INFLUENCE OF CIMETIDINE ON THE PHARMACOKINETICS OF PARACETAMOL IN HEALTHY SUBJECTS

BY
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DECLARATION

THIS IS TO CERTIFY THAT THE WORK REPORTED IN THIS THESIS WAS CARRIED OUT BY ME UNDER THE SUPERVISION OF DR. M. GARBA AND DR.[MRS] M.T. ODUNOLA IN THE DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY, FACULTY OF PHARMACEUTICAL SCIENCES, AHMADU BELLO UNIVERSITY,ZARIA.

THE WORKS OF OTHER INVESTIGATORS WERE ACKNOWLEDGE AND REFERRED TO ACCORDINGLY. I DECLARE THAT NO PART OF THIS THESIS HAS BEEN SUMITTED ELSE WHERE FOR THE PURPOSE OF A DEGREE.

BALA HINA AHMED.

CERTIFICATION

The thesis entitled "INFLUENCE OF CIMETIDINE ON THE PHARMACOKINETICS OF PARACETAMOL IN HEALTHY SUBJECTS" BY BALA HINA AHMED meet the regulations governing the award of the degree of Master of Science (Pharmaceutical and Medicinal Chemistry) of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my parents, whose love, encouragement, support and prayers mainly contributed to my success; and to all that died due to adverse effects of drug interactions.

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ABSTRACT

Considering the prevalence of peptic ulcer diseases in Nigeria and the popularity of paracetamol as an "household" analgesic, there is the possibility of patients being treated for peptic ulcer to take paracetamol. It is therefore possible for the drug(s) used in the peptic ulcer treatment to influence the pharmacokinetic parameters of paracetamol.

The influence of cimetidine (400mg) on the pharmacokinetics of oral single dose (1g) paracetamol was studied in 8 healthy subjects under two protocols (concomitant and delayed paracetamol administration 1 hour after cimetidine). The study was carried **out** in 3 phases: paracetamol alone (control); paracetamol and cimetidine concomitantly; and paracetamol 1 hour after cimetidine. A wash-out period of 2 weeks was allowed between the phases of the study. The method of residuals was used to obtain the pharmacokinetic parameters using the salivary concentration-time curves generated from the salivary concentration-time data as preliminary data. Statistical analysis was done by the student two-tailed t-test of paired data.

Following the single oral one gram dose of paracetamol, the relevant mean pharmacokinetics parameters obtained were in agreement with those in the established literature.

For the influence of cimetidine on paracetamol, there were no significant changes ($P<0.10$) when compared to the control in the salivary pharmacokinetics of paracetamol

when the two drugs were concomitantly administered. Delayed administration of paracetamol 1 hour after cimctidine, on the other hand, was associated with significant changes in the pharmacokinetic parameters. The peak salivary concentration (Cmax) and absorption rate constant (Kab) were significantly reduced (P<0.05), while the time to peak concentration (Tmax),

absorption half-life (t1/2ab) and lag time were all significantly increased (P<0.05). Elimination half life (t1/2el) and area under the curve (AUC o - oc) were significantly increased (P<0.05 and 0.10 respectively). Elimination rate constant (Kel) and clearance (C1) were significantly reduced (P<0.05 and 0.10 respectively). While Volume of distribution (Vd) was significantly increased (P<0.05).

These findings indicated that cimetidine does not affect the pharmacokinetics of paracetamol when the two drugs were concomitantly administered but impaired the absorption and inhibited the metabolism of paracetamol when the administration of the latter was delayed by 1 hour after cimetidine. The therapeutic implications of this interaction is that the efficacy of paracetamol may be affected when the two drugs arc used in combination.

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ABBREVIATIONS

ABBREVIATION	FULL MEANING
w/v	= Weight per unit volume
X	= initial Concentration of Standard paracetamol in saliva sample.
Y	= Mean Absorbance of Standard paracetamol in saliva sample.
α	= Alpha
β	= Beta
α-slope	= First order absorption slope
β-slope	= First order elimination slope
∝	= Infinity
λ	= Wavelength
λmax	= Wavelength of maximum absorbance
μg	= Microgramme
μl	= Microlitre
%	= Per cent
<	= Less than
>	= greater than
°C	= Degrees Centigrate
ADH	= Alcohol dehydrogenase
AUC	= Area Under Concentration – time Curve

B.P	= British Pharmacopoiea
CIM	= Cimetidine
CL	= Clearance
Cmax	= Peak Concentration Attained
CNS	= Central Nervous System
Conc.	= Concentration
Cp	= Plasma Concentration
CSF	= Cerebrospinal Fluid
DPH	= Diphenylhydantoin(Phenytoin)
Exp. Date	= Expiry Date
F	= Bioavailability
GIT	= Gastro Intestinal Tract
GS	= Glutathione Moeity
GSH	= Glutathione
ID	= Internal Diameter
I.M	= Intramuscular
I.P	= International Pharmacopoeia
I.V	= Intravenous
Kab.	= Absorption Rate Constant
M	= Molar
Manuf. Date	= Manufacturing Date
MAOI	= Mono amine oxidase inhibitor

Mol. Formular	= Molecular Formular
Mol. Weight	= Molecular weight
n	= Number of Observations
nm	= Nanometer
ng	= Nanogramme
N.P.H	= Not Potentially Harzadous Interactions
OTC	= Over The Counter
PAS	= Para amino salicylate
PCM	= Paracetamol
PH	= Potentially hazardous interaciton
pH	= Hydrogen ion concentration
Pka dissociation	= Negative Logarithm of the Constants for the acid form of drugs.
QID	= Four times daily
RSD	= Relative standard deviation
(R)	= Registered trade name
r	= Correlation coefficient
rpm	= Revolutions per minute
SAR	= Structure activity relationship
SD	= Standard Deviation
S.E.M	= Standard Error of Mean
Soln.	= Solution

T45	= Dissolution Rate at 45 minutes
$t_{1/2}$	= Half life
$t_{1/2}ab$	= Absorption half life
$t_{1/2}el$	= Elimination half life
TID	= Three times daily
Tmax	= Time to peak concentration
UV	= Ultra Violet
UV-VIS	= Ultra Violet – Visible
Vd	= Volume of distribution.

CHAPTER ONE

1.0 INTRODUCTION

A drug interaction occurs whenever the presence of one chemical substance changes the pharmacological effects of a therapeutically administered drug. The term 'chemical substance' in this context should be extended to include alcohol, foods, insecticides, possibly food additives, environmental chemical agents as well as drugs therapeutically administered, and drugs of abuse such as cannabis and tobacco (Griffin and D'Arcy, 1979). Drugs very rarely form direct chemical or physical bonds with one another as protamine does when it neutralises the activity of heparin. Drug-drug (and other agents) interactions take place because the interactions of drug with macromolecules changes the environment of a second or third drug molecule, thereby resulting in a modified effects.

Much of the detailed knowledge of drug interactions has been gained from animal experimentation and although such observations are of undisputed importance it should not be overlooked that the speed and pathway of drug metabolism in man may be quite different from that which has been determined in many species of laboratory animal. Indeed, it have been emphasised that trial in man is the only valid way of establishing drug interactions in man and that ideally such studies should be performed during the early stages of drug development (Brodie, 1962; Modell, 1964). New drug

combinations requires separate investigation with animal toxicity studies and clinical evaluation in a full scrutiny as afforded to a completely new drug.

While in the past, pharmacologist tended to consider drug interactions as phenomena taking place only at certain receptors, the current feeling is that there may be many other factors involved. This is based on findings that a number of drug interactions may occur outside the receptor sites. Drug interactions can occur inside or outside the body. They can occur before administration when drugs are added in-vitro to an intravenous fluid, or they can have origins in a tablet or capsule when one component of the formulation alters the subsequent bioavailability of the active drug (physicochemical interactions). They can occur in the intestine before absorption when a drug or food component modifies the absorption characteristics of another drug. They can occur after absorption either from drug competition at binding sites or antagonism at receptor sites in the tissues at which they arrive. Interactions may modify the degradation of a drug by inducing or inhibiting metabolic enzymes systems, especially those associated with liver microsomes. They may intervene in the excretory processes of the drug in the kidney tubules; indeed there is no phase from formulation to elimination where drug interactions are excluded (Griffin and D' Arcy, 1974).

Thus drug interactions may be quite varied and complex, and in some cases, more than one mechanisms may be involved (Hansten, 1979). However, experimental findings and clinical experience have shown that the great majority of interactions occur by a small number of relatively specific basic mechanisms (Evaluation of drug interactions, 1976). The mechanisms are

classified into pharmacokinetic, pharmacodynamic and miscellaneous interactions (Hansten, 1979). An understanding of the mechanism involved with a particular interaction is essential in interpreting, preventing, and treating specific interactions. An understanding of the mechanisms involved may also be useful, to a limited degree in predicting the possibility of previously unreported interactions by virtue of the drug's characteristics that are similar to other known interacting drugs (Evaluation of drug interactions, 1976).

A common cause of pharmacological sequel following multiple drug therapy is the alteration by one drug on the pharmacokinetics of another. Prescott (1969), has called these "Pharmacokinetic interactions". This group includes these interactions in which gastrointestinal absorption of a drug is increased or decreased, plasma protein and other macromolecules binding is affected, drug metabolism is stimulated or inhibited, or urinary excretion is enhanced or inhibited (Prescott, 1969). In contrast, pharmacodynamic interactions encompasses the numerous interactions between drugs at their site of action. This occurs when one drug directly alters the molecular, cellular or physiological action of another. In either of the instances action on the same receptor may or may not be involved (Hansten, 1979). Miscellaneous mechanisms on the other hand, comprised of these drug interactions which cannot be assigned to either pharmacokinetic or pharmacodynamic classifications.

Many adverse drug interactions can be considered necessary concomitants to therapy with potent drugs. Patients treated with phenothiazines, corticosteroids, antineoplastics and many other drugs must frequently be subjected to certain adverse effects in order to obtain therapeutic benefit. A possible correlation has been noted between the significant increase in adverse effects and the use of multiple drug therapy (Evaluation of drug interaction, 1979). Hurwitz (1969), also showed that the incidence of drug reactions increased with the number of drugs prescribed simultaneously and drug interactions make a small but significant contribution to the overall morbidity and mortality due to drugs (Hurwitz, 1969).

Outside hospital, patients may complicate the situation further by taking over-the counter (OTC) products, many of which are themselves combinations of drugs. Self-medication is encouraged by modern marketing and advertising, but any symptomatic benefit may be gained at the expenses of a prescribed therapeutic regimen. For example, potent pharmacological substances such as antihistamines and indirectly acting sympathomimetic amines may be among the constituents of cold and cough cures which may be implicated in interactions with prescribed drugs.

It is true to say that of the many reported drug interactions in the literature many are anecdotal and have not been confirmed, nor does there exist any well pharmacological principle for believing that they could occur (Griffin and D'Arcy, 1979). Nevertheless, individual variability is such that factors such as pharmacogenetic differences and effects of disease states may have contributed to a unique reaction. Environmental factors such as smoking and

atmospheric pollution or even the hardness of the water supply have also been reported to influence drug metabolism and may also be involved in contributing to a drug interaction as may also dietary factors and particularly herbal remedies which are increasing in their usage by the population due to the mistaken belief that they are free of adverse effects, when in fact their usage is surrounded by ignorance of their pharmacology and toxicology (Dukes, 1977).

Many drug interactions occur daily in clinical medicine which remain undetected with the adverse effect frequently attributed to patient idiosyncrasy or the underlying disease. As a striking example of this, it should be remembered that coumarin anticoagulant were given concomitantly with phenylbutazone and barbiturates to many patients over a period of several years before the adverse interactions were detected (Hansten, 1979).

It is quite clear that there has been a tendency to over-stress the importance of drug interactions and to ignore many other factors which may influence the response of a patient to a drug or combination of drugs. Even when specific interactions can be predicted, the clinical consequences will depend on drug administration factors and within the patients, genetic and pathophysiological factors which may alter drug metabolism, elimination and response. Thus, many such interactions could either be avoided or allowed for by intelligent adjustment of dose or dose interval.

1.1 INTERACTIONS AFFECTING DRUG ABSORPTION

To reach the bloodstream from the alimentary tract a drug must be absorbed. The absorption of a drug refers to its transfer from the site of administration (the GIT), to the systemic circulation. The absorbing system in the gastrointestinal tract (GIT) is the mucosal epithelium which extends from the oral cavity to the anus forming a continuous cellular barrier to orally administered substances. For drugs the most important process of absorption is that of passive diffusion (Binns, 1971), no energy is required, it is not saturable and the transfer is directly proportional to the concentration gradient and to the lipid-water partition coefficient of the drug.

In general sense, whenever a drug must penetrate biologic membrane(s) to gain entry into the vascular fluids there are three important factors governing the rate and extent of access ; the physicochemical characteristics of the drug and its dosage form; the nature of the biologic membrane(s) and the physiological characteristics of the biological system near the site of drug absorption. A knowledge of each of these factors and the interactions among these factors is essential for predicting and explaining their influence on absorption (Mayersohn, 1979).

Many drugs influence G.I.T function, thereby causing absorption drug interactions. Most of these interactions results in decreased rather than increased drug effects and they are not considered to be of much clinical importance. Interactions that cause therapeutic failure are obviously important but are not so markedly impressive as fatal haemorrhage,

uncontrolled hypertension or symptomatic hypoglycaemia, can readily pass unrecognised. Nevertheless, the consequences of failure of drug absorption can be very serious.

Drug interactions during the absorptive phase result in either or both of the following potential clinically significant effects (Barr, 1969).

(1) - Alteration in the rate of absorption.

(2) - Alteration in the amount of drug absorbed.

From a practical clinical point of view, the consequences of those effects may be quite different. A decrease in the fraction of drug absorbed is equivalent to a decrease in the dose given, with the obvious clinical implications. On the other hand, an increased in the amount absorbed could be dangerous for drugs with narrow therapeutic index such as digoxin or theophylline.

Although a drug may eventually be completely absorbed, it may be absorbed so slowly that: (a) It may never reach effective serum levels, (b) the rate of onset may be greatly delayed when prompt relief of acute symptoms or (c) the slow rate of absorption may act to sustain release and unduly prolong an effect.

As a general rule, a decrease rate of absorption is most often important for drugs that are given as a single dose in clinical situation requiring a rapid onset of activity (e.g. analgesics such as aspirin, antihistamines such as isoproteronol, and hypnotics such as pentobarbital), because delayed

absorption will defeat the object of the treatment (Prescott et al,, 1977). This is also important for rapidly eliminated drugs such as procainamide, as therapeutic levels might not be achieve if absorption rate is slowed.

Drug interactions resulting in decreased rate of absorption are usually not important for compounds that are given in multiple-dose regimens to achieve a constant serum level such as antibiotics, sedatives or tranquillisers. The reason for this is that the average steady-state serum levels in a multiple-dose regimen is affected by the fraction of the drug absorbed but not usually by the relative rate of absorption (Evaluation of drug interactions, 1976).

1.1.1 MECHANISMS OF INTERACTIONS AFFECTING DRUG ABSORPTION.

There are several ways in which the rate and amount of absorption of a drug can be modified. Most drug interactions during absorption are simple alteration of one of the steps involved in normal mechanisms of absorption (Evaluations of drug interactions , 1976). Thus, one drug may alter the absorption of other drug(s) by several different mechanisms. In many cases, however, the mechanisms are unknown (Prescott et al, 1977). Some possible mechanisms of interactions affecting drug absorption are listed in Table 1.1.1.1

The pH of the gastrointestinal lumen fluids can vary greatly and may affect the transport of drugs in some cases. Drug induced changes in the pH of GI fluids may have complex and unpredictable effects on absorption.

Table 1.1.1.1 Possible mechanisms of interactions affecting drug absorption.

- pH effects on drug ionisation and dissolution
- Changes in gastrointestinal motility
- Modification of gastric emptying rate
- Complex ion-pair and chelate formation
- Interference with active transport
- Disruption of lipid micelles
- Changes in splanchnic blood flow
- Toxic effects on gastrointestinal mucosa
- Changes in volume, composition and viscosity of secretions
- Effects on mucosal and bacterial drug metabolism
- Unknown mechanisms.

Most drug molecules are either weak acids or weak bases and these compounds will be ionised to an extent govern by the molecules dissociation or ionisation constant pKa and the pH of the fluid in which it is dissolved.

The importance of ionisation in influencing drug absorption is based upon the observation that the unionised form of a compound has a greater lipid-water partition coefficient (k L/W) than the ionised form. Early classic studies on drug absorption from solutions perfused through rat intestine clearly showed

that the rate of absorption was proportional to the fraction of unionised drug in solution (Schanker, 1964). Since k L/W is an important determinant of a compound's membrane penetrating ability, generally change in pH would be expected to influence absorption.

The Henderson-Hesselbatch equation was used to relate pH and pKa to the membrane transport of ionizable compounds (Jacobs 1940). Later extensive experiments by a group of investigators resulted in the integration and application of the above observations to drug absorption resulting to the pH - partition theory (Shore et al 1957, Schanker et al 1957).

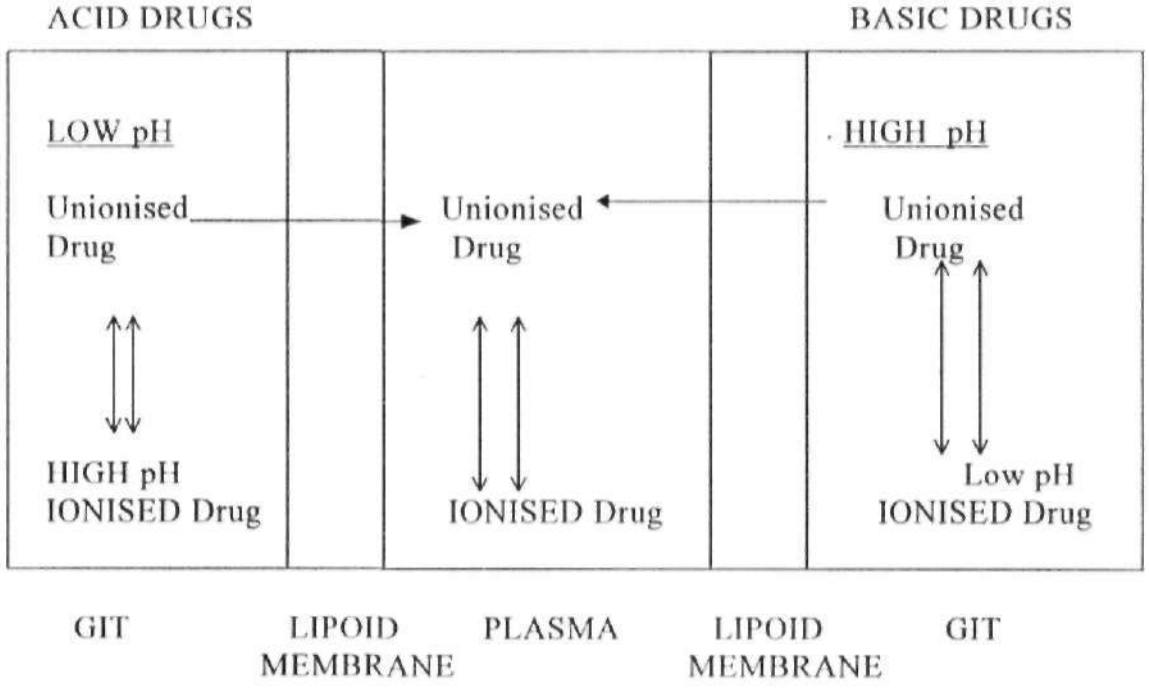


Figure 1.1.1.1 Effects of pH on the absorption of acidic and basic drugs(pH-partition theory).

In essence, the theory (pH-partition) states that ionizable compounds penetrate biologic membranes primarily in the unionized form (i.e. nonionic diffusion) and as a result acidic drugs should be best absorbed from acidic solution where pH is greater than pka, while basic drugs should be best absorbed from alkaline solution where pH is less than Pka (Mayersohn 1979).On this basis, the ionization of weak acids should be increased and the rate of absorption decreased by simultaneous administration of alkalis and antacids, while the reverse should apply with weakly basic drugs.

In practice, however, the pH partition theory rarely seems to be the case and in many instances exactly the opposite effect is seen. Thus, the absorption of ketoconazole was reduced by more than half with cimetidine but enhanced in acid solution (Vander Meer et al, 1980). Acetylsalicyclic-acid (ASA) is absorbed much more rapidly when taken in a well buffered alkaline solutions than from unbuffered solutions at pH 2.8 (Cooke and Hunt, 1970).The absorption of the free acid form of sulphadiazine is greatly enhanced by magnesium oxide; while tetracycline and quinine absorption is reduced by alkalis (Barr et al, 1971; Hurwitz, 1974). In general, in the usual clinical situation, antacids may tend to decrease absorption of basic drugs and increase absorption of acidic drugs. The explanation of this paradox lies in the effect of pH on drug solubility and dissolution i.e. acid drugs are sparingly soluble at low pH but dissolution is rapid in alkaline solution. The converse applies to the basic drugs.

ABUBAKAR TAFAWA BALEWA UNIVERSITY

The importance of gastric emptying with regard to drug absorption is readily apparent since most drugs, irrespective of pH and whether they are acidic, basic or neutral have been shown to be best absorbed from the small intestine (Levine, 1970; Prescott, 1974a). The relationship of gastric emptying to the drug concentration profiles in the body is described by scheme 1.1.1.2

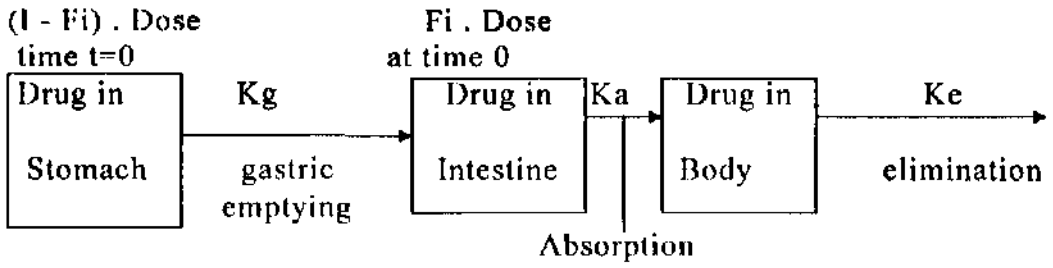


Figure 1.1.1.2: Compartment model for gastrointestinal absorption.

After oral administration of a drug, approximately 10-50% of the dose (Fi.Dose) passes rapidly into the intestine (Nimmo, 1976; Watanabe et al, 1977), and the remaining dose is emptied from the stomach at a rate given by the first order gastric emptying rate constant Kg. The drug is absorbed into the body at a rate given by absorption rate constant Ka, and then is eliminated from the body at a rate given by the first-order elimination rate constant Ke.

As a result, any factor influencing gastric emptying and therefore drug movement into the small intestine will alter the rate and possibly the extent of absorption. Therefore, in addition to the rate of drug dissolution and the inherent ability of the drug to be absorbed, gastric emptying may be a rate limiting process for absorption from the small intestine, and is particularly

important in the context of interactions since it can be influenced by food and several drugs. Table 1.1.1.2. lists some drugs that might influence gastric emptying (Prescott, 1974b).

Many drugs are absorbed more slowly when taken with food, and this is probably due largely to the inhibitory effect of food on gastric emptying. Drug effects can be reduced dramatically or even abolished if gastric emptying is retarded by food (Kojima et al, 1971). In contrast, absorption is more rapid and toxicity can be greatly increased when drugs are given orally in the same dose in dilute rather than concentrated solutions, and this effect has been attributed in part to rapid gastric emptying (Borowitz et al, 1971).

Table 1.1.1.2. Drugs that might influence gastric emptying.

- Atropine and anticholinergics
- Antihistamines
- Tricyclic antidepressants
- Phenothiazines
- Sympathomimetics
- Anti-parkinson drugs
- Narcotic analgesics
- Nitrites
- Iproniazid
- Chloroquine
- Metoclopramide
- Caffeine
- Prostaglandins
- Antacids
- Antihypertensives

A striking example of an absorption interaction involving gastric emptying is the influence of barbital on aminopyrine. Aminopyrine apparently inhibits gastric emptying in rabbits and is absorbed very slowly when given alone. Barbital itself has no significant effect on gastric emptying but it antagonises the inhibitory action of aminopyrine and greatly increases the rate of absorption of the Latter (Goto et al, 1972).

Propantheline and metoclopramide delay and accelerate gastric emptying respectively, and their effects on the absorption of an oral dose of 1.5g of acetaminophen were investigated (Nimmo et al, 1973). Simultaneous measurements of gastric emptying rate and acetaminophen absorption were made in six convalescent hospital patients on separate occasions before and after I.V. injection of 30mg of propantheline. The investigation indicated that propantheline delayed gastric emptying and slowed down the rate of acetaminophen absorption but did not reduce the total amount absorbed. In contrast, metoclopramide (10mg i.v.) significantly increased the rate of acetaminophen absorption in five healthy volunteers who were known to be slow absorbers of the drugs.

Propantheline and metoclopramide have also been shown to retard and accelerate, respectively, the absorption of tetracycline and pivampicillin in man (Gothoni et al, 1972a). On the other hand, propantheline and metoclopramide seemed to have opposite effect on the absorption of digoxin (Maninem et al, 1973). This paradox could be explained by rather slow dissolution and absorption of digoxin or absorption from a limited area of the

intestine. Drugs which are poorly soluble or actively absorbed from a limited area of the intestine may be less well absorbed if a gastrointestinal transit induced by metoclopramide could reduce the effective time for dissolution and absorption of digoxin where as propantheline would have the reverse effects (Manninen et al, 1973). Similar observations have been made with riboflavin, the absorption of which is delayed by propantheline, although the total amount absorbed is greatly increased (Levy et al, 1972).

A striking delay in the absorption of oral lignocaine in patients premedicated with 0.6mg of atropine (I.M) prior to laparoscopy was observed (Adjepon-Yamoah et al, 1973). The mean peak plasma concentration of lignocaine occured at 45 minute in healthy volunteers but was delayed until 3 hours in the patients. Similarly, the absorption of lidocaine in healthy volunteers was reported to be delayed by atropine, however, the area under the plasma concentration-time curve (AUC) for lidocaine was not altered (Adjepon-Yamoah et al, 1974), and the absorption of phenylbutazone was reported to be delayed by desmethylimipramine (Consolo et al, 1970). Those interactions are probably due to changes in the rate of gastric emptying. Other mechanisms may be involved however. For example changes in gastrointestinal motility and peristalsis could modify tablet disintegration and dissolution and increase or decrease the contact between drug and absorbing epithelium (Prescott, 1974b).

Studies have shown that antacids may alter the rate and extent of absorption of other orally administered drugs. Antacids do this if they affect the rate limiting steps in absorption, which may vary from one drug to another. For

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evidently by a different mechanism. In the presence of $NaHCO_3$, tetracycline dissolution is reduced, delaying its absorption (Barr et al, 1971).

For drugs that are slowly and incompletely absorbed and for which chemical interaction such as, complexation, chelation, or altered solubility cannot be shown, there is less likelihood of a significant interaction with antacids in the GIT. An example is ampicillin, which is slowly and incompletely absorbed and which is absorbed to a similar extent in the presence or absence of aluminium hydroxide in patients (Hurwitz 1974).

Magnesium-aluminium hydroxide antacid was found not to affect plasma warfarin levels when both agents were given together (Robinson et al, 1970). In the study, first blood sample was obtained 6 hour after drug administration, by which time the effects of delayed gastric emptying, if any, would have been missed. Also, retarded drug absorption, if not the result of irreversible binding in the gut, would be of no clinical significance for a drug with a long half life (42 hr for warfarin) if all the drug were eventually absorbed. This would result in similar steady-state blood levels on a multiple dosing schedule.

So far, those antacid-drug interactions that have documented occur due to changes in the amount of free drug in solution as affected by dissolution, chelation, or complexation, to changes in ionization of the drug caused by pH shifts, or to changes in gastrointestinal function, especially gastric emptying. Potential interactions due to changes in drug stability as pH is raised as may occur with penicillins, or to other cation induced changes in gastrointestinal physiology such as in transit time, stirring of intestinal contents, or cell

membrane permeability are possibilities that are currently being studied (Hurwitz, 1974)

Concomitant food intake may influence the rate and extent of drug absorption depending on the physicochemical properties of the drug, the physiological changes in the gastrointestinal (GI) tract resulting from the food and fluid ingested, and interactions between the food and the drug (Mayersohn, 1979). The effects of food on the absorption of drugs from the GI tract have been studied and reviewed extensively (Welling, 1977; Melander, 1978; Toothaker, and Welling, 1980).

Table 1.1.1.3 Lists the mechanism that appear to be the primary methods whereby food may cause drug absorption interactions (Mayersohn, 1979).

Table 1.1.1.3 Mechanisms of food-drug absorption interactions.

- Changes in gastric emptying rate
- Changes in gastrointestinal (GI) motility
- Food-induced secretion of GI fluids which may act on the drug molecule (e.g. secretion of enzymes)
- Food components which may compete with the drug for absorption.
- Adsorption of drug onto food
- Increased GI fluid viscosity which will reduce drug dissolution rate and drug diffusion to the absorbing membrane.
- Specific physicochemical interactions between the component(s) of the meal and a drug.

Changes in gastric emptying due to the presence of food and the resulting influence on the absorption of various drugs are summarized in Table 1.1.1.4. The effect of quantity and timing of food intake on alcohol absorption have been evaluated in male subjects (Lin et al, 1976). The results indicate that a large amount of solid food (e.g. heavy breakfast reduced alcohol absorption more than a small amount of solid food did.

Table 1 .1 .1. 4.The Influence of food on the absorption of various drugs in man.

Drug	INFLUENCE ON ABSORPTION	REFERENCE
Paracetamol	Reduction in rate but not extent of absorption	Jeff et al, 1971.
Aspirin	Reduction in rate and extent of absorption	Wood 1967; Volans 1974.
Bretylium tosylate	Reduction in rate and extent of absorption	Dollery et al, 1960.
Capuride	Reduction in rate but not extent of absorption	Johnson et al, 1973
Clindamycin	Reduction in rate but not extent of absorption	Dehann et al, 1972
Digoxin	Reduction in rate but not extent of absorption	White et al, 1971
Fenoprofen	Reduction in rate absorption	Chenish et al, 1972
Lincomycin	Reduction in rate and extent of absorption	McCall et al, 1967
Nitrofurantoin	Reduction in rate but increase in extent of absorption	Bates et al, 1974
Rifampicin	Reduction in rate and extent of absorption	Siegler et al, 1967

The effects of food intake on the oral bioavailability of propranolol and metoprolol were reported (Melander et al, 1977; Mclean et al, 1981). Greater bioavailability was observed for both drugs following drug ingestion together with a meal as compared with ingestion on an empty stomach. Since the gastrointestinal absorption of both drugs is virtually complete (Shand, 1974), the observed increase in bioavailability is likely due to an alteration of first-pass hepatic clearance resulting from the increase in splanchnic blood flow (Mclean et al, 1978; Mclean et al, 1981) caused by the presence of food.

An increase in oral bioavailability as a result of food intake has also been reported with nitrofurantoin (Bates et al, 1974; Rosenberg and Bates, 1976), riboflavin (Levy and Jusko, 1966) and griseofulvin (Crounse, 1961). The greatest enhancement in nitrofurantoin bioavailability is seen with the dosage forms that exhibit the poorest dissolution characteristic (Rosenberg and Bates, 1976).

For those drugs that have chemical structure similar to compounds required by the body and for which specialized absorption mechanisms exist, there is the possibility of competitive inhibition of absorption. L-Dopa whose absorption may be inhibited by certain amino acids obtained from the breakdown of ingested proteins may be cited as an example (Van Woert, 1971; Gillispie et al, 1973). Such an interaction may account for the Loss of therapeutic effects of L-Dopa in patients on a high protein diet.

Drug adsorption onto food materials and physicochemical interaction between drug and specific food components are also a means by which absorption drug

interactions occur. A classic example is that of the formation of a nonabsorbable complex between calcium present in dairy foods and tetracycline (Kunin and Finland, 1961). Foods high in iron content will also reduce tetracycline bioavailability by the same mechanisms (Neuvonen et al, 1970; Gothoni et al, 1972b). However, if iron is given not less than 3 hour before or 2 hour after tetracycline administration, there is no significant reduction in absorption (Gothoni et al, 1972b).

Some sugars, vitamins, aminoacids, purine and pyrimidine bases and related drugs are thought to be absorbed from the small intestine by active transport processes which may be blocked by metabolic inhibitors or inhibited by substrate competition. Such an interaction has been postulated between chlorpromazine and levodopa (Rivera-calimlim, 1972).

Some drugs have a toxic effect on the intestinal mucosa and may cause a malabsorption syndrome with impaired absorption of other drugs. Thus, colchicine may cause megaloblastic anaemia through interference with Vit-B12 absorption (Webb et al, 1968). Drugs can also influence the volume and composition of gastrointestinal secretions, and changes in viscosity may modify drug absorption (Mayersohn, 1979).

During oral therapy, the gastrointestinal mucosa is exposed intermittently to very high drug concentrations that may alter the permeability of the gastrointestinal epithelium. In this context, it is interesting to note that insulin and many polypeptides greatly enhance the membrane transport of pethidine,

isoniazid, salicylate, and chlorpromazine - drugs normally considered to cross cell membranes by passive diffusion. Thus the intestinal uptake of isoniazid is enhanced by insulin (Danysz and Wisnieski, 1970).

Some drugs are extensively metabolized by the gastrointestinal mucosa or by gut bacteria, and this process might be influenced by the concurrent administration of other drugs (Prescott, 1974b). Mucosal and bacterial drug-metabolizing enzymes are inducible (Pantuck et al, 1974), and the metabolism of levodopa and salicylazosulphapyridine in the gut is altered by simultaneous administration of antibiotics (Goldman et al, 1974).

Drugs may interact in the gastrointestinal tract to form complexes, ion pairs and chelates, which may be absorbed more rapidly or more slowly than the parent drugs. The absorption of tetracyclines is inhibited by the formation of insoluble chelates with metals such as calcium and iron (Neuvonen et al, 1970). Absorption of drugs may also be reduced by binding to ion exchange resins (Robinson et al, 1970) or adsorption onto Kaolin or charcoal (Binnion and McDermot, 1972). For example, as a result of anion exchange properties, cholestyramine interfere with the intestinal absorption of aspirin, phenyl butazone and warfarin (Gallo et al, 1965; Koch-Weser and Sellers, 1971).
On the other hand, absorption may be enhanced by the formation of more soluble complexes and ion pairs. Increased absorption of dicoumarol when taken with magnesium hydroxide (Ambre and Fischer, 1973) and increased absorption of a quaternary ammonium antiarrhythmic agent when taken with salicylate or trichloroacetate (Prescott, 1974b) may be cited as an examples.

Interference with micelle formation may limit the solubility of lipids, and inhibition of absorption of cholesterol, bile acids and vitamin A by neomycin has been attributed to this mechanism (Barrowman et al, 1973). Several substances can solubilize drugs which may alter absorption. Surfactants used as stool softeners, such as dioctyl sodium sulfosuccinate, may increase solubilization of some poorly soluble drugs such as digitalis.

In summary, the absorption of drugs from the gastrointestinal tract is a complex process that is influenced greatly by physiological and physicochemical factors. Absorption interactions are often unpredictable, and one drug may enhance the absorption of a second drug but interfere with the absorption of a third drug. Drugs are absorbed much more slowly from the stomach than from the small intestine and absorption may often be limited by the rate of gastric emptying. Atropine, propantheline, and metoclopramide alter the rate of gastric emptying in man and have significant effects on the rate of absorption of other drugs. Many other therapeutic agents influence gastrointestinal motility and gastric emptying and could cause absorption interactions through a similar mechanism.

1.1.2 CLINICAL SIGNIFICANCE OF INTERACTIONS AFFECTING DRUG ABSORPTION.

Many drug absorption interactions have been described, and the data obtained from formal studies often look very impressive. Yet virtually nothing is known of their clinical importance. Interactions are obviously likely to be more important when they involve drugs with a low margin of safety and

commonly used drugs, or when the drugs involved are often taken in combination. Inhibition of absorption of isoniazid and rifampicin by para-aminosalicyclic acid granules (Boman, 1974) is clearly a serious matter, and interactions involving antacids are probably significant (Hurwitz, 1974), if only because these agents are used very widely in the treatment of serious organic disease and non-specific complaints and are obtainable without prescription. But most other interactions affecting drug absorption pale into insignificance in comparison with the effects of food on drug absorption, about which doctors show very little concern (Prescott et al 1977).

1.2 INTERACTIONS AFFECTING DRUG DISTRIBUTION

Distribution is the act of apportioning or spreading out of a drug in an orderly manner once it reaches the circulation. Drug distribution is dependent on (Rawlins, 1977a); (a) the partition coefficient of drug between blood and tissues; (b) regional blood flow; (c) binding to plasma proteins and tissue macromolecules; and (d) active transport. However, for drug interactions, only the last three are important.

Drugs affecting liver blood flow by a direct effect on the splanchnic circulation or secondarily by effects on cardiac output can alter the pharmacokinetics of other drugs which are subject to extensive liver metabolism. Lignocaine extensively metabolised by the liver, and its plasma clearance is primarily limited by liver blood flow which may be affected by other drugs or factors operating within the patient. The use of lignocaine as an anti arrhythmic agent in patients provides an example of the effect of a

change in circulation altering drug kinetics. It has been noted by several authors that lignocaine concentrations were higher in shocked patients than in normal people given the same intravenous (i.v) infusion of the drug (Thomson et al, 1971).

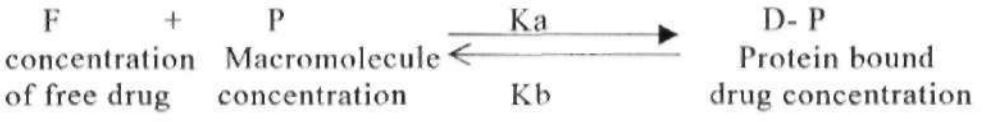
Certain diseases are associated with altered drug distribution. Lignocaine is cleared from the circulation to the extent of about 70 per cent at each transit of the blood through the Liver (Stenson et al , 1971). Thus, patients with cardiac failure eliminate lignocaine more slowly than normal subjects- presumably because of the reduction in liver blood which accompanies this disorders (Thomson et al 1973).

Propranolol is another drug with a high first pass clearance by the liver (George et al, 1976). Propranolol reduces both its own plasma clearance and that of lignocaine as a result of a dose-related reduction on cardiac output and therefore secondarily a reduction of liver blood flow (Branch et al, 1973).

1.2.1 DRUG-MACROMOLECULE BINDING AND DISPLACEMENT INTERACTIONS.

Most drugs and endogenous compounds are reversibly bound to a varying extent to proteins (principally albumin) circulating in the plasma (Brodie, 1965). Plasma protein binding is a very important factor in determining the intensity and duration of drug effect and important changes in drug distribution can arise from competition between drugs for protein-binding sites. Thus, plasma proteins are frequently site for drug interactions.

A completely reversible interaction of a drug with the unoccupied binding site of a macromolecule obey the law of mass action, i.e.



Where Ka/Kb is the measure of the affinity of the drug to the protein (affinity constant).

The fraction of bound drug decreases as the total serum concentration of drug increases or the concentration of protein decreases. Binding may be decreased due to displacement by a second drug which also binds at the same site, depending on the relative concentrations and affinity constants of the two drugs. Displacement might increase, decrease, or have no effect on the amount of drug at the receptor site, depending on the relative rates of tissue distribution and elimination of the drug.

Binding of drugs to non-receptor site macromolecules is important because such associations tend to limit the access of free drug both to the receptor sites and to the biotransformation enzymes and excretion processes. Binding may be beneficial in that it may result in a diminished incidence of side effects and a prolonged duration of action (Brodie, 1965). A number of acidic drugs are attached to only one or two sites on the albumin molecule; for these drugs, the protein has a limited carrying capacity (Brodie, 1965). Many of these acidic drugs appear to compete for the same limited number of nonspecific protein binding sites. Hence, competition between coadministered drugs for binding sites in the body can result in displacement of one drug by another, with a

resulting rise in the free and active fraction of a drug. It is widely believed that this displacement or redistribution phenomenon caused enhanced clinical effects and toxicity seen when certain drugs interact in man (Wardell, 1974). As specific examples, highly bound acidic agents such as phenylbutazone, oxyphenbutazone, ethylbiscoumarocetate, dicoumarol sulfinpyrazole and salicylic acid are able to displace the long-lasting albumin bound sulphonomides from plasma proteins. Since these sulphonamides are not rapidly metabolised or excreted, the displaced molecules diffuse from plasma into tissues with resultant enhanced antibacterial activity. By the same mechanism, phenylbutazone increases the antibacterial activity of acidic antibiotics such as penicillins (Hartshorn, 1973).

Displacement of a drug can be dangerous if it is bound so extensively to plasma proteins that the unbound moiety is only a small proportion of the total. Displacement of only a small proportion of the drug from protein may then double unbound concentration at the target site. For instance, displacement of warfarin, an anticoagulant from protein binding sites by phenylbutazone and its analogues has led to serious bleeding episodes (Eisen, 1964). Chloralhydrate metabolite, trichloroacetic acid displace warfarin from binding site, causing the overall increased in anticoagulant effect of warfarin (Sellers and Koch-Weser, 1970). Ilowever, the displalcement also causes increased metabolism of unbound warfarin which results in the subsequent return of the prothrombin time to previous (pre-chloral) levels or lower.

Tolbutamide is a highly protein bound drug which reportedly may be displaced from protein binding sites by the highly bound sulphonamides e.g.

sulphaphenazole, leading to severe hypoglycaemic crises (Cristensen et al, 1963). Similar results have been elicited by phenylbutazone and salicyclates (Cristensen et al, 1963). Methotrexate has been reported to be displaced from plasma protein by salicylates and sulphonamides (Dixon et al, 1965; Brodie, 1965). Since methotrexate is often used therapeutically in doses that are nearly toxic, the clinical implications are obvious (Dixon et al, 1965).

Premature babies have relatively low quantities of albumin and the acidic binding sites are readily saturated with bilirubin. A study (Silverman et al, 1956) showed that premature infants given prophylactic sulfisoxazole and penicillin had a high incidence of fatal kernicterus compared with a control group given oxytetracycline. The mechanism of the increased incidence of kernicterus as proposed was displacement of bilirubin from plasma protein binding sites by the sulfisoxazole (Odell, 1959). Because of this phenomenon, it is recognised that neonates should not be given sulphonamides, salicylates or other agents that can displace bilirubin from albumin (Odell, 1959).

Drugs may also be displaced from specific proteins binding sites in tissues. For example, displacement from tissue binding sites has been demonstrated for pamaquine by mepaquine from liver proteins (Bridges, 1977). Displacement of aldosterone by carbenoxolone from protein binding sites of the intestinal wall was also reported (Humphrey, 1973). A further example is with the sulphonylurea group of oral hypoglycaemics; these drugs displace insulin from protein-binding site in the pancreatic beta cells, plasma and tissues and this is probably their basic mechanism of hypoglycaemic action resulting in increased insulin secretion and raised plasma levels (Hartshorn,

1973). In addition to plasma and tissue proteins, there are other macromolecules with which some drugs are known to interact. Several drugs bind with nucleic acid either reversibly or irreversibly. The binding by nucleic acids of certain antimalarials, such as quinacrine, undoubtedly contributes to the parasiticidal actions as well as to toxicity (Hartshorn, 1973). Mucopolysaccharides have strong ionic groups which bind certain charged drug molecule. The body contains numerous lipid materials. The quantitative importance of drug-lipid interaction is very great, since the lipid contents of the body can be as high as 50% of the body weight.

Although in theory displacement phenomenon could produce potent drug interactions, it has been over-emphasized because unwarranted extrapolations have been made from in-vitro studies. If a drug "A" displaces a second drug "B" from its plasma protein binding sites, the increase in the unbound fraction of B which will occur in-vivo will always be less than that observed in-vitro. The reason for this is that a proportion of the displaced drug molecules will diffuse extravascularly into the drug's distribution volume (Rawlins, 1974a). Consequently, the greater the distribution volume, the less will displacement interactions result in clinically important effects.

For an interaction due to displacement to be of clinical significant the drug should have extensive protein binding (>90%) and small distribution volume; the increased free drug levels should not result in a significantly enhanced clearance from the body through increased availability to the biotransformation and excretory sites; there must be only a few plasma protein binding sites which are largely saturated with drug; and the levels of drug

reached before displacement should be close to those causing toxicity. Those conditions are more likely to be met at the steady state situation, where more surplus binding sites are approaching saturation than after a single dose (Bridges, 1977).

1.2.2 FORCES INVOLVED IN DRUG-MACROMOLECULE INTERACTION

Some drugs are relatively inert chemically and interact only minimally with macromolecules in the cell , but most drugs have one or more very reactive groups in their structures and enter into a variety of interactions. The type and degree of those interactions are strongly influenced by the polarity of the drug molecule. Generally, drug-macromolecule binding processes are reversible; although, increasing examples of irreversible binding are becoming apparent (Michell et al, 1975). The binding forces that hold a drug in combination with macromolecule arise from the numerous bonds existing in the body.

Covalent bonds, which are usually much stronger than other types of bonds are formed from two electrons, one contributed from each of the two atoms forming the bond between them. Because of the high bond strength, covalent bond complexes between drugs and macromolecules are not likely to be broken down (irreversible), unless specific enzyme is present to break the bond and this may lead to serious drug interactions. It has been proposed (Brodie, 1967), that chemically inert therapeutic agents may produce tissue damage by being metabolised in the body to highly reactive intermediates that combine covalently with tissue macromolecules.

Several authors have shown that compounds such as carbon tetrachloride, chloroform and trichlorobromomethane produce various liver and other tissue damage by being converted into highly reactive intermediates that combine covalently with various tissue macromolecules (Sipes et al, 1972; Krishna and Bonanomi, 1974). Also the alkylation and acylation of cell constituents by certain drugs that are, respectively alkylating and acylating agents involve the formation of covalent bonds. In a study (Krishna and Bonanomi, 1974), it was established that the binding of drugs to microsomes and other macromolecules is covalent by isolating the proteins containing the labelled drugs, hydrolysing them by pronase to amino acid derivatives of the reactive metabolites and separating these amino acid derivatives in an amino acid analyser.

In contrast to covalent bonds, electrostatic (ionic) bonds are formed between ions of opposite charge. This force increase in strength with increasing ionic charge and with decreasing distance of approach. Electrostatic bonds are stronger in a medium of low dielectric constant, such as the lipid medium of a membrane, than in a medium of high dielectric constant, such as water. Electrostatic forces plays an important role in interaction involving ionizable drugs, and macromolecules having an ionic group such as certain mucopolysaccharides.

Ion-dipole and dipole-dipole interactions are dipolar interactions which2 occur as a consequence of electronegativity differences between carbon and heteroatom like oxygen, Nitrogen and sulphur in a molecule. The result is an uneven electron distribution through out the molecule, and such molecules can

interacts with each other (dipole-dipole) or with ion (ion-dipole) interactions. Dipolar functions such as amides, carbonyl, ester and ether are often located in drugs and biological macromolecules. Thus, it has been shown that for some pharmacological agents, the extent of drug-macromolecule interactions is directly related to the dipolar character in the compound (Olaniyi, 1990).

On the other hand, Hydrogen bonding is a weak or loose bond between the electron-deficient hydrogen atoms of hydroxyl, amino and thiol groups, and the non bonding electrons of electronegative atoms such as oxygen, Nitrogen, sulphur and chlorine. e.g 0-H.....0; N-H.....N; S-H.....S; O-H.....CL; N-H.....0. Since many drugs contain hydroxyl, amino, carboxyl, thiol, and carbonyl groups and since there are many replaceable hydrogen atoms in proteins and other macromolecules, hydrogen bonds would be expected to play significant roles in drug-macromolecule interactions. Hydrogen bond formation may be inter-or intra-molecular. Intramolecular hydrogen bonding give rise to ring formation or chelation and this is usually when the formation of a 5-, 6-, or 7-membered ring is possible.

Van-der waals forces operates in all types of molecules, both polar and non-polar, are the most universal forces of attraction that arise between atoms and result from natural polarisation in electron clouds induced by atoms as they approach one another. The electron clouds are thereby distorted by this polarisation and as a result the nuclie of each atom are attracted to the electrons of the other atom. Although Van der waals forces are weak and short-acting, they are an important element of the binding processes. The

aromatic hydrocarbon portions of drug molecules are believed to interact with flat surfaces on receptors through Van-der waals attraction. Since several biologically active molecules contain aromatic rings, Van der waals forces can be considered as very important bond. Van-der waals attraction play a significant role in the binding of steroids to plasma proteins, in the enhancement of fat solubility, protein binding and hypnotic activity of hydrocarbon substituents in barbiturates (Olaniyi, 1990).

Hydrophobic interaction describes the Van der waals attractions between atoms in the non-polar parts of two molecules immersed in water. Hydrophobic bonding is very important for the binding of small molecules to biological macromolecules, thus, it plays an important role in stabilizing the conformation of proteins, in the transport of lipids by plasma proteins and in the binding of steroids to other receptors (Olaniyi, 1990).

1.2.3 TECHNIQUES USED FOR DRUG-MACROMOLECULE INTERACTION STUDIES.

To ascertain the likelihood of a drug's displacement from serum albumin (or any macromolecule) by a second drug, recourse to in-vitro model test system is necessary. Many techniques are in use, the most favoured being ultrafilteration, equilibrium dialysis and gel chromatography methods. Unfortunately, using these techniques free drug is measured, which creates analytical difficulties in assessing the binding and displacement of those drugs which are highly protein bound and in which displacement is most likely to have serious practical consequences (Bridges, 1977).

In recent years, spectroscopic techniques have played an important role in studies of drug binding to proteins and other biologically important macromolecules (Chignell, 1974) when compared to the more classical methods available for studying drug binding, spectroscopic techniques have two distinct advantages. Firstly, they are much more rapid than procedures such as equilibrium dialysis. Secondly, they cannot only be used to measure the amount of bound and free drug in a given system but can also yield additional information on the nature of the drug-protein interaction. For example, changes in the ultraviolet (UV) or visible absorption spectrum of a drug may be interpreted in terms of the polarity of a drug-binding site (Chignell, 1974). Nuclear magnetic resonance (NMR) spectroscopy often indicates which groups or parts of a\ drug molecule are involved in the binding process, whereas circular dichroism may yield information on the three-dimensional structure of the drug-binding site (Chignell, 1974).

Fluorescence spectroscopy is undoubtedly one of the most versatile techniques currently available for the study of drug interactions with proteins (Chignell, 1974). The fluorescence of a drug molecule may be characterised by such parameters as the wavelength of maximal activation and emission, quantum yield, fluorescence lifetime, and degree of polarization (Chen, 1972). Since these parameters are often exquisitely sensitive to changes in the micro-environment of the fluorophore, it is perhaps not surprising that the fluorescence of a drug molecule may be drastically altered when it binds to plasma albumin. Similarly, the fluorescence characteristics of plasma albumin may be altered by the binding of a drug, if neither the drug nor the protein has

suitable fluorescence properties, a drug-albumin interaction can often still be studied by complexing (Covalently or non-covalently) the protein with a fluorescent label (Chignell, 1974). Fluorescence spectroscopy is especially useful for measuring the binding of drugs to, and their displacement from, plasma proteins, since it is more rapid than equilibrium dialysis and is often more sensitive than other spectroscopic techniques, such as U.V. and visible absorption spectroscopy and N.M.R. In addition, fluorescence spectroscopy can also provide information on the structure of a drug binding sites and the nature of the binding forces involved (Chignell, 1974).

1.3 INTERACTIONS AFFECTING DRUG METABOLISM

1.3.1 BIOTRANSFORMATIONS OF DRUGS

Drug metabolism may take place in the gastrointestinal tract (G.I.T) contents, in the G.I.T. wall during absorption, in the liver, and in various tissues of the body, particularly in the Lung and Kidney. However, drug metabolism occurs mainly in the liver. The drug-metabolising enzymes of the liver cell occur in microsomes and in non-microsomal areas.

Rate of drug metabolism is controlled by genetic, environmental, and circadian rhythm factors. Genetic control is probably predominant. Environmental factors can be very important also. For example, certain chlorinated insecticides induce drug metabolising enzymes and cause a more rapid metabolism of certain drugs.

Drug metabolism is capacity-limited hence the kinetics of drug metabolism are non linear when the entire concentration range of substrate is considered. The limitation may arise in several ways. For example, because of a limited amount of co-factors involved in the biotransformation, or because of limited amount of a reactant such as the availability of sulphate in sulphate conjugation.

The pathways of drug metabolism have been divided into phase I and Phase II reactions.

Phase I (functionalization or non-synthetic) reactions include Oxidation, reduction and hydrolytic reactions in which a new functional group is introduced into the drug molecule or an existing functional group is modified, making the drug more polar, and therefore more readily excreted. Generally, most of these reactions occur at the more reactive centres of the molecules, such as hydroxyl, or amino groups, allylic carbons and aromatic rings. Oxidation is the most common and important Phase I reactions and include aromatic and aliphatic hydroxylation, oxide formation, desulfurization, deamination, dehalogenation, N-O-and S-dealkylation, and sulfoxidation. Reduction reactions include aldehyde reduction, azoreduction and nitroreduction, while hydrolytic reactions include de-esterification and deamination. Oxidative and reducing enzymes are found primarily in the liver microsomes. Hydrolytic enzymes are located in the plasma, liver microsomes and many other tissues.

Phase II (conjugation or synthetic) reactions are enzyme synthesis whereby a functional group is masked by the addition of an endogenous substance such as acetyl, sulphate, glucuronic acid, or certain amino acids, which increases the polarity of the drug. Conjugating enzymes occur mainly in the liver but also in other tissues. Most drugs undergo both phase I and Phase II reactions. Drugs that are resistant to drug-metabolising enzymes or that are highly hydrophilic are excreted largely unchanged. The basic pattern of drug metabolism is common to all animal species, including man, but there are species differences in details of the reaction. The activity of the enzyme systems involved are also subject to wide inter-individual variability (Remmer et al, 1972).

As a consequence of a drug's biotransformation, either more or less biologically active compounds may be produced. Because the majority of drugs, together with many non-nutritive constituents of food and endogenous substances (for examples, benzpyrene, eugenol, butylated hydroxytoluene (BHT), oestradiol, cholesterol) are metabolised by common enzyme systems, the potential for interactions among such compounds is considerable (Bridges, 1977). Interactions may arise through either competitive or non-comnpetitive inhibition or by enhancement of drug-metabolising enzyme activity. The biological implications of these enzyme activity alterations will depend not only on the extent of the change in the rate of metabolism of the drug produced by the enzyme concerned, but also on the alternative metabolic and distributional fates available and on the relative biological activities of drug and metabolites. Because inactivation and enhanced excretion is the most

common consequence inhibition potentially represent a clinically more important drug interaction than ENZYME induction (Bridges, 1977).

Interactions might be expected to assume greatest significance for drugs whose metabolic fate is determined by variations in the activity of a single rate limiting enzyme which becomes largely saturated at the therapeutic level. For drugs which are metabolised concurrently by several independent enzymes a change in the activity of one enzyme alone will not generally be important unless it is responsible for the formation of more active metabolites (Bridges, 1977).

1.3.2. CHARACTERISTICS OF CYTOCHROME P-450 ENZYMES.

The liver microsomal hydroxylating system is centred on the cytochrome named, from its characteristic absorption spectrum, cytochrome P-450. For this family of enzymes, a very wide variety of substrates appear to compete for the major binding site (known as the type I site) association with which is probably a prerequisite for the metabolism of some compounds. Lipophilicity rather than steric considerations is generally the major determinant of binding (Bridges 1977). The presence of ionisable groups or substituents capable of hydrogen bonding also tends to enhance binding.

A number of apparently different reactions are catalysed by the cytochrome P 450-dependant system other than hydroxylation; those include oxidation, dealkylation, deamination and sulphoxidation. Any compound possessing a sufficiently high chloroform to water partition ratio (high liphophilicity) will

be attracted by the lipoidal membrane and then will be metabolised; those enzyme systems therefore metabolised most xenobiotics, as well as steroids, hemin, bilirubin, indoles, thyroxine, sympathomimetic amines and fatty acids (Conney, 1967).

From this lack of specificity arises the ability of a single inducing agent to stimulate the metabolism of a host of other drugs and also of one drug to inhibit the metabolism of another structurally unrelated drugs (Davies, 1972; Pearson and Havard, 1974). So many drugs and environmental chemicals are implicated in cytochrome P450 enzymes induction and inhibition. Cytochrome P450 enzyme systems inducers include phenobarbital and many other drugs and environmental chemicals, including chlorinated hydrocarbons, insecticides, carcinogenic hydrocarbons, food additives, and cigarette smoke (Conney and Burns, 1972). Inhibitors of cytochrome P450 enzymes such as phenylbutazone and imidazole compounds have since been recognised (Powell and Donn, 1983).

The activities of the cytochrome P450 dependant systems are extremely sensitive to differences in sex, age, strain and species and to differences in the hormonal and nutritional state of the animal (Conney, 1967). Thus, it is not surprising that the rate of elimination for a drug that is primarily metabolised is far less predictable than for a drug that is eliminated unchange in the urine (Park and Breckenridge, 1981).

1.3.3 ENZYME INDUCTION-DRUG INTERACTIONS

Chronic administration or administration of several doses, of one drug, may cause stimulation of its own rate of metabolism or may lead to enhanced metabolism of one or more other drugs. The process has been called enzyme induction (Testa and Jenner, 1978).

As to how these augmenting action arise is a matter of contention and biochemical discussion, but it has been suggested that they are due to the synthesis of enzyme protein being stimulated by polycyclic hydrocarbons and drugs in virtually all pharmacological classes, and therefore increase the rate of metabolism, of many drugs (Conney, 1967; Gillette, 1967). Support for this view is given by the observation that the induction of increased microsomal enzyme activity is completely prevented by introducing the amino acid antagonist, ethionine, into the system. This prevents the incorporation of methionine and glycine into liver protein (Griffin and D'Arcy, 1979). Smoking, alcohol, pesticides and many drugs will act as enzyme inducers. Table 1.3.3.1. Lists some drug interactions due to enzyme induction (Griffin and D'Arcy, 1979).

TABLE 1.3.3.1. Some drug interactions due to enzyme induction.

ENZYME INDUCERS	DRUG ACTIVITY REDUCED BY ENHANCE METABOLISM
Alcohol	Phenytoin, Pentobarbitone, Tolbutamide, Warfarin
Barbiturates	Coumarin anticoagulants, cortisol, digoxin dipyrone, doxycycline,phentoin, testosterone
Chloral hydrate	Dicoumarol
Glutethimide	Dipyrone, warfarin
Griseofulvin	Warfarin
Phenylbutazone	Amodiaquine, cortisol
Phenytoin	Cortisol

The barbiturates as a drug group present a classic example of enzyme induction. When administered chronically they not only stimulate their own metabolism (auto-induction), but they also stimulate the metabolism of other drugs (cross-induction), notably phenytoin (Kutt et al, 1969) and griseofulvin (Bushfied, et al; 1964; Riegelman et al 1970) and decrease their action. The acceleration of coumarin metabolism and the resulting inhibition of coumarin-induced hypoprothrombineaemia has been well established for nearly all the barbiturates in use. A review of barbiturate-coumarin interaction has been published (Koch-Weser and Sellers, 1971) and the danger of suddenly withdrawing the barbiturate in such cases without adjusting the anticoagulant dosage has been emphasized (Williams et al, 1976; D'Arcy and Griffin, 1979). The barbiturates are also capable of accelerating the metabolic breakdown of endogenously secreted chemicals. Animal studies have shown that dosage

with phenobarbitone for several days increases the activity of liver enzymes that hydroxylate glucocorticoids, and androgenic, oestrogenic and progestational steroids; barbiturates have also been shown to accelerate the metabolism of synthetic oestrogens and progestational steroids used widely in oral contraceptives (Griffin and D'Arcy, 1979). Clinical reports of contraceptive failure in women on anticonvulsants have been documented (Coney, 1967; Azarnoff and Hurwitz, 1970; Janz and Schmnidt, 1974).

The metabolic fate of triated vitamin D_3 in phenobarbitone-treated volunteers and children with anti-convulsant rickets showed shortened biological half life of the vitamin. There is a considerable evidence accumulating that other enzyme-inducing drugs shorten the half life of vitamin D_3; and prolonged glutethimide administration has been shown to cause osteomalacia associated with vitamin D_3 (Greenwood et al; 1973).

It is known from animal studies, that barbiturates stimulate the enzymatic glucuronide conjugation of bilirubin by liver microsomes. It is therefore suggested that barbiturates might be effective in the treatment of hyperbilirubinaemia in man. A number of clinical studies have confirmed this (Crigler and Gold, 1966; Yaffe et al; 1966).

Antibiotics can also act as enzyme inducers. Example, it have been shown that rifampicin administered constantly at 600mg per day for 7 days gave significantly lower blood levels on the seventh day than it did on the first day of treatment (Acocella et al; 1972). This problem has particular relevance in long term treatment of infections. Also, it has been shown by several workers

(Burley, 1977) that rifampicin administration significantly lessens the hypothrombinaemia caused by the administration of certain anticoagulants such as acenocoumarol and warfarin.

Rate of metabolism of alcohol in alcoholics who have recently been drinking can be more than twice than in abstinent subjects (Kater et al, 1969). Thus, this aspect of stimulated metabolism is non-specific, and also extends to certain drugs. Example, serum level of phenytoin, falls more rapidly in alcoholics than it does in abstinent controls. Tolbutamide and warfarin have shorter half-lives in the blood of alcoholics, than in abstinent controls. These effects are due to the alcohol having an inductive effect on microsomal enzymes.

Cigarette smoke contains minute amounts of polycyclic aromatic hydrocarbons which are potent inducers of microsomal enzymes (Conney, 1967). The link between smoking and enzyme induction was strengthened by the work which established that smoking enhanced the metabolism of nicotine (Beckett and Triggs, 1967). This probably explains why smokers develop tolerance to the effects of nicotine. It was also shown that the rate of metabolism of the analgesic pentazocine was more rapid in smokers (Kerri-Szanto and Pomeroy, 1971).

Controlled studies have shown that insecticide residues in animals and man stimulate drug-metabolising enzymes in the liver. It was reported that the metabolism of phenylbutazone in dogs was increased after a single dose of chlordane (Hart et al, 1963). Also, metabolism of phenazone was reported to accelerate in farm workers exposed to dichlorodiphenyltrichloroethane (DDT

or dicophane) and gamma benzene hexachloride (Lindane) (Kolmodin et al; 1969).

In conclusion, enzyme induction might lead to serious drug-interactions of therapeutic significance. The therapeutic implications are that doses of drugs may need to be increased to obtained satisfactory therapeutic benefits and also that there is a real danger of toxicity after enzyme inducer withdrawal if reduction of drug dosage is not made when enzyme-inducing activity wins.

1.3.4 **ENZYME INHIBITION-DRUG INTERACTIONS.**

The slowing or inhibition of the metabolism of one drug by another is an effect already well documented in animal studies and there are many examples of drugs and other substances inhibiting the metabolic degradation of other drugs. SKF-525A (β-diethylamino ethyl diphenylpropyl acetate) was reported to inhibit a wide diversity of microsomal enzymes (Skovsted et al, 1974), thereby prolonging the action of drugs, such as hexobarbital, aminopyrine and ephedrine.

Drugs causing enzyme inhibition include chloramphenicol, (Christensen and Skovsted, 1969) monoamine oxidase inhibitors (MAOIs) (Marley, 1977), para amino salicylic acid (PAS) (Kutt et al, 1966), pheniprazine, triparanol and the two norcotics, pethidine and morphine (Griffin and D'Arcy, 1979). Several other example of drugs causing inhibition of drug metabolism have been reported (Skovsted et al, 1974). Other compounds, for example, chlorcyclizine, glutethimide and phenylbutazone, can inhibit or stimulate drug

metabolism by enzymes depending upon whether they are administered acutely or chronically, (Griffin and D'Arcy, 1979).

Inhibition of hepatic microsomal enzymes lead to reduced drug clearance and increased steady state plasma levels. Inhibition of microsomal enzymes is therefore liable to precipitate toxicity. Several cases of severe, even life-threatening, drug intoxication due to interactions of this type have been observed and examples are listed in table 1.3.4.1. below.

However, metabolic inhibition can lead to decreased toxicity when the toxicity is due to a metabolite or can produce no change in drug activity when the metabolite has a similar pharmacologic profile to the parent drug. Enzyme inhibition is especially important in relation to the therapeutic use of monoamine oxidase inhibitors (MAOIs) and the tricyclic antidepressants, the coumarin anticoagulants, the oral hypoglycaemic agents and the xanthine oxidase inhibitors, such as allopurinol.

Table 1. 3. 4. 1. Example of serious drug-intoxication cases caused by inhibition of drug biotransformation (Skovsted et al, 1974)

INTOXICATING DRUG	INHIBITORY SUBSTANCE	SIGN OF TOXICITY
Tolbutamide	Sulfaphenazole	Hypoglycaemic collapse
Tolbutamide	Phenylbutazone	Hypoglycaemic collapse
Tolbutamide	Bishydroxycoumarin	Hypoglycaemic collapse
Tolbutamide	Chloramphenicol	Hypoglycaemic collapse
Chlorpropamide	Phenylbutazone	Hypoglycaemic collapse
Chlorpropamide	Bishydroxycoumarin	Hypoglycaemic collapse
Phenytoin	Isoniazid,para amino-Salicyclic acid.	Cerebellar symptoms
Phenytoin	Bishydroxycoumarin	Vertigo, anorexia
Phenytoin	Phenylbutazone	Vertigo, anorexia
Phenytoin	Disulfiram	Vertigo, anorexia
Phenytoin	Sulthiame	Vertigo, anorexia
Phenytoin	Sulfamethizole	Vertigo, anorexia, Cerebellar symptons

The mechanism by which inhibitors exert their effect is not well understood. Even in-vitro experiments have not given any complete answer to the problems. However, the predominant types of inhibitions are competitive and non competitive.

Some enzyme inhibitors may bind avidly to the major binding site (type I site) of cytochrome P-450 enzyme system without being effectively metabolised by

the enzyme, because of a low maximum velocity of the enzyme-substrate reaction (Vmax) and a low Michelis Menten constant (Km). Such compounds competitively prevent the drug substrates from binding to the type I site of the enzyme system, association with which is probably a prerequisite for the metabolism of the substrates. The consequences of which is an inhibition or slowed metabolism of the drug substrates due to microsomal drug-metabolising enzyme inhibition of the competitive nature. Oxyphenbutazone potentiates the anticoagulant action of warfarin, and also inhibit metabolism of phenylbutazone by this mechanism.

Other compounds, especially those with several basic nitrogen atoms, may bind directly to the haem iron (type II site) of the cytochrome P-450 enzymes thus interfering with the ability of the enzymes to ligand oxygen which is a prerequsite for oxidation of a drug. Examples of such compounds include some imidazoles and triazoles (Wilkinson et al, 1974a). Other inhibitors may inhibit by destroying the intergrity of the cytochrome-for example, sedormid and carbon tetrachloride (Bridges, 1977). A further category of potentially potent inhibitors comprises those which are metabolised to intermediates which then bind more avidly to cytochrome P-450. Many hydrazines and methylene dioxyphenols appear to inhibit in this manner (Bridges, 1977).

Many interactors cause a biphasic effect on drug metabolic enzymes, producing an initial inhibition generally due to competition for the enzyme sites, followed by stimulation of protein synthesis to generate new enzymes,

thus permitting enhanced drug metabolism activity. Enzyme inhibition can also be stereo-specific.

1.4: INTERACTIONS AFFECTING DRUG EXCRETION

Change in renal function can modify a number of pharmacokinetic processes in the body and thereby lead to unanticipated drug effects or interactions (Rendenberg, 1974). Those observations lead one to conclude that the study of renal function and drug action can help one to understand factors that modify drug action and also lead to improvements of drug therapy for azotaemic and uraemic patients. Drug excretion is slowed in patients with impaired renal function. Various methods have been proposed for reducing approximately the usual doses of drugs eliminated from the body by the renal excretion of unmetabolised drugs or active metabolites, thus correcting for dosage for the slowed drug excretion in uraemia (Reidenberg, 1974). Often a combination of dosage reduction with prolongation of dosage interval is used. This modification of dose is essential for safe, effective use of excreted drugs in patients with impaired renal function. When a drug is excreted by extra-renal as well as renal pathways, impaired renal function can decrease urinary excretion significantly without necessarily changing the extra renal excretion rate constant. Thus, the overall elimination rate constant will fall and the major pathway of elimination may shift from renal to extra renal. Excretion of drugs by the kidneys is a net effects of 3 processes. Passive glomerular filtration, active tubular secretion and passive tubular diffusion. Glomerular filtration is not greatly influenced by other drugs, although displacement from

protein binding sites may lead to increased concentrations of drug in glomerular fluid and enhanced renal elimination (Rawlins, 1977a).

Tubular secretion of acidic drugs is mediated by a relatively non-specific active transport system, and a variety of endogenous (for examples, bilirubin glucuronide, uric acid) and exogenous (for example, salicylates, penicillins, probenecid, phenylbutazone, indomethacin, thiazide diuretics and frusemide) compounds are potential substrates. Competition for tubular secretion may lead to a major drug interaction involving the kidney for substances whose major route of elimination is via this pathway. Thus, probenecid impairs the elimination of salicylate, penicillins and indomethacin (Rawlins, 1977a), by competition for tubular secretion. Another mechanism of drug interaction affecting drug excretion is the effect of a drug on tubular reabsorption of another drug into the blood as the glomerular filtrate passes down the nephron. Tubular re-absorption is passive and depends upon the lipid solubility with which a drug can cross the tubular epithelium and on the concentration gradient between tubular fluid and plasma water. Thus changes in urinary pH induced by ingestion of ammonium chloride, sodium bicarbonate, carbonic anhydrase inhibitors etc. may have a profound influence on the excretion of weak acids and bases (Milne, 1965). In general, acidic or basic drug may be expected to show the phenomenon of pH-dependent excretion if the un-ionized fraction is lipoid-soluble and if the pKa is within a favourable range of 7.5-10.5 for weak bases, and 3.0-7.5 for weak acids. Weak acids are excreted at a higher clearance in highly alkaline urine, and weak bases in acidic urine. Drugs which are known to show the phenomenon of pH-

dependent excretion include the weak acids salicylic acid, phenobarbital, nitrofurantoin, nalidixic acid and some sulphonomides. The weak base include quinacrine, chloroquine, procaine, mecamylamine, meperidine, levorphanol, quinine, imipramine and amitriptyline (Hartshorn, 1973).

Drugs which alter the pH of the tubular urine can markedly affect the excretion of other drugs. Examples would include the hastened excretion of streptomycin and the reduced excretion rate of mecamylamine in an alkaline urine, and the enhanced effectiveness of the mercurial diuretics in an acidic urine (Harstshorn, 1973). This phenomenon has been extensively investigated with phenobarbital and alkalinization of the urine and put to use treating patients with phenobarbital poisoning (Rendenberg, 1974).

1.5 PHYSIOLOGICAL AND PATHOLOGICAL FACTORS AFFECTING DRUG INTERACTIONS.

Many physiological factors exert their influence on drug action and interaction by affecting the drug-metabolizing enzymes. The metabolism of a drug is a biochemical reaction. It is quite aware that increase in temperature speeds up chemical reactions, hence, this is also true with the metabolism of drugs. Things are not quite so simple, however, for temperature also influences other physiological functions in the body, such as the rate of distribution, binding and excretion of drug. During starvation there is a lack of nutrients including amino acids, and consequently there is a reduction of enzyme protein. Thus, a person in a poor nutritional state might by expected to be extra-sensitive to drug action because the relative lack of drug-metabolizing enzymes results in

depressed drug metabolism. Similarly, a lack of substances essential to enzymatic activity, such as ascorbic acid, may increase the sensitivity to drugs (Burns and Conney, 1974).

The newborn have a relatively lower glomerular filtration rate and renal plasma flow than adults and are also seriously deficient in drug-metabolizing enzymes for at least the first month after birth. These deficiencies are enhanced in premature babies; thus neonates may fail to metabolize effectively Vitamin K analogues, sulphonamides, chloramphenicol, barbiturates, morphine and curare (Griffin and D'Arcy, 1979). Penicillin excretion is also delayed.

In the geriatric patient drug overdose is particularly likely to occur if the drug remains active in the body until it is excreted by the kidneys. This is so because renal function diminishes with increasing age even in the absence of clinically detectable disease. Indeed at 90 years of age the functional capacity of the 'normal' kidney may be only half what it was at 30 years (Agate, 1963). Diminished renal function may be made worse by dehydration, congestive heart failure (C.H.F), urinary retention or diabetic nephropathy, all of which are more frequent in the elderly patient. Under these circumstances drugs such as streptomycin, digitalis and oral hypoglycemic agents may prove a real hazard in some elderly patients (Griffin and D'Arcy, 1979). Slower metabolism of digoxin in the elderly makes them particularly liable to interactions between hypokalemia-producing diuretics and the cardiac glycosides (Goodman and Gilman, 1965a).

The metabolic handling of a drug may be altered by the presence of various pathological conditions. The absorption of a drug may be altered by the presence of gastrointestinal disease either due to increased or reduced transit time through the gut, but also may be altered by conditions such as coeliac disease where the degree of sulphation of certain drugs in the gut wall may be considerably less than in normal patients. The rate of metabolism or elimination of a drug may be reduced in the presence of hepatic or renal disease but may also be reduced where the blood flow or cardiac output is reduced as in shock or cardiac failure. Also one might expect Mono amine oxidase Inhibitors or other microsomal depressants to have a potentiated effect in conditions such as obstructive jaundice where microsomal activity is already depressed. Disease may alter the incidence of adverse reactions to drugs for reasons that are not apparent at present, e.g; vincristine-induced neuropathy occurs more commonly in patients with lymphoma than in patients with non-lymphoma malignancies (Watkins and Griffin, 1978).

1.6 DRUG INTERACTIONS *IN VITRO*

Drug may interact prior to administration. This was a frequent, but well-recognized hazard in the bygone days of the elaborate compound prescription. Incompatibility was the only drug interaction that caused concern then, but times have changed, and the unit dosage from the bottle of medicine has been largely replaced by the tablet, capsule or injection of the newer therapeutic agent. This specific problem of *in vitro* drug interaction has been forgotten for over a quarter of a century. It has now come back into prominence as a result of the common vogue of adding multiple drugs at patient level in the

ward to commercially prepared standard intravenous (I.V) infusions, and also as a result of the gradual realization that the formulation of the solid dosage form, the tablet or capsule, can have pronounced effects on the bioavailability of the active component (Griffin and D'Arcy, 1979).

There are many reasons for the practice of haphazard addition of drugs to I.V. fluids (Griffin and D'Arcy, 1979). There is a good reason to believe, however, that in many instances it is just a matter of convenience to use the infusion for the administration of another drug. There is also good reason to believe that many drugs are given via an infusion bottle without due regard to the stability and therapeutic integrity of such combinations.

It is inevitable that the number of situations in which drugs are added to infusion fluids is increasing; this practice reflects the expanding use of a wide range of I.V. fluids. In a survey of 10 hospitals in Ulster, carried out during a 1-month period in January 1972 or January 1973, it was found that of a total of 7900 separate I.V. infusions documented, 3096 (39.2%) had drugs injected into the infusion container, and in a considerable proportion of these the additives were multiple (D'Arcy and Thompson, 1974). In a ward study of I.V. additives, it was reported that 44% of I.V. solutions used contained drug additives, and that multiple additives were present in 17 (24%) of the fluids containing additives (Harrison and Lowe, 1974). Interestingly, it was found that single drugs were added to fluids for medical patients, whereas surgical patients, 15% of the additives contained two or more drugs (Brodie et al, 1974).

There is growing evidence that some drugs will not retain their integrity and efficacy in certain fluids if they are added alone or with other drugs (Griffin and D'Arcy, 1979). When drugs are added to infusion solutions a number of unwanted changes may occur; there may, for example, be an interaction between the drugs and the infusion fluid, or there may be an interaction between drugs themselves. In either of these situations, the drug or drugs and the fluid may be degraded. The rate at which these processes occur will vary from situation to situation and, like all chemical and physicochemical reactions, it will be influenced by temperature, time, pH, exposure to light, presence of electrolytes and the inherent instability of those fluids which are emulsions or contain components in saturating or near saturating concentration (Griffin and D'Arcy, 1979). There are two further problems associated with the addition of drugs to infusion fluids; the first of these is sterility (Lancet, 1974). The second problem is that for many drugs the original trial work was done on injection solutions of a few milliliters given I.M. or I.V. The pharmacokinetic implications of diluting a drug to 500-1000ml with the infusion fluid are thus not always established or even realized. For instance, in a study (French, 1972), it was reported that the peak plasma level of gentamicin (60mg) when injected over a period of 60 minutes only just attained the lowest plasma level of the drug when the same dose (60mg) was injected over a period of 5 minutes.

In the majority of drug administrations, the clinical efficacy of the drug will be directly related to the infusion rate, and yet when added to an I.V. drip, the drug can enter the circulation in high dilution at a rate that is virtually

arbitrary and certainly not at which it is predetermined to provide optimal blood levels (Griffin and D'Arcy, 1979). Awareness of problems of compatibility of drugs *in vitro* and of the implications of diluting drugs in infusion fluids is in itself a step forward in making such administrations safe and effective (Griffin and D'Arcy, 1979).

For the practice of mixing injection solutions of drugs in the same syringe, all the known hazards of an invitro drug interaction apply and may be easily be enhanced by the relatively stronger concentrations of drugs involved. Moreover, it is unlikely that the pH of two injection solutions will be similar and the resultant pH or solubility characteristics of the mixture optimal for both drugs. An example of this type of practice is the mixing of soluble insulin injection and protamine zinc insulin injection together in the same syringe. The soluble insulin interacts with excess zinc and protamine so that the onset of action of the added insulin is delayed. In such circumstances the insulins are best injected separately at different sites (Alstead et al, 1971; Goodman and Gilman, 1965b).

Excluding the obvious and usually established incompatibilities, there is now a growing awareness that the composition of solid dosage formulations may influence the release and subsequent absorption of the active ingredient(s) in the intestine and the plasma levels it achieves. 'Bioavailability' is a word frequently used, but it should realized that the interference of a formulation ingredient, whether it be a diluent or an active principle, with the optimal

activity of another active components is just one further facet of drug interaction in its widest sense (D'Arcy, 1973).

1.7 PRINCIPLES OF PHARMACOKINETICS

Pharmacokinetics is concerned with quantitatively accounting for the whereabouts of a drug after it has been introduced into the body. It involves the studies of these rates of absorption from the various Loci of administration, of distribution in the body, of metabolic transformation and of elimination (Van de Kleijn and Vree, 1979). The application of the information obtained from theoretical and experimental pharmacokinetics for the treatment of patients is further specified by the adjective clinical (Van der Kleijn and Vree, 1979).

The rates at which pharmacokinetics processes occur simultaneously are characteristic for every drug with the restrictions of a number of variables. Often a well chosen drug may fail or result in toxicity, because too little or too much is present at the site of action for too short or too long a time. For most drugs, the extent and duration of drug action depends on how much of the drug gets to the receptor sites and how long it stays there (Paxton, 1981). Thus, it is the aim of clinical pharmacokinetics to describe the relation between the dose administered to an individual patient, and the concentration that results in blood, plasma or any other body fluid available for sampling, in organs and in tissues and if possible, in the so-called biophase near or on the receptors, as a variable of time.

1.7.1: COMPARTMENT MODELS IN PHARMACOKINETICS

Once absorbed, a drug disperses to a variety of locations, within the body. The rate of distribution into a tissue depends on its vascular perfusion, the permeability of the tissue membranes to the drug and the relative partitioning of drug between tissue and blood. These complex procedures may be simplified by considering the body to consist of a number of compartments. These are postulated to account for the experimental observations that drugs distributed into various fluids and tissues at different rates (Paxon, 1981).

A compartment is defined as a kinetically distinguishable "pool" in terms of the drug concentration - time profile. If data indicate loss of a certain fraction of drugs per unit time, this site would constitute a compartment regardless of whether or not the anatomical or physiological significance were known (Notari; 1975). Thus, the number of compartments which can be defined in any particular case will ultimately depend on the number of areas with different rates of drug penetration. Experimentally it is difficult to demonstrate more than 3 compartments, and usually only 2 can be differentiated (Paxon 1981).

The basic approach used in pharmacokinetics is to fit experimental data on drug concentrations in plasma to mathematical equations, that represent the flow of drugs and their metabolites through the discrete compartments of a model system. although each individual drug possesses its own unique properties, it is possible to apply certain general principles to the manipulation of pharmacokinetic data, resulting in a precise description of drug disposition.

Before any equations may be derived from these models, several assumptions have to be made (Paxon, 1981). Firstly, drugs enter the system only via the central compartment, and are eliminated only from that compartment. Secondly, reversible transfer occurs between central and peripheral compartments, and thirdly, the exit of drugs from all compartments in the system is described by first- order kinetics. Under this last assumption the rate at which a drug is removed from a compartment is directly proportional to the drug concentration in it.

If the distribution of drug throughout the body is very rapid relative to the rate of elimination, then absorption rate constant (k_{ab}) >> Elimination rate constant (k_{el}) and this condition can be described by the single compartment open model. Single compartment is the most simplest model, and depicts the body as a single homogenous unit in which the drug entering the body distributes instantly between the blood and the body fluids or tissues. Single-compartment open model is actually an approximation or simplification used to describe the two-compartment open model when $k_{ab.} >> K_{el}$ (Notari; 1975). The characteristic of single-compartment open model may be illustrated as in Figure 1.7.1.1

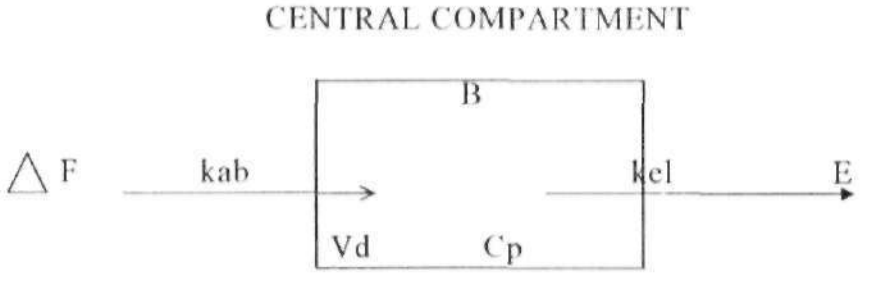


FIGURE 1.7.1.1. Characteristics of a single-compartment open model.

D= Dose of drug administered

F= Bioavailability

Kab= Absorption rate constant

B= Body, composing of blood, body fluids and tissues

Vd= Volume of distribution

Cp= Drug concentration in plasma

Kel= Elimination rate constant

E= Routes for drug elimination

Assumptions that the body behaves as a one compartment 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, than an intravenous (I.V) Injection of a drug into this model (Figure 1.7.1.1), the concentration (C_o) in the plasma immediately after injection is equal to the dose (D) divided by the Volume of the compartment (Vd), i.e.

$$C_o = D/ Vd$$

This apparent volume of distribution (Vd) 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 (Paxon, 1981).

After instantaneous distribution, the concentration will fall according to first order kinetics which means that a constant fraction of drug present is eliminated per unit time. Thus a plot of plasma drug concentration against time on the abscissa with each unit representing the time for one half of the

drug to be removed, results in the exponential curve shown in figure 1.7.1.2. Replotting this curve with concentration on a logarithmic scale, results in a straight line (Figure 1.7.1.3).

This plot is easily extrapolated back to zero time to give C_o, the theoritical intial concentration. The elimination half life ($t_{1/2}$ el) of the drug is represented by $t_{1/2}$ (Figure 1.7.1.2 and 3).

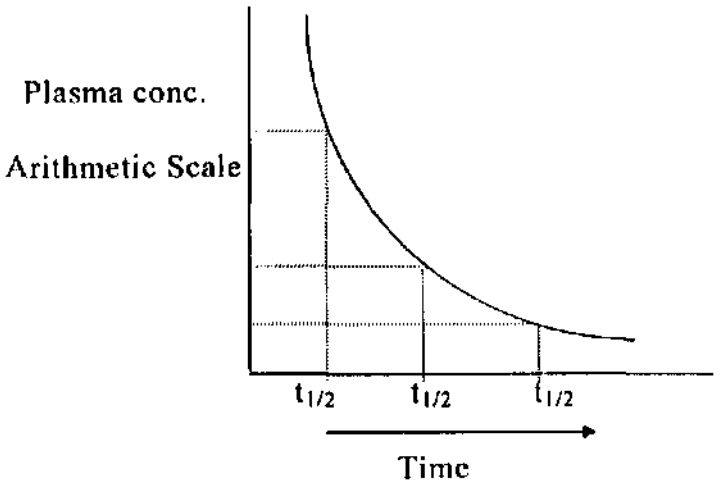


FIGURE 1.7.1.2: Plasma concentration-time plot on a liner (arithmetic) scale following a rapid I.V. Injection of a drug into a single-compartment open model.

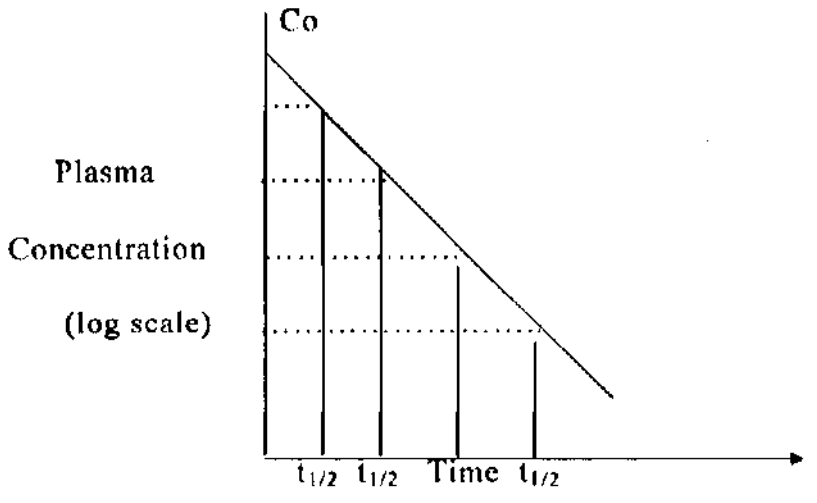


FIGURE 1.71.3: Plasma concentration-time plot on a logarithmic scale following a rapid I.V. Injection of a drug into a single compartment open model.

If distribution of the drug is so slow that it cannot be disregarded; a model (Two-compartment) must then be considered which contains a central and peripheral compartment. The two- compartment open model may be illustrated as in Figure 1.7.1.4.

The central compartment, composed of blood and well perfused tissues, while the peripheral compartment composed of the tissues or the rest of the body. All drug removed from the body, regardless of the route of elimination, is from the central compartment. The central compartment is open in the sense that elimination occurs from it. Thus, the entire system is open, since the drug passes reversibly from blood to tissue. With an open system, drug is always being lost from one compartment, so that, depending on the rate of equilibration, the ratio of drug in the tissues to drug in the plasma may or may not reach its equilibrium value (Notari, 1975).

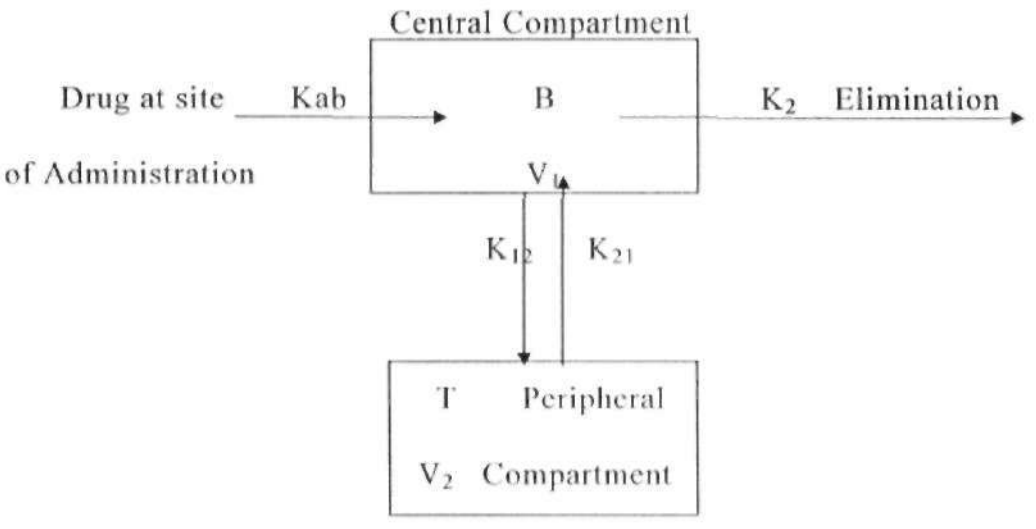


FIGURE 1.7.1.4.: Characteristics of Two-compartment Open Model.

Where,

B = Blood and well-perfused tissues

T = Less perfused tissues or the rest of the body

K_{12} and K_{21} = Rate constants for drugs transfer between the two compartments

K_2 = Rate constant for elimiation of drug from the blood

V_1 and V_2 = Distribution volume for central and peripheral compartment respectively.

Rate constants for drug transfer between the central and peripheral compartments are represented by K_{12} and K_{21} and K_2 is the rate constant for elimination. It is important to realize that K_2 is not the same as Kel; the overall rate constant for elimination from the body (Notari, 1975

It has been noted (Garrett, 1970; Wagner, 1971) that the preferred compartmental model is the one containing the fewest compartments which adequately describe the data. While one - and two-compartment models accommodate a great many drugs, there are a number of cases where these are not sufficient. Significant distribution of drug in deep tissues such as bone or fat, or strong binding to any tissue, may result in the appearance of a triexponential blood level curve, indicating the presence of a third compartment (Notari, 1975), thus the three-compartment open model (Figure 1.7.1.5).

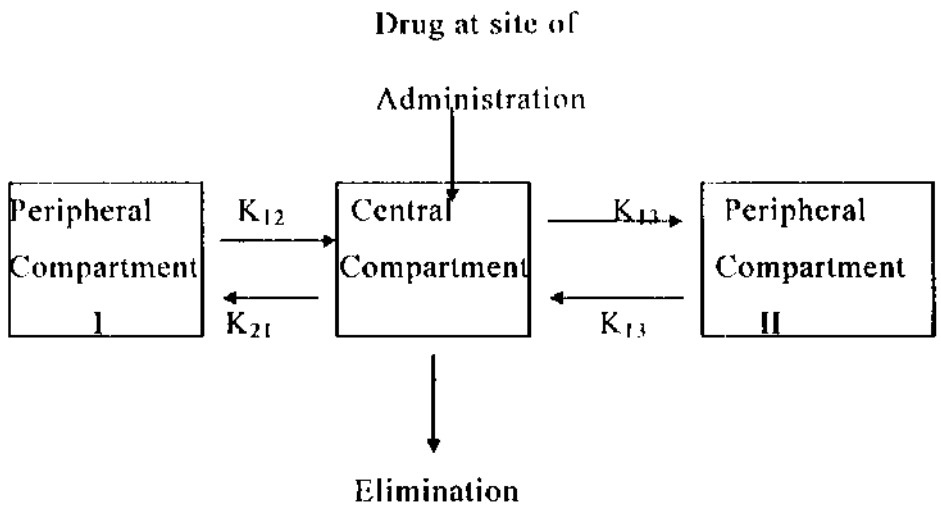


FIGURE 1.7.1.5: Three-compartment open model for a rapid I.V. administered drug.

A modified three-compartment open model in which one of the peripheral compartment is an absorption compartment is shown in Figure 1.7.1.6, and is used to described drug distribution in the body after oral dosing.

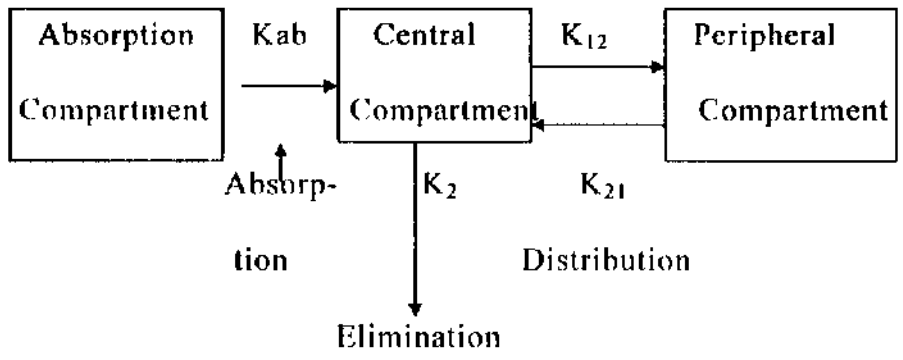


FIGURE 1.7.1.6: Three-compartment open model for an orally administered drug.

In this modified model, it is assumed that the entire dose is rapidly introduced into the absorption compartment, from which it is absorbed into the central compartment and then distributed into the peripheral compartment.

More than three compartments are also possible, but there are practical limits on the detection of new compartments. The addition of each new compartment requires an additional phase in the first-order plots employed in the classical pharmacokinetic approach (Notari, 1975). Deciding how many phases actually exist can be a problem. One alternative approach is based upon the rate at which the plasma flow perfuses various organs. Methotrexate, thiopental and arabinosylcytosine are drugs which have been analyzed using this approach (Notari, 1975).

1.7.2. PHARMACOKINETIC PARAMETERS

Drug absorption and disposition are characterized by the following pharmacokenitic parameters: Volume of distribution (Vd), rate constants for absorption and elimination, and the biological half life of the drug. Mathematical formulas have been developed for calculating these parameters when the concentrations of drug in the plasma and urine are known (Levy, 1963). Other important parameters are area under the curve, clearance and the absorption half life.

Elimination (plasma) half life.

This is the time required to change the amount of drug in the body by one-half during elimination (Holford and Benet, 1995), and can be calculated using the equation:

$$t_{1/2} = 0.693 / Kel \qquad (2)$$

Where Kel is the slope of the first order plot based on the equation for a one-compartment model or the final slope of the biphasic plot based on the equation for a two-compartment model. Half-life (elimination) can be determined graphicallly from a plot of the drug concentration in the blood (on a logarithmic scale) against time. It is related to volume of distribution (Vd) and clearance (Cl) by the following relationship:

$$t_{1/2} = 0.693 \, Vd / Cl \qquad (3)$$

The elimination half-life of a drug will be affected by any factors which change kel. As might be imagined, therefore, Inter-subject variations may be quite large (Notari, 1975). Renal insufficiency is one obvious cause of an increased half-life. Metabolic differences because of age or disease are also important. Changes in the pH of the urine or co-administration of drugs which stimulate or inhibit metabolism can also change the half-life (Notari, 1975). Values of *elimination half life* are frequently quoted in the drug literature and are important in establishing a proper dosage schedule so that neither accumulation of drug to the point of toxicity nor periods when drug levels are below the minimum required for effective therapy will occur (Gourley, 1970). As an indicator of drug elimination, half-life alone can be misleading. Disease states can affect both the physiologically related parameters, volume of distribution and clearance; the derived parameter, half-life will not necessarily reflect the expected change in drug elimination (Holford and Benet, 1995).

Area under the curve (AUC)

Area under the plasma concentration curve from time zero to infinity (AUC) is a measure of the extent of drug absorbed into the systemic circulation (i.e. measure of bioavailability). AUC may be obtained in several ways. One is by use of a planimeter. Another method involves the cut and weigh method. In this case, AUC may be calculated from the weight if the weight of one unit area of the paper is known. In a third method, the trapezoidal-triangular method may be employed. In this method, AUC is estimated by dividing the plasma concentration-time curve into sections that approximate a series of trapezoids with a triangle at each end. The individual areas of the trapezoids and triangles are summed to obtain the AUC (Notari, 1975). It is necessary to have the small units of concentration and time in order to compare AUCs from different curves. However, it is not necessary to have the same scale. In fact, one of the advantages of the trapezoidal method is that the curves can be drawn to occupy the maximum amount of space on the graph paper, therefore improving the estimates of the lengths of the sides involved in the calculations. In the method involving cutting and weighing, a small blood level profile would be less accurate than the larger one that might be used for comparison (Notari, 1975).

Apparent volume of distribution.

Apparent volume of distribution (Vd), is not literally a volume at all. It might be hypothetically defined as the volume of body water which would be required to contain the amount of drug in the body if it were uniformly present

in the same concentration in which it is in the blood or plasma. Vd. relates the amount of drug in the body to the concentration (C) in blood or plasma:

$$Vd = \frac{\text{Amount of drug in body}}{C} \qquad (4)$$

The area under the blood level vs time curve (AUC) following I.V. injection can be used to calculate Vd. Equation (5) below is used for this calculation.

$$Vd = \frac{Do}{(AUC)\ Kel} \qquad (5)$$

Where Do = Dose of drug administered

Kel = elimination rate constant

AUC from time zero to infinity is employed. This equation is also usable for other routes, since AUC is independent of the route of administration if absorption of the dose is complete . If absorption is incomplete but the fraction absorbed, F, is known, this may be applied as a correction to yield I". Do in the numerator (Equation 5).

Drugs with very high volumes of distribution have much higher concentration in extravascular tissue than in the vascular compartment, i.e., they are not homogeneously distributed. Drugs that are completely retained within the vascular compartment, on the other hand, have a minimum possible Vd equal to the blood compartment in which they are distributed (Molford and Benet, 1995).

Clearance (CD

At the simplest level, clearance of a drug is the ratio of the rate of elimination by all routes to the concentration of drug (C) in a biologic fluid:

$$CL = \frac{\text{Rate of Elimination}}{C} \quad (6)$$

Clearance has dimensions of volume per unit time and expresses the efficiency with which a drug is eliminated. The total clearance is made up the sum of all the clearances due to individual organs. Example, for a drug that is eliminated by Liver, Kidney and other tissues, the total body clearance (CL) for that drug is given by:

$$CL = CL\ Liver + CL\ Kidney + CL\ Others \quad (7)$$

The "other" tissues of elimination could include the lungs and additional sites of metabolism, e.g. blood or muscle. If only one organ is involved, e.g. the kidneys, the total body clearance is equal to renal clearance.

For most drugs, clearance is constant over a plasma or blood concentration range encountered in clinical setting, i.e. elimination is not saturable (Holford and Benet, 1995) and the rate of drug elimination is directly proportionate to the concentration. This is sometimes referred to as "first order" elimination. When clearance is first-order, it can be measured by calculating the AUC after a dose (Holford and Benet, 1995). Clearance is proportionate to the product of the dose (Do) and bioavailability (F) divided by the AUC, i.e.

$$CL = \frac{D_o F}{AUC} \quad (7)$$

For drugs that exhibit capacity-limited elimination, clearance will vary depending on the concentration of drug that is achieved. Capacity-limited elimination is also known as saturable, dose-or concentration-dependent, non linear, and Michaelis-Menten elimination. Most drug elimination pathways will become saturated if the dose is high enough.

1.7.3: METHOD OF RESIDUALS IN PHARMACOKINETICS

Residual method is also known as feathering and it refers to a graphical method for the separation of exponents such as separating absorption rate constant (Kabs. or α-slope) from the elimination rate constant (Kel or β-slope). Thus it can be applied to drugs that exhibit one-compartment kinetics and for which the absorption and elimination are first-order (Tozer, 1979). Under these conditions, the rate of change of drug in the body is simply the difference between the rate in and the rate out.

For this graphical approach, the drug concentration data are plotted on a log.scale versus time. In one compartment open model, the mono-exponential elimination slope is back-extrapolated to the ordinate. The differences (i.e. the residuals) between the measured plasma concentrations and the corresponding values on the back-extrapolated line at each sampling time point at the absorption phase are calculated. These residuals are then plotted semi-logarithmically versus time on the same graph. The slope of this plot yields an estimated absorption rate constant (kab). This method of residuals is probably a reasonable means of graphically approximating the absorption rate constant if the data are well described by the difference between two exponential terms and if the distribution of the drug in the body is truly uni-compartmental (Tozer, 1979). The lag time can also be determined from this method, and is determined from the point of interception of the line of residual and the back extrapolation

1.8 SPECTROPHOTOMETRIC ASSAY OF DRUGS IN BIOLOGICAL FLUIDS

Ultraviolet - visible (UV-VIS) spectrophotometry is best known as a tool for quantitative analysis. Calculations for the drug or metabolite concentrations using Beer's Law are performed at the wavelength of maximum absorbance (λmax) of the desired compound. At the λmax, optimum sensitivity is obtained. Because change in absorbance is minimal for small changes in wavelength, errors are minimized. As a result, good accuracy and precision are achieved (Smith and Steward, 1981).

Speed, simplicity, and sensitivity have made UV-VIS. spectrophotometry a popular tool among analysts for the quantification of drugs and metabolites in biologic samples. One of the main reasons for its popularity is that the sensitivity of the methodology is in the 1-10 uglml range, which is comparable to the concentration level of many drug substances in biologic samples (Smith and Steward, 1981). Some specific applications of spectrophotometry in pharmaceutical bioanalysis follows:

A U.V. spectrophotometric assay has been developed to evaluate the absorption, distribution and excretion of a single 600mg dose of Tolmetin in human subjects (Smith and Steward, 1981)). In the assay, 3ml of plasma tolmetin contained in pH 7 phosphate buffer is read at 323nm. The procedure determines tolmetin concentrations of as low as 500ng/ml of plasma. The method is relatively free of interference from a major metabolite of tolmetin, even though the metabolite has a similar absorption spectrum.

Mustapha et al, (1996) reported a U.V. method to study the effect of Tamarindus indica L. on the bioavailability of aspirin in healthy human volunteers. According to the method, plasma samples (1ml) were placed in a 20ml extraction tube and 2ml of 0.05M HCl was added. 10ml of ethylacetate was then added and the mixture shaken for 15 min at 3000g. The supernatant (5ml) was transferred into a second extraction tube and evaporated to dryness on a water bath maintained at 40 ± 10^0C under a gentle stream of nitrogen passing through the tube. The residue was reconstituted in 5ml methanol and absorbance taken at 231nm and 276nm, respectively. Concentrations of both aspirin and its metabolite, salicylic acid in the plasma were estimated using the simultaneous equation for multiple component mixtures.

Drug can be determined either as an intact compound or as a derivative formed by chemical reaction with suitable reagents (Smith and Steward, 1981). An advantage of chemical derivatization as an aid to U.V. analysis is that the sensitivity of the U.V. procedure is increased significantly. Example, it has been reported that the sensitivity of the U.V. procedure for amitriptyline was increased significantly by oxidation of the drug to anthraquinone. Anthraquinone shows stronger absorption around 250nm than the drug itself (Hamilton et al, 1975).

U.V. absorption measurements utilizing chemical derivatization procedure has been developed for fenclorac, a drug that is used in the treatment of developing or established adjuvant arthritis (Smith and Steward, 1981). The assay used 1.5ml of human serum containing fenclorac, and the reactions in the assay is the conversion of fenclorac to the highly U.V. absorbing aldehyde.

The product of the reaction is extracted with 2.5 ml hexane, and after centrifugation, absorbance of the hexane phase is measured at 252nm. The minimum detectable concentration of fenclorac in serum using this method was 1 ug/ml (Smith and Steward, 1981).

A microprocedure for U.V. assay for phenytoin was reported (Fellenberg et al, 1975). The procedure involving dichloromethane as the extraction solvent for blood and plasma, back-extraction into alkali, permanganate oxidation to benzophenone, and absorbance measurement for the n-heptane extract. Using only 0.1ml plasma, these assays have a sensitivity of 1 ug phenytoin per ml.

Hamilton et at (1974), reported a U.V. assay for methadone. The procedure involves an alkaline extraction into n-hexane and back-exraction into a ceric sulphate-H_2S0_4 solution. After refluxing, the generated benzophenone is measured in the n-heptane layer by U.V. spectrophotometry. Amounts of methadone as low as 5 ug can be detected in biologic specimens.

Method has been developed which measured phenothiazines (e.g. chlorpromazine) and their sulphoxide metaoblite in biologic samples via oxidation with C_O(III) (Wallace and Biggs, 1971) and Phenylbutazone by initial permanganate oxidation to azobenzene and subsequent measurement of absorbance at 3145 nm (Wallace, 1968).

Another type of chemical derivatization method utilises conversion of a drug to a stronger U.V. absorbing species by an acid-catalysed re-arrangement (Fellenberg and Pollard, 1976). In this procedure, carbamazepine is extracted from blood, with dichloromethane, the solvent evaporated to dryness, and the residue treated with HCI at 150°C. After extraction of the acidic solution with n-heptane, its absorbance is measured at 258nm.

Difference (differential) spectrophotometry, is as convenient and precise as conventional spectrometry but offers the advantage of increase specificity. It provides automatic cancellation of interference present in biologic samples yet retains the inherent advantage of direct absorbance readout for the drug present in a sample. It is especially useful when isolation of a drug from a biologic sample is difficult or impractical because of solubility characteristics (Smith and Steward, 1981). The methodology requires that a drug exist in two forms that differ in their absorption spectra. The forms are usually generated by pH effects when reversible ionization of an auxochrome attached directly to chromophore in a drug can occur and thus affect the absorption spectrum for that drug (Smith and Steward, 1981).

A differential spectrophotometric method has been reported that utilizes initial extraction of theophylline from blood followed by difference measurements of the drug solution at acidic Vs basic pH (Gupta and Lundberg, 1973). The method is useful in the determination of theophylline levels in overdose cases, but is not as valuable in the assay of normal therapeutic levels (2 to 10 ug/ml), because of its limited sensitivity.

Second-derivative U.V. spectrophotometric method for the determination of naproxen in the presence of its metabolite, 6-0-desmethylnaproxen was reported (Panderi and Parissi, 1994). According to the procedure, plasma (1ml) was acidified with 0.5ml of IM-HCl and 5ml of diethyl ether were added. The mixture was vortex-mixed for one minute and centrifuged at 4000 revolutions per minute (rpm) for 10 minutes. After refrigeration at -17°C, the organic layer was placed in a glass vial and evaporated to dryness at 37°C,

under a stream of nitrogen. The residue was dissolved in 2ml of 0.1M-NaOH and the second-derivative U.V. spectrum of the solution was recorded from 280 to 350nm and the amplitude at 328.2nm was measured. The calibration graph was linear from 5-100 mg/l of naproxen, with a determination limit of 2.42 mg/l. The results obtained agreed with those obtained by HPLC (Panderi and Parissi, 1994).

Colorimetry (also known as visible spectrophotometry) is based on a chemical conversion of a colourless drug to a coloured derivative via a chromatogenic reagent. The resulting derivative absorbs in the visible region and often posseses more absorptivity than the non derivatised drug. Colorimetric analysis is also used for determination of drugs in samples in which interference from other UV-absorbing species is obviated because derivatization only affects the drug to be analysed (Smith and Steward, 1981). The Bratton-Marshall reaction forms the basis for one of the most frequent colorimetric assays (Wolfgang and Geertruida, 1980). It is applied to the assay of primary amines, e.g. chlordiazepoxide, after hydrolysis of procainamide (Sitar et al, 1976), but can also be adapted to aromatic nitro compounds after quantitative reduction of the corresponding aromatic amines, e.g. chloramphenicol (Wolfgang and Geertruida, 1980). Phenyl-subsitituted drugs can be nitrated and similarly treated, e.g. phenytoin. Moreover, some drugs release nitrous acid upon acid treatment, which is another component of the Bratton-Marshall reaction, e.g. metronidazole . Finally, aromatic compounds capable of coupling to diazonium ions to form azo dyes are equally detectable(Wolfgang and Geertruida, 1980)..

Ion complex formation with dyes, followed by organic Ion-pair extraction and spectrometric analysis of the organic phase is a widely applicable colorimetric method ((Wolfgang and Geertruida, 1980).). It is applied for the colorimetric analysis of chloroquine, Ethambutol, Meperidine, Nitrofurantoin and Succinylcholine (Wolfgang and Geertruida, 1980).

Sitar et al, (1976), described a colorimetric method for the determination of procainamide in plasma. The method, using 1ml quantity of plasma containing procainamide involves diazotization and coupling reaction to produce a derivative that absorbs at 550nm. Plasma concentrations of procainamide in the range of 0.05 to 25mg/l are determined with good accuracy and precision. The method is free of interference from N-acetyl-procainamide, a metabolite of the drug (Sitar et al, 1976). A colorimetric assay for nitrazepam in urine samples has been developed (Wassel and Diab, 1973). The procedure involves formation of highly coloured ferric hydroxamate derivative from the chemical reaction of nitrazepam, hydroxylamine and ferric Ion. Absorbance is measured at 550nm and quantified using Beer's law and standard curve.

Steward and Settle (1975), described a colorimetric assay method for isoniazid levels in plasma. The procedure involves reaction of isoniazid with 9-chloro acridine. The assay procedure detect plasma levels of isoniazid even in the presence of its metabolites (Steward and Settle, 1975).

CHAPTER TWO

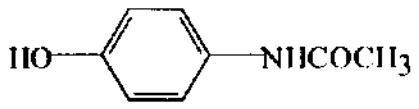
LITERATURE REVIEW

2 .1 PARACETAMOL

2. 1. 1 INTRODUCTION AND CHEMISTRY

Paracetamol (acetamenophen) was first used in medicine by Von Mering in 1893. However, it has gained popularity since 1949, after it was recognised as the major metabolite of acetanilide and phenacetin (Goodman and Gilman, 1990). Paracetamol was introduced in America in 1950, as a compound analgesic containing paracetamol 125mg, aspirine 230mg and caffeine 30mg but was soon withdrawn because of a suspicion that it could cause aggranuolocytosis. The complete prohibition of the use of phenacetin in 1979 has made paracetamol the most widely accepted alternative to aspirin as an antipyretic - analgesic. It's use has been further stimulated by the perceived risks of the other previously popular O.T.C analgesic aspirin, notably it's gastrointestinal effects and its association with Reyes Syndrome in children. Because paracetamol is well to tolerated, lack many of the side effects of aspirin and is available without prescription it has earned a prominent place as a common household analgesic.

Paracetamol is a para-aminophenol derivative with the chemical name : 4' - hydroxyacetamide; N- (4- hydroxyphenyl) acetamide; N- acetyl - p - aminophenol, and a graphic (structural) formular presented as below.



Paracetamol

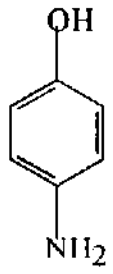
The physico chemical characteristics of paracetamol are represented in Table 2.1.1.1.

Table 2.1.1.1: Physicochemical characteristics of paracetamol (Acetaminophen).

1. Molecular formula : $C_8H_9NO_2$
2. Description : White, odourless crystalline powder with a bitter taste.
3. Melting point : 169 - 172°C
4. Pka : 9.5 (weak acid)
5. Molecular weight : 151.2
6. pH : 6 (a saturated aqueous solution) which is stable with half-life of over 20years. Stability decreases in acid or alkaline conditions.
7. Solubility: Insoluble in benzene. Very sparingly soluble in chloroform, sparingly soluble in water and ether, soluble in boiling water (1:20), freely soluble in 96% ethanol (1:10), acetone and solutions of alkali hydroxides.

The structural activity relationship among the P-aminophenol denvatives is as follows (Williette 1982):

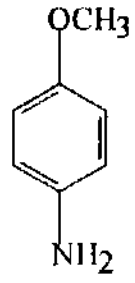
The parent compound (II) possess a strong antipyrentics and analgesic action. However, it is too toxic to serve as a drug (Williette, 1982).



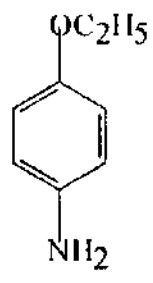
II p - aminophenol

In general, any type of substitution on the amino group that reduces its basicity results also in a lowering of its physiological activity (including toxic effect). Acetylation is one type of substitution that accomplishes this effect (Willette, 1982).

Etherification of the phenolic - OH group produces stronger analgesics but too toxic to use due to the free amino group, example include anisidine (III) and phenetidine (IV) which are the methyl and ethyl ethers, respectively (Willette, 1982).

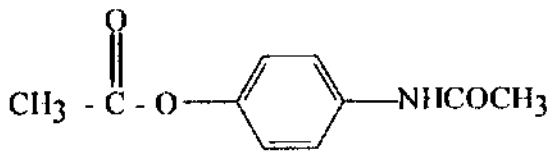


iii Anisidine



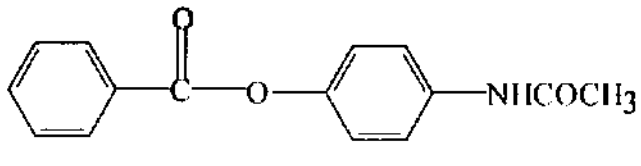
iv Phenitidine

Esterification of the OH-group with an acetyl moiety produced analgesic which has the same activity and disadvantages as the free phenol e.g. P-acetoxyacetani lid (V) (Willette, 1982).



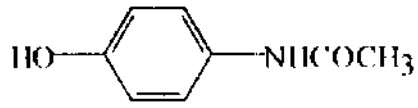
v P-acetoxyacetanilide

However, the salicyl ester (VI) exhibits a diminished toxicity and an increased antipyretic activity.



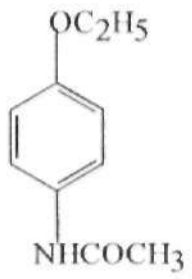
VI Salicyl ester (phenetsal)

Acylation of the amino groups with free OH-group produced the best P-aminophenol analgesics e.g. paracetamol (I) (Willette, 1982).

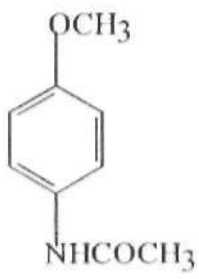


I Paracetamol

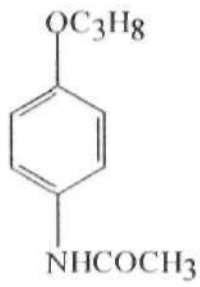
Among the alkyl ethers of the N - acetyl - P - aminophenol derivatives, the ethyl ether was found to be the best and is now the official phenacetin (VII). The methyl and propyl homologs were undesirable from the stand-point of causing emesis, salivation, diaresis and other reactions (Willette, 1982).



VII Ethyl ether
(Phenacetin)



viii Methyl ether



ix Propyl ether

2.1.2 PHARMACOKINETICS

Absorption

Paracetamol is administered orally. It is rapidly and almost completely absorbed from the gastrointestinal tract. After oral administration of paracetamol, plasma levels of the drug reach their peak readily. The time required for maximum drug concentrations in the plasma varies between 15 to 90 minutes but may be decreased significantly by the coadministration of an effervescent buffer.

Paracetamol is a weak acid with Pka of 9.5. It is largely unionized in both the stomach and the small intestine and should be well absorbed from both sides (Schanker et al, 1957). In humans, however, the absorption of paracetamol is very slow in the stomach and the primary site for absorption resides in the intestine. The rate of absorption has been reported to be directly related to the rate of gastric emptying (Heading et al, 1973).

It was reported (Nimmo et al, 1973) that i.v. injection of metoclopramide increased the mean maximum plasma concentration of paracetamol. The time to

reaching the peak plasma concentration was reduced, however, the total amount of drug absorbed was not changed. These results may be explained by the fact that metoclopramide accelerates the gastric emptying leading to a significant increase in the rate of absorption with no effect on the bioavailability (Nimmo et al, 1973). On the other hand, it has been shown that drugs which delays gastric emptying such as propantheline (Nimmo et al, 1973), pethidine and diamorphine (Nimmo et al, 1975) also delays paracetamol absorption.

There is a wide inter subject variability in plasma concentrations after paracetamol administration. For instance, it was reported (Prescott, 1974c) that under practical clinical conditions, the variation among the plasma concentrations of paracetamol in 43 fasting patients one hour after oral administration of a therapeutic dose can be as much as 80 - fold. It was suggested that this intersubject variation might be due to individual differences in the rate of gastric emptying.

The presence of food in the stomach has been reported to reduce the rate of absorption of paracetamol (Heading et al, 1973; Jaffe et al, 1971). This is probably due largely to the inhibitory effect of food on gastric emptying (Welling, 1977). Certain specific dietary components can significantly alter the absorption pattern of orally administered paracetamol (Jaffe et al, 1971) .

The effect of food and sleep on the absorption and excretion of paracetamol was studied (McGilveray and Mattok, 1972). Concomitant administration of food produced little effect on the extent of its absorption. Less paracetamol is

absorbed and excreted when the drug was taken before sleep than after morning ingestion. Workers were, however, not sure of the cause of the reduced absorption (McGilveray and Mattok, 1972).

In a study (Pottage et al, 1974), it was reported that alterations in gastric pH (e.g. in patients with achlorhydria) have no appreciable effect on paracetamol absorption. Although paracetamol is rapidly absorbed unchanged from the gastrointestinal tract, it is incompletely available to the systemic circulation after oral administration, a variable proportion being lost through first-pass metabolism (Chiou, 1975; Perucca and Richens 1979). It was suggested that differences in the contribution of the first-pass effect for rectal and orally administered paracetamol would not be of clinical significance (Chiou, 1975).

Distribution

Paracetamol is relatively well distributed throughout the body fluid (Flower et al, 1985). It was demonstrated that paracetamol is distributed evenly in all tissues with the exception of fat in dogs (Gwilt et al, 1963). In human, usual analgesic doses produce total serum concentrations of 5 to 20 μg/ml; a good correlation between serum concentration and analgesic effect has not been reported (Drug facts and comparison, 1989a).

Binding of the drug to plasma proteins is variable. In humans, plasma protein binding is negligible with plasma concentrations which correspond to the therapeutic doses. However, 15-20% of paracetamol is protein bound at concentrations of about 280ug/ml, corresponding to the levels observed after human overdose (Gazzard et al 1973). In neonates, a slightly higher proportion

of the drug is bound to plasma protein. The sulphate and glucuronide conjugates of paracetamol do not bind to plasma protein even in the relatively high concentrations found in anepheric patients (Lowenthjal et al, 1976).

Paracetamol has been found in human saliva in concentrations which correlate well with plasma levels (Glynn and Bastain, 1973). This observation has led to the suggestion that in therapeutic drug monitoring, or in pharmacokinetic studies in general, saliva might be substituted for plasma (Danhof and Breimer, 1978). Paracetamol is also secreted in breast milk (Bennet, 1983; Findlay, 1981), though its presence is probably without clinical significance.

Metabolism

Paracetamol is metabolised via a variety of pathways. Figure 2.1.2.1. illustrates the metabolic pathways of paracetamol (Correia, 1995).

It normally undergoes glucuronidation (major) and sulphation (minor) to the corresponding metabolites (X and XI respectively; Figure 2.1.2.1), (Ameer and Greenblatt, 1977). These conjugates are pharmacologically inactive and comprise 95% of the total excreted metabolites. The alternative cytochrome P450 dependant glutathione (GSH) conjugation accounts for the remaining 5% (Correia, 1995).

In newborn infants glucuronide formation of paracetamol may be delayed because the enzyme synthesising systems are not completely developed, perhaps leading to enhanced toxicity (Vest and Streiff, 1959).

When paracetamol intake far exceeds therapeutic doses, the glucuronidation and sulphation pathways are saturated, and the cytochrome P450 - dependant

pathway becomes increasingly important. Little or no hepatotoxicity results as long as glutathione is available for conjugation. However, with time, hepatic glutathione is depleted faster than it can be regenerated, and accumulation of a reactive and toxic metabolites occurs. In the absence of intracellular nucleophiles such as glutathione, this reactive metabolite (thought to be an N-hydroxylated product or an N-acetylated iminoquinone) reacts with nucleophillic groups on cellular macromolecules such as protein, resulting in hepatotoxicity (Correia, 1995).

Consistent with this view, the effects of various pre-treatments that alter the availability of glutathione also altered hepatic necrosis by paracetamol (Mitchell and Jallow, 1974). Depletion of glutathione by diethylmaleate pre-treatment dramatically potentiated paracetamol - induced hepatic necrosis (Mitchell et al, 1973). Conversely, treatment with cystein, a precursor of glutathione decrease hepatotoxicity. Since neither diethylmaleate nor cysterne inhibited the metabolism of paracetamol, they apparently altered the hepatic damage via their effect on glutathione availability (Mitchell et al, 1973).

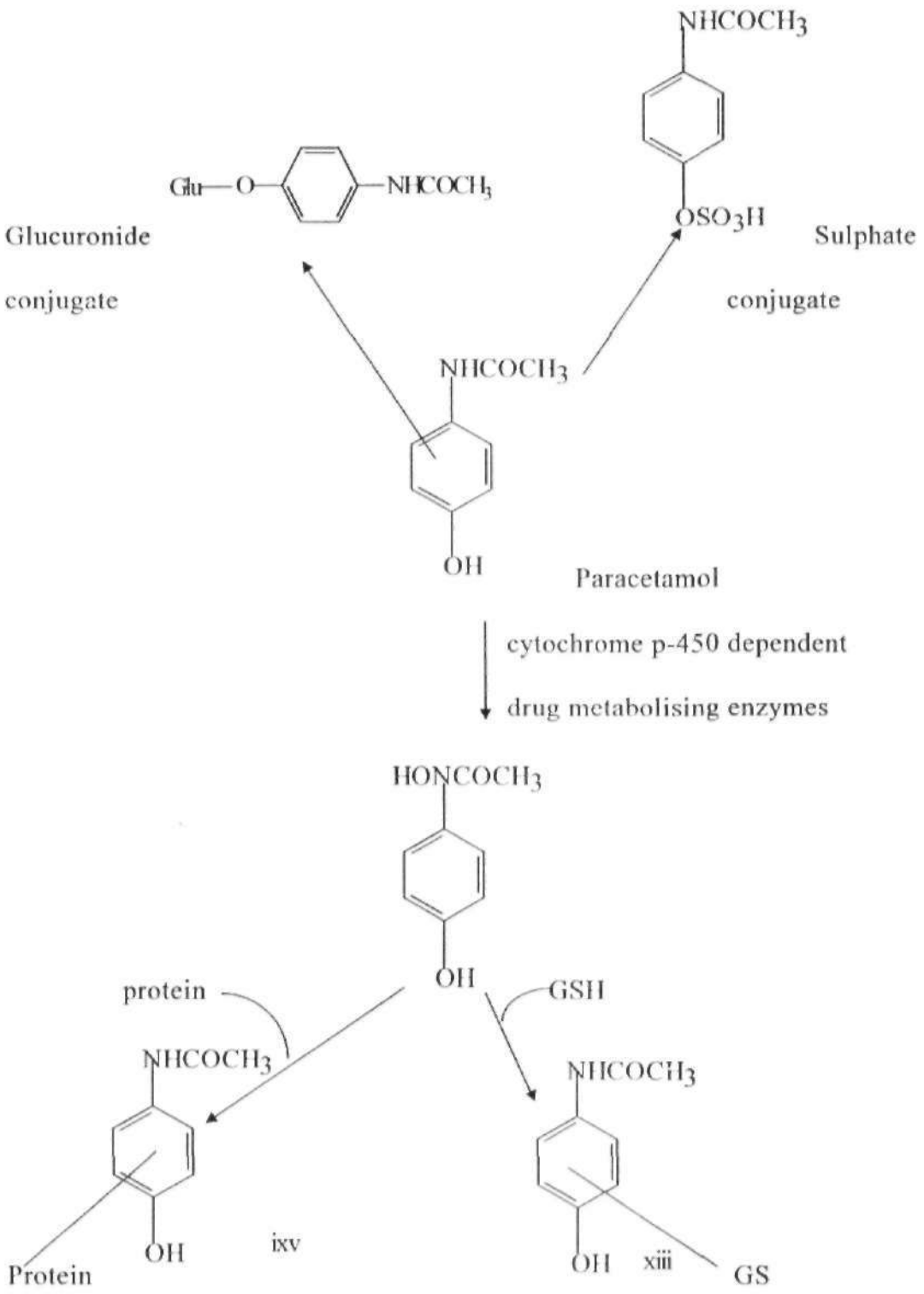


FIGURE 2.1.2.1: Pathways of paracetamol metabolism (GSH, glutathione; GS, glutathione moiety, GLU, glucuronide moiety. Adapted from Correia, (1995)).

The chemical and toxicological characterisation of the electrophilic nature of the reactive paracetamol metabolite has led to the development of effective antidotes - cysteamine and N-acetyl cysteine for paracetamol overdose (Correia, 1995). Administration of N-acetyl cysteine within 8-16 hours following overdose has been shown to protect victims from fulminant hepatotoxicity and death (Correia, 1995).

Excretion

Paracetamol is excreted in the urine primarily as inactive glucuronate and sulphate conjugates. Studies have been conducted on the elimination of paracetamol and its metabolites. Results indicated that after oral administration of lg dose of paracetamol, the average recovery of drug in all forms was 52%, mostly as glucuronide and sulphate which are non toxic (Levy and Yamada, 1971).

When paracetamol is taken in normal doses, the urinary excretion is as follows: unchanged drug (5%), glucuronide conjugate (55%), sulphate conjugate (30%), and mercapturic acid conjugate (8%) (Thomas, 1993). In overdose without liver damage, there is an increased percentage in the mercapturic acid and glucuronide conjugates, reflecting saturation of the sulphation pathway and increased metabolic activation of paracetamol. Glucuronidation is not saturated, but consumption of glutathione by the mercapturic acid conjugation pathway prevents detoxification and excretion of the reactive intermediate metabolite, N-acetyl-P-benzo-quinone imine (Thomas, 1993).

The normal plasma elimination half-life of paracetamol is about 2hours, but can be considerably longer after massive dose (Prescott et al, 1971). Half-life is slightly prolonged in neonates and in cirrhotics (Drug facts and comparison, 1989a).

2.1.3 PHARMACODYNAMICS

The pharmacological properties of paracetamol have been reviewed (Clissoid, 1986). It is the principal active metabolite of phenecetin and acetanilid, but is associated with less toxicity in usual recommended dosages. Although the mechanism and site of action have not been definitely established (Goodman and Gilman, 1980), it appears to involve central prostaglandin inhibition (Randall, 1963; Brodie and Axelrod, 1949). Paracetamol reduces fever, a direct action on the hypothalamic heat regulating centres, which increases dissipation of body heat (Via dilation of the cutaneous vessels and increased sweating) (Drug facts and comparisons, 1989a). Although the drug have little influence on normal temperature, the antipyretic response occurs also when the temperature is only slightly elevated. The action of endogenous pyrogen on heat-regulating centres is inhibited. Paracetamol is almost as potent as aspirin in inhibiting prostaglandin synthetase in the central nervous system (CNS), but its peripheral inhibition of prostaglandin synthesis is minimal, perhaps because of the high concentrations of peroxides that are found in inflammatory lesions (Marshall et al, 1987).

Although comparative analgesic effectiveness is difficult to establish because of the nature of existing clinical testing procedures, paracetamol is similar in

potency to aspirin as an analgesic and antipyretic (Beaver, 1965). However, a single 1000mg paracetamol dose is less effective than 600mg of aspirin in relieving pain associated with rheumatoid arthritis when it is given as an analgesic supplement to regular anti inflammatory drug therapy (Tyle, 1986). Although there are reports of minimal anti inflammatory activity with paracetamol, the P-aminophenols have no therapeutic use as anti inflammatory drugs (Goodman and Gilman, 1980; Beaver, 1965; Randall, 1963).

Paracetamol is without major effect on other organ systems with the exception of the blood and perhaps the kidney. Depression of the heart or a decrease in blood pressure is unlikely even after large doses. The CNS as well as most other organs of experimental animals appears to be more sensitive to the drug than is the heart. Respiratory effects after therapeutic doses are uncommon. Toxic doses may depress respiration, and in fatal poisoning the immediate cause of death is respiratory failure. The liver and spleen are not injured with paracetamol except, perhaps, after repeated large doses. A few cases of death due to liver necrosis have been reported after the consumption of huge doses of the drug. Paracetamol does not inhibit platelet aggregation, affect prothrombin response or produce gastrointestinal ulceration.

2.1.4 INDICATIONS ADMINISTRATION AND DOSAGE.

Paracetamol is equivalent to aspirin as an effective analgesic and antipyretic agent (Styrt and Sugarman, 1990). Thus, it is probably the best substitute for aspirin as an antipyretic and analgesic drug but not as an anti inflammatory compound. The drug is useful in mild to moderate pain such as headache,

myalgia, post partum pain, analgesic adjunct to anti inflammatory therapy, anti pyresis in certain bacterial and viral infections; and other circumstances in which aspirin is an effective analgesic. For mild analgesia, paracetamol is the preferred drug in patients allergic to aspirin or when salicylates are poorly tolerated. It is preferable to aspirin in patients with hemostatic disturbances (including anti coagulant therapy), bleeding diatheses (e.g. hemophilia) or a history of peptic ulcer and in those in whom bronchospasm is precipitated by aspirin. Since paracetamol does not antagonise the effects of uricosuric agents, it may be used concomitantly with probenecid in the treatment of gout. Paracetamol is preferred to aspirin in children with viral infections (Payan and Katzung, 1995).

Paracetamol is given orally. There is no reason under ordinary circumstances for any other method of administration. Oral adult dose is 325 to 650mg every 4-6hours, or lg, 3 or 4 times per day, and not exceeding 4g per day (Drug facts and comparisons, 1989a). For children, a 10mg/kg/dose schedule has been recommended. This dose may be repeated 4 or 5 times daily; and not exceeding 5 doses in 24hours (Drug facts and comparisons, 1989a).

2.1.5 TOXICOLOGY

When used as directed, paracetamol rarely causes severe toxicity. Side effects associated with paracetamol may include hypoglycaemia, jaundice and hematologic disorders like neutropenia, leucopenia, pancytopenia. Allergic reactions such as skin eruptions, urticarial and erythematous skin reactions; fever may also occur. Therefore, the major contraindication of paracetamol is

the history of hypersensitivity reactions to the drug. Hemolytic anaemia and methaemoglobinaemia, reported with the use of phenacetin, have rarely been noted with paracetamol. Interstitial nephritis and papillary necrosis, serious complications of phenacetin, although anticipated with widespread chronic use of paracetamol, have not occurred despite the fact that about 80% of phenacetin is rapidly metabolised to paracetamol (Payan and Katzung, 1995). Gastrointestinal bleeding does not occur. However, hepatotoxicity and severe hepatic failure occurred in chronic alcoholics following therapeutic doses. The hepatotoxicity is believed to be caused by induction of hepatic microsomal enzymes resulting in an increase in toxic metabolites; or by the reduced amount of glutathione responsible for conjugating toxic metabolites (Drug facts and comparisons, 1989a).

When used in therapeutic doses, paracetamol appears safe for short-term use during all stages of pregnancy. Continues high daily dosage caused severe anaemia in a mother, and the neonate had fatal kidney disease. Although there is no evidence of a relationship between paracetamol ingestion and congenital malformations, three cases of congenital hip dislocation may have been associated with paracetamol (Drug facts and comparisons, 1989a). No adverse effects of paracetamol in nursing infants were reported.

Effect of overdosage

Because it lacks many undesirable effects produced by aspirin, paracetamol is gaining favour as the "common household analgesic" (Tyle, 1986). However, there is also growing concern that increasing household availability and the

public lack of recognition of paracetamol toxicity will produce a new health hazard (Sutton and Soyka, 1973; Goulding, 1973). Acute paracetamol poisoining may produce fatal hepatic necrosis (Tyle, 1986; Clark et al, 1973). Chronic excessive use of paracetamol (greater than 5g per day) for several weeks can produce hepatotoxicity, which is potentiated by chronic alcohol consumption (Barker et al, 1977; McClain et al, 1980). In addition, the kidneys may undergo tubular necrosis; the myocardium may be acutely damaged. In adults, symptoms of acute toxicity may occur following the ingestion of 10-15g of paracetamol (Proud and Wright, 1970). A single oral ingestion of 15-25g is seriously hepatotoxic and potentially fatal (Koch-Weser, 1976; Ambre and Alexander, 1977; McJunkin et al, 1976). However estimates of the ingested paracetamol dose are not reliable predictors of potential hepatotoxicity. Plasma paracetamol levels should be determined following ingestion of a potentially toxic amount, and monograms have been established to relate plasma paracetamol levels to the likehood of hepatoxicity (Prescott et al, 1976). For instance, plasma paracetamol levels greater than 300ug/ml at 4 hours post ingestion are always associated with hepatic damage, minimal hepatic damage is anticipated if plasma levels at 4 hours are below 120ug/ml. Hepatotoxicity is also likely if plasma levels at 12 hours are greater than 50ug/ml. In addition, if the plasma paracetamol half-life is greater than 4hours hepatic necrosis is likely to occur (Prescott et al, 1971). If greater than 12 hours, hepatic coma is likely to occur. The progression of symptoms with acute paracetamol poisoning include: Vomiting within a few hours; anorexia, nausea, and stomach pain within 24 hours; evidence of hepatotoxicity in 2-4days with jaundice; and death at any time in 2-7 days (Goodman and Gilman, 1980; Proud and Wright, 1980).

In addition, kidney damage, disturbances in clotting mechanisms, metabolic acidosis, hypoglycaemia, and myocardial necrosis may occur (Goodman and Gilman, 1980; Sutton and Soyka, 1973).

Management of paracetamol poisoning

Emergency first aid treatment of paracetamol poisoning should include emesis with syrup of ipecac (Krenzelok et al, 1977). Activated charcoal will reduce paracetamol absorption significantly, but is most effective if given immediately after emesis within the first 30minutes following paracetamol ingestion (Levy and Houston, 1976). Activated charcoal should not be employed if oral administration of acetylcysteine is anticipated since the charcoal may prevent adequate absorption of the antidote (Rumack and Peterson, 1978). Fluid and electrolyte therapy may be required in the early stages of poisoning, if nausea and vomiting have been a feature. Occasionally, i.v glucose is needed to prevent hypoglycaemia. I.V sodium bicarbonate is often given, but there is no evidence that this affect outcome.

In addition to supportive therapy, measures that have proved extremely useful are the provision of sulfhydryl groups to neutralise the toxic metabolites (Payan and Katzung, 1995). Acetylcysteine is used for this purpose. The oral administration of solution of acetylcysteine is effective without significant toxicity in preventing the hepatic necrosis of paracetamol poisoning and is being evaluated for this purpose (Rumack and Paterson, 1977). The initial dose of acetylcysteine should be given within 24hours of overdose of 140mg/kg, followed in 4 hours with a maintenance dosage regimen of 70mg/kg every 4

hours for 17 doses or until serum paracetamol level is zero. Dilute with a soft drink or unsweetened grapefruit juice to 5% (Scalley and Conner, 1978).

2.1.6 ASSAY METHODS

Several methods for the quantitative determination of paracetamol as such, in pharmaceutical dosage forms and in biologic fluid are available. The earliest colorimetric method for paracetamol in plasma was proposed by Brodie and Axelrod (1949). According to the method, after acid hydrolysis, the formed P-aminophenol is diazotised and coupled to alpha-naphthol, and the azo dye is measured by spectro-photometric method. However, the method is non specific and time consuming (Wallace et al, 1973). Other procedures utilise the reaction of P-aminophenol with $NaNO_2$ - HCl to form the coloured 2-nitro-P-aminophenol (Chambers and Jones, 1976) and with vanillin to a stable imine with an absorbance maximum at 395nm (Plankogiannis and Saad, 1978). These procedures resulted in good selectivity for paracetamol.

Dasgupta and Kinnaman (1993), reported a method for micro wave - induced rapid hydrolysis of paracetamol and its conjugates in urine for emergency toxicological screen. According to their procedure, paracetamol was detected by a method involving hydrolysis, by boiling with HCl, to 4 - aminophenol which is reacted with O - cresol and NH_4OH to form a distinctive indophenol blue colour, the absorbance of which is measured at 620nm. The hydrolysis is still incomplete after 10 minutes, so that hydrolysis is the time-limiting step in the procedure. Paracetamol could be rapidly hydrolysed by microwave

irradiation for 2 minutes at 120 watts. Results agreed with those by conventional hydrolysis for 20 minutes (Dasgupta and Kinnaman, 1993).

A validated HPLC method for the determination of paracetamol and its major metabolites in urine was reported by Goicoechea et al, (1995). According to the method, paracetamol and its glucuronide and sulphate metabolites were diluted with water and filtered to 0.45um. Analysis was carried out using a waters Radial-PAK column (10cm x 8mm I.D). Elution (2.5ml/min) was with aqueous 0.1M - KH_2PO_4 / methanol / acetic acid (95:4:1) and detection was at 248nm. Detection limits were 2ug/ml for paracetamol and 5ug/ml for sulphate and glucuronide metabolites.

Gilar (1995), reported a HPLC method for the determination of paracetamol in serum with dialytic sample pretreatment. According to the procedure, a centrifuged sample of serum was dialysed and trace enriched on an automated Gilson ASTED system operated according to manufacturer's instructions. Then the sample was analysed on a 4um Nova-pak C-18 column (15cm x 3.9mm ID) and eluted (0.5ml/min) with detection at 254nm. Calibrahon graphs were linear from 0.5 - 10mg/l paracetamol with 8-chlorotheophylline as internal standard. Recoveries were 95.8 - 101.7% (Gilar, 1995).

A rapid method for the determination of paracetamol in blood serum samples by first - derivative U.V. absorption spectroscopy was reported (Damiani et al, 1995). According to the method, serum (lml) was deproteinized. Following centrifugation, lml of the supernatant solution was treated with 0.2ml 10M - NaOH. The U.V. absorption spectrum was recorded and the first derivative at

292nm was calculated. Paracetamol was determined from a calibration graph of the first-derivative intensity versus the original paracetamol concentrations in the serum samples. The calibration graph was linear from 100-1500 ug/ml of paracetamol. Other anti-inflammatory drugs except salicylate did not interfere. Recoveries of the method ranged from 97.7 - 107%.

Assay of paracetamol and its metabolites in urine, plasma, and saliva of children with chronic liver disease was reported (AL-Obaidy et al, 1995). Urine, plasma or saliva containing 10uglml 3-acetaminophen (internal standard) were analysed by HPLC on a 5um sphensorb ODS-2 column (25cm x 4.6mm ID.) and U.V. detection. The mobile phases were acetonitrile/10mM-H_3PO_4 (1:24) of pH 3.5 for urine, methanol/acetonitrile/10mM-H_3PO_4 (1:2:47) of pH 3.2 for plasma and a solvent similar to that described by Sommers et. al (Human Toxicol. (1987) 6,407) but containing 6% acetonitrile and not methanol, for saliva. Calibration graphs were linear for 0.625-40ug/ml paracetamol and four of its metabolites in urine.

Christie et al (1993), reported a direct method for the electrochemical determination of paracetamol in plasma. According to the method , direct measurements were made at 21°C with a probe comprising of platinum (Pt) electrode covered with a cast cellulose acetate membrane over which was affixed a nuclepore polycarbonate membrane to limit diffusion; the nuclepore membrane was dip-coated with dimethyldichlorosilane to minimise signal attenuation after repeated exposure of the probe to solution containing proteins. The electrode was maintained at 1650mV vs. Ag/AgCl to monitor the oxidation

of paracetamol. A calibration graph for plasma with added paracetamol was rectilinear from 0.2 - 2mM with detection limit of 0.1mM (Christie et al, 1993).

A method (Peng et al, 1994) for the direct injection of urine and determination of paracetamol by micellar liquid chromotography was reported. Paracetamol was detected in a flow through detector with a large volume wall-jet cell at a preconditioned carbon fibre micro electrode. The peak due to paracetamol was well separated from those due to ascorbic acid, citric acid and 4 - aminophenol. Calibration graphs were linear from 0.02 (detection limit) to 70ug/ml of paracetamol.

Spectrofluorimetric method for the determination of paracetamol in pharmaceuticals and biological fluids was reported (Vilchez et al, 1995). According to the procedure, pharmaceuticals containing paracetamol were dissolved in water, filtered and the filtrate was heated for 2 minutes at 80°C with 0.4M - Na_2CO_3 / H_3BO_3 buffer at pH 10 and 10mM - NaOCl, cooled and diluted with water. If salicylic acid or acetylsalicylic acid was present, samples were extracted in acid medium with ethyl ether prior to analysis. Human plasma or urine was mixed with water and 2M - NaOH, extracted with ethyl acetate, the extract was evaporated to dryness and the residue was treated with NaOCl as described above (Vilchez et al, 1995). To determine glucuronidated and sulphated paracetamol, samples were treated with β- glucuronidase and aryl-sulphatase prior to extraction. Portions of the resulting solutions were analysed by fluorimetry at 427nm (excitation at 335nm). Recoveries from plasma and

urine were satisfactory. The results for pharmaceuticals agreed well with those obtained by voltammetry.

Nese Dogan (1996), reported a simultaneous spectrophotometric method for the determination of paracetamol and caffeine in tablets by absorbance ratio technique. In the method, powdered tablets equivalent to 94.4mg paracetamol and 5.6mg caffeine were sonicated for 20 minutes with 80ml of water and the solution was diluted to 100ml, filtered and 10ml portion of the filtrate was diluted with water to 100ml. The absorbance of the resulting solution was measured at 241.6, 259.7 and 272nm and the quantity of drugs were calculated from a given equation. Calibration graphs were linear for 1-18ug/ml paracetamol and 1-17ug/ml caffeine (Nese Dogan, 1996).

A method for the simultaneous estimation of ibuprofen and paracetamol in combined dosage formulation by high performance thin-layer chromatography (HPTLC) was reported (Shirke et al, 1993). According to the procedure, powdered tablets were dissolved in methanol and the solution filtered. The filtrate was applied to silica gel GF 254 HPTLC plates which were developed with hexane/ethylacetate/anhydrous acetic acid (18:5:2); densitometric scanning at 265nm was used for quantitation. Calibration graphs were linear for 10-50 and 10-60ug of paracetamol and Ibuprofen, respectively.

The application of first derivative fluorescence spectrometry to the simultaneous determination of paracetamol and salicylamide in pharmaceuticals was reported (Murillo and Garcia, 1996). The method was based on the "Zero crossing"

technique. Samples were treated with 5ml 2M-NH4Cl/NaOH buffer of pH 8.5 and 1ml of 0.5g/l hexacynoferrate (III) solution. After 30 seconds, 2ml of 2.5g/L ascorbic acid and a suitable amount of water were added. Fluorescence spectra of the samples were recorded at 20°C at 426nm (excitation at 321nm) and the first derivatives were calculated. The absolute value of the first derivatives was measured at 327 and 321 nm for paracetamol and salicylamide respectively, for simultaneous determinations.

2.1.7 PARACETAMOL -DRUG INTERACTIONS.

There are few reports of pharmacokinetic drug interactions involving paracetamol in the literature and in most cases occur at the sites of metabolism and gastrointestinal tract (GIT) absorption. Paracetamol absorption was reported to be considerably slower in the presence of propantheline than in its absence (Nimmo, 1973; Nimmo et al, 1973). Other agents with anticholinergic activity also probably delay paracetamol absorption (Heading et al, 1973; Nimmo et al, 1973; Prescott, 1974b). This interaction is unlikely to be clinically important in a patient receiving paracetamol several times daily, because extent of absorption is not affected (Hansten and Horn, 1989d). In contrast, the rate of paracetamol absorption has been shown to be hasten by metoclopramide, with the extent of absorption unaffected (Nimmo et al, 1973; Nimmo, 1973). Food, especially if high in carbohydrates, has been shown to delay paracetamol absorption, an effect associated to delayed entry of the drug into the intestine or to delay in tablet disintegration or dissolution (McGilveray and Mattok, 1972). Similarly, activated charcoal in large oral doses (5-10g)

(Levy and Houston, 1976) and oral cholestyramine (Hansten and Horn, 1989d) were reported to reduces the GIT absorption of paracetamol.

The hypoprothrombinemic effect of oral anticoagulants may be increased by paracetamol, but may depend on dose and duration of action (Drug facts and comparisons, 1989a; Udall, 1970; Jones, 1976). A combination product containing paracetamol and D-propoxyphene (distalgesic) was reported to be associated with enhanced hypoprothrombinemic responses and bleeding in a few patients (Jones, 1d976). Paracetamol has been reported to increase, decrease and have no effect on the clearance of chloramphenicol (Hansten and Horn, 1989b). Reasons for the desperate results probably result from differences in study design, number of subjects, assay techniques or route of paracetamol administration. Definite studies of this interaction will be difficult to do, since patients have an acute, changing illness (making a crossover design of questionable value) and there may be ethical reservations about giving healthy subjects chloramphenicol (Hansten and Horn, 1989b). Preliminary report on paracetamol-diazepam interaction indicates that paracetamol reduces diazepam bioavailability (Hansten and Horn, 1989d), but the clinical importance of this effect is questionable. Thus, additional study is needed to confirm these results and to define the magnitude of the interaction (Hansten and Horn, 1989d).

In a study (Perucca and Richen, 1979), the fate of paracetamol (Orally and I.V.) was compared in healthy subjects and epileptic patients receiving chronic anticonvulsant (barbiturates, primidone, carbamazepine, phenytoin) therapy. Results indicated a lower oral paracetamol bioavailability and shorter serum half

life of paracetamol following its I.V. administration in epileptic patients on anticonvulsants. Phenobarbitone has been shown to increase the hepatotoxicity and nephrotoxicity of paracetamol overdose in rats (Hansten and Horn, 1989a), but evidence from humans is limited to isolated case reports (Boyer and Rouff, 1971). Caffeine enhances the analgesic of paracetamol, while administration of diflunisal with paracetamol resulted in significantly increased paracetamol plasma levels (Drug facts and comparisons, 1989a). Substantial evidence indicate that chronic, excessive alcohol ingestion increases the toxicity of excessive therapeutic doses or overdoses of paracetamol (Barker et al, 1977; McClain et al, 1980). These findings are consistent with the known ability of chronic alcoholism to result in enzyme induction and with the likelihood that enzyme induction results in accelerated formation of hepatotoxic paracetamol metabolites. In contrast, acute alcohol intoxication tends to inhibit hepatic microsomal drug metabolism and thus might be expected to reduce the formation of toxic paracetamol metabolites. Oral contraceptives may increase the hepatic metabolism of paracetamol, decreasing the elimination half life by 20-30% (Drug facts and comparisons, 1989a). The magnitude of this effects is probably sufficient to inhibit the effect of paracetamol, in at least some patients (Hansten and Horn, 1989c).

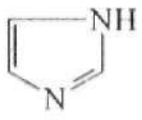
2.2 CIMETIDINE

2.2.1 INTRODUCTION AND CHEMISTRY.

Cimetidine is one of a new class of drugs which has specific antagonistic activity on histamine H_2 - receptors. Its principal action is on parietal cell

histamine H_2 - receptors. The discovery of these antagonists demonstrated the important role of histamine in the physiological control of gastric acid, and the introduction of cimetidine into market was a remarkable clinical break-through. Although other agents were and are being developed, cimetidine is the first histamine H_2 -receptor antagonist. With its remarkable safety profile, cimetidine has become extensively used in the treatment of ulcer disease and acid-related disorders. The drug was introduced into market in 1976, became available in the United Kingdom (U.K) in November, 1976 and in the United State of America (U.S.A) in August, 1977, and by the end of 1978, it was available for clinical use in more than 90 countries (Duncan and Parsons, 1980).

Chemically cimetidine is a substituted imidazole compound with a chemical nomenclature: 2-cyano-1-methyl-3-[2-(5-methylimidazole-4-ylmethylthio)ethyl] guanidine (B.P., 1993), and a graphic (structural) formular as below



Imidazole ring



Cimetidine

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Briefly, cimetidine is a weak base with a high degree of water solubility. These properties affect many of the drug's pharmacokinetic characteristics. The

solubility of cimetidine in water is greatly increased with the addition of dilute acid which protonates the imidazole ring.

Table 2.2.1.1 Lists some of the physicochemical properties of cimetidine.

Table 2.2.1.1

PHYSICOCHEMICAL CHARACTERISTICS OF CIMETIDINE.

1. Appearance : White to off-white crystalline powder.
2. Polymorphism : Polymorphic and exist in three polymorphic forms.
3. Odour : Odourless or with a faint odour
4. Mol. Formular : $C_{10}H_{16}N_6S$
5. Mol. Weight : 252.34
6. Melting point : 141 - 143°C
7. Pka : 6.80
8. pH-Value : pH of a 5.0mg/ml in carbondioxide free water is 8.0-9.5(basic)
9. Loss on drying : When dried to constant weight at 100°C to 105°C, loses not more than 0.5% of its weight.
10. Solubility : Solubility in acetonitrile is 0.27% at 24°C. Slightly soluble in water (1.14% at 37°C, but Hydrochloride is more soluble water. Very soluble in methanol (14.1% at 37°C). Practically insoluble in dichloromethane and in ether. Cimetidine dissolves in dilute mineral acids.
11. Stability : Stable for 48 hours at room temperature when added to commonly used i.v. solutions (e.g. 5% Dextrose injection)

Cimetidine hydrochloride has been found to be both visually and chemically stable for at least 1 week at ambient room temperature when combined with commonly used i.v. fluids (Rosenberg et al, 1980; Yuhas et al, 1981). As well, cimetidine hydrochloride when added to 5% dextrose and then frozen for 30days

was found to be stable and sterile for at least 8 days after thawing when kept at 4°C (Walker et al, 1981).

The synthesis of cimetidine was described by Durant and co-workers in 1977 (Olaniyi, 1990).

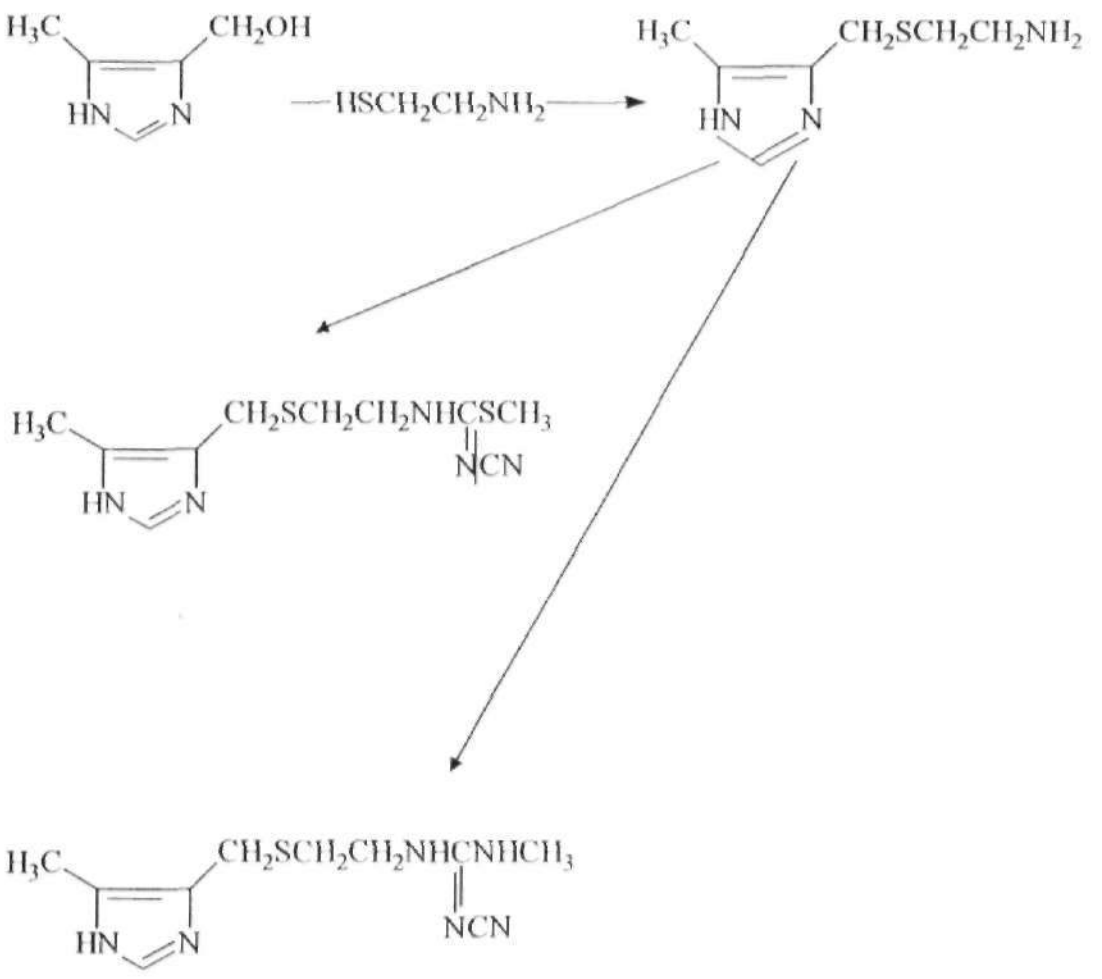


Figure 2.2.1.1 Schematic representation of the synthesis of cimetidine (Olaniyi, 1990).

2.2.2 DEVELOPMENT OF CIMETIDINE AS AN HISTAMINE H_2-ANTAGONIST.

Based on the suspected existence of different types of histamine receptors, rational molecular modification led to the introduction of cimetidine (Berkowitz and Katzung, 1995). The discovery of cimetidine (and other H_2 - receptors blockers) began with the observations that histamine is a potent stimulant of gastric acid secretion and that the classic antihistamines did not inhibit this action. These observations suggested that there might be distinct subtypes of histamine receptors serving different functions and that these receptors could be stimulated or blocked in a selective manner by drugs (Black et al, 1972).

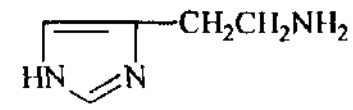
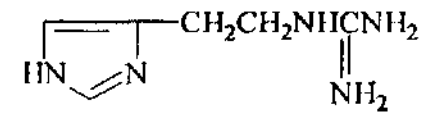
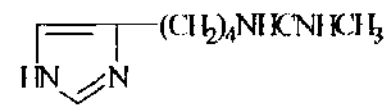
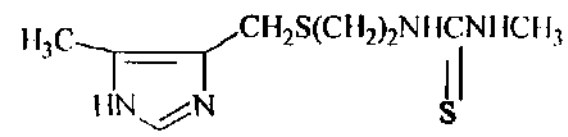
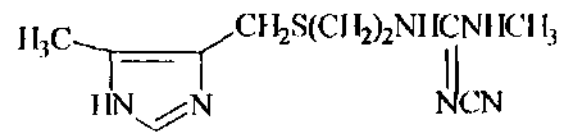
The clinical need was that: Peptic ulcers are very common and can be treated by suppressing gastric acid secretion. The need for effective acid suppression was not met by the drugs or the surgery then in use to treat ulcer disease.

Two hypothesis (biological and chemical) were then postulated. The biological hypothesis was that histamine could be selectively blocked at the receptors that mediate histamine's effects on acid secretion. The chemical hypothesis was based on the fact that the then existing antihistaminic drugs were ineffective for treatment of acid-related gastrointestinal disorders and had no obvious chemical similarity to the structure of histamine. It was then reasoned that chemical modification of histamine itself might result in a selective antagonist for the postulated gastric histamine receptors (Berkowitz and Katzung, 1995).

A large number of compounds based on histamine's structure were synthesized and tested (Black, 1989). The first selective H_2 -antagonist, burimamide, lack

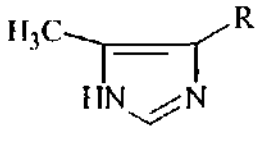
adequate potency and clinical activity. The structure of burimamide was further altered to produce the more potent metiamide. Clinical trials with metiamide revealed good efficacy but also unexpected and serious toxicity, granulocytopenia. Further rational analysis finally yielded cimetidine, which was approved as the first H_2 - receptors antagonist in 1974 (Berkowitz and Katzung, 1995).

TABLE 2.2.2.1 A history of the development of H_2-receptor blockers (Berkowitz and Katzung, 1995).

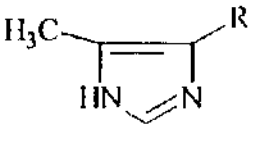
COMPOUND AND CHARACTERISTICS	STRUCTURE
Histmine(Agonist) The starting material	
N-Guanylhistamine The first lead compound. A weak partial agonist.	
Burimamide Thiourea compound with a longer side chain. Weakly active.	
Metiamide Active in humans but toxic	
Cimetidine: Replaces the thiourea with an N-CN substituent. Retains high potency with decreased toxicity.	

2 . 2. 3 SARs OF IMIDAZOLE H_2-RECEPTOR ANTAGONISTS

IMIDAZOLE RING: The Imidazole ring in the compounds is capable of undergoing a 1,3-prototropic tautomerism (Olaniyi,1990).



N^3-H tautomer



N^1-H tautomer

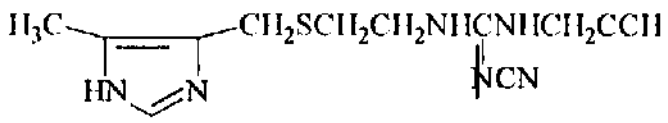
The N^3-H Tautomer is found to be predominant in all the species studied and it seems to be necessary for maximal H_2 -receptor antagonism .Substitution with electron -donating groups like Methyl, etc. favour the N^3-H tautomer, while electron -withdrawing group favour N^1-H tautomer. In burimamide , N^1-H tautomerism is found to be more prevalent whereas N^3-H tautomerism predominated in metiamide. Metiamide is about 5 times more potent than burimamide (Brimble Combe, et al 1978).

SIDE CHAIN: For optimal acitivity, the ring should be separated form the N-group by the equivalent of a four-carbon chain. A shorter chain drastically lowers activity. Example, N-Guanylhistamine is only a weak partial agonist (Berkowitz and Katzung, 1995), because the ring is separated from the N-group by only two carbons. More active compounds contain isosteric thioester (-S-) link in place of methylene group in the side chain.

N-GROUP: The terminal N-group should be a polar, non-basic substituent for maximal activity. A positively charged group should bind more tightly to the

receptor, but it seems to permit the molecule to mimic histamine and act as an agonist as examplified by N-Guanylhistamine which contains guanidine group which becomes protonated at physiological pH and acts more as an agonist.

The addition of ethinyl group at the N-methyl position in cimetidine resulted in etintidine, and was found to be more potent than cimetidine. This was shown in invitro and invivo animal studies (Cavanagh et al, 1980), as well as in clinical studies (Meyers and Peterson, 1980; Brater et al, 1982; Cavanagh et al, 1983).



Etintidine

2.2.4 ASSAY METHODS IN BIOLOGICAL FLUIDS

Cimetidine has been assayed in various biological fluids by high-performance liquid chromatography (HPLC) employing both normal and reverse phase columns (Randolph et al, 1977; Larsen et al, 1979; Soldin et al, 1979; Chiarmonte and Schentag, 1979; Cohen et al, 1980; Lorenzo and Drayer, 1981).

Because of its high degree of water solubility, the extraction of cimetidine into most organic solvents is not complete and averages about 60%, though Ziemniak et al, (1981), reported almost 100% extraction. The method (Randolph et al, 1977) is laborious and results in a mobile phase pH of 10.8, which reduces the life-time of the column; this can be overcome by reducing the

amount of ammonium hydroxide. The method of Chiarmonte and Schentag (1979) is a modification of that of Randolph et al (1977).

The only method which measures the metabolites in plasma is that of Ziemniak et al (1981). The methods of Cohen et al (1980) and Lorenzo and Drayer (1981) are simple reverse-phase methods and that of Kunitani et al, (1981) involves protein precipitation and direct injection of the supernatant.

A method for the measurement of cimetidine sulphoxide in blood and urine has also been reported (Lee and Osborne, 1978) and Larsson et al (1982) have developed a method for measuring both cimetidine and its sulphoxide metabolite in plasma using an ion exchange column

22 5 PHARMACOKINETICS

Absorption and bioavailability.

Cimetidine is rapidly absorbed from the gastrointestinal tract (GIT) and is approximately 60 to 70% bioavailablc after oral administration. When cimetidine is administered orally in tablet or in liquid form in the fasting state, the blood/plasma concentration-time profile is discontinuous, in that frequently, 2 'peaks' are observed, the first at about 1 hour and the second after about 3 hours (Somogyi and Gugler, 1983). It was proposed, based on a model that the 2-peak effect was due to the drug being stored in hepatic parenchymal tissue, with the second peak resulting from rapid release of cimetidine and possible conjugates from this tissue with subsequent re-absorption (Veng pedersen and

Miller, 1980). This release could occur spontaneously or be triggered by food intake (Somogyi and Gugler, 1983).

A study (Logan et al, 1978) showed a good correlation between the gastric emptying rate of a liquid meal and cimetidine absorption at 1 hour in healthy subjects, suggesting that the individual variation in gastric emptying was responsible for the variation in cimetidine absorption. Absorption of cimetidine may be affected by various antacids, metoclopramide and anticholinergic agents (Somogyi and Gugler, 1982), but is unaffected by food (Somogyi and Gugler, 1983).

Cimetidine concentrations between 0.5 and 1.0ug/ml are required to suppress gastric secretion under basal conditions or stimulated by pentagastrin or food. Attempts to correlate plasma concentrations of cimetidine to duodenal ulcer healing have so far failed, and thus prediction of therapeutic response from pharmacokinetic data appears disappointing (Festen et al, 1981). Blood concentrations remain above those required to provide 80% inhibition of basal gastric acid secretion for 4 to 5 hours following a 300mg dose (Drug facts and comparisons, 1989b).

Distribution

Cimetidine distributes widely and extensively throughout the majority of body fluids, organs and tissue in man. Schentag et al, (1981) reported that there is extensive uptake of cimetidine into selected organs (e.g kidney, lung) and tissues (e.g . lung). However, the amount distributed depends upon the skeletal muscle uptake of cimetidine. Skeletal muscle plays the dominant role in the

physiological estimation of the volume of distribution of the drug, which is as low as 1L/kg body weight (Somogyi and Gugler, 1983).

Cimetidine distributes into the cerebrospinal fluid (CSF) at a ratio of 0.1 to 0.2 compared with plasma. Higher ratios have been observed in patients with liver disease (Schentag et al, 1981) which may help to explain some of the CNS adverse effects of cimetidine in this patient group. The mean saliva to plasma ratio is 0.2 (Somogyi and Gugler, 1983). According to the pH partition hypothesis, a ratio of about 1.3 is expected, about 10 times that observed. This suggests that it is the lipophilicity of cimetidine which controls its penetration across membranes into both saliva fluid and CSF, with the degree of lonization being of much lesser importance.

Between plasma concentrations of 0.05 and 50ug/ml, cimetidine was found to be bound between 18 and 26.3% (Taylor et al, 1978). There is no relevant effect of changes in binding on the pharmacokinetics of cimetidine because the plasma protein binding is well below the critical value of 80% at which changes in the unbound concentration of drug due to altered protein binding become unimportant (Sjoqvist et al, 1980). Cimetidine is concentrated in the red blood cells (Somogyi et al, 1980). This uptake into erythrocytes is much greater than that into both CSF and Saliva (Somogyi and Gugler, 1983).

Cimetidine crosses the placenta and is detectable in the foetus in considerable concentrations. In a study (Howe et al, 1981), it was reported that the ratio of foetal to maternal blood concentrations of cimetidine was time dependent. The

highest concentration of cimetidine in infant blood occurred within 1 hour after administration. Cimetidine also is secreted into breast milk of nursing mothers and may reach the infant in amounts in several milligrams daily (Somogyi and Gugler, 1979).

Elimination

Cimetidine is eliminated from the body by renal, metabolic and biliary processes. However, the principal route is by renal elimination (Taylor et al , 1978; Grahnen et al, 1979; Walkenstein et al, 1978). Between 50 and 80% of the dose administered i.v. is recovered in urine as unchanged cimetidine. This fraction is less after oral doses, but is independent of the amount of the dose (Somogyi and Gugler, 1983). In ulcer patients, 40% is recovered unchanged in urine after oral administration (Somogyi et al, 1980; Bodemar et al, 1981). The high urinary excretion of cimetidine coupled with low plasma concentrations results in a high renal clearance of the drug (Somogyl and Gugler, 1983).

Biliary excretion of cimetidine accounts for only 2% of the dose (Gugler et al, 1981). Thus, the elimination of cimetidine into bile is negligible and of no clinical significance.

Cimetidine is metabolised to 3 known products, cimetidine sulphoxide, hydroxymethyl cimetidine and guanylurea cimetidine (Figure 2.2.5.1), although the latter may be formed non-enzymatically and may be an in vitro degradation product (Taylor et al, 1978). In man metabolism represents only 25-40% (depending on age) of the total elimination of cimetidine, the sulphoxide being the major metabolite (10-15%) and the hydroxymethyl derivative being the minor metabolite (4%) (Somogyi and Gugler, 1983).

In the presence of both rat an human faecal homogenates, cimetidine sulphoxide has been shown to be reduced back to cimetidine (Taylor et al, 1978; Grahnen et al, 1979; Mitchell et al, 1982a). As well, in a study using ^{14}C-labelled cimetidine sulphoxide administered orally, to two healthy volunteers; the results suggested that apart from the gut microflora, the kidney may play an important role in reductive metabolism (Mitchell et al, 1982b).

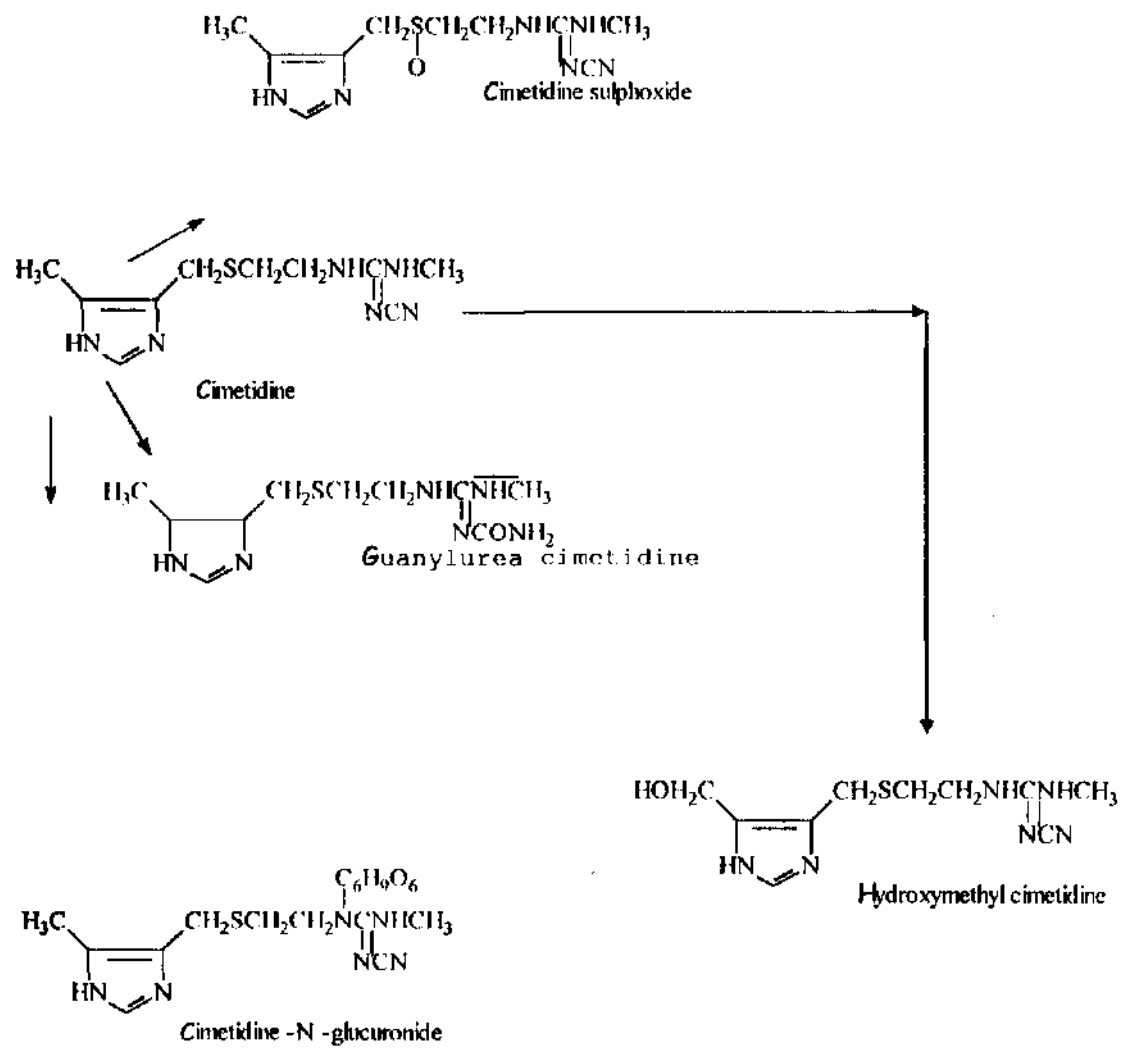


FIGURE 2.2.5.1: Schematic representation of the metabolic pathways for cimetidine.

The line to guanylurea cimetidine indicates that the metabolite can be formed non enzymatically (Somogyi and Gugler, 1983).

Elimination of cimetidine is accelerated by an average of 15% in the presence of phenobarbitone, due to induction of its metabolism by phenobarbitone (Somogyi et al, 1981). Elimination half-life of cimetidine is approximately 2 hours in healthy adults with normal kidney and hepatic function (Somogyi and Gugler, 1983). Half-life increases in renal impairments, hepatic impairment and in the elderly. Cimetidine is not significantly removed by hemodialysis or peritoneal dialysis (Drug facts and comparisons, 1989b).

2.2.6 PHARMACOLOGICAL PROPERTIES AND INDICATIONS

Cimetidine competively and selectively inhibits the action of histamine at the histamine H_2-receptors of the parietal cells. Thus it inhibits the secretion of gastric acid stimulated by histamine, pentagastrin, acetylcholine, insulin, caffeine, food and other secretagogues.

Cimetidine is used in conditions where inhibition of gastric acid may be beneficial such as in the following:

- Short term treatment of active duodenal ulcer.
- Maintenance therapy for duodenal ulcer patients at reduced dosage after healing of active ulcer.
- Pathological hypersecretory conditions, such as in Zollinger - Ellison syndrone, systemic mastosytosis, multiple endocrine adenomal.
- Unlabeled uses: Oral or i.v cimetidine, 60 to 90 minutes before anesthesia has been used to prevent aspiration pneumonitis. Doses of 1g/day have been

used with variable success to treat primary hyperparathyroidism and to control secondary hyperparathyroidism in chromic hemodialysis patients. Cimetidine has been used in the prophylaxis of stress-induced ulcers and of acute upper gastrointestinal bleeding, in gastro esophageal reflux, tinea capitis, herpes virus infection and hirsute women (Drug facts and comparisons, 1989b).

2.2.7 ADMINISTRATION AND DOSAGE

Cimetidine is available for oral use as tablets containing 200, 300, 400 or 800mg and as a liquid containing 200mg/5ml. Solution of hydrochloride salt of cimetidine containing 100mg/ml is available for intramuscular (1.m) and i.v. administration.

For treatment of active duodenal or benigh gastric ulcers, pathological hypersecretory conditions, dosage is 800mg at bedtime. Alternate regimens are 300mg, 4 times a day, with meals and at bedtime or 400mg twice daily. Treatment should be continued for 4 to 6 weeks unless healing is demonstrated by endoscopy. A dose of 400mg may be given at bedtime for prevention of recurrence of duodenal ulcers.

In severely impaired renal function, accumulation may occur, so dosage reductions is recommended. When liver impairment is also present, further dosage reductions may be necessary.

For hospitalised patients with pathological hypersecretory conditions or intractable ulcers, or patients unable to take oral medication, parenteral form

may be used. Usual dose is 300mg i.m or i.v every 6 to 8 hours. Dosage may be increased if necessary, but not exceeding 2400mg/day.

2.2.8 ADVERSE EFFECTS OF CIMETIDINE

Cimetidine is among the safest of drugs currently available. Most adverse effects of cimetidine are of minor nature and are usually promptly reversible upon stopping treatment.

Side effects of cimetidine include altered bowel habit, interstitial nephritis, acute pancreatis, tiredness and hypersensitivity. Reversible liver damage and hematologic disorders reported. Cardiac arrhythmias and arrest following i.v. bolus may occur. Gynaecomastia is also an occasional problem with cimetidine (but usually only in high dosage), and reversible impotence has also been reported.

CNS adverse effects associated with cimetidine include dizziness, somnolence, headache, confusional states, hallucinations and delirium; symptoms of brainstem dysfunction, peripheral neuropathy. The apparent causes of cimetidine induced CNS toxicity appear to be due to the prescribing of large doses of cimetidine in patients who have a reduced ability to eliminate the drug (Somogyi and Gugler, 1983).

2.2.9 CIMETIDINE - DRUG INTERACTIONS.

Because of the ability of cimetidine to produce fundamental changes in the absorption and disposition of other drugs; and its widespread (but often uncritical) use, there is considerable potential for interactions to occur with other drugs. Interactions with cimetidine occur primarily through 3 different mechanisms:

(a) The ability of cimetidine to raise gastric pH may have influence on oral drug bioavailability, as the disintegration of some tablet formulations and, the dissolution and ionization of many drugs is dependent on the pH of gastric fluid (Mayersohn, 1979). Change in pH may also increase the stability and presumably the oral bioavailability of acid-labile drugs (Mayersohn, 1979).

(b) Cimetidine, like other substituted imidazoles inhibits various microsomal drug metabolising enzymes in animals as well as human liver (Wilkinson et al, 1974a). Thus, in man cimetidine interacts with various clinically important drugs (Somogyi and Gugler, 1982; Klotz and Reiman, 1984), which are extensively metabolised in the liver.

(c) Due to the ability of cimetidine to undergo renal tubular secretion via the renal cationic transport system, competition with other basic compounds renally secreted may occur as in the case of triamtrene (Somogyi et al, 1989), chlorpropamide (Maeda et al, 1994) Zidovudine (Fletcher et al, 1995) and creatinine (Zeimniak et al, 1986).

Effects of cimetidine on absorption of other drugs.

In a study (Fairfax et al, 1978), the absorption of sodium benzylpenicillin (600mg) in 5 healthy volunteers before and after 1 day's treatment with cimetidine were investigated. No differences were observed in 4 of the 5 subjects between the 2 occasions in the peak serum concentrations; the concentration at 4 hours and the 6 hours urinary excretion. However, in 1 subject the peak concentration increased significantly with cimetidine, and in urine an 8-fold increase in excretion occurred, which was confirmed upon repeating the experiment. Thus, workers (Fairfax et al, 1978) concluded that increased absorption of acid-labile drugs can occur in some patients taking cimetidine.

Reports on the interaction between cimetidine and tetracycline are conflicting. In 1 study a 400mg dose of cimetidine reduced significantly the absorption of tetracycline (as measured by the AUC, and urinary excretion). However, chronic dosing with cimetidine for 5days had no effect on tetracycline disposition (Fisher et al, 1980). In another study, chronic treatment with cimetidine reduced the absorption of tetracycline (as measured by urinary excretion) from a capsule formulation, but had no effect when tetracycline was given in solution form (Cole et al, 1980). In a study by Garty and Hurwitz (1980), a single 300mg dose of cimetidine did not reduce the absorption of tetracycline (as measured by AUC, and urinary excretion), although intragastric pH rose from 2 to 5 at 1 hour after cimetidine dosing. These discrepancies between results may be due to failure to standardise food intake, which has a marked effect of tetracycline absorption (Welling et al, 1977). Tetracycline is

also metabolised by the liver, and since cimetidine inhibits drug metabolism, this may have masked any interaction at the level of drug absorption.

Cimetidine has been shown to slightly increase serum salicylate concentrations and bioavailability following administration of enteric-coated aspirin (Paton et al, 1983; Willoughby et al, 1983). However, the magnitude of the increases does not appear to be sufficient to produce adverse effects. In another study of 6 subjects, cimetidine appeared to increase the absorption rate of aspirin in 3 subjects, but there was no significant change in the group as a whole (Khoury et al, 1979). The type of aspirin used was not stated. Thus, there is little evidence that cimetidine has a clinically important effect on aspirin absorption, although an effect of cimetidine on aspirin cannot yet be ruled out.

In a report, six normal healthy subjects received 400mg of cimetidine or placebo every 6 hours, and on the 6th day 500mg of ampicillin (Rogers et al, 1980). As assessed by the peak plasma concentration (Cmax), the time of its occurrence (Tmax), the AUC and the terminal half-life, cimetidine induced no alterations in ampicillin disposition. In the same study design as for ampicillin, the subjects took cotrimoxazole (160mg trimethoprim plus 800mg sulphamethoxazole) with and without cimetidine (Rogers et al, 1980). No significant differences in the pharmacokinetics of sulphamethoxazole or trimethoprim were observed, although plasma concentrations of the latter tended to be higher under cimetidine treatment.

In a randomised double-blind crossover study design involving 6 healthy volunteers (Morrison et al, 1980), Prednisolone (10mg) tablets were given following 5 days of treatment with cimetidine (1g daily) or matching placebo in divided doses and continued for 24 hours after the dose of prednisolone. Cimetidine had no overall statiscally significant effect on the prednisolone pharmacokinetic parameters (i.e AUC, Cmax, Tmax, $t_{1/2}ab$). However, a large inter individual variation in the above parameters was noted which may have masked any possible interaction. There was also a significant order of treatment effect, in that the 3 subjects who received placebo treatment first developed lower plasma prednisolone concentrations than those who received cimetidine first. Since cimetidine is a most potent inhibitor of drug metabolism, the results could be interpreted as being due to inhibition of prednisolone metabolism in the group receiving cimetidine first, despite the conclusion by Morrison et al. (1980) that prednisolone absorption and disposition is unaltered during cimetidine treatment when given as enteric -coated tablets (Somogyi and Gugler, 1982).

In a case report a patient was found to be unresponsive to orally administered Ketoconazole whilst receiving cimetidine and antacids concomitantly (Van der meer et al, 1980). The absorption of the antifungal agent was subsequently studied in 3 healthy volunteers. When 400mg cimetidine was given 2 hours prior to administration of Ketoconazole, the AUC of Ketoconazole was reduce by 65% over the control, but was increased by 52% when Ketoconazole was given in an acid solution, again 2 hours after cimetidine. Thus, an apparently clinically significant interaction can occur between cimetidine and

Ketoconazole, which can be avoided when ketoconazole is given in an acid solution.

Summarising the studies on drug absorption interactions related to cimetidine, it appears that some are contradictory or inadequately designed, and most of them (except for ketoconazole) show no significant interaction. However, there are a number of areas of concern which have not been handled well in most studies to date (Somogyi and Gugler, 1982). Such areas include the effect of food, diet, fluid volume and intrasubject variation on absorption; the inhibitory effect of drug metabolism by cimetidine which may masked any interaction at the level of drug absorption, etc.

Effect of cimetidine on disposition of other drugs.

Cimetidine and ranitidine have been found to inhibit the renal tubular secretion of triamtrene in isolated perfused rat kidney and produced a significant change in the tubular secretion of triamtrene which will be of clinical significance if extrapolated to man (Somogyi et al, 1989). Cimetidine was also reported to significantly influence the kinetics of creatinine by inhibition of renal tubular secretion (Zeimniak et al, 1986).

The effects of oral cimetidine on the kinetics of I.V enoxacin was reported (Misiak et al, 1993). In the study, 10 healthy subjects received a single 400mg i.v dose of enoxacin alone with 300mg (QID) oral cimetidine. Serial blood and urine samples were collected over a 48hours period. Plasma and urine enoxacin concentrations were determined using a validated HPLC method. Mean

enoxacin plasma concentrations were higher after administration of enoxacin with cimetidine than those measured after enoxacin alone.

Maeda et al (1994), described the inhibitory effects of cimetidine on renal tubular secretion of chlorpropamide in rabbits. The effects of cimetidine treatment on the kinetic behaviour of chlorpropamide, were investigated in rabbits with the standard renal clearance method. The renal clearance ratio and the renal tubular secretion volume of chlorpropamide were found to be markedly decreased by the treatment of cimetidine.

Furthermore, the results of experiment using renal cortical slices demostrated that cimetidine of the cationic drug decreased the accumulation of chlorpropamide. The observations indicate that the treatment of cimetidine may depress the renal excretion of chlorpropamide by inhibiting the tubular secretion.

In a study (Somogyi et al, 1994), the in-vitro potencies of histamine H_2 - receptor antagonists on tetraethylammonium uptake in rat renal brush-border membrane vesicles were determined. Cimetidine, ranitidine, famotidine are organic bases that are cleared from the body by active renal tubular secretion involving the organic cation transporter in the proximal tubule. To determine the potential form competition for the transporter between these drugs and other drugs, their inhibitory potencies were assessed in-vitro using rat renal brush-border membrane vesicles and tetraethylammonium as the substrate. The concentration - dependant effect of cimetidine, ranitidine and famotidine on the 15-S proton stimulated uptake of tetraethyl ammonium into the membrane

vesicles were studied using 5 different rat kidneys. The order of inhibition potencies was: cimetidine > famotidine > ranitidine. The result indicate the potential for drug interactions in the kidney especially for cimetidine and famotidine.

In a report (Fletcher et al, 1995), the effect of cimetidine administration with zidovudine was described. The study was carried out to evaluate the possibility of a drug interaction with zidovudine and histamine H_2-receptor antagonists in individuals affected with the human immunodeficiency virus (HIV), using the randomized crossover study design. Six HIV - infected individuals are involved in the study. The subjects received 7 days regimens of zidovudine 600mg daily alone, and zidovudine with cimetidine 1200mg daily. The renal clearance of zidovudine when given alone was 0.41 L/kg/hr and was reduced to 0.18 L/kg/hr (P=0.002) when given cimetidine. In the presence of cimetidine the urinary excretion of zidovudine decreased from 89.5 to 53.7 micro ML (P = 0.01), the urinary ratio of metabolite to parent drug increased from 5.16 to 9.96 (P = 0.0001), and the fraction of zidovudine converted to metabolites increased from 0.86 to 0.92 (P = 0.0025). It was concluded that cimetidine presumably inhibits the renal clearance of zidovudine by competing for renal tubular secretion (Fletcher et al, 1995).

Antipyrine has been used as a model to investigate alterations in drug metabolism caused by physiological, pharmacological and environmental factors (Vesell, 1979). Several studies have been conducted on investigating the influence of cimetidine on antipyrine pharmacokinetics (Serlin et al, 1979;

Klotz and Reimann, 1980a; Henry et al, 1980; Puurunen et al, 1980; Daneshmend et al, 1980; Robert et a;, 1981). The results of all studies clearly indicated reduced elimination, and hence metabolism, of antipyrine when taken concomitantly with chronic administration of cimetidine (Somogyi and Gugler, 1982).

Cimetidine may produce a clinically important increase in the hypoprothrombinemic response to oral anticoagulants (Serlin et al, 1979; Puurunen et al, 1980). In patients receiving warfarin, the addition of cimetidine usually results in a gradual increase in hypoprothrombinemia over 1-2 weeks; it takes about 1 week for the prothrombin time to return to pre-cimetidine levels when cimetidine is discontinued. The interaction between cimetidine and warfarin is dose related. Example, cimetidine doses of 800mg nightly tend to affect warfarin less than larger doses given two or more times daily, and 400mg cimetidine daily may be insufficient to produce clinically significant effects on warfarin in some patients. Phenprocommon undergoes glucuronide conjugation and does not appear to be affected by cimetidine.

Phamacokinetic studies have consistently shown that cimetidine reduces theophylline plasma clearance ,increases theophylline half -life and increases plasma theophylline levels (Hansten and Horn 1989d). Moreover, several case reports have appeared describing elevated plasma theophylline levels and/or theophylline toxicity during cimetidine therapy (Wood et al 1980; Weinberger et al, 1981). The magnitude of this interaction increases as the dose of cimetidine increases. Since cimetidine begins to reduce theophylline elimination as soon

as therapeutic serum levels of cimetidine are achieved, a new steady-state serum theophylline level will usually be observed by the second day of cimetidine therapy. However, it may take longer in some patients. Factors that may increase the magnitude of this interaction include high pre-existing serum theophylline concentration, TID, or QID dosing of cimetidine, smoking and protein-deficient diets (Hansten and Horn, 1989d).

Calandre et al (1981) studied the pharmacokinetics of orally administered phenytoin in 5 healthy subjects before and after chronic dosing with cimetidine. The elimination half-life of phenytoin was reported to prolonged by between 46 and 118% with a similar increase in the AUC. They also observed an increase in urinary excretion of phenytoin and a decrease in urinary excretion, of phenytoin major metabolite, 5(P-hydroxyphenyl) - 5 - phenylhydantoin (P-HPPH). In another study (Hetzel et al, 1981), chronic dosing with cimetidine increased the steady-state phenytoin plasma concentration by between 16 and 33%. The greatest increase in phenytoin plasma concentration was associated with the highest initial concentration and vice versa, a reflection of the non linear elimination of phenytoin. An increase in the urinary excretion of phenytoin and, unexpectedly, its major metabolite, P-HPPH was explained by cimetidine inhibiting other metabolic pathways, or by increased phenytoin absorption.

Interactions of cimetidine with 5 different benzodiazepines - Diazepam (Klotz and Reimann, 1980b), chlordiazepoxide (Desmond et al, 1980), Oxazepam (Klotz and Reimann, 1980c; Patwardhan et al, 1980) and Lorazepam

(Patwardhan et al, 1980) were reported. The total plasma clearance values for diazepam, desmethyl diazepam, and chlordiazepoxide were significantly reduced by an average of 43, 28 and 63% respectively. The terminal half-lives of these 3 benzodiazepines were significantly prolonged by an average of 53, 40 and 135%, respectively, with concomitant cimetidine intake. Their volumes of distribution remained unaltered, as well as their degree of protein binding. Hence, the plasma clearances of free drug were also significantly reduced by cimetidine. In contrast, cimetidine produced no alterations in the pharmacokinetic parameters of oxazepam and lorazepam, which are eliminated almost entirely as glucuronide conjugates. Total (and free) plasma clearance, terminal half-life and volume of distribution of these benzodiazepines (Oxazepam and lorazepam) remained unchanged under cimetidine treatment.

In two studies (Schoerlin et al, 1991; Zimmer et al, 1990), it was reported that the pharmacokinetics of moclobemide are significantly modified by cimetidine. Pretreatment of healthy volunteers with cimetidine 1000mg daily for 2 weeks resulted in increase in the peak concentration (39%), terminal elimination half-life (35%) and AUC (120%) values of a single oral dose of moclobemide 100mg.

One interaction with different H_2 - receptor antagonists which has received considerable attention recently concerns alcohol (Howden, 1993). The first report of cimetidine increasing blood alcohol levels antedated the isolation and characterization of gastric alcohol dehydrogenase (ADS) (Feely and Wood, 1982). Since then, there have been a number of studies examing the effects of

different H_2 - receptor antagonists on blood alcohol levels. For studies with cimetidine, there is most evidence for the drug increasing blood alcohol levels over a wide range of doses of alcohol (Feely and Wood, 1982; Caballena et al, 1989; Roine et al, 1990; Guram et al, 1991).

Ahmad et al (1992), reported the effect of cimetidine on the LD50 of paracetamol in mice. Mice were injected with 250, 500, 750 and 1000mg per kg paracetamol.

After 1 hour, either saline (control) or an aqueous solution of cimetidine (50mg/kg) was injected. The LD50 of paracetamol was increased form 392 to 550mg/kg by the administration of cimetidine. Thus, cimetidine protects from liver injury induced by paracetamol and could be used clinically in patients intoxicated with paracetamol (Ahmad et al, 1992).

In a report (Tateishi et al, 1992), the influence of diltiazem versus cimetidine on propranolol metabolism was described. Six healthy male volunteers received a single oral dose of 20mg propranolol with pretreatment with placebo, 60mg diltiazem or 400mg cimetidine three times daily for 4 days. Diltiazem and cimetidine increased the AUC of propranolol and it's glucuronide. Cimetidine also increased the urinary excretion of propranolol glucuronide. There were no significant differences in the AUC of 4-hydroxypropranolol (4-OH PPL) and its conjugates or the urinary excretion of conjugated 4-OH PPL. Diltiazem increased the AUC of naphthoxylactic acid (NLA) and the urinary excretion to NLA. After cimetidine pre treatments, there was the trend toward a decrease in the partial metabolic clearance to 4 - OH PPL and a significant decrease in that

of NLA. Results suggest that diltiazem and cimetidine inhibit the oxidation pathways of propranolol in different manners. Cimetidine might inhibit both oxidative pathways to 4 - OH PPL and NLA, whereas diltiazem might not inhibit the pathway to NLA.

Ohashi et al (1993) reported the effect of diltiazem or cimetidine pre treatment on the inhibition of theophylline metabolism in nine healthy male non smokers. Diltiazem 60mg, cimetidine 400mg, or placebo was given orally TID for 3 days in a randomized 3-way crossover manner. Both diltiazem and cimetidine pretreatment decreased the mean theophylline clearance, resulting in prolonged mean theophylline half-life with no change in volume of distribution. The mean metabolic clearances for 3 major theophylline metabolites, 1 - methyluric acid (1 -Mu), 1,3 - dimethyluric acid (1,3 - DMU) and 3 - methylxanthine (3-MX), were reduced significantly by cimetidine. Conversely, diltiazem significantly reduced only the mean metabolic clearance of 1,3 - DMU by 21% without changes in that of 1 - MU or 3 - MX. These results suggest that cimetidine inhibited both N-demethylation and 8-hydroxylation of theophylline, whereas diltiazem exerted little influence on N-demethylation in spite of inhibition in 8 - hydroxylation (Ohashi et al, 1993).

Forgue et al (1996) reported the inhibition of tacrine oral clearance by cimetidine in 12 healthy elderly subjects. Subjects received 40mg tacrine alone and during multiple dose cimetidine (300mg QID) administration. After coadministration of cimetidine with tacrine, plasma tacrine concentrations were approximately one third higher than values after administration of tacrine alone,

metabolites concentrations were also higher. Mean tacrine oral clearance was reduced by 30%. However, mean absorption rate and elimination half-life values were not affected by cimetidine. It was concluded that cimetidine inhibits first pass hepatic extraction of tacrine by cytochrome P450 enzymes but has little effect on systemic drug clearance. Clinical considerations may dictate a reduction in tacrine dosage when it is co-administered with cimetidine.

Conflicting data exists regarding the effect of cimetidine on hepatic perfusion, another important determinant of hepatic drug clearance (Grasela et al, 1987). A study was conducted to investigate the effect of cimetidine 1g/day on the oral disposition of propranolol 80mg in 6 peptic ulcer patients (Heagerty et al, 1981). The AUC of propranolol was significantly ($P<0.05$) increased by an average of 61% when cimetidine was administered concomitantly with a similar increase in the peak plasma concentration. The data were interpreted as a decrease in the first-pass extraction of propranolol by cimetidine. Following a single 600mg dose of cimetidine in six healthy, male volunteers, a mean decrease of approximately 25% in hepatic blood flow (HBF) as estimated by indocyanine green (ICG) and propranolol clearances was reported (Feely et al. 1981). The result of this work implied that cimetidine-induced reductions in HBF could result in excessive accumulation of drugs whose systemic clearances were limited by hepatic perfusion (Nies et al, 1974). In another study (Reimann et al, 1981), the interaction between cimetidine and propranolol during chronic dosing with both drugs was investigated. Cimetidine administration resulted in doubling of the steady-state propranolol concentration; thus a decreased in oral clearance of propranolol.

Investigators have attempted to examine the effects of cimetidine on the elimination of drugs with perfusion-limited clearances, including Lidocaine (Knapp et al, 1983; Powell et al, 1983; Wing et al; 1984.) and Morphine (Mojaverian et al, 1982). Lidocaine clearance following single doses of cimetidine has been reported to be decreased by 12-30% following cimetidine administration (Wing et al, 1984). Similar results were found in study of patients receiving Lidocaine infusions (Knapp et al, 1983). However, another study in seven patients did not find an effect of I.V cimetidine on the disposition of Lidocaine given as a continous I.V infusion (Powell et al, 1983). Also, chronic cimetidine administration was reported to have no effect on the systemic clearance of Morphine (Mojaverian et al, 1982). The studies thus far reported on interactions between cimetidine and high clearance drugs have been somewhat contradictory (Grasela et al, 1987), the one area of agreement being a reduction in oral clearance of a number of drugs by cimetidine. Alterations in hepatic blood flow (HBF) or systemic clearance have not been consistently observed (Grasela et al, 1987). The inconsistency in results is due to the fact that most studies failed to mention how such variables as intake and type of food, exercise, posture and age of the patients or subjects were controlled, all of which alter HBF (Wilkinson, 1975), and these variables must be strictly controlled (Somogyi and Gugler, 1982).

To assess the effects of chronic cimetidine (300mg Q.I.D. x 4 days) on estimated HBF while controlling posture, a double-blind, placebo-controlled, repeated measures study was performed in 9 healthy males (Grasela et al,

1987). Indocyanine green (ICG) plasma clearance after an I.V dose was used to calculate HBF. ICG plasma concentrations were measured by HPLC. The results of the study suggest that cimetidine does not produce a uniform, clinically significant effect on HBF, and that H_2 - receptors may not be intimately involved in HBF regulation. Thus cimetidine-induced alterations in the systemic clearance of drugs appears to be primarily due to its effect in inhibiting the cytochrome P450 system (Grasela et al, 1987).

Clinical significace of cimetidine- drug interactions.

Drug interactions caused by cimetidine have stimulated the interest of clinicians and pharmacologists due to the large number of drugs affected. However, much more has been made of this than is probably justified, given that most such interactions are of purely pharmacokinetic interest (Howden, 1993). Before an effect of cimetidine interaction can be regarded as significant, it is suggested that the change caused by the interaction should be between 20 and 25% (Upton et al, 1982). Certainly some of the effects of cimetidine interactions are not significant at clinical level. Moreover, many of those which are potentially harmful only occur in a small proportion of patients. Thus, long lists of drugs with which cimetidine has been shown to interact are likely to have served the commercial objectives of rival pharmaceutical companies than to have usefully informed prescribers (Howden, 1993). Table 2.2.9.1 show the effects of cimetidine on various drugs and their relative clinical importance (whether potentially hazardous or not).

The potential of cimetidine to affect the absorption of other drugs is small; in most cases the changes are minor and seem to be inconsistence (Somogyl and Gugler, 1982). However clinically important interactions do occur between cimetidine and certain other drugs which are extensively metabolised in the liver and have a narrow therapeutic index. For all practical purposes, this list is limited to phenytoin, warfarin and theophylline (Howden, 1993). As an example, a decrease in elimination of benzodiazepines during cimetidine administration may not necessitate dosage changes in most patients, since benzodiazepines have a wide therapeutic concentration range. However, cimetidine-induced inhibition of metabolism of, for example, anticoagulants can have serious consequences (Somogyi and Gugler, 1982). Patients at increased risk from cimetidine - drug interactions include the elderly and those with impaired renal or liver function.

TABLE 2.2.9.1 Influence of cimetidine on the pharmacokinetics of drugs (BNF, 1994).

DRUG CLASS	INFLUENCE OF CIMETIDINE	REMARK
Analgesics	increases plasma concentrations of opioid analgesics notably pethidine by inhibiting metabolism.	N.P.H
Anti-arrythmics	Increases plasma concentrations of amiodarone Flecainide, Lignocaine, procainamide, propafe -none and quinidine.	P.H
Antibacterials	Reduces absorption of cefpodoxine. Increases plasma concentration of metronidazole by inhibiting its metabolism.	N.P.H
Anticoagulants	Enhances anticoagulant effect of nicoumalone and warfarin by inhibiting their metabolism.	P.H
Antidepressants	Increases plasma concentrations of amitriptyline, desipramine, doxepin, imiprmine, nortriptyline, moclobemide and probably other antidepressants by inhibiting their metabolism.	N.P.H
Antidiabetes	Increases plasma concentration of metformin by inhibiting its renal excretion.	N.P.H
Antiepileptics	Increases plasma concentrations of phenytoin and carbamazepine by inhibiting their metabolism.	P.H
Antifungals	Reduce absorption of itraconazole and Ketoconazole. Increases plasma concentration of terbinafine.	N.P.H
Anxiolytics and Hypnotics	Increases plasma concentrations of benzodiazepines and chlormethiazole by inhibiting their metabolism.	N.P.H
Antimalarials	Increases plasma concentrations of quinine and chloroquine by inhibiting their metabolism.	N.P.H

Antipsychotics	Enhances effect of chlopromazine, clozapine and possibly other antipsychotics.	N.P.H
Beta-Blockers	Increases plasma concentrations of labetalol and propranolol by inhibiting their metabolism.	N.P.H
Calcium-channel blockers	Increases plasma concentrations of some calcium-channel blockers by inhibiting their metabolism	N.P.H
CYCLOSPORIN	Possibly increases plasma cyclosporin concentration.	P.H
Cytotoxics	Increases plasma concentration of fluorouracil.	N.P.H
Mebendazole	Enhances plasma concentration by inhibiting its metabolism.	N.P.H

KEY:
P.H = Potentially Hazardous Interaction.
N.P.H = Not Potentially Hazardous Interaction.

2.3 AIMS AND OBJECTIVES OF PRESENT STUDY

In Nigeria today, there are millions of peptic ulcer patients including many with endoscopically proven cases of peptic ulcer as personal investigation revealed (Garba, 1992). The histamine H_2-receptor antagonists are among the most widely prescribed of all drugs. By any standards, they have been an enormous therapeutic and commercial success and have become regarded as standard treatment for peptic ulceration in most countries (Howden, 1993), and cimetidine is the one mostly used (Somogyi and Gugler, 1993). However, in man cimetidine interacts with various clinically important drugs (Somogyi and Gugler, 1983; Klotz and Reimann, 1984) which are extensively metabolised in

the liver. On the other hand, in studies on drug interactions with cimetidine related to drug absorption, it appears that some are contradictory or inadequately designed (Somogyi and Gugler, 1982).

Paracetamol, an over-the-counter drug is widely used in the treatment of mild to moderate pains of various aetiology. Most often it is prescribed as an analgesic adjunct to ulcer patients who are on cimetidine, It is evidently clear that other analgesic (the non-steroidal anti inflammatory drugs) are to be avoided in ulcer conditions (Howden and Holt, 1991). Furthermore, since paracetamol is well tolerated and can be obtained without prescription, many patients on cimetidine take paracetamol for analgesia and other complaints without being prescribed for them. Therefore, a great potential exists, and needs to be thoroughly investigated, for interaction to occur between cimetidine and paracetamol since the two drugs are commonly used in combination.

The proposed study therefore, is aimed at understanding the nature and extent of the possible interactions (if any) of cimetidine with paracetamol. This combination was specially chosen because of the importance of peptic ulcer diseases in Nigeria, and the frequent use of cimetidine - paracetamol combination in ulcer patients. Also the literature cited contained scanty information on cimetidine and paracetamol interaction. In most cases, the reported effects of cimetidine on paracetamol are inconsistent, probably because of inadequately designed study protocol.

On the whole, this study is expected to address itself to the following objectives:

1. To determine the salivary pharmacokinetic profile of paracetamol in healthy subjects in our local community.
2. To determine the influence of cimetidine on the salivary pharmacokinetic profiles of paracetamol under two study protocols (i.e. concomitant and delayed administration of paracetamol).
3. To investigate the relevance of the study protocol on the pharmacokinetic interaction between paracetamol and cimetidine in healthy subject in our local community.

2.3.1 METHOD TO EMPLOY

The study is going to be executed in 3 phases and saliva specimen will be sampled for the pharmacokinetic analysis. A wash-out period of two weeks will be allowed between the phases of the study. The following gives the arrangement of the methodology:

PHASE I

The phase I of the study will involve the administration of paracetamol (orally) after overnight fasting and determining the pharmacokinetic profile in healthy volunteers .

PHASE II

Phase II will involve the concomitant administration of paracetamol and cimetidine after overnight fasting and determining the influence of cimetidine on the pharmacokinetic profile of paracetamol in healthy volunteers.

PHASE III

This phase of study will involve the delayed administration of paracetamol one hour after cimetidine after overnight fasting, and determining the influence of cimetidine on the pharmacokinetic profile of paracetamol in healthy human volunteers.

Saliva samples obtained will be analysed by direct U.V spectrophotometer (Chapter 3). The method of residuals will be used to obtain the pharmacokinetic parameters using the salivary concentration - time curves on a semilog scale as preliminary data. The pharmacokinetic parameters generated will then be analysed statistically and the influence of cimetidine on the absorption and disposition kinetics of paracetamol under the two study protocols (concomitant and delayed administration) interpreted accordingly.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

The following materials were used in carrying out the project:

3.1.1 GLASSWARE

- 10ml extraction tubes, Pyrex, England
- 20ml burette, Pyrex, England
- Screw-capped sample bottles: 5ml, 10ml and 20ml sizes
- Pipettes: 0.02ml. 0.1ml. 1ml, 5ml and 10ml sizes, Pyrex, England.
- Test tubes: 10ml and 20ml sizes, Pyrex, England.
- Measuring cylinders: 5ml, 10ml, 50ml and 100ml sizes, Pyrex, England.
- Volumetric flasks; 25ml, 50ml, 100ml, 250ml, 500ml and 1000ml sizes, Technico, England.
- Glass weighing pan
- Crucible
- Conical flasks: 25ml, 50ml, and 250ml sizes, Pyrex, England.
- Beakers: 50ml, 100ml and 250 sizes, Pyrex, England.
- Reflux condenser, Pyrex, England.
- Round bottom flask, Pyrex, England.
- Syringes and needles (2ml, 5ml and 20ml sizes syringes and 21g needles).
- Glass funnel and filter papers (Whatman).

- Pasteur pipette.

3.1.2 EQUIPMENT

- Auto-vòrtex mixer, Stuart, England
- Centrifuge, junior, Gallenkamp, England
- Flask shaker, Gallenkamp, England
- Disintegration rate study apparatus, Erweka, Germany
- Dissolution rate study apparatus, Erweka, Germany
- Gilson pipetteman, France
- Hot air oven BS size one, Gallenkamp, England
- HPLC priming syringe, Hamilton, England
- Injection microsyringe, Hamilton, England
- Electronic balance, mettler, A.E 240
- Thermocool refrigerator, premier, Nigeria
- Solvent filters, 0.45um, Waters, Massachusetts, USA.
- SP8-100 U.V. spectrophotometer, pye unicam.
- Melting point apparatus, Gallenkamp, England
- Hot plate, B212, Bibby, U.K.
- Water bath, compenstat, Gallenkamp, England
- Friabilator, Erweka, Germany.

3.2.3 CHEMICALS AND STANDARD SAMPLES

Chemicals

- Methanol, Analar, BDH Chemicals, England
- Perchloric acid, Analar, BDH Chemicals, England

- Glacial acetic acid, Harris reagent, England
- Ethylacetate, May and Baker, England
- Distilled and filtered water
- Sodium hydroxide pellets, May and Baker, England
- Acetone, May and Baker, England
- Hydrochloric acid, Analar, BDH Chemicals, England
- Potassium dichromate, May and Baker, England
- Ferric chloride, May and Baker, England
- Acetic anhydride, Analar, BDH Chemicals, England
- Crystal violet, Harris reagent, England
- Lead Nitrate, May and Baker, England
- Potassium hydrogen Phthalate, BDH Chemicals, England
- Potassium hydroxide, May and Baker, England
- Bismuth oxynitrate, Analar, BDH Chemicals, England

Standard Samples

Standard pure samples used in the project were obtained as gift samples. Standard paracetamol powder (control number 914810) was obtained from Zazzau pharmaceutical (Nigeria) Limited, Zaria, while the standard cimetidine powder was obtained from Smithkline Beecham (Nigeria) Limited, Lagos.

3.1.4 TABLET SAMPLES

The following are the particulars of the tablet samples used in the study:

Paracetamol Tablets

SOURCE: Purchased from a registered pharmaceutical shop

BRAND NAME: Panadol (R)

MANUFACTURER: Sterling Products (Nig) PLC

51, Town Planning Way,

Ilupeju Industrial Estate, Lagos.

MANUF. DATE: 03, 1997

EXP. DATE: 03, 2000

STRENGTH: 500mg per tablet

BATCH No. C23

Cimetidine Tablets

SOURCE: Purchased from a registered Pharmaceutical Shop

BRAND NAME: Tagamet (R)

MANUFACTURER: Smithkline Beecham (Nig) PLC

20 Industrial Avenue, Ilupeju, Lagos.

MANUF. DATE: 06, 1994

EXP. DATE: 06, 1999

STRENGTH: 200mg per tablet

BATCH No. 296460

3.2 METHODS

3.2.1 PREPARATION OF REAGENTS, SOLUTIONS AND DISINTEGRATION / DISSOLUTION MEDIUM

5% Crystal violet

10ml of 5% crystal violet solution in glacial acetic acid was prepared by weighing accurately and dissolving 0.5mg crystal violet in 10ml of glacial acetic acid.

Ferric chloride test solution

10ml of ferric chloride test solution used for the identification test for paracetamol tablet was prepared by weighing accurately and dissolving 0.45g ferric chloride in sufficient distilled water to produce a solution of 10ml.

Lead nitrate paper

Lead nitrate paper used for the identification test for cimetidine was prepared by the following procedure:

10% lead nitrate solution was first prepared by dissolving 5g of lead nitrate powder in 50ml distilled water. Filter paper (Whatman qualitative) was then soaked into the solution, removed and allowed to dry.

0.1M Perchloric acid

250ml of 0.1M perchloric acid was prepared according to the method described in I.P. (1979) volume I, general method of analysis.

To 225ml glacial acetic acid, 2.05ml of 70% w/w perchloric acid was added, mixed and to the content was added 8ml of acetic anhydride and thoroughly mixed. The solution

was then cooled and sufficient glacial acetic acid was added to produced 250ml. The solution was allowed to stand for 24 hours.

The solution was then standardised with potassium hydrogen phthalate (previously dried at 120°C for 2 hours) dissolved in acetic acid, and was stored, pending use.

Potassium iodobismuthate / acetic acid test solution.

Potassium Iodobismuthate/acetic acid test solution used for the identification test for cimetidine tablet was prepared by dissolving 4g of potassium hydroxide in 2ml of water. The solution was then added to another solution containing 0.085g of bismuth oxynitrate dissolved in 4ml of water and 1ml of glacial acetic acid.

0.1M Sodium hydroxide

0.1M solution of sodium hydroxide was prepared by dissolving accurately weighed 4.5g of sodium hydroxide pellets in sufficient distilled water to produce 1000ml solution.

Preparation of dissolution / disintedration medium.

0.1M hydrochloric acid was used as the disintegration and dissolution medium and was prepared as follows:

8.5ml of concentrated hydrochloric acid was accurately measured and was diluted with distilled water sufficiently enough to produce 1000ml.

3.2.2 QUALITY CONTROL

Identification Tests

A - PARACETAMOL TABLETS: Both B.P (1993) and I.P. (1988) methods were used for identification of paracetamol.

For B..P. (1993) method, powdered tablets (0.59 g) containing 0.5g of paracetamol was extracted with 20ml of acetone. It was then filtered and the filtrate evaporated to dryness. The residue obtained was dried at 105°C and then subjected to the following test:

(i) Melting point determination

(ii) 0.1g of the residue was boiled with 1ml of concentrated hydrochloric acid for 3 minutes, 10ml of water was then added and the content cooled. 0.05ml of 0.0167M potassium dichromate was then added and observed for the appearance of a violet colour or any other change.

For the I.P. (1988) method, powdered tablet (0.12 g) containing 0.1g paracetamol was extracted with acetone. The residue was dissolved in 10ml of water and 0.05ml of ferric chloride test solution added. The content was then observed for the appearance of a violet-blue colour.

B- CIMETIDINE TABLET: The method described in basic tests for Pharmaceutical dosage forms (WHO, 1991) was followed for the identification test for Cimetidine.

One tablet was weighed and the amount (0.12 g) equivalent to 60mg Cimetidine calculated. The tablet was finely powdered and the amount equivalent to 60mg Cimetidine weighed, divided into 2 equal parts and used directly as the test substance. The test substance was subjected to the following tests.

(1) - One part of the test substance was ignited. Lead nitrate paper was then exposed to the vapour evolved and observed for the darkening of the lead nitrate paper.

(2)- To another part of the test substance was added 10ml of water, and stirred. Few drops of potassium iodobismuthate/acetic acid test solution was added and observed for an orange precipitation.

Test for Uniformity of Weight

B..P. (1993) procedure was followed:

Twenty (20) tablets of paracetamol were selected randomly and their average weight determined. The tablets were then weighed individually and the percentage weight deviation of each tablet from the mean (average) weight calculated.

The same procedure above was repeated using sample of Cimetidine tablets. The results for the test are presented in chapter four.

Friability Test

Ten (10) tablets were weighed and introduced into a friabilator (Erweka, Germany) which was set to rotate at 25 rpm for 4 minutes. At the end of 100 revolutions (4 minutes), the tablets were removed and re-weighed and compared to the initial weight. The procedure was carried out for both samples of paracetamol and Cimetidine tablets. The percentage loss in weight was subsequently calculated and represents the friability;

..

$$\text{Percentage friability} = \frac{\text{Loss in weight}}{\text{Initial weight}} \times 100$$

Assay for Content of Active Ingredient

A - PARACETAMOL TABLET: B.P. (1993) method was adopted

Twenty (20) tablets of paracetamol were randomly selected, weighed and crushed into a fine powder. A quantity of the powder (0.178 g) equivalent to 0.15g of paracetamol was accurately weighed on an analytical balance and was added to 50ml of 0.1M sodium hydroxide in a 200ml volumetric flask. The content was diluted with 100ml of water shaken for 15 minutes and sufficient water added to make 200ml. It was mixed, filtered and 10ml of the filtrate was diluted to 100ml with distilled water 10ml of the resulting solution was added to 10ml of 0.1M sodium hydroxide, diluted to 100ml with distilled water and the absorbance of the resulting solution was measured at the wavelength maximum at 257nm. Content of paracetamol was calculated taking 715 as the value of A (1%, 1cm) at the Wavelength of 257nm. Thus,

A (1%, 1cm) of paracetamol at 257nm = 715.

Expected conc. of sample after dilutions = 0.00075% w/v

$$\therefore \text{expected absorbance} = \frac{0.00075 \times 715}{1}$$

$$\text{So percentage content} = \frac{\text{Absorbance given by sample}}{\text{Expected absorbance}} \times 100$$

The results for the assay are presented in chapter 4.

B - CIMETIDINE TABLET : Non-aqueous titrimetric method, using 0.1M perchloric acid as titrant was adopted to assay for content of active ingredient of the cimetidine tablet.

0.1M perchloric acid was prepared by mixing acetic acid, 70%w/w perchloric acid and acetic anhydride, and allowed to stand for 24 hours. It was then standardised by titrating

it with potassium hydrogen phthalate (previously dried at 120°C for 2 hours) dissolved in glacial acetic acid.

Twenty (20) tablets of cimetidine were randomly selected accurately weighed and finely powdered. Powder (0.501 g) equivalent to 0.25g cimetidine was weighed and dissolved (by gentle warming and cooling) in 30ml glacial acetic acid. The content was then titrated with the previously prepared and standardized 0.1M perchloric acid using 5% crystal violet dissolved in glacial acetic acid as indicator for end point determination. The titration was performed 5 times and the average value calculated.

Cimetidine content was determined by using the relationship: Each ml of 0.1M perchloric acid consumed in the titration is equivalent to 25.23mg of cimetidine. The results for the assay is presented in chapter 4.

Disintegration Rate Test

The test was performed for both paracetamol and cimetidine tablet samples.

Six (6) tablets were placed in 6 tubes (one tablet per tube) of the tablet disintegration apparatus (Erweka, Germany). The apparatus was operated in accordance with Appendix XII procedure of B.P. (1993) to determine the disintegration time of the tablets as follows:

The assembly of tubes was suspended in a beaker containing the disintegration medium (0.1M HCl) that was maintained at 37°C $\pm$ 1°C by an electrically heated water bath. The assembly of tubes was made to move up and down in the medium so that the tablets were constantly agitated. The time taken for all the particles of the disintegrated tablets

to pass freely through the mesh at the lower end of the tubes is usually considered to be the disintegration rate time.

The procedure was repeated, but with cimetidine tablets. The results for the disintegration rate tests for both paracetamol and cimetidine tablets are presented in chapter 4.

Dissolution Rate Test

The rotary basket method described in B.P. (1993), and using 0.1M HCl as the dissolution medium was adopted.

One tablet of paracetamol was placed in the basket and the assembly was placed in the flask containing 1000ml of 0.1M Hydrochloric acid, warmed and maintained between 36.5 and 37.5°C by an electric heater. The stirrer was set at 100 r.p.m. and the apparatus operated for 45 minutes. Sample of the dissolution medium was then withdrawn, filtered and assayed spectrophotometrically using a U.V. spectrophotometer at wavelength of 257nm. The test was further repeated 4 times and the average determined. The amount of paracetamol released after 45 minutes was calculated by comparing the absorbance of the dissolution medium obtained with the absorbance given by a known concentration of paracetamol in 0.1M hydrochloric acid at 257nm.

The percentage of paracetamol released from the dosage form after 45minutes was calculated using the relationship below:

$$\text{Percentage released} = \frac{\text{Amount of sample released}}{\text{Content of standard paracetamol tablet}} \times 100$$

The dissolution rate test was also carried out for cimetidine tablet, in 5 replicate using the same operating conditions and procedure as above for paracetamol. Sample of dissolution medium was also analysed spectrophotometrically at a wavelength of 218nm. The amount of cimetidine released after 45 minutes was calculated by comparing the absorbance given by the sample of the dissolution medium with the absorbance given by a known concentration of cimetidine in 0.1M hydrochloric acid at 218nm.

The percentage of cimetidine released from the dosage form after 45minutes was calculated using the relationship below:

$$\text{Percentage released} = \frac{\text{Amount of sample released}}{\text{Content of standard cimetidine tablet}} \times 100$$

3.2.3 PREPARATION AND VALIDATION OF CALIBRATION CURVE

Preparation of paracetamol Stock solution

A stock solution of pure paracetamol powder in methanol with a concentration of 4mg/ml w/v (4000ug/ml) used for the construction and validation of calibration curve was prepared as follows:

A 100mg of pure paracetamol powder was accurately weighed using an electronic analytical balance. The powder was carefully and completely transferred into a 25ml volumetric flask and dissolved with about 15ml of methanol. The volume of the solution was than made to 25ml by the addition of a sufficient quantity of methanol. The stock solution was then stored in a refrigerator, pending the time of its use.

Construction of Calibration Curve

Calibration Curve of Concentration of paracetamol in human saliva based on absorbance was prepared by spiking drug-free (blank) saliva with standard solution of paracetamol to give a concentration range of 0 to 50ug/ml. The procedure involved for the construction of the calibration curve is as follows:

Sufficient quantity of blank (drug-free) saliva sample was collected with the aid of chewing a piece of semi-solid paraffin. The collected saliva sample was pooled and then distributed into twelve 10ml extraction (centrifuge) tubes in two milliliter volumes, using an auto-pipette. Two of the twelve tubes were kept as blanks (Drug-free), and the remaining ten tubes were spiked with different (increasing) concentrations of paracetamol in methanol from the standard stock solution, using a microlitre Hamilton syringe. Each concentration was spiked in duplicate (as indicated in Table 3.2.3.1) and each duplicate was repeated four times on different days, resulting in 8 replicate data for each concentration and blanks.

Table 3.2.3.1 shows the volumes of stock solution of paracetamol spiked into 2ml saliva samples and the concentrations of paracetamol in saliva sample obtained. The amount (volume) of paracetamol stock solution spiked to 2ml saliva to obtained a desired concentration was determined from the following relationship:

$$C_1,V_1, = \quad C_2,V_2$$

Where,

C_1 = Concentration of paracetamol stock solution (i.e. 4mg/ml or 4000ug/ml)

V_1 = Volume of stock solution to be spiked (unknown) to saliva sample.

V_2 = Volume of saliva samples (i.e. 2ml)

C_2 = Concentration ranges of paracetamol in saliva samples (i.e. 0, 10, 20, 30, 40 and 50ml), for the construction of the calibration curve.

In order to obtain absorbance readings for the various paracetamol concentrations in saliva, the following procedure was followed.

Five milliliters (5ml) of ethylacetate was added to each of the twelve saliva samples and the extraction (centrifuge) tubes were well stoppered. The mixture in the tubes were vortex-mixed for 1 minute and centrifuge at 2500 rpm for 5 minutes. Ethyl acetate layer (upper) was removed from each of the tube using a Pasteur pipette and its absorbance measured at 262nm, using 1cm silica cuvette, using a double beam SP8-100 U.V. spetrophotometer. The absorbance for the blank (drug-free) saliva samples were then subtracted from those of the samples containing serial concentrations of paracetamol in order to obtain a set of absorbance readings corresponding to the serial concentrations of paracetamol in saliva samples (i.e. 0.00, 10.00, 20.00, 30.00, 40.00 and 50.00ug/ml concentrations).

The calibration curve was constructed by plotting the total mean absorbance readings obtained against the corresponding concentrations of paracetamol (10-50 µg/ml) in saliva samples, and correlation coefficient calculated.

Table 3.2.3.1. Volumes of stock solution of paracetamol spiked to saliva samples and the concentrations obtained for the construction of the calibration curve.

SAMPLE SERIAL No	VOLUME OF STOCK SOLUTION SPIKED TO 2ML SALIVA SAMPLE (µL)	CONCENTRATION OF PARACETAMOL IN SALIVA SAMPLE OBTAINED (ug/ mL)
1	0.00	0.00
2	0.00	0.00
3	5.00	10.00
4	5.00	10.00
5	10.00	20.00
6	10.00	20.00
7	15.00	30.00
8	15.00	30.00
9	20.00	40.00
10	20.00	40.00
11	25.00	50.00
12	25.00	50.00

Validation of the calibration curve.

The calibration curve constructed was validated using the following procedure:

Sufficient quantity of blank (drug-free) saliva was obtained with the aid of chewing a piece of semi-solid paraffin. The saliva sample was then pooled and distributed into Eight, 10ml extraction (centrifuge) tubes into 2ml-volumes using an auto-pipette. Two of the tubes were kept as blanks and the remaining six (6) tubes were spiked with different volumes of paracetamol stock solution to give a concentration range of 5 to 55ug/ml (as indicated by Table 3.2.3.2). The concentrations used for the construction of the calibration curve (i.e. 10, 20, 30, 40 and 50ug/ml concentrations) were not selected.

Each sample spiked with the stock solution was then extracted with ethylacetate and the ethylacetate extract analysed using the same analytical procedure as described for the construction of calibration curve.

Table 3.2.3.2 Volumes of paracetamol stock solution spiked to saliva samples and the concentrations obtained for the validation of the calibration curve.

SAMPLE SERIAL No	VOLUME OF STOCK SOLUTION SPIKED TO 2ML SALIVA SAMPLE (μL)	CONCENTRATION OF PARACETAMOL IN SALIVA SAMPLE OBTAINED (μg/mL)
1	0.00	0.00
2	2.50	5.00
3	7.50	15.00
4	12.50	25.00
5	17.50	35.00
6	22.50	45.00
7	27.50	55.00
8	0.00	0.00

Absorbance readings obtained were then converted directly to the corresponding concentrations of paracetamol in saliva using the calibration curve. The concentrations estimated from the calibration curve were then compare with the actual concentrations spiked to the saliva samples. The results are presented in chapter four.

3.2.4 *IN VIVO* PHARMACOKINETIC STUDIES

Volunteers and Protocol of Study

Eight healthy male volunteers aged between 28 - 32 (mean ± SD; 29.63 ± 1.41) years, weighing between 60 and 69 (mean ± SD; 64.25 ± 3.32) kg participated in the study. The volunteers were clinically certified fit for the study and were asked to abstain from taking any drug for at least two weeks before the commencement of, and during the

study. They were all non-smokers and did not take alcohol at all before and during the study period. The volunteers were fully briefed on the study and their informed consent was obtained before the commencement of the study.

Cross-over study fashion was used and the study was approved by the ethical committee of the Ahmadu Bello University Teaching Hospital, Tudun Wada, Zaria. The protocol of the study was divided into 3 phases and a wash-out period of 2 weeks was allowed between the phases of the study.

The first phase of the study involved the ingestion of 1g dose of paracetamol tablets with about 100ml of water after overnight fasting. Food but not water was withheld for the next 4 hours. Saliva samples (4ml) were immediately collected prior to the drug ingestion and at 0.25, 0.50, 1.00, 2.00, 3.00, 4.00, 5.00 and 6.00 hour intervals with the aid of chewing a semi-solid parafilm. The saliva samples were then stored at -20°C, pending analysis.

The second phase involved the concomitant ingestion of 1g of paracetamol and 400mg single dose of cimetidine after overnight fasting. Food but not water was withheld for the next 4 hours as before and saliva samples were collected and stored as described before.

The third phase of the study involved ingestion of 400mg single dose of cimetidine, followed by 1g oral dose of paracetamol tablets one hour after, still under fasting state. Food but not water was withheld for the next 4 hours and saliva samples (4ml) were collected and stored as before.

Extraction and analysis

The method used for the extraction and analysis of paracetamol in saliva samples in this study was adopted from Bello (1990).

Saliva samples (2ml) were placed in 10ml extraction (centrifuge) tube using auto-pipette. Ethylacetate (5ml) were added to the content of the tubes and well stoppered. The mixture was vortex-mixed for one minute and centrifuged at 2500 rpm for 5 minutes. The ethylacetate layer (upper) was removed with pasteur pipette and its absorbance measured at 262nm, using 1cm silica cuvettes using a double beam SP8-100 UV-spectrophotometer.

Data Handling

The values for the concentration of paracetamol in saliva samples were plotted against time on a logarithmic scale. The method of residuals was then used to determine the following pharmacokinetic parameters, using salivary concentration-time curves as preliminary data: Lag time, Absorption and elimination half-lives ($t_{1/2}ab$ and $t_{1/2}el$ respectively), Absorption and elimination constants (Kab and kel respectively).

The remaining pharmacokinetic parameters were determined as follows:

(i) AUC (from time 0 to 6 hours) were calculated using the triangular-trapezoidal method.

(ii) AUC (From time 0 to infinity) were calculated as follows:

$$AUC(0 - \infty) = AUC(0 - 6) + AUC(6 - \infty)$$

$$AUC\ (6 - \infty) = \frac{\text{Conc. at 6 hours}}{\text{Kel}}$$

iii) Volume of distribution (Vd) = $\frac{F.D}{AUC.Ke}$

(iv) Clearance (Cl) = Vd.ke = $\frac{0.693.\ Vd}{t_{1/2}\ el}$

Where,

AUC = Area under the salivary concentration - time curve

Kel = Elimination rate constant

$t_{1/2}$ el = Elimination half life

F = Bioavailability (assumed to be 100%)

D = Dose of paracetamol ingested

The mean pharmacokinetic parameters for the study were then calculated from the results obtained for the 8 volunteers for the 3 phases of the study.

All the mean pharmacokinetic parameters obtained in the study were analysed statistically using the student two tailed t-test of paired data. Values of $p < 0.05$ were considered as significant and $p > 0.05$ insignificant for the differences between the controls and treated groups, unless other levels of significant stated.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 QUALITY CONTROL ASSESSMENTS

The quality control assessments carried out for paracetamol and cimetidine samples showed conformity with the B.P (1993) requirements (Table 4.1.1). The identification tests are based on colour reactions. The tests were performed to verify the identity of the samples. Paracetamol gave a violet colour with 0.0167M potassium dichromate test solution and a melting point range of 168-170°C. Those results were in conformity with the B.P (1993) and I.P (1988) requirements for paracetamol identification in dosage form. Cimetidine on the other hand gave an orange precipitation with potassium iodobismuthate / acetic acid test solution and when ignited under a lead nitrate paper, the vapour evolved darken the paper. Those are positive results for identification of cimetidine in tablet dosage form as specified in the basic tests for pharmaceutical dosage forms (WHO, 1991).

Test for uniformity of weight was performed to ensure that accurate administration of doses will be achieved during the study. Mean weights for paracetamol and cimetidine tablets were found to be 0.5933 and 0.4007g respectively. The maximum percentage deviations of a tablet individual weights from mean weights were 1.22 and 2.72% for paracetamol and cimetidine

Table 4.1.1 QUALITY CONTROL ASSESSMENT RESULTS FOR PARACETAMOL AND CIMETIDINE TABLET SAMPLES.

QUALITY ASSESSMENT	PARACETAMOL	CIMETIDINE	REMARK.
1. IDENTIFICATION TESTS	POSITIVE	POSITIVE	PASS
2. WEIGHT UNIFORMITY			
Mean (±M.P.D) weight(g)	0.593(1.22)	0.402(2.72)	PASS
3. FRIABILITY			
Mean(±S.E.M) percentage Friability after 100 falls	0.752	0.589	PASS
4. ASSAY FOR CONTENT			
Mean (±S.E.M) percentage Content	99.21(0.645)	98.89(0.08)	PASS
5. DISINTEGRATION RATE			
Mean (±S.E.M) disintegration Rate (minutes)	6.00(0.354)	10.40(0.430)	PASS
6. DISSOLUTION RATE			
Mean (±S.E.M) percentage Released after 45 minutes	86.5(0.963)	87.34(0.646)	PASS

Key:

M.P.D = Maximum percentage deviation of tablet weight from the mean weight.

S.E.M = Standard error of mean.

respectively (see appendix I). B.P (1993) states that for tablets of average weight of 250mg or more to pass the test, not more than 2 of the individual weight deviates from mean weight by more than 5% and none deviates by more than twice that percentage. From this interpretation, the samples passed the uniformity of weight test.

Friability test, though less important as a quality assessment in bioanalytical study was carried out using a friabilator. The tablets were subjected to self – abrasion as the cylinder section rotates, and shock as they fall 6 inches on each turn of the friabilator. The test was performed to verify the ability of the tablets to resist shock and frictional forces that most often cause tablets to chip, cap or breaking during handling. Weight loss after 100 falls in 4 minutes (friability) for paracetamol and cimetidine were found to be 0.752 and 0.589% respectively. Friability values are usually considered satisfactory when the product exhibit a weight loss of less than 0.8% (Gunsel et al, 1970).

Results for assay for content of active ingredients were found to be 99.21 and 98.89% for paracetamol and cimetidine respectively (Table 4.1.1 and Appendix I). Those are within the range (95-105%) considered satisfactory as specified by B.P (1993). The assay was performed to verify that accurate administration of doses will be achieved during the in vivo study.

The disintegration rate tests were found to be satisfactory (6.00 and 10.40 minutes for paracetamol and cimetidine respectively). B.P (1993) specify a period of 15 minutes or less for uncoated tablet to disintegrate. In disintegration

rate test, attempts are made to simulate in-vivo conditions. Nonetheless, a careful perusal of all the in-vitro procedures show that no available disintegration test actually simulates physiologic conditions (Gunsel et al, 1970). However, the test ensures that the tablets will disintegrate within a reasonable time in the GIT to increase the surface area of particles and consequently hasten the total process of absorption.

The percentage released of active ingredients from the dosage forms after 45 minutes were found to be 86.56% for paracetamol and 87.26% for cimetidine, indicating a good dissolution rate. The results complied with the official (B.P 1993) specification of 70% (or above) release of active ingredients from dosage forms after 45 minutes. The in-vitro dissolution rate test may help to predict the in-vivo bioavailability performance of the dosage form. 0.1 M – HCl was used as the medium for both disintegration and dissolution rate studies to simulate the pH of the stomach. The rotating effect (100rpm) of the dissolution rate apparatus simulate the intestinal motility which enhances both disintegration and dissolution of dosage forms.

4.2 CONSTRUCTION AND VALIDATION OF CALIBRATION CURVE

4. 2.1 CALIBRATION CURVE

Linear salivary calibration curve for standard paracetamol powder over the range of concentrations 10-50ug/ml, using the analytic method (extraction with ethylacetate and U.V. spectrophotometric detection at 262nm) is shown in Figure 4.2.1.1. Mean data obtained for construction of the calibration curve are

presented in Table 4.2.1.1. Correlation coefficient (r) for the curve was found to be 0.999, showing a very good correlation of response of the detection system (U.V spectrophotometer) at the concentrations of paracetamol in saliva used. Thus, with a good precision in measurement, accurate quantitative estimation of the drug (paracetamol) in saliva will be obtained using the calibration curve.

Table 4.2.1.1 Calibration Data for spiked saliva samples containing paracetamol.

Initial concentration (ug/ml) [1]	Mean Absorbance of saliva sample		Mean Absorbance of of sample minus Absorbance due to blank[3]
(X)	ABSORBANCE[2]	SD	(Y)
0.00	0.032	0.0316	0.000
10.00	0.240	0.0631	0.208
20.00	0.482	0.0339	0.450
30.00	0.752	0.0940	0.720
40.00	1.022	0.0570	0.990
50.00	1.228	0.1273	1.196

NB:

1 = Based on 2-ml saliva sample.

2 = Data represents 8 replicate absorbances of calibration solutions.

3 = Absorbance (mean) due to blank saliva samples is 0.032.

S.D = Standard deviation.

In most analyses, construction of a standard or calibration curve for a drug or metabolite using known concentrations is an important pre-requisite. In such a curve, a line is graphed showing the relationship between an independent

variable, X (usually drug concentration) and a dependent variable Y (some analytic measurement resulting from X concentration). Because of recovery losses and the variability inherent in measurements in biologic fluids, calibration curves constructed using the identical analytic parameters that will be used for biologic samples (standard assays in relevant biologic media) containing unknown quantities of the drug are always required. That is, it is generally not acceptable to develop a standard curve (response Vs concentration of drug) from analysis of "clean" systems such as buffer or water and to use such a curve to determine amounts of drug or metabolite in a biologic sample like plasma, urine, saliva or any other biologic fluid (Smith and Stewart, 1981).

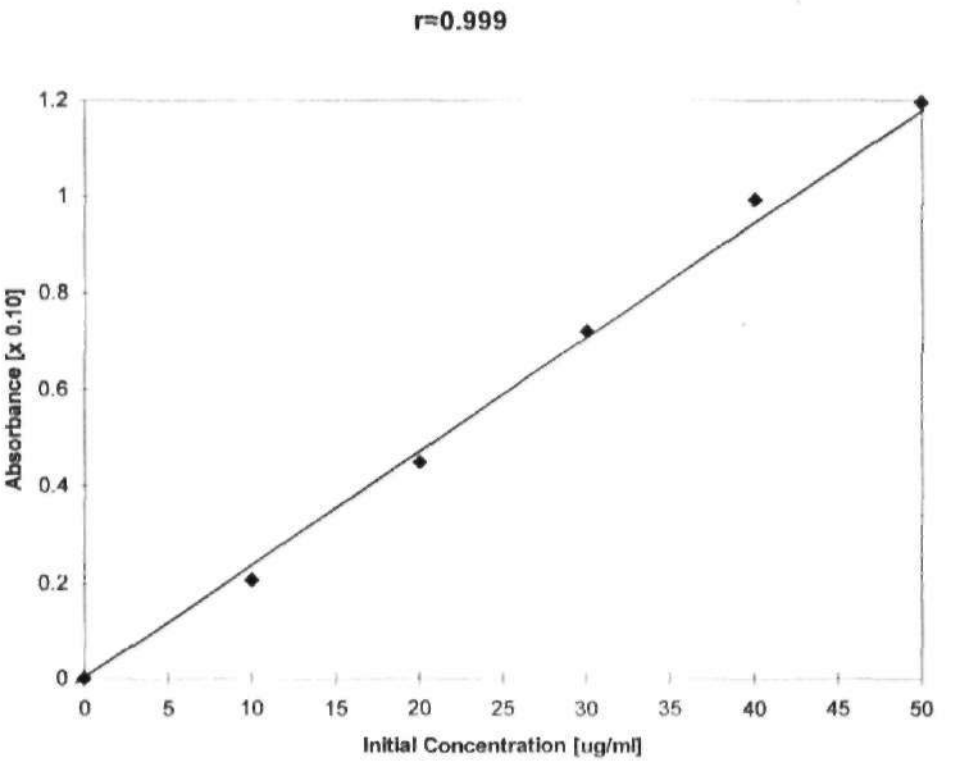


Figure 4.2.1.1 Calibration curve for paracetamol in saliva over a concentration range of 10-50ug/ml.

4.2.2 VALIDATION OF THE CALIBRATION CURVE.

Table 4.2.2.1

Results obtained from the validation of the calibration curve (n = 5).

Saliva sample S/N	Vol. of stock sol. Spiked to 2ml saliva (ul)	Absorbance obtained at wavelength 262nm	Conc. of paracetamol in saliva sample (ug/ml) A	Estimated conc. from the calibration curve (ug/ml) B	Remark
1	0.00	0.00	0.00	0.00	G.R.
2	2.50	0.125	5.00	5.20	G.R
3.	7.50	0.330	15.00	14.50	G.R
4	12.50	0.591	25.00	25.20	G.R
5	17.50	0.828	35.00	34.60	G.R
6	22.50	1.089	45.00	45.50	G.R
7	27.50	1.259	55.00	52.80	G.R
8	0.00	0.00	0.00	0.00	G.R

Key:

G.R = Good correlation between the actual concentration of paracetamol in spiked saliva sample (A) and the estimated concentration from the calibration curve (B).

After a simulated analyses, the results obtained for the validation of the curve clearly demonstrated a good correlation between the spiked concentrations and

the ones estimated directly from the calibration curve. The results compares well with those obtained by Bello (1990) and Bukhari (1996).

Table 4.2.2.2 Percent Recovery of Analytic method as calculated after reference to the calibration curve (% relative recovery).

Conc. Spiked (ug/ml)[1]	Conc. Estimated from calibration curve(ug/ml)[2]	RSD (%)	Percent Relative Recovery
5.00	5.20	8.60	104.00
15.00	14.50	4.22	96.67
25.00	25.20	3.01	100.80
35.00	34.60	3.13	98.86
45.00	45.50	2.58	101.00
55.00	52.80	2.47	96.00

NB: 1= Based on 2ml saliva sample.
2 = Mean of 5 determinations.
RSD = Relative Standard Deviation (Coefficient of Variation)

Recoveries (relative) calculated after reference to the calibration curve are presented in table 4.2.2.2. The values ranged from 96.00 and 104% and are good enough for accurate quantitative determination of paracetamol in saliva samples in a concentration range of the calibration curve. The relative recovery is a measure of how close the results obtained from the calibration curve are to the actual levels of drug in the spiked biologic samples. In other words, it is the measure of the accuracy of the assay method (Smith and Stewart, 1981).

Accuracy in any bioanalytic method is achieved through precise measurements (precision) and calibration (by calibration curve).

4.3 IN-VIVO PHARMACOKINETIC STUDIES

The influence of cimetidine (400mg) on the pharmacokinetic parameters of paracetamol following a single oral dose of paracetamol (1g) under two study protocols concomitant and delayed treatment were studied in eight(8) healthy volunteers in after overnight fasting.

Table 4.3.1 shows the mean ± SD salivary concentration – time data for paracetamol alone (control), and for the influence of cimetidine on paracetamol under the two study protocols (concomitant and delayed administration of paracetamol 1 hour after cimetidine). Figure 4.3.1 shows the mean salivary concentration – time profiles for paracetamol alone (control), an for the influence of cimetidine on paracetamol under the two study protocols (concomitant and delayed treatment).

Table 4.3.1 Mean (± SD) Salivary concentration – time data for paracetamol alone (control), and for the influences of cimetidine on paracetamol (n=8).

	SALIVARY CONCENTRATION (ug /mL)		
TIME (hour)	PARACETAMOL ALONE	CONCOMITANT TREATMENT	DELAYED TREATEMENT.
0.25	11.60 ± 2.84	11.00 ± 3.33	4.20 ± 1.20
0.50	21.50 ± 6.72	21.00 ± 6.52	8.80 ± 2.26
1.00	19.40 ± 4.43	19.80 ± 4.30	13.50 ± 2.66
2.00	15.00 ± 3.04	15.60 ± 3.06	12.70 ± 2.44
3.00	7.80 ± 1.61	8.10 ± 1.42	11.20 ± 2.10
4.00	4.00 ± 0.89	3 .70 ± 0.75	9.00 ± 1.95
5.00	2.50 ± 0.66	2.90 ± 0.61	6.40 ± 1.28
6.00	1.20 ± 0.27	1.60 ± 0.33	4.50 ± 0.93

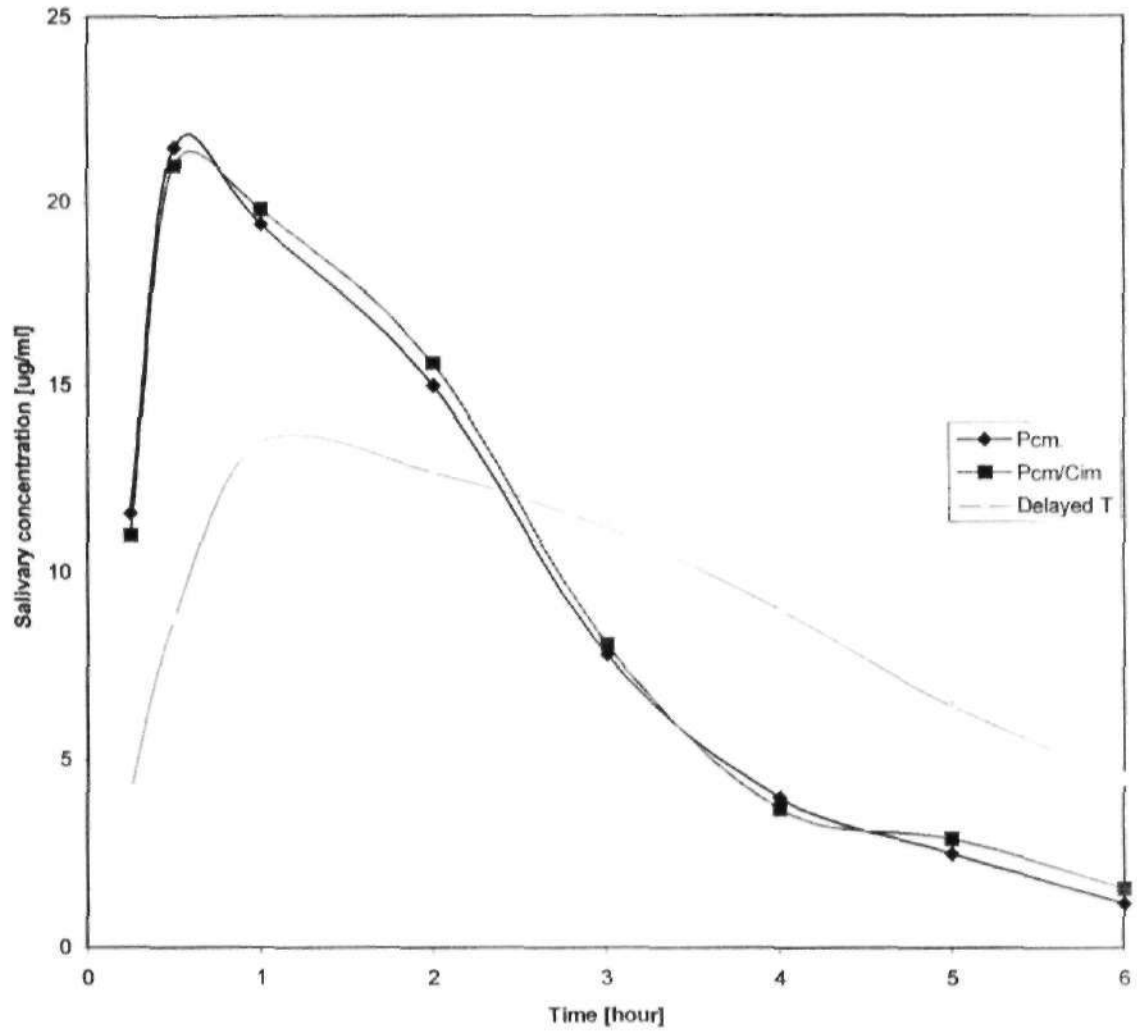


Figure 4.3.1 Mean salivary concentration – time profiles for the control (paracetamol alone) and the two study protocols (concomitant and delayed treatment).

4.3.1 SINGLE DOSE SALIVARY PHARMACOKINETICS OF PARACETAMOL.

The salivary pharmacokinetic profile of paracetamol alone was studied in 8 healthy volunteers in fasting state. Table 4.3.1.1 shows the mean ± S.E.M. salivary pharmacokinetic parameters of paracetamol following 1g paracetamol tablets. The mean ± SD salivary concentration – time data and the mean salivary concentration – time profile were presented in Table 4.3.1 and Figure 4.3.1 respectively.

The absorption half life ($t_{1/2}$ ab) was found to be 0.2656 hr in saliva with the corresponding absorption rate constant (Kab) of 2.7693 ug/ml/hr. The Kab is in agreement with the Kab of 2.100 ug/ml/hr in plasma reported by Garba et al (1997). However, the $t_{1/2}$ab (0.2656 hr) was found to be shorter than the 0.34hr in saliva found by Bukhari (1996). Thus, the absorption phase in this study is fairly rapid. The difference might be due to inter-subject variation in paracetamol absorption. The time to peak concentration (Tmax) was 0.8438hr. This agree with the Tmax of 0.88hr in saliva found by Bukhari (1996). In addition, the Tmax is within the range of 0.5 – 1.0hr reported by Ameer and Greenblat (1977), and in most established Literature, (Payan and Katzung, 1995; Goodman and Gilman, 1990). The peak salivary concentration (Cmax) was found to be 22.96 ug/ml.

Table 4.3.1.1 Mean ± S.E.M salivary pharmacokinetic parameters of paracetamol following 1g paracetamol in 8 healthy volunteers.

S/N	PARAMETER (UNIT)	MEAN ± S.E.M
1	Cmax (ug/ml)	22.96 ± 2.30
2	Tmax (hr)	0.8438 ± 0.08
3	$t_{1/2}$ ab (hr)	0.2656 ± 0.02
4	Kab (ug/ml/hr)	2.7693 ± 0.29
5	$t_{1/2}$ el (hr)	1.100 ± 0.04
6	Kel (ug/ml/hr)	0.6403 ± 0.03
7	Vd (L)	29.1931 ± 3.16
8	Clearance (L/hr)	18.1861 ± 1.51
9	Lag time (hr)	0.1406 ± 0.01
10	AUC (ug.hr/ml)	57.7367 ± 4.81

NB: S.E.M = Standard error of mean.

The Cmax of 22.96 ug/ml in saliva is lower than the 30.00 ug/ml reported by Garba et al (1997), using plasma levels. Cmax of 29.45 ug/ml was found by

Bukhari (1996) and 15.29 ug/ml by Bello (1990), both using saliva levels. This discrepancies might be due possibly to inter-subject variability or the influence of food. The lag time found in the study (0.1406hr) is in close agreement with 0.150hr reported by Garba et al (1997). Elimination half life ($t_{1/2}$el) was found to be 1..100hr, with the corresponding elimination rate constant (Kel) of 0.6403 ug/ml/hr. The $t_{1/2}$el is within the range of 1-2hr reported in most established Literature (Clissold, 1986; Drug facts and comparisons, 1989a; Goodman and Gilman, 1990; Payan and Katzung, 1995). However, the $t_{1/2}$el is shorter than the 2.4 and 2.69hr found by Bello (1990) and Bukhari (1996) respectively using saliva levels. Volume of distribution (Vd) was 29.1931 liters, with the corresponding clearance of 18.1861 L/hr. Bello (1990) and Bukhari (1996) reported a Vd of 40.75 and 43.012 L/kg respectively using salivary levels. However, the clearance in this study (18.1861 L/hr) is higher than the 12.07 and 12.904 L/hr reported by Bukhari (1996) and Bello (1990) respectively. Area under the curve from time 0 to infinity (AUC) was found to be 57.7367 ug.hr/ml. This is much lower than the 128.98 and 77.497 ug.hr/ml reported by Bukhari (1996) and Bello (1990) respectively.

The pharmacokinetics of paracetamol have been extensively investigated (Prescott, 1974c; Rawlings, 1977b; Thomas, 1993). All have reported that there are individual variability in the absorption and disposition of paracetamol when subjects are given the same dose. Example, in a study (Prescott 1974c), it was reported that under practical clinical conditions, the variation among the plasma concentrations of paracetamol in 43 fasting patients 1 hour after oral administration of a therapeutic dose can be as much as 80-fold. Bello (1990)

and Bukhari (1996) have demonstrated inter-subject variability in salivary pharmacokinetics of paracetamol. This has also been shown in this study. The variation in paracetamol pharmacokinetics among individual might be due to individual differences in the rate of gastric emptying (Heading et al, 1973). Other physical and environmental factors, such as age, body weight, sex, smoking, variations in diet and disease, and the like, can also contribute to inter-subject variation in paracetamol pharmacokinetics. The pharmacokinetics of paracetamol after a single dose of 1g paracetamol tablets was linear (first-order kinetics) in this study and the properties were adequately described by the biexponential function. In this study, the salivary pharmacokinetics of paracetamol determined in 8 healthy Nigeria subjects are within the range of values reported in established literature.

4.3.2 INFLUENCE OF CIMETIDINE ON THE SALIVARY PHARMACOKINETICS OF PARACETAMOL.

The mean estimated salivary concentration – time data, and the salivary concentration – time profiles for the two study protocols were presented in Table 4.3.1 and figure 4.3.1 respectively (section 4.3).

From the mean ± S.E.M salivary pharmacokinetic data for paracetamol concomitantly administered with cimetidine (Table 4.3.2.1), there were no significant changes to control ($P>0.100$). These findings indicated that cimetidine did not have any effect on the pharmacokinetics of paracetamol when the two drugs were administered concomitantly.

Table 4.3.2.1 Influence of cimetidine on the salivary pharmacokinetics of paracetamol in healthy subjects (concomitant administration).

[Values are mean (± S.E.M); n = 8].

PARAMETER (UNIT)	CONTROL	CONCOMITANT TREATMENT	CHANGE (%)	P-VALUE
Cmax (ug/ml)	22.96 (2.30)	22.120 (2.14)	3.66	P>0.100
Tmax (hr)	0.8438 (0.08)	0.7625 (0.03)	9.63	"
$t_{1/2}$ab(hr)	0.2656 (0.02)	0.2663 (00.03)	0.26	"
Kab (ug/ml/hr)	2.7693 (0.29)	2.8326 (0.032)	2.29	"
$t_{1/2}$el (hr)	1.1000 (0.04)	11.1438 (0.03)	3.98	"
Kel (ug/ml/hr	0.6463 (0.03)	0.6088 (0.02)	4.92	"
Vd (L)	29.1931 (3.16)	30.0157 (2.64)	2.82	"
CL (L/hr)	18.1861 (1.51)	18.1121 (1.40)	0.41	"
Lag time (hr)	0.1406 (0.01)	0.1438 (0.01	2.28	"
AUC(ug.hr/ml)	57.73677 (0.001)	57.6164 (4.47)	0.21	"

NB: S.E.M = Standard error of mean.

The reason might be that for a pharmacokinetic drug interaction to be observed, a reasonable concentration of each of the interacting drugs should appear in the plasma and other biological fluids at the same time. The time to reach peak plasma concentration for paracetamol is between 0.5 – 1 hr (Ameer and Greeblat, 1977; Payan and Katzung, 1995), whereas for cimetidine, it is between 1-2 hours (Walkenstein et al, 1978). Thus, when the two drugs are concomitantly administered, paracetamol will appear at a reasonable concentration in the plasma and other biological fluids earlier enough than

cimetidine and the effects of cimetidine on pharmacokinetics of paracetamol will not be manifested. Recent work (Garba et al, 1997) revealed that the effects of cimetidine on paracetamol absorption were only evident when the H_2 - antagonist was given 1 hour before ingestion of paracetamol. This provided evidence that the study design adopted had determined the interaction that was observed.

Table 4.3.2.2: Influence of cimetidine on the salivary pharmacokinetics of paracetamol in healthy subjects (Delayed administration of paracetamol).

Values are Mean ± S.E.M.) n = 8

Parameter (unit)	Control	Delayed Treatment	Change (%)	P - Value
Cmax (ug/ml)	22.96 (2.30)	14.58 (2.14)	-36.50	P<0.05
Tmax (hr)	0.8438 (0.08)	1.375 (0.13)	62.95	"
$t_{1/2}$ab (hr)	0.2656 (0.02)	0.4156 (0.03)	56.48	"
Kab (ug/ml/hr)	2.7693 (0.29)	1.8721 (0.24)	-32.40	"
$t_{1/2}$el (hr)	1.1000 (0.04)	1.85 (0.04)	68.18	"
Kel (ug/ml/hr	0.6463 (0.03)	0.3767 (0.009)	-41.17	"
Vd (L)	29.1931 (3.16)	40.873 (3.60)	40.01	"
CL (L/hr)	18.1861 (1.51)	15.3984 (1.40)	-15.33	P<0.10
Lag time (hr)	0.1406 (0.01)	0.2344 (0.01)	66.71	P<0.05
AUC (ug.hr/ml)	57.73677 (4.81)	68.5319 (5.76)	18.70	P<0.10

NB: S.E.M = Standard error of mean.

When paracetamol administration was delayed by 1hr after cimetidine, there were significant changes in the mean pharmacokinetic data. (Table 4.3.2.2).Cmax and kab were significantly reduced by 36.50 and 32.40% respectively (P< 0.05). T_{max}, $t_{1/2}$ab and lag time were significantly increased by 62.92, 56.50 and 66.7%respectively (P<0.05). On the other hand $t_{1/2}$el were significantly increase by 68.18% (P< 0.05). While kel was significantly reduce by 41.17%(P< 0.05). The total body clearance (CL) was reduce by 15.33%, P<0.10), While the corresponding volumes of distribution (Vd) was

significantly increased by the 40.01% (P< 0.05). AUC was significantly increase by the 18.70% (P<0.10).

The study clearly indicated that cimetidine impaired the absorption of paracetamol under the protocol described (delayed administration of paracetamol by one 1hr after cimetidine). Also the reduced elimination and to some extent, the reduced clearance and increased AUC, indicated that cimetidine inhibited the metabolism of paracetamol even though elevation in salivary paracetamol concentration was not observed in the results of the study. This might be most probably due to the fact that the impairment of absorption and the inhibition of the metabolism of paracetamol were taking place simultaneously. Cimetidine is a substituted imidazole, and these compounds are known to be potent inhibitors of drugs oxidation (Wilkinson et al, 1974b). An effects which can be explained by the interactions with the microsomal cytochrome P-450 system (Puurunen and Pelkonen, 1979). The impairment of absorption in this study was indicated by the significant decreased in Cmax and Kab, and increased in Tmax, $t_{1/2}ab$ and lag time. The therapeutic implications of the impairment of absorption of paracetamol by cimetidine are that: the decrease rate of absorption can alter pharmacological effects of paracetamol in that it may be absorbed so slowly that the minimal effective concentration (MEC) may never be reached. In addition, a decrease rate of absorption is important for paracetamol, requiring a rapid onset of analgesic activity. This is because delayed absorption will defeat the aim of the treatment.

The explanation for the reduce in Cmax would seem to go along side Heading et al, (1973) who showed that paracetamol absorption is related to gastric emptying. This is further supported by Nimmo et al (1973) who showed that propantheline reduced gastric emptying for the paracetamol with associated reduced rate of absorption. Cimetidine a H_2-recepter blocker might have acted as an anticholinergic to cause delayed gastric emptying of the paracetamol as did propantheline. If were to be the case, then another mechanism of action of cimetidine would have to unraveled. The increase Tmax, Kab and Lag time in this study strongly support this observation. Some workers (Kwanashie et al.1992) reported that cimetidine at higher concentrations (500 to 1000 ug/ml) cause relaxation of the guinea pig ileum, a factor that might further explain the delayed absorption of paracetamol.

Slattery et al, (1989)have reported lack of effect of cimetidine on paracetamol kinetics in human and also reviewed earlier methods which showed inconsistent effects of cimetidine on paracetamol pharmacokinetics. The conclusion from the workers was that cimetidine had no effects on the urinary unchanged drugs and metabolites. The present study however, contradicts this conclusion and suggests that cimetidine does have some effects on the metabolism of paracetamol. The discrepancies was due to the differences in the study protocol and this according to Powell and Donn (1984)would have the effects of masking or bringing into prominience, the interactions with the cimetidine. In the present study, the delayed administration of paracetamol by 1hr after cimetidine brought the interaction to prominence.

Ziemniack et al (1986) reported that cimetidine inhibit the metabolism of paracetamol, while Slatery et al (1989) reported lack of any effect on paracetamol disposition. The present study agrees with the former and disagrees with the latter. However the study revealed difference in the pharmacokinetic interaction if the study design conforms to the one suggested by Powell and Donn (1984). The present study seem to go along side Garba et al (1997), who showed that paracetamol absorption was delayed by cimetidine, an effects that was attributed to the indirect action of cimetidine on gastric emptying and by its direct action on gut smooth muscle. In that study, paracetamol was administered 1hr after cimetidine and after 3 days pretreatment with cimetidine.

4.4: GENERAL DISCUSSION

The tablets in this study were branded products, i.e. cimetidine (Tagamet, Smith Beecham), and paracetamol (Panadol, Sterling). They were pre-analyzed before the commencement of the study and found to conform with the B.P (1993) standards on dissolution at T45, disintegration time, weight uniformity and percentage content of the active ingredients. Paracetamol was assayed by spectrophotometric method, while cimetidine by non-aqueous titration method.

The analytical method used was adopted from Bello (1990). The method, using saliva as the fluid of sampling involved extraction with ethyl acetate and U.V. spectrophotometric determination of the ethylacetate Extract. The method was found to have minimal interference from U.V. absorbing endogenous materials. Calibration curve obtained from the simulated analytical method over concentration range of 10-50ug/ml was linear (Beers Law obeyed) and the correlation coefficient (r) calculated from the data was very satisfactory (r = 0.999). The accuracy of the analytical method was tested by validation of the calibration curve, using the identical analytical parameters that was used for the construction of the calibration curve and for the analysis of the saliva sample containing unknown quantities of the drugs. A good correlation between the concentration spiked and the estimated concentration from the standard curve was obtained. Relative recoveries for the analytical method calculated in reference to the curve was also satisfactory.

The doses of the test drugs used in this study were the recommended single oral doses used in a clinical setting and in most pharmacokinetic works involving the drugs as cited in the literature. Plasma is the most commonly used biologic fluid for drug assays. However, the observation that drugs concentrations in saliva are often proportional to the concentrations in plasma has let to the suggestion that in therapeutic drugs monitoring, or in pharmacokinetic studies in general, saliva might be substituted for plasma (Danhof and Breimer, 1978). Saliva sampling appears to be attractive when a large number of samples is indicated in cases of "pharmacokinetic profile" determination (Vander Kleijn and Vree, 1978). Saliva can be obtained without the discomfort, possible hazard and necessary attendance of trained personnel associated with repeated Venipuncture as in the case for plasma sampling. It can be simply obtained by non-invasive techniques generally following the stimulation of salivary flow by chewing on some sami-solid materials or by spraying citric acid on the tongue (Breimer, 1979). Glynn and Bastin (1978) have established a correlation between saliva and plasma concentrations of paracetamol after ingestion of the tablets. Therefore, the data obtained from the salivary sampling in this study represents the pharmacokinetic profiles of the paracetamol under the protocols adopted for the study.

Paracetamol was shown in this study to be well observed after oral administration. The rate and extent of absorption, and other Pharmacokinetic properties were found to be in agreement with those in the established literature. The lack of effects of cimetidine on paracetamol pharmacokinetics when the two drugs were concomitantly administered has been predicted from the pharmacokinetic properties of the two drugs. However, the prominence

absorption drug interaction when paracetamol administration was delayed by 1hr after cimetidine was in conformity with the study protocol. The effects of cimetidine on the absorption of paracetamol need to be thoroughly investigated in other to elucidate the exact mechanism involved. Since the present work attributed the effects of cimetidine on paracetamol absorption to the modification of gastric emptying further work in this aspect should attempt to compare this effects of cimetidine with drugs that have been known to modify gastric emptying like metoclopramide and propantheline. To some extent, the inhibition of paracetamol metabolism by cimetidine, when paracetamol administration was delayed by 1hr was also predicted (going by the side that paracetamol is metabolized in the body and cimetidine is a powerful drug metabolic inhibitor). However, since paracetamol metabolites were not determined further work would be necessary for a conclusion to this aspect.

Etintidine, a substituted imidazole, is a new H_2-receptor antagonist with a structure very similar to cimetidine, has been reported to interact with rat liver microsomol Cytochrome P-450 (Schulz and Schmoldt, 1987). Therefore, indication was given by workers that etintidine may cause mainly the same type of drug interactions as cimetidine but seems to be a more potent inhibitor (Schulz and Schmoldt, 1987). It is therefor of relevant concern to investigate the effects of the etintidine along with cimetidine on commonly used drugs in healthy subjects as extrapolation from animal findings might inherently have some limitations.

Ethnic and genetic factors have been reported to affect the Pharmacokinetics of cimetidine (Powell and Donn,1984; Somogyi and Gugler, 1983). Also individual variability in the pharmacokinetics of paracetamol have been severally reported. The present study design has been prepared to take care of these factors by using a relatively large number of subjects for the study.

4.5 CONCLUSION

From the findings of this study, it can be concluded that cimetidine have no effects on the pharmacokinetics of paracetamol, when the two drugs were concomitantly administered. However, cimetidine impaired the absorption and inhibited the metabolism of paracetamol, when the study protocol was change, in such a way that the administration of paracetamol was delayed by one 1hr after cimetidine. The delayed absorption of paracetamol observed in the study might possibly be due to the modification of gastric emptying caused by cimetidine. The impairment of absorption and the inhibition of metabolism have been significant and this might have some important clinical implications in situations where the two drugs are used together. thus there is the need to thoroughly evaluate the exact mechanism involve in this drugs interaction.

Drug-drug interactions are complex phenomena and only after a carefully designed protocol has been followed could the conclusions be relevant. The insidious nature of drug interaction may further be complex as demonstrated by reduced Cmax, and clearance, and increased Tmax and $t_{1/2}$el in this study and this is due to the fact that impairment of absorption and inhibition of metabolism were possibly taking place at the same time. Also from the results of this study, indications were given that effects of cimetidine on paracetamol pharmacokinetics can only be evaluated if the study design is suitable for the purpose.

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APPENDIX I

ANALYSIS OF TABLETS USED

All tests are based on the recommendation of BP 1993, except for the assay of Cimetidine tablet, where non-aqueous titrimetric method (WHO 1991) was adopted.

UNIFORMITY OF WEIGHT
PARACETAMOL TABLET 500mg

S/No	WEIGHT OF TABLET (g)	% Deviation from Mean weight
1	0.5925	0.1305
2	0.5936	0.0516
3	0.5892	0.6901
4	0.5881	0.8687
5	0.5921	0.2080
6	0.5931	0.0310
7	0.5942	0.1595
8	0.5878	0.9244
9	0.5954	0.3567
10	0.6000	1.2163
11	0.5928	0.0816
12	0.5923	0.1591
13	0.5944	0.1797
14	0.5935	0.0230
15	0.5910	0.3799
16	0.5944	0.1814
17	0.5949	0.2690
18	0.5970	0.6280
19	0.5927	0.1035
20	0.5963	0.5050
Mean weight =	0.5933g	Remark = pass

CIMETIDINE TABLET 200MG

S/No	Weight of tablet (g)	% deviation from mean weight.
1	0.4091	2.1015
2	0.3978	0.7260
3	0.3972	0.8682
4	0.4000	0.1794
5	0.4025	0.4519
6	0.3981	0.6461
7	0.4010	0.0801
8	0.4050	1.0684
9	0.4001	0.0172
10	0.3937	1.7566
11	0.3940	1.6643
12	0.4058	1.2605
13	0.3971	0.8957
14	0.4014	0.1650
15	0.4033	0.6491
16	0.4104	2.4160
17	0.3912	2.3830
18	0.3929	1.9488
19	0.4116	2.7204
20	0.4014	0.1724

Mean weight = 0.4007g Remark = pass

ASSAY OF CONTENTS

PARACETAMOL TABLET 500mg

S / No	Percentage Content	Content per Tablet found (mg)	Remark
1	98.09	490.45	**Pass**
2	98.65	493.25	**Pass**
3	101.63	508.12	**Pass**
4	99.39	496.95	**Pass**
5	98.28	491.40	**Pass**
Mean ± SEM	99.21 ± 0.645	**496.04 ±3.225**	**Pass**

Cimetidine Tablet 200mg

S / No	Percentage Content	Content per Tablet found (mg)	Remark
1	98.91	197.82	**Pass**
2	99.11	198.22	**Pass**
3	98.71	197.42	**Pass**
4	99.01	198.02	**Pass**
5	98.71	197.42	**Pass**
Mean ± SEM	**98.89 ± 0.080**	**197.78 ± 0.160**	**Pass**

DISSOLUTION RATE TESTS

Basket Method in 0.1MHCl at 37 ± 0.5 ^{O}C

PARACETAMOL TABLET 500mg

S/No	Percentage released after 45min.	Amount released after 45min	Remark
1	83.82	419.10	Pass
2	87.45	437.25	Pass
3	85.12	425.60	Pass
4	89.41	447.05	Pass
5	86.78	433.9	Pass
Mean ±SEM	86 .56 ± 0.963	432.58 ± 4.82	Pass

CIMETIDINE TABLET 200mg

S/No	Percentage released after 45min.	Amount released after 45min	Remark
1	88.50	177.00	Pass
2	86.70	173.40	Pass
3	88.92	177.84	Pass
4	85.32	170.64	Pass
5	87.26	174.52	Pass
Mean ± SEM	87 .34 ± 0.65	174.68 ± 1.30	Pass

DISINTEGRATION RATE TEST

	PARACETAMOL TABLET500mg		CIMETIDINE TABLET 200mg	
S/No	**Disintegration time (min)**	**Remark**	**Disintegration time (min)**	**Remark**
1	6.00	Pass	10.50	Pass
2	5.50	Pass	9.00	Pass
3	6.50	Pass	11.50	Pass
4	5.00	Pass	11.00	Pass
5	7.00	Pass	10.00	Pass

APPENDIX II

CALIBRATION CURVE

1. **DATA OBTAINED FOR** THE CONSTRUCTION OF **THE** CALIBRATION CURVE

ABSORBANCE OF SAMPLE AT WAVELENGTH 262nm

	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
Conc. of Stock soln. Of Paracetamol in Saliva (ug/ml)	**1A**	**1B**	**2A**	**2B**	**3A**	**3B**	**4A**	**4B**
0.00	0.024	0.040	0.010	0.020	0.002	0.002	0.082	0.076
10.00	0.247	0.205	0.167	0.143	0.289	0.252	0.305	0.311
20.00	0.503	0.497	0.433	0.431	0.471	0.491	0.516	0.514
30.00	0.808	0.930	0.652	0.626	0.732	0.732	0.763	0.769
40.00	1.055	1.055	0.942	0.978	0.949	1.033	1.077	1.083
50.00	1.395	1.274	1.043	1.030	1.290	1.229	1.289	1.277

II. VALIDATION OF CALIBRATION CURVE

CONC. OF PARACETAMOL SPIKED (ug/mL)	CONC. OF PARACETAMOL DETERMINED FROM THE CALIBRATION CURVE (ug / mL)				
	1	**2**	**3**	**4**	**5**
5.00	5.50	5.00	4.50	5.50	5.50
15.00	15.50	14.00	14.50	14.50	14.00
25.00	25.00	26.00	24.50	26.00	24.50
35.00	36.00	34.00	35.50	34.00	33.50
45.00	43.50	46.00	45.50	46.50	46.00
55.00	51.50	54.50	53.50	53.00	51.50

APPENDIX III

PARTICULARS OF SUBJECTS USED FOR STUDY

S/No	Code Name	Age (years)	Weight (Kg)	Sex	Remark
1	**AA**	30	65.00	Male	Healthy/Non smoker
2	BA	32	62.50	"	"
3	GM	29	66.00	"	"
4	FD	28	60.50	"	"
5	HL	28	60.00	"	"
6	KP	31	63.00	"	"
7	MM	29	68.00	"	"
8	YB	30	69.00	"	"

Mean age ± SD = 29.63 ± 1.41
Mean weight ± SD = 64.25 ± 3.32

APPENDIX IV

PHARMACOKINETIC DATA OF SUBJECTS

1. Pharmacokinetic parameter of paracetamol in saliva of individual subjects following oral 1g paracetamol

		Healthy Subjects							
S/No	**Parameter (unit)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	Cmax (ug/ml)	18.30	20.00	27.40	31.70	15.00	16.50	31.00	23.80
2	Tmax (h)	0.50	0.75	1.00	1.00	1.00	0.50	1.00	1.00
3	$t_{1/2}$ ab (h)	0.15	0.225	0.25	0.300	0.30	0.35	0.30	0.25
4	Kab(ug/ml/h)	4.62	3.080	2.772	2.310	3.310	1.98	2.310	2.7720
5	$t_{1/2}$ el (h)	1.25	1.250	1.0000	1.000	1.200	0.950	1.050	1.100
6	Kel (ug/ml/h)	0.5544	0.5544	0.693	0.693	0.578	0.7595	0.6600	0.6300
7	Vd (L)	43.811	34.4197	21.9620	19.6250	37.7039	30.3520	19.6978	25.9730
8	Cl (L/h)	24.2888	19.0823	15.2196	13.6000	21.7929	22.1418	13.00	16.3630
9	Lag time (h)	0.100	0.150	0.200	0.1250	0.150	0.150	0.150	0.1000
10	AUC (ug.h/ml)	41.1715	52.4047	65.7059	73.5288	45.8866	45.1635	76.9189	61.1135

II. Pharmacokinetic parameter of paracetamol in saliva of individual subjects following concomitant oral 1g paracetamol and cimetidine (400mg)

		Healthy Subjects							
S/No	**Parameter (unit)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	Cmax (ug/ml)	20.40	19.20	26.30	32.40	14.32	15.80	26.80	21.70
2	Tmax (h)	0.75	0.50	0.75	1.00	0.90	0.50	0.75	0.95
3	$t_{1/2}$ ab (h)	0.16	0.220	0.200	0.350	0.350	0.30	0.35	0.20
4	Kab (ug/ml/h)	4.331	3.150	3.465	1.980	1.980	2.310	1.980	3.465
5	$t_{1/2}$ el (h)	1.200	1.200	1.050	1.050	1.200	1.050	1.150	1.250
6	Kel (ug/ml/h)	0.5775	0.5775	0.6600	0.6600	0.5780	0.6600	0.6026	0.5544
7	Vd (L)	40.653	33.6118	23.6707	20.2528	38.5442	32.1653	23.0520	28.1757
8	Cl (L/h)	23.477	19.4108	15.6226	13.3668	22.2786	21.2291	13.8911	15.6206
9	Lag time (h)	0.150	0.125	0.200	0.150	0.100	0.175	0.125	0.125
10	AUC (ug.h/ml)	42.5943	51.5177	64.0097	74.8120	44.8862	47.1051	79.9884	64.0180

III. Pharmacokinetic parameter of paracetamol in saliva of individual subjects following delayed administration of oral 1g paracetamol one hour (1h) after administration of cimetidine (400mg)

		Healthy Subjects							
S/No	**Parameter (unit)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	Cmax (ug/ml)	12.10	12.00	18.00	20.50	8.25	9.20	21.80	14.72
2	Tmax (h)	1.00	1.25	1.50	1.50	1.50	0.75	2.00	1.50
3	$t_{1/2}$ ab (h)	0.225	0.350	0.400	0.500	0.450	0.450	0.50	0.45
4	Kab (ug/ml/h)	3.080	2.772	1.733	1.386	1.540	1.540	1.386	1.540
5	$t_{1/2}$ el (h)	1.850	1.850	1.950	1.750	1.950	1.600	1.900	1.950
6	Kel (ug/ml/h)	0.3746	0.3746	0.360	0.396	0.355	0.4331	0.3647	0.3554
7	Vd (L)	60.121	40.084	37.7360	29.8045	49.5341	43.4527	29.9140	36.3379
8	Cl (L/h)	22.522	15.0155	13.6189	11.8026	17.5846	18.8193	10.9096	12.9145
9	Lag time (h)	0.200	0.200	0.350	0.250	0.250	0.250	0.200	0.175
10	AUC (ug.h/ml)	44.4024	66.9250	73.1280	84.7263	56.8678	53.1368	91.6622	77.4333