: hypersensitivity reactions, a history of any penicillin reaction in a patient is to be regarded as a contraindication to the use of these drugs since they show cross allergy. In the gastrointestinal tract, cloxacillin causes a minimum shift or disturbance of the enteral bacteria (Kasik and Thompson,1970).

At present, ampicillin, one of the most prescribed drug in the world, causes several unwanted effects (Kerrebijn,1970). In 1967, Scientist of Beecham research laboratory in the U.K announced the discovery in commercial penicillins of proteinaceous, a substance with high allergenic properties (Dursch,1968). These impurities would be

largely responsible for the high incidence of allergic reactions observed upon administering these penicillin. This impurity partly originated from the bacterial amidase preparation, used in the preparation of 6-APA from benzyl penicillin, and partly also from residues being present in the benzyl penicillin itself (Hills, 1970).

After oral administration of Ampicillin by 90% of patients with infectious mononucleosis (glandular fever, Pfeiffer's disease) they develop a rash (Kerrebijn,1970). Mononucleosis is often associated with sore throat and positive throat culture for haemolytic streptococci. In some individuals, ampicillin may cause depression of the bone narrow, which is at least in some patients, has been fatal. This rare form of bone marrow aplasia has also occasionally been observed with methicillin (Atkinson,1969). Agranulocytosis during therapy with ampicillin occured in at least two patients (Reisman and Arbesman, 1968). Much about the cutaneous reactions following oral ampicillin has been reported; a distinction has to be made between true penicillin reactions, which are usually of the urticaria type, and the reactions specific for Ampicillin and perhaps some other semi-synthetic penicillins that are macular or maculo-papular in type. The latter start most commonly over the elbows and knees, and often spread symmetrically to most areas of the body. They usually give rise to much less irritation than urticaria due to penicillin (Hills,1970). Ampicillin causes a far higher incidence of contact dermatitis than other penicillins (Samuels et al,1968). Patients receiving oral ampicillin may complain of a number of gastrointestinal tract reactions, such as loose stools, diarrhea, pruritis ani, nausea and vomiting. Mouth ulcers and sorethroat following oral treatment with ampicillin have also been observed. Abnormal serum transaminase level were found after parenteral administration of ampicillin (Reisman and Arbesman, 1968). Ampicillin may promote the selective multiplication of resistant organisms such as

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candida, pseudomonas, enterobacter, and certain group of proteus. Especially in debilitated or malnourished patients, and those receiving corticosteroids, immunosuppressive agents, or irradiation are at risk of developing infections or superinfections (Hills,1970). Bacterial resistance to Ampicillin/cloxacillin is usually of the extra chromosomal type. It may occur in enterobacteriacaea, and in staphylococcus aureas. the resistance is due to the formation of enzymes (β-lactamases,penicillinases) destroying the antibiotic (Duguid, 1946).

2.2.2.5 DRUG INTERRACTIONS WITH AMPICILLIN/CLOXACILLIN

In 1976, the American Pharmaceutical Association in conjunction with National Professional Society of Pharmacists in America through evaluation of drug interactions (EDI) documented the interaction of the penicillins and its analogues with drugs such as Aspirin, chloramphenicol, Colistimethate, Decamethonium, erythromycin, Gallamine, triethiodide, Gentamycin, Kanamycin, Metocurine, neomycin, pancuronium, probenicid, Salicylate, Streptomycin, Succinylcholine, tetracycline, tobramycin and tubocurarine (Ivan, 1994).

Aspirin in large doses, increases the serum level and half-life of penicillin when these drugs are given concurrently. Although concurrent use of high dose of Aspirin has been suggested to increase the clinical benefit of penicillins, the possible toxicities due to high dose of Aspirin administration discourages such therapy (Moskowitz, et al,1973). The mechanism of action is unclear, but it appears to involve two separate effects: Aspirin may displace penicillin from its plasma protein binding site. The acetylgroup of Aspirin binds colvalently to the amino group of lysine residues on albumin, resulting in an altered secondary structure of the protein. The altered secondary structure may

result in altered protein binding to other drugs (Kunin,1966). Penicillin and Aspirin also may compete for the same renal secretory sites. Aspirin and the other acidic drugs are secreted by the same proximal tubular active transport system as penicillin. The increase in the penicillin half-life that occurs when penicillin and Aspirin are administered concurrently may be the result of competition between the two drugs for the same excretory system (Moskowitz et al, 1973).

Concurrent administration of penicillin and probenicid results in higher and more sustained serum antibiotic levels (Ivan,1994). One possibility is that probenicid actively competes with other weak organic acids for the renal transport process and thus, diminishes tubular secretion of such compounds (Hills,1970). A second possibility is that probenecid decreases the apparent volume of distribution of the penicillins, resulting in a larger penicillin fraction in the central compartment (plasma and extracellular). Concurrent therapy however, should be limited to certain infections where high single - dose level of penicillin are desirable as in veneral diseases (Ivan, 1994).

Bacteriostatic action of chloramphenicol antagonised the bactericidal action of penicillin in the early growth phase of an organism (Wallace,1967). The mechanism involved has been described by Ivan (1994). Chloramphenicol inhibits protein synthesis, specifically by inhibiting the function of messenger RNA, at the 50S ribosomal submit. Bacteriostatic action occurs because of immediate ceasation of new protein formation within the cells, thus preventing bacterial multiplication. Penicillins exerts a bactericidal effect by inhibition of a specific step in cell wall synthesis, and exerts its greatest activity during the early phase of bacterial growth. When the static phase of bacterial

growth is reached, the activity of penicillin is considerably reduced (Park and Strominger,1957).

2.2.2.6 PREPARATIONS, PRESENTATIONS, DOSAGES AND CLINICAL USE OF AMPICILLIN/CLOXACILLIN.

Ampicillin/cloxacillin are formulated as a single capsule of Ampiclox by Beecham Laboratories as: Ampicillin 125mg or 250mg plus cloxacillin 125mg or 250mg per capsule. Also available is the suspension where 5ml of such liquid suspension is equivalent to one 125/125mg capsule in powdered form in 60ml and 100ml volumes.

Injection of Ampicillin 125mg or 250mg plus cloxacillin 125mg or 250mg in powder is also available together with neonate injection of Ampicillin 50mg, cloxacillin 25mg in powder. Neonatal suspension of Ampicillin 60mg, cloxacillin 30mg per 0.6ml is also available (MIMS, Africa,1996).

An adult dosage consist of 500mg - 1g every 6 hours or more frequently according to the Severity of infection. In severe infection, dosage may be increased. Children needs ¼ and ½ of the adult dose if they are below 2 years and 10 years respectively (Petersdort and Turck, 1966).

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Ampiclox is a formulation of broad spectrum ampicillin and penicillinanse-stable cloxacillin. Ampiclox exhibits bactericidal activity against a wide range of Gram positive and Gram-negative organisms, including: streptococci, staphylococci (including penicillinase-producing strains), Neisseria SPP., H. influenzae, Escherichia coli, proteus mirabilis and Salmonella SPP. Both ampicillin and cloxacillin are acid stable and are

well absorbed, producing good serum and urine concentrations. Ampiclox is predominantly excreted by glomerular filteration and renal tubular secretion (Craig and Vogelman,1984).

Indications specific of Ampiclox include: Respiratory tract infections, Ear, Nose and Throat infections, urinary Tract infections, Gastro-intestinal infections, skin and soft Tissue infections, pelvic infections, septicaemia, Endocarditis, and Orthopaedic infections (Hills,1970). Ampiclox Neonatal is indicated for the prophylaxis or treatment of bacterial infections in premature babies or neonates (MIMS Africa,1996).

2.3.0 ANALYTICAL METHODS

The science of drug analysis is an extremely active one in terms of research on newer, more reliable, or more sensitive methods of analysis (Olaniyi and Ogunlana, 1995). The various analytical methods that have been developed in the overall assessment of drugs include the physicochemical methods which comprise the following: Titrimetry and gravimetry; chromatography; spectrophotometry (uv/visible,IR, N.M.R.); Mass spectra; Atomic absorbtion spectra and Flame photometry; Refractometry and Polarimetry (Olaniyi and Ogunlana, 1995).

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Ultraviolet and Visible Spectroscopy

Even with the availability of more sensitive and sophisticated instruments for drug analysis nowadays, U.V - spectrophotometric technique still remain one of the most basic and fundamental technique used for ease of handling, maintenance, and availability (Blanchard et al,1979).

Before now, theophylline concentrations in serum of patients and volunteers were measured by HPLC. Using spectrophotometric assay in peadiatric patients and in normal subjects, good agreement was found between the two assay methods (Blanchard et al,1979).

Using differential Scanning Colorimetry (DSC), the relationship between the three crystalline hydrates, (Pentahydrate,heptahydrate, and decahydrate) of a bivalent metal salt, nedocromil magnesium, and their characterisation were established (Schirmer,1982).

Using U.V spectrophotometric method, (Ngo and Kinget,1996), measured the concentrations of artemisinin according to the method described by Zhao and Zeng,1985, in which artemisinin was transformed into a U.V absorbing compound with an absorbance maximum of 290nm after heating the solution with sodium-hydroxide.

By the use of spectrophotometric and fluorometric method, study on the hydrolysis of Aspirin was carried out recently and the results obtained were identical to those obtained using traditional constant - temperature Kinetic runs, thus reducing the amounts of time and chemicals usually spent in collecting Kinetic data in mechanistic studies in solutions by an order of the same magnitude (Bello,1998).

Over 80% of drugs in current use contain conjugated chromophores which make them suitable for spectrophotometric analysis. Chromophores are unsaturated groups in a molecule responsible for absorption e.g. C=C; C=O; C=N; -N=N-; these groups are therefore responsible for the colours present in organic molecules. These groups

contain loosely bound pi (ii) electrons which are mobile. One of such electrons can easily absorb electromagnetic radiation and get promoted to the next energy level.

Theory/Principle

The use of absorption spectrophotometry in the ultra violet and visible regions in the assay of drugs is based upon the fact that the absorptivity of a substance, A (1%,1cm), is usually a constant independent of the intensity of the incident radiation, pathlegth and concentration. Because of these factors, concentrations may be determined photometrically based upon Beer-Lambert's Law (Olaniyi and Ogunlana, 1995).

The laws made by Lambert and Beer govern the phenomenon of absorption of light and form the basis for quantitative analysis of drugs.

Lambert's Law: This states that the decrease in the intensity of light due to its absorption by a sample, is related exponentially to the thickness of the absorption layer for a monochromatic light.

Beer's Law: The decrease in the intensity of light due to its absorption by a sample is related exponentially to the concentration (C) of the absorbing substance in solution.

The two laws when put together is known as Beer-Lambert's Law which is defined as : The Absorbance of a solution of a substance is related to the pathlenght (l) of solution through which the light passes and to its concentration (C), expressed mathematically as:

$$Log_{10}\ I_o/I = KCl$$

Where k is a constant for a particular substance; the constant k is called absorptivity. I_o and I are the incident and transmitted light respectively (Olaniyi and Ogunlana, 1995).

For the analysis of plasma glucose, it has been established that glucose oxidase, a specific enzyme that promotes the oxidation of glucose to acid, is involved in a reaction that produces an equivalent amount of hydrogen peroxide. In the presence of a second enzyme, peroxidase, oxygen from the peroxide can be transferred to a suitable acceptor with the production of a coloured complex compound. The intensity of the colour is proportional to the concentration of glucose in the sample. In this way, glucose concentration can be estimated by colorimetry (Stryer,1988).

Glucose Oxidade + Gluconolactone + H_20 ⟶ Gluconic acid + $2H_20_2$ + 0_2.

$2H_20_2$ + Oxygen acceptor peroxidase coloured complex + H_20.

Figure 2.9 Showing enzymatic method for glucose estimation, (Williams,1974).

2.3.1 OBJECTIVES AND SCOPE OF THE STUDY

Staphylococcal infections and various skin infections are common features of diabetes and are frequently early signs of type II diabetes mellitus (Aguwa,1986). Other infections proven and suggested to be associated with diabetes are urinary Tract infections, Mucocutaneous Candidiasis, pneumonia, foot ulceration, tuberculosis and fungal infections associated with poorly controlled diabetes. Uncontrolled diabetes significantly alters the efficiency of body defense mechanisms; vascular insufficiency and neuropathy contributes to infections of the lower extremities (FaJans, 1972).

Infections can generally affect diabetic patients such that previously controlled diabetes often reemerges with exacerbation of the disease. In such cases, insulin becomes necessary for non-insulin treated patients, and Ketoacidosis may be precipitated (Oakley et al, 1975).

Successful treatment of diabetes is usually measured by the degree to which various infections and complications of the disorder are prevented. It is on this basis that multiple drug therapy in diabetics can not be ruled out. Treatment of infections in diabetes with standard drugs must be prompt and thoroughly adequate. The prescription pattern for infected diabetic patient show a combination of Ampiclox together with an oral hypoglycaemic agent. Such therapeutic goal is beneficial in diabetes mellitus type II and in diabetics placed on sulphonylureas who constitute the majority of out patient attending diabetic clinic (Aguwa,1986).

Prescription of two drugs as observed above in the clinics is not uncommon, but when two drugs are taken together, possibility of drug interraction is also not uncommon.

An interraction is said to occur when the effects of one drug are changed by the presence of another drug, food, drink, or by some environmental chemical agent (Ivan,1994). The outcome may be harmful if the interraction causes an increase in the efficiency or toxicity of the drug. For example, patients on Warfarin may begin to bleed if given azapropazone or phenylbutazone unless the Warfarin dosage is reduced appropriately; Also, patients taking Monoamine Oxidase inhibitor antidepressants may experience an acute and life-threatening hypertensive crisis if they eat tyramine-rich foods (Ivan,1994). A reduction in efficacy as a result of an interraction may also be

harmful. Thus, patients on Warfarin given rifampicin will need an increase in the dosage of Warfarin to maintain adequate ,anticoagulation while patients taking Tetracycline antibiotics should avoid antacids and milky foods because the antibacterial effects can be drastically reduced (Ivan,1994).

Numerous interactions of hypoglycaemic sulphonylureas have been reported with other drugs and alcohol. A recent review of these interactions lists more than 70 relevant publications (Balant,1981). Outline of major class of drugs implicated is given in table 2.2.

Drug	Interraction	Effect on glucose
Sulphonamides	Increased action of chlorpropamide	Mark decrease in glucose concentration
Monoamine oxidase inhibitors	Potentiate the effect of chlorpropamide	Decreased level of glucose
Alcohol	Disulfiram-type reaction	Decrease glucose concentration
Aspirin	Potentiate the effect of chlorpropamide	Decreased level of glucose following high dose of Aspirin
Dicoumarol	Increased action of chlorpropamide	Reduction in glucose concentration
Chloramphenicol	Potentiation of chlorpropamide action	Reduced level of glucose
Halofenate	Raised serum level of chlorpropamide	Marked reduction in glucose concentration

Table 2.2 Showing the interraction of chlorpropamide with some drugs or class of drugs.

All these interractions highlighted above can occur by any or combinations of the following mechanisms: Displacement or competition for common plasma protein binding site; Enzyme inhibition, Enzyme induction and competition for renal tubular excretion route (Balant,1981). These examples suffice to show that the posibility of interactions of practical clinical importance is a real one.

This study is to determine the effect of Ampiclox on the pharmacokinetics of chlorpropamide. Since the incidence of drug interractions are sometimes not only undesirable but could be fatal, it will be of interest to understand the interraction between chlorpropamide and Ampiclox to increase our knowledge in this area of research to prevent unwanted toxicity and interference with therapeutic objective. To assess the clinical significance of such interractions, the following criteria will be adopted in the selection of diabetic patients:

(i) Diagnosis of non-insulin dependent diabetes mellitus by a clinician.

(ii) The patient will be ruled out for uncontrolled severe hypertension, congestive heartfailure, Gastrointestinal disease or alcohol abuse.

This study will involve the quality control study and the pharmacokinetic studies by selecting type II diabetic patients according to standard criteria (ADA,1986).

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Participating subjects will undergo two treatments:

First, single dose of 250mg chlorpropamide will be administered orally and after a washout period of two weeks, each subject will receive 250mg chlorpropamide together with 250mg Ampicillin/250mg cloxacillin in 500mg Ampiclox Capsules.

Blood samples will be taken from each patient following treatments with chlorpropamide tablets alone and concurrent treatment with chlopropamide tablets and Ampiclox capsules as outlined above. Analysis of the samples will be carried out using Colorimetric method and also for the glucose level analysis. Results will be analysed by logarithmic method and then subjected to statistical analysis using student t - test for paired data where p - values less than 0.05 indicates a significant difference at 95% selected level of confidence.

On comparative basis, it is envisaged that results obtained from this study will benefit both diabetic patients and health professionals involved in the management of diabetes mellitus besides its contribution to knowledge.

CHAPTER THREE

MATERIALS AND METHOD

3.1 MATERIALS

3.1.1 TEST DRUGS

3.1.1.a Clorpropamide (Diabenese$^{(R)}$ tablets

BATCH NO. 52417003Y

Manufacturing date : December,1996

Expiry date : December,2000

Manufacturer: Pfizer Products Plc, Plot 16, A. Doherty Layout, Oregun, Lagos, Nigeria.

3.1.1.b Ampicillin / Cloxacillin (Ampiclox$^{®}$) Capsules

Batch no : 51864B

Manufacturing date : February,1997

Expiry date : February,2000

3.1.2. REFERENCE COMPOUNDS

Chlorpropamide standard powder (Pfizer international, N.Y,U.S.A).

Ampicillin/cloxacillin standard powder (Beecham, England).

3.1.3 SOLVENTS/REAGENTS

-Acetic acid (Glacial)-Analar grade (BDH Chemical Ltd., P. England).

-Acetic acid- analar grade (BDH Chemicals Ltd., P. .England).

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

-Brilliant green- BPC (Imperical Chem. Pharm. Ltd, England).

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

-Hydrochloric acid-Analar grade (BDH Chemicals, Ltd P, England).

-Heparin-Sigma Chemical Co.

-Methanol -Analar grade (BDH chem. Ltd P. England.

-Potassium dihydrogen phosphate

-Perchloric Acid-Analar grade (BDH chem. Ltd, P. England).

-Phenol-Analar grade (BDH chemicals Ltd, P. England).

-Sodium hydroxide pellets -(BDH chem. P. England).

-Sodium Nitrate – (May and Baker Ltd, D. England).

-Sodium carbonate anhydrous (GPR, Hopkin and William, E. England).

-Sodium acetate anhydrous (GPR, Hopikn and Williams E. England).

-Sodium chloride - Analar grade (BDH Chem. Ltd, P. England).

-Sulphuric Acid - Analar grade (BDH, Chem. Ltd, P. England)

-Iso-amylalcohol -Analar grade (BDH, Chem. Ltd P. England).

-Iso-amylacetate -Analar grade (BDH, Chem. P. England).

- 1-Fluoro-2,4- dinitrobenzene- Analar grade (BDH, Chem. Ltd P. England),

-4-aminophenazine- Analar grade (BDH, chem. Ltd ,P. England).

-Imidazole-mercury reagent

-Chromotropic acid Sodium salt

-Potassium carbonate

-Potassium curpric tartarate solution

-Potassium antimonate solution

-Nitric Acid

-Silver nitrate solution

-Phosphate buffer (pH 6.8)

3.1.4 EQUIPMENTS

-Centrifuge, Econospin model 120010 (Sorvail instruments, Republic of Germany, Biotechnology System Division, U.S A).

-Dissolution apparatus and accessories (Erweka apparatus, G . M B. H. 6056, housesentamm Kr, offenbuch. Main).

-KENT pH meter model 7060 with a combination pH electrode Model EL 887 (Electronic Instruments Ltd, Surrey, England).

-Sensitive Mettler Balance Model AE 240 (Mettler Instruments Ltd, Buckinghamshire, U.K).

-SP8 - 100 Ultraviolet spectrophotometer (PYE - Unicam Ltd, York street, Cambridge, England, CBI, 2PX).

- MAINS STUART Auto Vortex Mixer (Gallenkamp, England).

- Cannula

-Disintegration apparatus and assessories (Erweka apparatus, G.M.B.H, 6056, H. Kr, offenbuch. Main).

3.200 METHODS

3.2.1 IDENTIFICATION OF CHLORPROPAMIDE TABLETS

A 1.919 g quantity of the powdered tablets equivalent to 1g chlorpropamide was extracted with acetone. The extract was filtered and evaporated to dryness on a water bath. 0.1g of the residue obtained was boiled with 8ml of a 50% w/w sulphuric acid solution for 30 minutes under a reflux condenser. The solution was cooled and filtered. The melting point of the resulting precipitate was taken.

The U.V- Spectrum of the sample was run using sp 8 - 100 - ultraviolet spectrophotometer from 200-320nm.

A 0.1g of the residue was heated with 1g of anhydrous sodium carbonate at a dull red heat for ten minutes, it was allowed to cool. The residue was extracted with water and filtered. The filtrate was acidified with 2ml of Nitric acid and silver nitrate solution was then added. This procedures was repeated using the reference standard supplied by Pfizer internationals (B.P,1988).

3.2.2 CHEMICAL ASSAY OF CHLORPROPAMIDE TABLETS

Twenty chlorpropamide tablets were accurately weighed and the average weight determined. The tablets were powdered and a quantity (0.479g) of the powder equivalent to 0.25g of chlorpropamide was weighed and added to 40ml of methanol in a volumetric flask, and sonicated for 20 minutes. Sufficient methanol was added to make the volume up to 50ml. This was filtered and 5ml of the filtrate was diluted to 100ml with 0.1M hydrochloric acid. The absorbance of the resulting solution was measured at 232nm. The percentage content of chlorpropamide was calculated taken 598 as the percentage of A(1%, 1cm) at 232nm (B. P, 1988).

3.2.3. DISSOLUTION RATE AND DISINTEGRATION TESTS OF CHLORPROPAMIDE TABLETS.

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Dissolution Rate Test

One litre of physiological buffer (PH 6.8)prepared as outlined in the B. P (1988) was introduced into the vessel of the dissolution apparatus and immersed in a constant temperature water bath maintained at 37^0C. One of the chlorpropamide tablet was introduced into a dry basket assembled in the dissolution apparatus. The apparatus was operated at 100 rpm. After 45 minutes 10ml of the sample was withdraw from the solution half-way between the surface of the medium and the top of the rotating basket. The

sample was filtered at 37^{0}C and the amount of active ingredient present in the sample was determined by measuring the absorbance of the diluted sample at 232nm. The total content of chlorpropamide in the medium was calculated taken 469 as the value of A (1%, 1cm) at the maximum of 232nm. Complete operation, was repeated four times.

Disintegration Test

Erweka disintegration tablet machine was used for the disintegration test of chlorpropamide tablets. One tablet was introduced into each hole of the machine. The basket holes containing the tablets were lowered into the beaker containing 1000cm^3 of water maintained at 37.5^{0}C, and the apparatus was operated for twenty minutes. At the end of the operation, very negligible particles of the tablets were found within the basket mesh to indicate a complete disintegration of the tablets.

3.3.1. IDENTIFICATION OF AMPICILLIN/CLOXACILLIN IN AMPICLOX CAPSULES

0.038g of the powder, equivalent to 2mg of the drug was transferred into a test-tube. 2mg of Chromotropic acid sodium salt and 2ml of Sulphuric acid (96%w/w) were then added to the content of the test tube, which was then immersed in paraffin oil-bath at 150^{0}C. The solution was shaken and observed for colour change at an interval of 30 seconds.

Also, 20mg of powdered capsules was suspended in 1ml of water and a mixture of 6ml of water and 2ml of potassium curpritartarate solution was then added to the contents of the test tube.

In another qualitative test, 0.2g of the powdered capsule was dissolved in 2ml of water contained in a test-tube. No precipitate was observed by adding 2ml of 15% w/w solution

of potassium carbonate even after being heated to boiling. The test continued by adding 4ml potassium antimonate solution to the contents of the test-tube and then heated to boiling (B.P.1988). The same procedures was adopted using the reference standard of Ampicillin/ Cloxacillin powder supplied by Beecham Laboratories, England.

3.3.2 CHEMICAL ASSAY OF AMPICILLIN/CLOXACILLIN IN AMPICLOX CAPSULES

CLOXACILLIN:

From the contents of 20 capsules, 0.1g of the powdered drug was accurately weighed and added to 70ml of water; this was shaken for 15minutes with sufficient water to produce 500ml. 25ml of the solution was diluted to 100ml with water. 2ml of the resulting solution was placed in separate stoppered tubes. To one of the tube, 10ml of imidazole - mercury reagent was added and mixed. The tube was then tightly covered and heated on water bath at 60°C for 25 minutes, with occasional swirling. The solution was cooled rapidly to 20°C. 10ml of water was added to the second tube and the absorbance were measured at 346nm, with which the contents of Cloxacillin was determined with reference to a calibration curve.

AMPICILLIN:

Using different sets of capsules 0.17g of drug was dissolved in sufficient water to produce 500ml. 10ml of Boric buffer pH 9.0 and 1ml of acetic anhydride solution were added to 10ml of the resulting solution in a 100ml flask with sufficient water to produce 100ml. 2ml of the resulting solution was placed in separate stoppered tubes. To one of the tube, 10ml of imidazole-mercury reagent was added and mixed. The tube was tightly covered, immersed in a water bath at 60°C for 25 minutes with occasional swirling. From the water bath, the solution was cooled rapidly to 20°C. 10ml of water was added to the second tube

and the absorbances of the two solutions were measured at 325nm with which the content of Ampicillin was determined with reference to a calibration curve (B.P. 1988).

3.3.3 DISSOLUTION RATE AND DISINTEGRATION TESTS OF AMPICLOX CAPSULES.

Using the same apparatus as in the case of chlorpropamide tablets and following the procedure described therein, the dissolution rate test was carried out for Ampiclox capsule. Using different sets of the same batch of Ampiclox capsules, the rates for each component of the capsules were determined in accordance to B.P 1988 specification. The operations were repeated four times in each case.

Employing the same disintegration apparatus as used for chlorpropamide tablets, disintegration test of the Ampiclox capsules was carried out using the same procedure described for the tablets according to B.P 1988 method, and repeated four times to obtain the mean.

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3.400 IN VIVO ABSORPTION STUDY

3.4.1 SUBJECTS

Seven type II diabetes mellitus patients, (three females and four males) freshly diagnosed, participated in the study. The criteria for selecting the participants were based on the National Diabetes Data Group's recommendation of 1979 and the selection was done by a practising clinician.

None of the participants was below the age of 35 years. Age range of 35-52 years (mean ± S.D 45 ± 6.5 years) with height range of 1,65 - 1.78m, (Mean ± S.D 1.72 ± 0.10M), and they weighed between 55 - 70kg, (Mean ± S.D 62.50 ± 7.50kg).

3.4.2 STUDY PROTOCOL

The protocol of the study was explained to each of the patients and an informed consent was obtained. They were not allowed any drug including alcohol, two weeks to the study, and each patient fasted overnight before taking part.

On the day of each test, prior to oral ingestion of the drugs, each subject attended the trial room and were allowed to relax. Each patient received the following two treatments at an interval of two weeks using cross over design. First, each received one 250mg chlorpropamide tablet administered in a fasting state; Secondly, each received one 250mg cholrpropamide tablet with one 500mg Ampiclox capsule concomitantly administered orally in a fasting state, with about 200ml of water. Prior to each dosing schedule, 5ml of blood were taken through a cannula placed in an antecubital vein. During each treatment, the drugs were taken on an empty stomach, and the patient remained in such fasting state for 4 hours after the administration of drugs. At the end of the 4 hours, each patient was given cooked "jollof" beans meal (350g) and further blood sampling continued. The blood samples taken were at times 0, 0.5, 1,2,4,6,8,24,48,72,144, and 168 hours intervals. The blood samples collected were centrifuged for ten minutes at 100 revolutions per minutes (rpm), and the plasma transferred into a bijoux bottle with a Pasteur pipette. These stoppered bottles were then stored in the freezer at - 20^{0}C for analysis. Glucose levels were determined for the samples taken at an interval of 0, 0.5, 1,2,4,6, and 8 hours.

3.5.0 DRUG LEVEL ANALYSIS

3.5.1 PREPARATION OF STANDARD SOLUTIONS

Stock solutions of chlorpropamide were always freshly prepared. 10mg chlorpropamide powder was weighed and dissolved in 10ml of methanol. This was transferred into a 100ml volumetric flask and diluted to volume with methanol to obtain standard chlorpropamide solution (100μg/ml). 0.05, 0.1, 0.25, 0.4 and 0.5ml of the standard solution were transferred to 1ml blank plasma and treated. Various concentration (5-50μg) were obtained.

3.5.2 COEFFICIENT OF VARIATION

1ml of drug free human plasma was placed in different volumetric flasks. These were spiked differently with 0.01, 0.02, 0.03ml of 1mg/ml stock chlorpropamide solution.
The Coefficient of Variation (CV) at three different concentrations of plasma for six samples at each level were studied for within assay and day to day assay.

3.5.3. PROCEDURE OF PLASMA ANALYSIS

1ml of phosphate buffer (pH 6.8) was added to 0.5ml of plasma in a test tube. This was extracted with 10ml 0.5% of Isoamylalcohol in hexane. The resulting solution was centrifuged and allowed to separate. 7ml of hexane extract was then transferred into another test tube containing 1ml of 1N NaOH with pipette. The content of this test tube was shaken while the hexane layer was removed. 1ml of 2NHCl and 3ml of pentylacetate were added to the basic layer left. This was shaken for 2 minutes and then centrifuged. 2ml of the pentylacetate extract was pipetted out into a test-tube containing 1ml of 0.05% Dinitrofluorobenzene solution. This test tube was lightly stoppered and heated for 1 hour

at 125^{0}c on a constant temperature paraffin oil bath. After cooling, the absorbance was read at 420nm against pentylacetate as the blank.

The concentration of chlorpropamide was calculated by reference to the calibration curve prepared (Matin and Rowland, 1973).

3.6.0 PLASMA GLUCOSE ANALYSIS

3.6.1 PREPARATION OF REAGENTS

18.02g of glucose was weighed and dissolved in 100ml of benzoic acid saturated with distilled water to produce stock glucose solution.

1ml of stock glucose solution was taken and diluted to 10ml with benzoic acid saturated with distilled water.

Sodium acetate acetic acid buffer (pH 6.8) was prepared by dissolving 12.88g of sodium acetate (anhydrous) in 200ml distilled water, while 320ml of glacial acetic acid was added. Then, 15mg peroxidase, 300mg of 4-aminophenazone and 15ml of glucose oxidase (Rercozyme) were mixed in a 1000ml volumetric flask. The solution was made up to volume with the prepared buffer and was kept stable for 8 weeks at 4^{0}C, producing colour reagent.

Also, 1.94g of colourless phenol crystals was weighed out and dissolved in 1000ml of 0.9% sodium chloride, producing 21.25mol/l phenol in 0.15M sodium chloride solution.

3.6.2 PROCEDURE

The plasma, the standard glucose, the distilled water, phenol reagent and coloured reagent were all mixed together in the order shown in table 3.1 in three sets of tubes labelled as test, standard and blank.

Reagent	Test (ml)	standard (ml)	Blank (ml)
Plasma	0.05	-	-
standard (10mmol/l)	-	0.05	-
Distilled water	-	-	0.05
Phenol reagent	2.50	2.50	2.50
Coloured reagent	2.50	2.50	2.50

Table 3.1 Showing the orders involved in plasma glucose analysis procedure employed.

The tubes were then incubated at 37^0C for 20 minutes and left to cool at room temperature for 45 minutes. The absorbances were measured at 520nm using the reagent blank. The amount of glucose in the samples were calculated using the following formulae (Bello, 1998).

$$\text{Glucose (mmol/l)} = \frac{\text{Absorbance of test - blank}}{\text{Absorbance of standard-blank}} \times \frac{10}{1}$$

3.700 DATA ANALYSIS

Following single Oral dose of chlorpropamide, the pharmacokinetic parameters were determined from the semi-logarithmic plots of concentration versus time data. Assuming a three –compartment model involving a compartment for absorption. That is, with an oral dose, an additional absorption compartment is incorporated into the model to represent

the volume (V_a) from which absorption occurs at a first-order rate (Figure 1.9). The equation for this model which include absorption factor is given below:

Where K at is the absorption rate constant, and are the distribution and elimination rate constants respectively. A' and B' are intercept terms and depends on the rate and completeness of absorption (Paxton, 1981).

The following parameters were obtained from each plot:

- Lag time (time at which absorption commenced)
- C_{max} (maximum concentration attained by the drug)
- T_{max} (time taken to reach the maximum concentration)
- $t_{½\alpha}$ (absorption half-life)
- $K\alpha$ (Rate constant of absorption)
- $t_{½\beta}$ (Elimination half-life)
- $K\beta$ (rate constant of elimination).
- AUC (Area under the curve from $0 \rightarrow \infty$).

Other parameters were calculated (Paxton, 1981) based on the derived parameters from the plots, and these include:

- Volume of distribution (V_d) $= \frac{F.D}{AUC} \times K\beta.$

F= fraction of the dose absorbed (assumed as 1).

D = dose of the drug administered.

AUC = Area under the curve

$K\beta$ = Elimination rate constant.

The Area under curve was calculated by trapezoidal rule (Gibaldi and Perrier,1975). The AUC derived, is from 0 to infinity (i.e. $AUC_{0\to\infty}$), consisting of $AUC_{0\to 168}$ hours using trapezoidal rule and $AUC_{168\to\infty}$ hr obtained by dividing the last concentration in the plasma level-time plot, by the elimination rate constant of the slow phase.

Therefore, $AUC_{0\to\infty} = AUC_{0\to 168}hrs + AUC_{168\to\infty}hr.$

- Clearance (cl) $= K_\beta \times V_d.$

- Absorption rate (K_α) $= \frac{0.693}{t_{1/2\alpha}}$

-Elimination rate (K_β) $= \frac{0.693}{t_{1/2\beta}}$

(Paxton,1981).

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All the results were expressed as standard error of mean (SEM). They were analysed for statistical significance using student t - test for paired data. Statistical analysis were carried out with 'prism graph pad" computer programme. P values less than 0.05 (i.e., $P<0.05$) were considered of statistical significance while $P>0.05$ were considered not to be statistically significant, (Haper,1984 and Akuezuilo,1993).

CHAPTER FOUR

RESULTS

4.1. a IDENTIFICATION TESTS OF CHLORPROPAMIDE TABLETS (B.P. 1988)

The extracted drug treated with anhydrous sodium carbonate and treated with silver nitrate solution produced a white precipitate. This reaction is characteristic of chlorpropamide (B.P 1988).

The melting point of recrystallized product of the extracted chlorpropamide was 140^{0}C.

The scan of chlorpropamide gave a maximum at 232 nm as shown in Figure 4.1.

4.1. b CHEMICAL ASSAY OF CHLORPROPAMIDE TABLETS

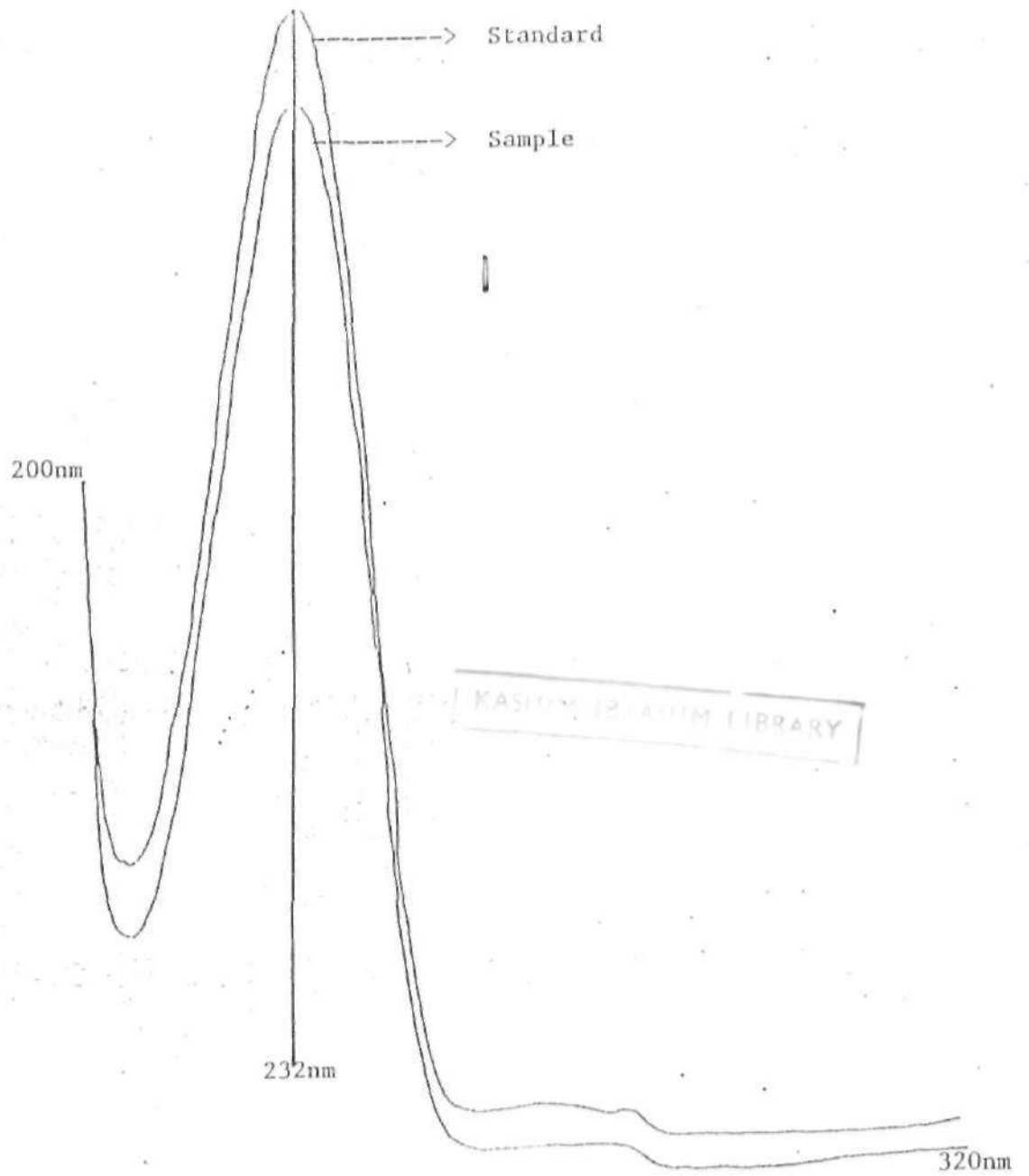
The chlorpropamide tablets analysed contained 99.40% of the active ingredient Using A(1%, 1cm) 598 value at 232nm. Thus, the sample complied with the official specification of drug content (92.5 - 107.5%), B.P. 1988.

The content values and the percentage composition are shown in Table 4.1.

4.1. c DISINTEGRATION AND DISSOLUTION RATE TESTS OF CHLORPROPAMIDE TABLETS.

Complete disintegration of chlorpropamide tablets occured in 30 minutes in the disintegration medium. The result complied with the official standard of between 25 - 40 minutes, (B.P. 1988).

The percentage of the active ingredient released in solution during the dissolution rate tests are shown in Table 4.3. The mean amount released was 99.99%. This result complied with the official specification of not less than 70% after 45 minutes, (B.P 988).



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FIG. 4.1 Absorption spectra for Chlorpropamide Standard Powder and Tablet.

Table 4.1 Quantitative determination of chlorpropamide Tablets Active Content (B.P. 1988).

Sample No	Drug content (mg)	% Content
1	248.44	99.38
2	247.79	99.12
3	248.21	99.30
4	249.10	99.64
Mean	248.39	99.40

Table 4.2 Dissolution rate tests of chlorpropamide tablets (B.P, 1988)

Sample No.	Amount released (mg)	% Released
1	235.70	99.00
2	238.95	100.37
3	240.08	100.84
4	237.56	99.76
Mean	238.07	99.99

4.2. a IDENTIFICATION TESTS OF AMPICILLIN / CLOXACILLIN IN AMPICLOX CAPSULE (B.P 1988).

Chemical test with Chromotropic acid sodium salt produced a purple colour after two (2) minutes confirming the presence of Ampicillin (B.P. 1988).

Chemical test with potassium cupritartarate solution produced an instant violet colour characteristic of Ampicillin.

A white dense precipitate was observed in the chemical test involving potassium carbonate and potassium antimonate solution, confirming the presence of cloxacillin in the sample (B.P. 1988).

4.2. b CHEMICAL ASSAY OF AMPICILLIN / CLOXACILLIN IN AMPICLOX CAPSULES

The Ampiclox capsules assayed contained 97.65% of Ampicillin and 98.20% of cloxacillin. The sample complied with the official specification of 92.5% - 107.5% for each of the active ingredients (B.P, 1988). The values of the contents and their percentage composition calculated for each of the ingredient are shown in Table 4.2.

4.2. c DISINTEGRATION AND DISSOLUTION RATE TESTS OF AMPICLOX CAPSULES

Complete disintegration of Ampiclox capsule was observed within 5 minutes in the medium. The result is in agreement with official specification of not more than 10 minutes for gelatinous capsules, (B.P 1988).

From the dissolution rate test of Ampiclox capsules, mean value of Ampicillin was found to be 98.56% while that of cloxacillin was found to be 99.25% released before 45 minutes. These results complied with official specification for each active ingredient, (B.P 1988). Table 4.4 shows detail result.

Table 4.3 Quantitative determination Of Ampicillin / Cloxacillin (Ampiclox ®) Capsules active content (B.P.1988).

	Ampicillin (mg)		Cloxacillin (mg)	
Sample	Drug content	% content	Drug content	% content
1	248.43	99.37	249.54	99.82
2	249.15	99.66	249.12	99.65
3	248.75	99.50	248.85	99.54
4	248.90	99.56	249.43	99.77
Mean	248.80	99.52	249.24	99.70

Table 4.4 Dissolution rate test of Ampicillin/Cloxacillin in Ampiclox Capsules (B.P, 1988)

	Ampicillin (mg)		Cloxacillin (mg)	
Sample No.	Content released	% released	Content released	% released
1	245.50	98.20	248.22	99.29
2	247.27	98.90	247.80	99.12
3	245.86	98.34	248.35	99.34
4	246.98	98.80	248.10	99.24
Mean	246.40	98.56	248.12	99.25

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4.3.0 IN VIVO ABSORPTION TESTS OF CHLORPROPAMIDE TABLETS

Linear calibration curve of chlorpropamide with good correlation coefficient (r = 0.999) obtained is shown in Figure 4.2.

Table 4.5 show the precision with which the plasma concentrations of chlorpropamide were determined within and day to day run.

Mean recovery of chlorpropamide from seven diabetic patients after Oral administration of 250mg dose is summarized in Table 4.6 at the sampling time intervals indicated. A: represents mean values obtained after chlorpropamide administration while B: represents mean values obtained after concomitant administration of 250mg chlorpropamide tablets with 500mg Ampiclox capsules. The difference between the two sets of values can be observed in Table 4.6 with their standard mean error(SEM).

The plasma concentration versus time curves of chlorpropamide from the seven diabetic patients are shown in Figure 4.3 for both chlorpropamide alone (A) and concurrently with 500mg Ampiclox capsules (B).

The mean pharmacokinetic parameters of chlorpropamide tablets for seven diabetic patients are shown in Table 4.7 following the ingestion of 250mg chlorpropamide tablets (A) and 250mg chlorpropamide tablets concurrently with 500mg Ampiclox capsules (B). There were significant differences in the values of C_{max}, AUC, V_d, $t_{½el}$, and clearance (cl). Other parameters (T_{max}, $t_{½ab}$, K_{ab}) were not significantly altered. Comparing this result with that obtained following the study involving healthy volunteers (Adebosin, 1998), Ampiclox capsules exhibit similar influence on the pharmacokinetics of chlorpropamide.

The mean plasma glucose concentrations of seven diabetic patients following 250mg chlorpropamide ingestion (A) and when the same dose were concurrently administered with 500mg Ampiclox capsule (B) are shown in Table 4.8. From the results in Table 4.8, the significantly lower mean plasma glucose concentrations in the presence of Ampiclox capsules can be observed among the seven diabetic patients. Mean plasma glucose concentrations versus time curves following 250mg chlorpropamide ingestion (A) and 500mg Ampiclox capsules concurrently administered with the same dose of chlorpropamide tablets (B) are shown in Figure 4.4. From the figure, decreased plasma glucose concentrations were observed following concurrent administration of Ampiclox capsules with chlorpropamide tablets compared with the values following the administration of chlorpropamide tablets alone.

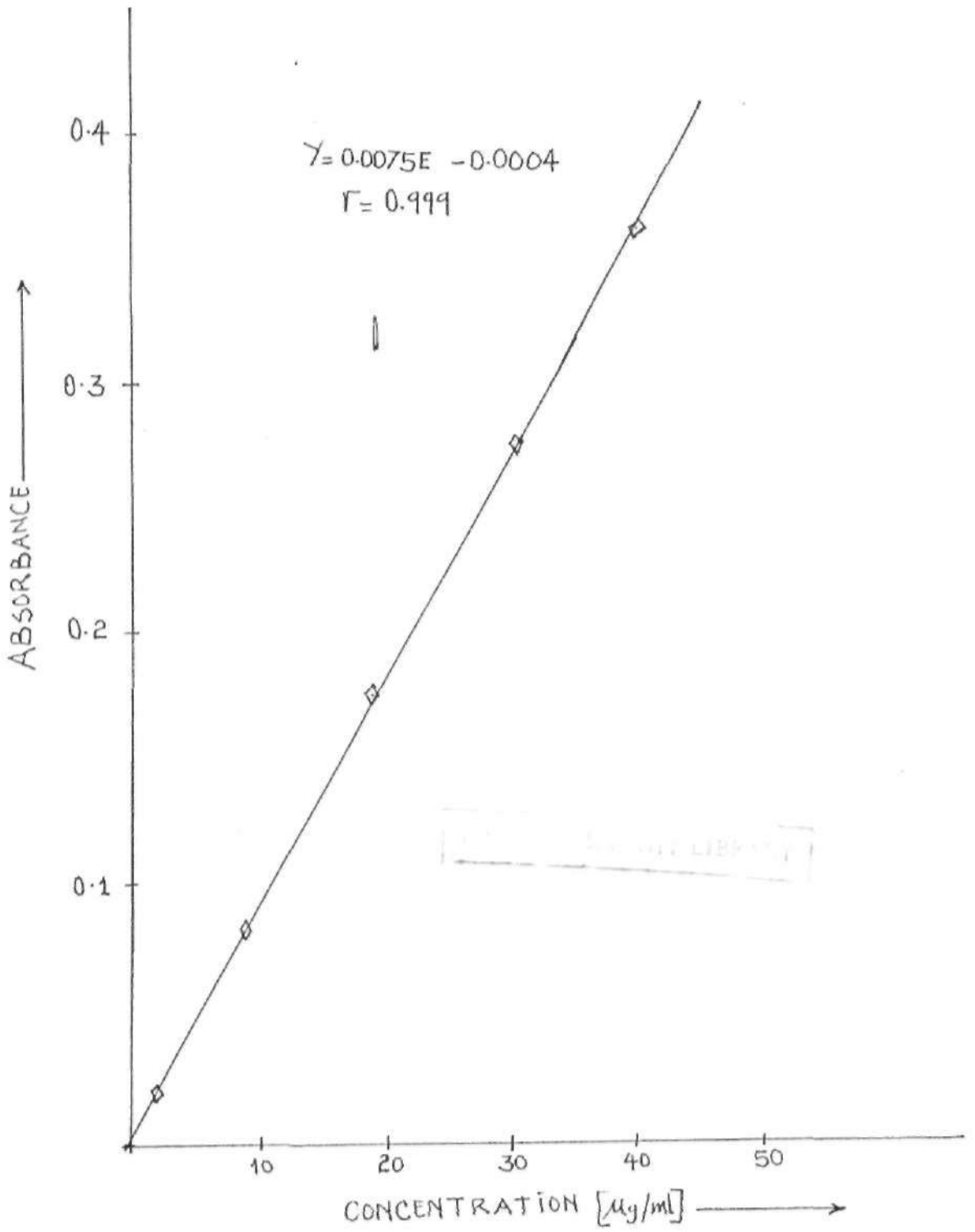


Fig.4.2. Standard curve for the Assay of Chlorpropamide obtained from spiked plasma.

Table 4.5 Day to day and within - day precision of plasma chlorpropamide determination

Biological fluid (plasma)	Concentration (μg/ml)	% Coefficient of Variance (C. V)	N
Within-run	10	4.80	6
	20	3.60	6
	30	2.50	6
Day to day	10	4.50	6
	20	3.37	6
	30	1.88	6

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Table 4.6 *Mean (S.E.M) plasma concentration of chlorpropamide in 7 (seven)* patients with type II diabetes mellitus following Oral administration of 250mg chlorpropamide tablets (A) and concomitantly with 500mg of Ampiclox capsules (B).

Time hrs	Concentration (μg/ml) ±S.E.M	Concentration (μg/ml) ±S.E.M
	A	B
0.5	20.137 ± 8.76	24.16 ± 10.50
1	25.80 ± 4.22	29.22 ± 6.74
2	37.70 ± 9.52	47.15 ± 11.87
4	23.22 ± 6.43	28.35 ± 7.08
6	19.19 ± 4.55	23.90 ± 5.53
8	12.88 ± 5.96	16.87 ± 4.33
24	9.97 ± 5.31	14.38 ± 5.12
48	7.10 ± 4.55	10.57 ± 4.43
72	4.33 ± 2.15	5.66 ± 1.95
144	3.02 ± 2.60	4.14 ± 2.56
168	1.92 ± 0.79	1.74 ± 0.60

S.E.M = standard Error Mean

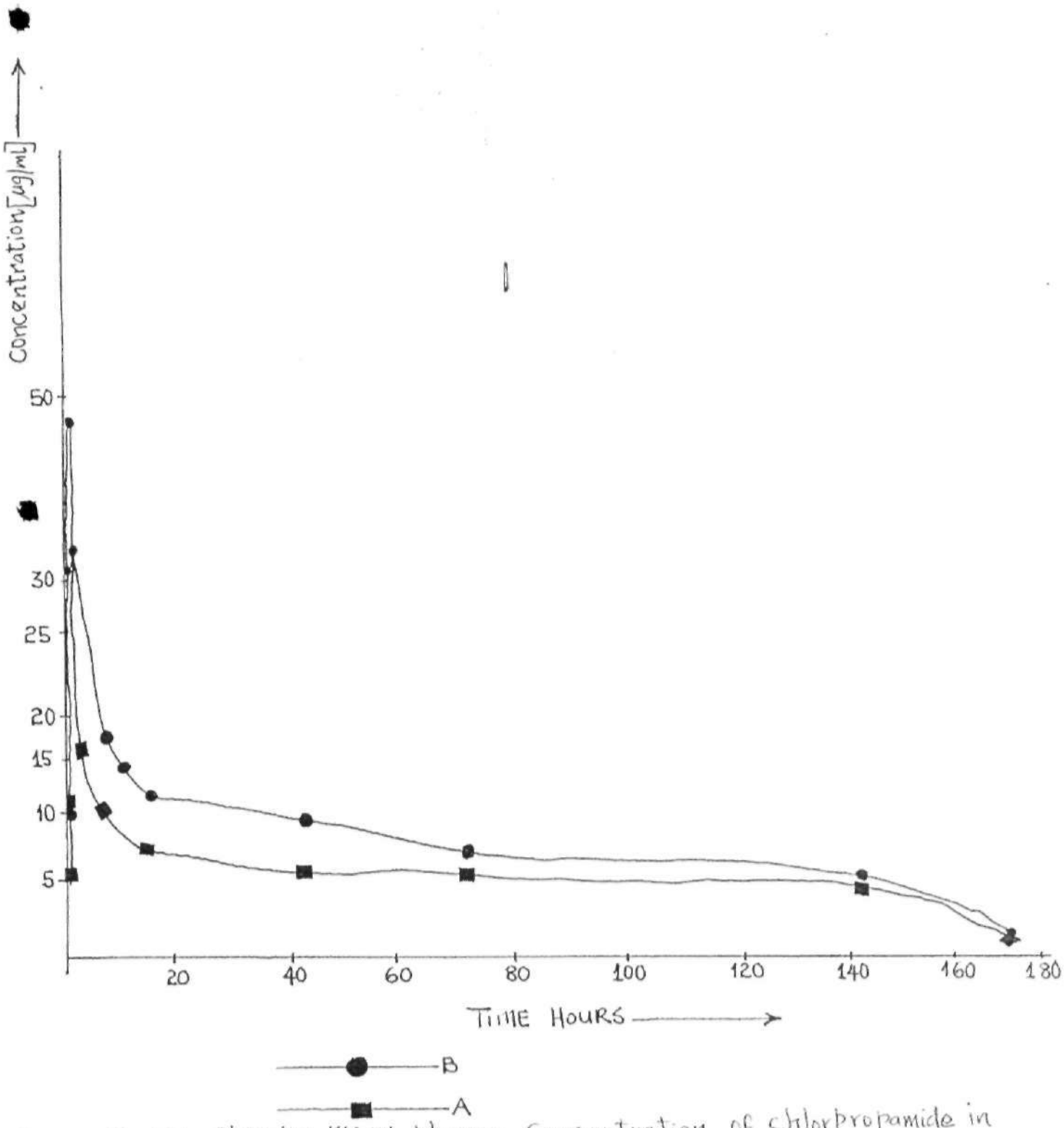


Fig. 4.3. Curves showing mean plasma concentration of chlorpropamide in seven diabetic patients following Oral administration of 250mg chlorpropamide tablets (A) and Concomitantly with 500mg Ampiclox Capsules (B) Versus time.

TABLE 4.7 Mean (± S.E.M) of pharmacokinetic parameters derived from plasma concentration of chlorpropamide in 7 (Seven) patients with diabetes mellitus (type II) following 250mg chlorpropamide tablets (A) and 500mg Ampiclox capsules (B) concomitantly administered.

Mean Pharmacokinetic Parameters ± S.E.M.	A	B	T – test (paired)
Lag-time (hr)	0.53 ± 0.052	0.74 ± 0.083	P<0.05
Maximum concentration C_{max} (μg/ml)	38.09 ± 8.92	47.33 ± 11.85	P<0.05
Time to peak concentration T_{max} (hr)	2.50 ± 0.39	2.80 ± 0.077	P>0.05
Absorption half-life $t½_{ab}$, (hr)	0.64 ± 0.097	0.67 ± 0.19	P>0.05
Elimination half-life $t½_{el}$, (hr)	29.14 ± 4.66	36.27 ± 3.99	P<0.05
Area under curve $AUC_{o\to\infty}$(μ g/ml/hr)	1095.26 ± 178.95	1445.94 ± 61.12	P<0.05
Absorption rate constant $K_{ab\ or\ \alpha}$, (h^{-1})	1.131 ± 0.090	1.117 ± 0.31	P>0.05
Elimination rate constant $K_{el\ or\ \beta}$, (h^{-1})	0.0242 ± 0.0033	0.0193 ± 0.0019	P<0.05
Volume of distribution V_d, (l)	10.331 ± 3.21	9.30 ± 2.00	P<0.05
Clearance cl (l/hr)	0.25 ± 0.087	0.178 ± 0.032	P<0.05

S.E.M = Standard Error Mean

TABLE 4.8 Mean (± S.E.M) Plasma Glucose Concentration in Seven Patients with type (II) diabetes Mellitus following Oral administration of 250mg Chlorpropamide tablets (A) and Concomitantly with 500mg Ampiclox Capsules (B).

Time (hrs)	Plasma glucose conc. [m. mol/l] ± S.E.M		T – Test *(paired)*
0	15.36 ± 5.91	12.58 ± 4.47	P<0.05
0.5	16.03 ± 7.15	12.50 ± 5.28	P<0.05
1	14.33 ± 4.57	11.61 ± 4.18	P<0.05
2	12.48 ± 2.84	8.71 ± 3.19	P<0.05
4	11.04 ± 3.07	9.40 ± 2.68	P<0.05
6	15.68 ± 5.63	10.84 ± 1.77	P<0.05
8	18.54 ± 5.67	11.46 ± 1.30	P<0.05

S.E.M = Standard Error Mean

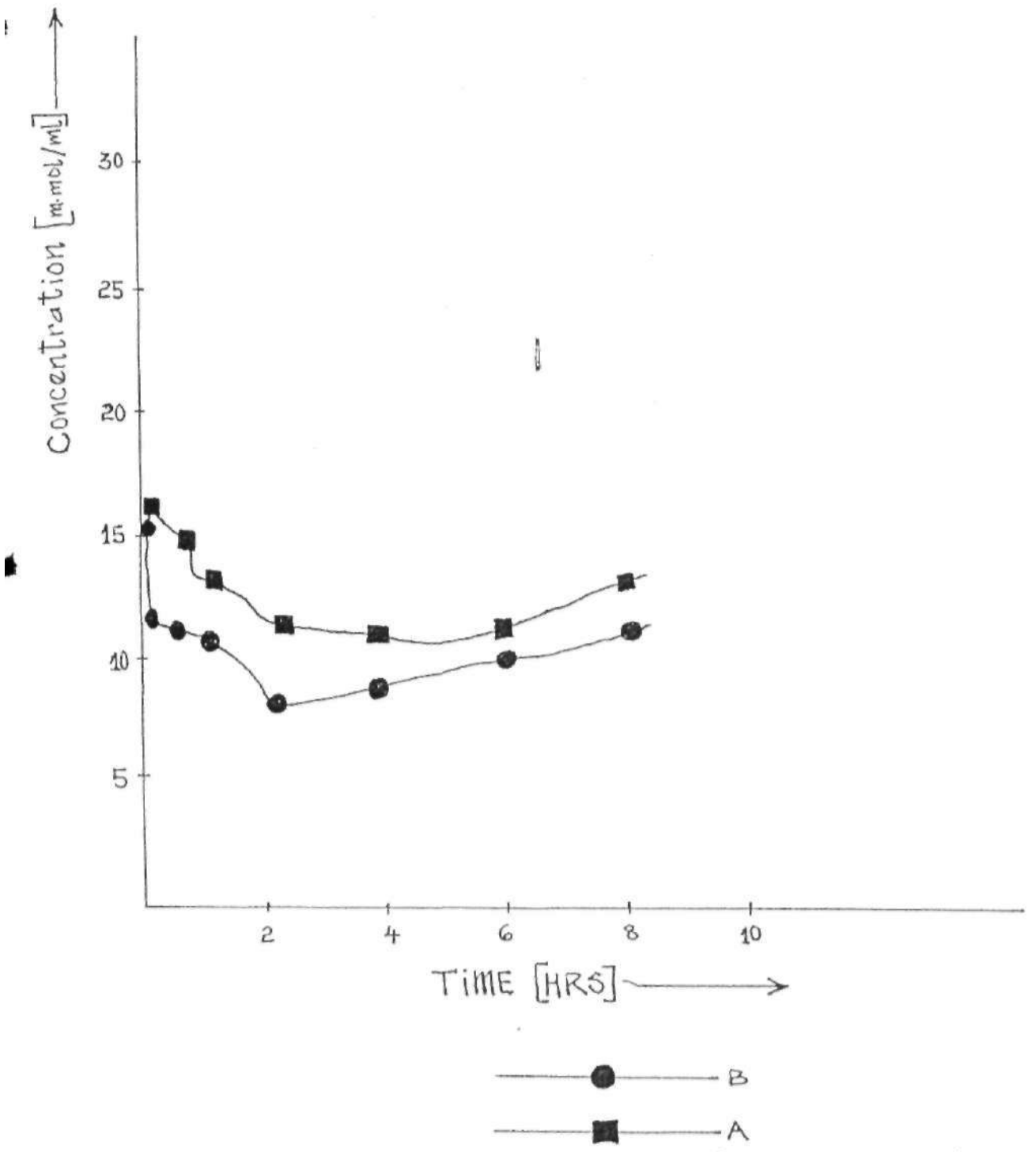


Fig. 4.4. Mean glucose concentration in seven Diabetic Patients following Oral administration of 250mg Chlorpropamide tablets (A) and Concomitantly with 500mg Ampiclox Capsules (B).

CHAPTER FIVE

DISCUSSION

5.10 QUALITY CONTROL OF CHLORPROPAMIDE TABLETS AND AMPICLOX CAPSULES

Before the commencement of the in vivo study, chlorpropamide tablets and Ampiclox capsules were subjected to quality control Tests (Identification, assay, disinlegration and dissolution rate tests).

The World Health Organisation (WHO) have always maintained the rationale for such tests and the necessity for such tests was strongly emphasised after the Bangladesh epidemic (Hannif et al, 1995).

Chlorpropamide tablets and Ampiclox Capsules evaluated were found to contain chlorpropamide and Ampicillin/ Cloxacillin respectively.

From the assay of chlorpropamide tablets, it was found to contain 99.40% of the active ingredient which complied with the official specification of between 92.50% to 107.50% (B.P, 1988).

Chemical assay of Ampiclox Capsules yielded 97.65% Ampicillin and 98.20% cloxacillin in the Capsules, and this conformed with Official specification in accordance with B.P, 1988.

Dissolution rate tests of chlorpropamide tablets released 99.99% chlorpropamide from the tablets within 45 minutes into the dissolution medium. This result conformed with

B.P, 1988 standard that required 70% of the drug to be released into solution in 45 minutes. Values of 98.56% Ampicillin and 99.25% Cloxacillin were released into the dissolution medium from Ampiclox Capsules within the sampling times of 45 minutes (B.P, 1988). This result conformed with the stated amount in official monographs. While it took chlorpropamide tablets 30 minutes before complete disintegration could take place, if took Ampiclox capsules only 10 minutes. From the results of these studies, the two batches of the drugs (chlorpropamide tablets and Ampiclox capsules) complied with the official specification of B.P, 1988.

Although this study does not aim at comparing the dissolution rates of the tablets and capsules, it was however observed that in solution, the dissolution and disintegration rates of Ampiclox capsules were found to be far higher than that of the chlorpropamide tablets. The reason is not far from the fact that while Ampiclox capsules contained powdered granules, chlorpropamide tablets are compressed tablets with binders, and disintegrating agents besides other excipients which could delay its rate of disintegration and dissolution.

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5.20 IN VIVO ABSORPTION STUDY

From the analysis of plasma concentration of chlorpropamide following Oral ingestion of 250mg chlorpropamide tablets by the seven diabetes mellitus patients, the influence of Ampiclox capsules on the pharmacokinetics of chlorpropamide was clearly revealed. Looking at the values of the Pharmacokinetic parameters (Table 4.6), there were significant ($P < 0.05$, using student t - test for paired data) differences in the C_{max}, AUC, V_d, $t\frac{1}{2}_{el}$, K_{el}, Cl and lag - time while other parameters were not significantly different in their values, ($P > 0.05$).

The amount of drug absorbed (AUC), the C_{max} and the elimination half - life ($t½_{el}$) were all significantly increased ($P < 0.05$) following treatment with Ampiclox capsules, while the elimination rate constant (K_{el}), the clearance rate (Cl), and the apparent volume of distribution (V_d) were all significantly reduced ($P < 0.05$) in the presence of Ampiclox capsules. Following treatment with Ampiclox capsules, Significant decrease ($P < 0.05$) in the plasma glucose levels were observed among the diabetic patients. However, the difference in the values of k_b, $t½_{ab}$, and T_{max} of chlorpropamide observed following Ampiclox treatment were not statistically significant ($p > 0.05$).

The significant increase ($P < 0.05$) in the C_{max} of chlorpropamide following Ampiclox treatment was confirmed by the value of AUC in table 4.6. There was a significant increase ($P < 0.05$) in the AUC value of chlorpropamide from 1,095 ± 178.95 to 1,445.90 ± 61.12 μg/ml/hr in the presence of Ampiclox capsules. AUC being the measure of the quantity of drug absorbed (bioavialability) in the body (Paxton, 1981), the high AUC value of chlorpropamide in the presence of Ampiclox capsules is most likely responsible for the decreased plasma glucose concentration following treatment with the two drugs.

Other pharmacokinetic parameters of chlorpropamide that were also significantly affected in the study include half - life of elimination ($t½_{el}$), clearance (cl), and the apparent volume of distribution (V_d).

Half-life of elimination being the time taken for the post - absorption plasma concentration of the drug to be reduced by 50% of its original concentration (Wagner, 1967), ($t½_{el}$) of chlorpropamide in this in vivo study showed a significant increase ($P <$

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0.05) from 29.14 ± 4.66 to 36.27 ± 3.99 hours in the presence of Ampiclox capsules. This means, an increased elimination time of chlorpropamide and consequently a decreased elimination rate of chlorpropamide. The significant decrease ($p < 0.05$) in the value of K_{el} (elimination rate constant) of chlorpropamide from 0.0242 ± 0.0033 to 0.0193 ± 0.0019 hours in the presence of Ampiclox capsules as observed in the study (Table 4.6) is a confirmation of this fact. The significant increase in the $t½_{el}$ must have arisen from a decreased clearance of the drug, chlorpropamide.

From the result shown in table 4.6, there was a significant decrease ($p < 0.05$) in the clearance (a direct index of drug elimination, Paxton, 1981) of chlorpropamide from 0.25 ± 0.087 to 0.178 ± 0.032l/hr in the presence of Ampiclox capsules. The observed decrease in clearance rate is an indication of decreased elimination of chlorpropamide in the presence of Ampiclox capsules. The effect on clearance suggest that this drug, Ampiclox most probably must have inhibited the hepatic enzyme involved in the metabolism of cholrpropamide. The observed influence of Ampiclox capsules on the pharmacokinetics of chlorpropamide is as a result of pharmacokinetic interraction between the two drugs.

Drug interactions could be pharmacokinetic or pharmacodynamic (Balant, 1981). While pharmacokinetic interractions are those which can affect the process by which drugs are absorbed, distributed, metabolised and excreted; pharmacodynamic interractions involve changes in the response of the patient to a drug combination without alterations in the serum concentration or pharmacokinetics of the object drug (Goodman and Gilman, 1990). Peak blood level of Ampicillin/Cloxacillian appear in 1-3 hours and detectable amounts persist for about 6 hours (Hills, 1970). Peak blood levels of

chlorpropamide appear within two hours with t½el of about 36 hours (Burgen and Mitchell, 1985).

Both drugs are tightly bound to plasma protein, and there exist the possibility of common binding sites (Ptalsky, 1980 and Hills, 1970). Competition for binding sites when both drugs are concurrently administered may lead to the displacement of chlorpropamide from its binding site. This may be the most likely reason for the high bioavailability of chlorpropamide observed by a significantly increased value of AUC and C_{max} following concomitant administration of these drugs. Since activity, that is, biological activity of any drug is usually a function of the fraction of the unbound drug, high level of chlorpropamide and thus, an enhanced activity in the presence of Ampiclox capsules must have accounted for the significant decrease in plasma glucose level observed following their concurrent administration.

Greater percentage of both drugs are excreted by the renal - tubular secretion mechanism (Burgen and Mitchell, 1985). Thus, there is a possibility that both drugs competes for the same renal - tubular secretion. When this happens, there is every tendency that Ampicillin/Cloxacillin known to be rapidly eliminated from the system will be secreted first in preference to chlorpropamide when they are co-administered. By such mechanism, the clearance rate of chlorpropamide will be reduced as well as its volume of distribution as shown in table 4.6. In this study, there was a significant decrease ($p < 0.05$) in the volume of distribution (V_d) of chlorpropamide from 10.331± 3.21 to 9.30 ± 2.00 litres in the presence of Ampiclox capsules which agrees with the result of Adebosin, 1998.

It can thus be said that Ampiclox capsules have an influence on the pharmacokinetics of chlorpropamide when they are concurrently administered, revealing a pharmacokinetic interraction between these drugs.

The significant increase ($p < 0.05$) in lag - time of chlorpropamide following treatment with Ampiclox capsules however did not affect its overall high bioavailability nor its mean plasma concentration (C_{max}). Delayed absorption is also seen from the increased half- life of absorption (t½ab) and the decreased absorption rate constant (K_{ab}). The greater the total amount of drug absorbed the greater the AUC will be and the greater is the bioavailability (Abdel-Monem & Henkel, 1978). Absorption may be rapid (large K_a) with a low bioavailability or, conversely, the absorption may be slow (small K_a) with a bioavailability near Unity.

Drugs have their own dissociation constant (pk_a) which describes the pH of the solution in which the drug is 50% ionised. Thus, the pk_a of the drug and the pH of the medium in which it is dissolved, govern the extent of ionisation which is a key factor determining the available drug (Paxton, 1981).

Chlorpropamide with an amide side chain has a pk_a of 7.8 and penicillins on the other hand can be classified as monobasic and Zwitterionic; Ampicillin with side chain amino group has pk_a of 7.4 and Cloxacillin has a pk_a of 7.2 (Olaniyi, 1989).

The pk_a range of both drugs thus favours their absorption, distribution,and elimination. This is because drugs that are substantially unionised at the mean pH of 7.4O ± 0.04 will be able to diffuse across membrane boundaries and becomes widely distributed (Paxton, 1981).

In this study, the interaction is clearly seen as pharmacokinetic and pharmacodynamic. Ampiclox must have competitively displaced chlorpropamide from the binding site, thus giving rise to a high bioavailability (AUC) and C_{max} of chlorpropamide compared to treatment with chlorpropamide alone. The Concurrent administration of Ampiclox capsules with the long - acting chlorpropamide may also affect the renal - tubular secretion of the later drug thereby slowing down its tubular - secretion leading to its reduced clearance rate. Through any of these mechanisms or by the combination of the two, the overall result is an increased bioavailability of chlorpropamide with longer half - life of elimination in the presence of Ampiclox capsules.

5.30 CONCLUSION

From this study, the quality of the drug products evaluated were confirmed to satisfy Good manufacturing practice standard certifying them safe for patient's consumption.

It has been established in the course of this study that Ampiclox capsules have an influence on the pharmacokinetics of chlorpropamide and strongly suggest a pharmacokinetics interraction between the two drugs. The bioavailability of chlorpropamide or the amount of chlorpropamide absorbed (AUC), its elimination half-life ($t½_{el}$), and the lag - time were all significantly increased ($p< 0.05$, using student t-test for paired data) when concurrently administered with Ampiclox capsules. The elimination rate constant (K_{el}), the clearance rate (cl), and the apparent volume of distribution (V_d) of chorpropamide were significantly reduced ($p < 0.05$) in the presence of Ampiclox capsules. Other pharmacokinetic parameters of chlorpropamide such as T_{max}, $t½_{ab}$, and k_{ab}, were not significantly different ($p > 0.05$) in the presence of Ampiclox capsules.

Since hypoglycaemic sulphonylureas are strongly bound to plasma proteins (Balant, 1981) as well as Ampiclox (Ptalsky, 1980) it would be appropriate to propose an interraction mechanism of competition for binding site between the two drugs. It can be said therefore, that Ampiclox must have competitively displaced chlopropamide from the binding site, thus giving rise to higher bioavailability (AUC) and C_{max}. The effect of higher bioavailability of chlorpropamide in the presence of Ampiclox capsules therefore produced significant decrease ($p < 0.05$) in the plasma glucose level of these patients. It is therefore suggested that this study protocol be altered to minimise the interraction and to confirm the mechanisms of the interractions. This can be done by taking Ampiclox capsules one hour before the administration of chlorpropamide. If the effect of single dose of Ampiclox can be so pronounced, then the effect of multiple doses of Ampiclox should also be of interest.

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APPENDICES

APPENDIX 1: Individual results of Seven subjects (a,b,c,d,e,f,g)

i: Results following the administration of 250mg Chlorpropamide tablet

ii: Results following the administration of 250mg Chlorpropamide tablet and 500mg of Ampiclox Capsule.

TIME (hrs)	a (i)			a (ii)		
	Absorbance	Concentration (ug/ml)	Glucose level (m.mol/ml)	Absorbance	Concentration (ug/ml)	Glucose level (m.mol/ml)
0			15.9			11.1
0.5	0.156	25.00	17.3	0.183	30.00	12.4
1	0.141	24.00	15.6	0.155	24.50	12.2
2	0.280	46.00	14.9	0.360	58.40	4.2
4	0.099	15.00	12.7	0.115	18.75	8.9
6	0.092	15.00	9.3	0.105	17.02	8.5
8	0.055	10.00	10.8	0.075	12.04	9.8
24	0.057	10.08		0.065	10.50	
48	0.047	6.05		0.065	10.20	
72	0.056	8.00		0.051	9.05	
144	0.055	8.00		0.050	9.00	
168	0.014	2.08		0.022	2.50	

TIME (hrs)	b (i)			b (ii)		
	Absorbance	Concentration (ug/ml)	Glucose level (m.mol/ml)	Absorbance	Concentration (ug/ml)	Glucose level (m.mol/ml)
0			12.2			9.3
0.5	0.130	20.60	13.1	0.160	26.00	10.2
1	0.122	21.85	12.9	0.150	24.50	10.9
2	0.144	23.75	12.9	0.220	36.08	10.2
4	0.092	15.00	9.8	0.1320	21.40	13.7
6	0.090	15.00	15.6	0.125	20.67	12.0
8	0.086	14.50	22.9	0.090	15.00	12.0
24	0.0742	11.15		0.086	14.50	
48	0.0701	11.05		0.075	12.20	
72	0.025	3.50		0.042	5.70	
144	0.0158	2.50		0.025	3.56	
168	2.00	2.00		0.012	2.15	

TIME (hrs)	c (i)			c (ii)		
	Absorbance	Concentration (ug/ml)	Glucose level (m.mol/ml)	Absorbance	Concentration (ug/ml)	Glucose level (m.mol/ml)
0			8.5			7.8
0.5	0.130	21.40	8.7	0.132	22.05	8.0
1	0.134	22.05	7.9	0.139	22.15	5.3
2	0.164	26.18	7.4	0.172	28.00	4.5
4	0.140	24.50	5.4	0.164	26.18	5.0
6	0.144	24.50	11.3	0.155	25.00	10.9
8	0.142	24.50	14.6	0.155	25.00	12.6
24	0.135	20.50		0.141	24.40	
48	0.095	15.40		0.110	18.80	
72	0.0152	2.35		0.025	3.50	
144	0.0144	2.30		0.017	2.15	
168	0.0124	2.15		0.015	2.10	

TIME (hrs)	d (i)			d (ii)		
	Absorbance	Concentration (ug/ml)	Glucose level (m·mol/ml)	Absorbance	Concentration (ug/ml)	Glucose level (m·mol/ml)
0			11.2			9.5
0.5	0.184	30.00	12.3	0.048	36.05	8.8
1	0.195	31.00	11.4	0.259	40.05	8.5
2	0.259	40.05	10.2	0.280	46.00	8.6
4	0.145	23.00	9.8	0.164	26.18	8.4
6	0.080	13.18	15.3	0.110	18.80	9.5
8	0.066	10.00	17.7	0.100	16.05	9.8
24	0.040	6.05		0.050	8.15	
48	0.035	5.00		0.0502	8.56	
72	0.035	5.00		0.041	6.05	
144	0.034	5.00		0.035	5.00	
168	0.021	3.08		0.0156	2.05	

TIME (hrs)	e (i)			e (ii)		
	Absorbance	Concentration (ug/ml)	Glucose level (m·mol/ml)	Absorbance	Concentration (ug/ml)	Glucose level (m·mol/ml)
0			19.8			15.5
0.5	0.024	3.05	29.5	0.026	3.15	23.3
1	0.130	22.10	18.6	0.160	26.00	16.7
2	0.222	36.00	15.3	0.0280	46.00	12.4
4	0.160	26.00	14.8	0.223	36.50	10.8
6	0.145	24.00	24.7	0.160	26.00	10.5
8	0.046	6.05	26.7	0.85	14.8	10.8
24	0.045	6.00		0.085	14.5	
48	0.023	3.05		0.046	6.05	
72	0.033	5.00		0.045	6.00	
144	0.015	1.05		0.025	3.08	
168	0.013	1.05		0.016	1.05	

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APPENDIX 2

A: Pharmacokinetic parameters of chlorpropamide in seven (7) diabetic patients given 250mg of chlorpropamide tablets.

Kinetic Parameters	Subjects						
	a	b	c	d	e	f	g
Lag time (hr)	0.50	0.45	0.55	0.61	0.64	0.52	0.43
C.max (ug/ml)	46.00	26.18	26.40	40.05	36.00	47.20	44.80
T.max (hr)	2.80	2.90	2.60	2.00	2.30	2.00	2.80
t ½ ab (hr)	0.90	0.70	0.73	0.50	0.60	0.65	0.43
t ½ el (hr)	29.60	38.90	29.80	25.40	25.21	25.75	27.34
AUCo →∞ (ug/hr.ml)	1469.43	1158.05	1498.25	1122.64	764.26	1036.19	617.76
Kab (hr^{-1})	0.77	0.99	0.94	1.386	1.155	1.066	1.612
Kel (hr^{-1})	0.0234	0.0178	0.0232	0.272	0.0275	0.0250	0.0253
Vd (l)	7.270	12.128	7.194	8.185	11.90	9.65	15.99
Clearance (l/hr)	0.170	0.216	0.167	0.223	0.327	0.241	0.405

B: Pharmacokinetic parameters of chlorpropamide in seven (7) diabetic patients given 250mg of chlorpropamide tablets and 500mg of Ampiclox Capsule.

Kinetic Parameters	Subjects						
	a	b	c	d	e	f	g
Lag time (hr)	0.80	0.60	0.80	0.75	0.65	0.82	0.73
C.max (ug/ml)	58.40	36.08	28.00	46.74	46.50	58.25	57.32
T.max (hr)	2.83	2.75	2.95	2.81	2.84	2.68	2.72
t ½ ab (hr)	0.40	0.48	0.57	0.69	0.86	0.83	0.89
t ½ el (hr)	35.40	43.27	35.10	31.56	32.35	37.72	38.50
AUCo→∞ (ug/hr.ml)	1604.94	1448.51	1745.24	1374.8	1284.57	1602.32	1061.22
Kab (hr^{-1})	1.733	1.444	1.216	1.004	0.806	0.835	0.780
Kel (hr^{-1})	0.0195	0.0160	0.0197	0.022	0.0214	0.0184	0.0180
Vd (l)	7.99	10.78	7.30	8.30	9.10	8.48	13.10
Clearance (l/hr)	0.156	0.172	0.144	0.183	0.195	0.156	0.24