CHAPTER 1

ROLE OF PHARMACOKINETICS IN DRUG DEVELOPMENT: A REVIEW
A drug may be defined as an agent which when administered into the body would selectively kill a foreign causative organism without affecting the host or correct a non-physiological situation to a physiological one through some mechanism. A contraceptive agent would therefore, not come under the strict definition of "drugs" but a study of pharmacokinetics and metabolism for contraceptive agents has the same role to play as in "drugs".

The mechanism of action of a hormonal contraceptive involves its availability at the hormone receptor site, interaction with receptors leading to hormonal or antihormonal responses, its metabolism and finally elimination from the body. It is also likely that some of these agents may act as prodrugs and are only effective on their metabolism to an active species. Thus a study of the transport of these agents to the receptor site as evidenced by their interaction with various carrier proteins, their distribution kinetics in different body tissues, isolation and study of active and inactive metabolites and their length of retention in the body (biological half-life of the compound) provides information regarding mode and duration of drug administration. This study enables us to workout the exact requirement
of the agent and its time course for maximum efficacy and minimize side effects. Different components of pharmacokinetic study are briefly described below.

CLASSIFICATION OF DRUGS:

Some terminology has been coined for the classification of drugs mainly on the basis of the fate of the drug on administration. A soft drug undergoes metabolism to an inactive nontoxic moiety after attaining its therapeutic role. These are mainly used in systemic and topical applications. In the case of an antedrug the active molecule is metabolized into an inactive product upon its entry into general circulation such compounds are used only for local application. A prodrug changes into an active molecule only after its transformation in vivo.

The concept of prodrug finds application in two areas, the first is to improve the overall properties of a parent drug with respect to its absorption, bioavailability, duration of action, safety, solubility, stability or taste. The second is to provide targeting or site specific drug delivery by enhancing selective concentration in the target tissue and/or by selective conversion to its active moiety by an enzyme which is present in the target tissue (Bundgaard and Hansen, 1981, Notari,
Prodrugs have been further classified in three groups. A *mutual prodrug* has two active moieties linked in a way so that each acts as a prodrug for the other (Baltzer *et al.*, 1980). A *tripartate prodrug* is provided with a spacer or connector group for a selection and control over the enzymatic system or the system required to release the drug (Carl *et al.*, 1981). In a *polymeric prodrug* the active moiety is attached to a polymeric backbone with a chemical bond.

**DRUG CARRIERS OR VEHICLE:**

Development of drug delivery system has become an important area of drug research in the recent past. The main objective is to effectively deliver the drug in its active form at the site of action for better efficacy and lower side effects. These have been classified by Gregoriadis (1979) from a biological viewpoint as:

(i) macromolecular (albumin, antibodies and glycoproteins)
(ii) cellular (erythrocytes, fibroblasts) and
(iii) synthetic (synthetic polymers and liposomes).

Langer and Peppas (1981) have classified these systems from the chemical point of view as:

(i) *diffusional controlled* (liposome membrane systems, non-
bioerodible matrices)

(ii) chemically controlled (bio-erodible matrices and pendent chain systems)

(iii) swelling controlled and

(iv) magnetically controlled.

They have also classified polymers as being either hydrophilic or hydrophobic.

CHEMICAL VEHICLES

Substances like small synthetic glycolipids or glycopeptides, polysaccharide derivatives, oxidised cellulose, co-polymer of vinyl pyrrolidine and maleic anhydride, polymers of organometallic compounds etc. have been used as drug carriers. This approach provides control over the rate of drug release depending upon whether the derivative is bound or attached rapidly by hydrolysis or over a longer period of time by enzymatic action.

BIOMATERIAL VEHICLES

A better control over the rate and site of drug action is provided by biocompatible carriers. The nature of drug carrier interactions ranged from true covalent bonds to weak or ill defined bonds to simple entrapment or encapsulation of the drug by macromolecu-
lar materials like albumin, antibodies, artificial cells, DNA, glycoproteins, erythrocytes, synthetic polymers and liposomes (Gregoriadis, 1979). Sezaki and Hashida (1984) have reported the importance of macromolecular drug conjugates in target cancer chemotherapy.

DEVICES, IMPLANTS, INFUSION PUMPS AND CONTROLLED RELEASE SYSTEMS

These systems find their most promising applications with drugs of low molecular weight or low daily doses. Implantable drug delivery devices, including contraceptive implants, narcotic antagonist implants, devices to provide zero order release of macromolecules and implantable infusion pumps have been reviewed by Langer and Peppas (1981), Langer et al (1981).

The magnetically controlled devices use biocompatible polymers, such as ethylene-vinyl acetate copolymers, to form matrices containing drug and magnetizable materials. After implantation, the rate can be adjusted by applying an external magnetic flux.

Infusion pumps, whether implanted or worn externally, are finding more frequent application in the treatment of diabetes, cancer and other conditions. Other controlled release systems of interest include a
hydrodynamically balanced system for diazepam which is designed to float on the contents of the stomach while releasing drug for up to 10 hrs. (Bogentoft, 1982), a polymeric film in which the rate of diffusion of non-ionic species varies with the pH of the gastrointestinal environment (Alhaique, 1981), the pennkinetic system which utilizes polymer coated drug/ion exchange resin complexes to provide sustained oral drug delivery for up to 12 hrs. (Raghunathan, 1981) and finally an oral preparation which utilizes an acrylic based coating to deliver drugs such as prednisolone and 5-aminosalicylic acid to the colon (Dew et al, 1982).

ROUTES OF DRUG ADMINISTRATION

Route of administration of the drug into the body plays an important role in pharmacokinetics. Commonly used routes are oral, intravenous, intramuscular subcutaneous, intraperitoneal, intranasal etc. The choice of the route depends upon the nature of the compound, site of action, onset and duration of action needed and also on the subject. In the most convenient oral route drugs are absorbed through the upper small intestine, a few through the stomach and the colon. The action begins slowly and persist for a considerable period of time because of the continuous absorption as gastrointestinal
movements bring fresh portion of drug into contact with the absorbing surfaces. The possibility of drug being metabolized to an active or inactive species is very bright in this case. In the intravenous (i.v) route the drug is directly carried through the blood and comes in immediate contact with the tissues and cells. The onset of action is minimized but in the case of side effect there is no possibility of drug withdrawal once administered. The chances of drug metabolism before reaching the site of action is low in this case. Only water soluble preparations which will not precipitate by blood plasma are given by i.v. route. Sufficiently potent soluble drugs effective in low doses are administered through subcutaneous (s.c.) route, provided they are not irritant to the tissue. In the intramuscular (i.m.) administration generally water or oil soluble preparations are introduced into the muscular tissue. Intraperitoneal (i.p.) route of administration is generally practiced in experimental laboratory. Some of the antirabies preparations are introduced by i.p. route. Another convenient route of administration is intranasal, however it is so far used only in limited cases such in asthma as nasal spray. Other routes of administration are intrathecal, intradermal etc., which are used under different situations.
A drug after administration undergoes absorption, distribution, biotransformation and finally excretion. These are the main parameters in the study of pharmacokinetics and are discussed below.

**ABSORPTION OF DRUG**

There are discrete steps involved in the process of absorption of a drug. These are (i) dissolution (ii) diffusion into the multicellular membrane (iii) transport of drug within membrane (iv) transport from membrane into the blood (v) translocation into site of action. Any of these steps can be rate limiting.

**PASSAGE OF DRUG ACROSS THE MEMBRANE**

There are number of mechanisms by which a drug is transported across the membrane. The three main mechanisms are as follows:

**PASSIVE TRANSPORT**

This is the most common mechanism by which a lipid soluble non-electrolyte and lipid soluble non-ionised fractions of weak electrolytes diffuse into the lipid bilayer of cell membrane and are carried across the membrane along a concentration gradient.
ACTIVE TRANSPORT

Drugs with large molecular size are absorbed by this mechanism. In this process the substance moves across the membrane against electrochemical gradient or if the substance is charged against a potential gradient or the combination of the two. It involves metabolic energy and a characteristic feature of it is that, it can be blocked by metabolic inhibitors. Active transport often show the specificity for a particular type of chemical structure and the transport mechanism can be saturated when the concentration of the substance gets too high. Active transport is often envisaged in terms of the carrier mechanism. At the outer surface of the membrane the inactive form of the carrier is converted into its active form which combines with the drugs or endogenous compounds to form a diffusible complex. The complex then diffuses into the inner surface of the membrane where active form of the carrier is converted into its inactive form. Energy is required during the interconversion of the two forms but immediate source of energy depends on the system e.g., absorption of amino acids from the intestine.
FILTRATION

In this process small hydrophilic molecules like urea and small ions such as chloride filter through water filled membrane pores as a result of hydrostatic or osmotic difference across the membrane. This process is of importance in the absorption of intramuscularly or subcutaneously injected poorly lipid soluble and highly water soluble drugs.

DISTRIBUTION OF DRUG

Passage of the drug to different parts of the body is greatly facilitated by the body fluids. The total body water which is about 50 to 70% of the body weight can be considered to be distributed in four parts (i) plasma water (4.5%) (ii) interstitial water (16%) (iii) cellular water (30-40%) and (iv) transcellular water (1-3%). The first two along with the lymph is also referred to as extracellular fluid while the third comes under intracellular fluids. Transcellular fluids comprise cerebrospinal, intraocular, peritoneal, pleural, synovial fluids and digestive secretions.

After a drug is absorbed or injected into a bloodstream it may be distributed between interstitial and
cellular fluids. Lymph also serves as additional compartment. Initially heart, liver, kidney, brain and other highly perfused organs receive most of the drugs within first few minutes after absorption, while the diffusion of the drug in muscle, skin and fat is slower. For drugs entering into fat, not only the lipid volume but also the lipid water distribution co-efficient has to be taken into account. Superimposed on the pattern of distribution of blood flow are factors that determine the rate at which the drugs diffuse into the tissue. Diffusion into interstitial compartment occurs rapidly because of the highly permeable nature of the capillary endothelial membrane. Lipid insoluble drugs that permeates membrane poorly are restricted in their distribution and hence in their potential sites of action. Distribution is also inhibited by the drug binding plasma proteins particularly albumin. An agent that is strongly bound has limited access to cellular sites of action and it can not be metabolized or and eliminated. Drugs that accumulate in the given tissue e.g., fat, serves as reservoir that prolongs drug action. Thus, binding of a drug in blood and tissues has an important role involving drug distribution, elimination and therefore drug action. A drug bound to macromolecules such as plasma proteins and to blood cells crosses biological
membrane only with difficulty. The unbound drug moiety is considered to be the species that diffuses freely between blood and tissue. Once the unbound drug has penetrated into the tissue, binding to cellular components may result. The rate and extent of distribution into the tissue depends on physical properties of the drug including the pKa and its partition characteristics, the pH of the milieu, the organ perfusion rate and the affinity of the binding component in the tissue for the drug. Since the pH difference between intra and extracellular fluids is small (7.0 Vs 7.4), this factor can result in only a relatively small concentration gradient of drug across the plasma membrane. Weak bases are concentrated, slightly inside the cell, while concentration of weak acid is slightly lower in the cell than in the extracellular fluid.

The animal body may be conceived of as made up of a number of hypothetical compartments depending upon the availability of the drug after its administration. The central compartment blood and highly perfused tissues such as liver, heart, kidney, brain, etc. are grouped together as one compartment whereas poorly perfused tissues such as skin, muscle, fat etc. are considered as separate peripheral compartments. Sometimes the distribution pattern of the drug in the body behaves in such a
fashion that a further subdivision of the peripheral compartments such as a shallow and a deep compartment may be considered.

**BIOAVAILABILITY OF DRUG**

Biological effect of a drug depends upon its bioavailability. The percentage of a drug available, following oral administration, to produce pharmacological action, which is also the fraction of the oral dose that reaches the left ventricle in an active form, determines the availability of the drug. Formulations which produce equivalent biological or therapeutical effects are said to have similar order of bioavailability. Dosage forms of a drug from different manufacturer and even different lots of the same preparations may differ in their bioavailability. Availability of the drugs have also been measured for routes of administration other than oral e.g., rectal absorption of acetaminophen (Shangraw and Walking, 1971), buccal absorption of barbiturates (Beckett and Moffat, 1971), eyes (Sorenson, 1971), skin (Ostrenga et al, 1971), peritoneal (Lukas et al, 1971) and through lymph (Beermann and Hellstrom, 1971). As the effect of the drug is related to its absorption, factor that mainly control bioavailability is the dispersion or dissolution of the drug which in turn depends upon the
particle size (Miller and Fincher, 1971), and influence of associated components present, such as binders etc. Susceptibility of the drug to hydrolytic degradation also affects bioavailability of the formulation.

Kakemi and coworkers (1971) studied the effect of buffer component for osmotic pressure, injection volume, buffer capacity and pH of the formulated intramuscular injections. Low osmotic pressure and low pH solution yielded decreased absorption of non ionised drug due to morphological changes in the muscle. Physiological factors in drug interactions also influence drug absorption. Nightingale and coworkers (1971) found that stimulation of the bile flow in the rat increases the gastrointestinal absorption of sulfadiazine. Kojima et al, (1971), found that the presence of food decreased pharmacological activity of phenobarbital by decreasing the rate of absorption in the rat and this decreased absorption rate is due primarily to slowed gastric emptying. Drug-drug interaction have also been shown to influence bioavailability by reducing the rate of absorption (Hayton and Levy, 1971., Hurwitz and Sheehan, 1971., Barr et al, 1971., Robinson et al, 1971).
BIOTRANSFORMATION OF DRUG

Biotransformation is essentially a part of the drug elimination process. Lipid soluble compounds of drugs undergo biotransformation to polar products in order to leave fatty tissues and escape reabsorption in renal tubules. Drugs which mimic in their structure that are native to the body may undergo biotransformation through specific enzyme systems which are required by endogenous counterparts. However, structures which are totally foreign, which is usually the case, are metabolized by relatively nonspecific enzyme systems.

The liver is the major organ for drug biotransformation although the gut (Scheline, 1973), lung (Hook and Bend 1976), skin (Ando et al, 1977), kidneys (Szerfler and Acara, 1979) and blood (Fehske et al, 1981) are known to possess drug metabolising enzymes. Since the unbound drug is susceptible to metabolism by enzymes hence clearance of the poorly excreted compound can be altered by changing its binding characteristics.

The biotransformation products which are generally more polar than the parent compound are easily eliminated through urine. However, some of the biotransformation products are less polar than the parent drug e.g., sulfonamides (Hirom et al, 1972).
EXCRETION OF DRUG

Excretion, is the final phase of the fate of the drug. Drugs are eliminated by all the channels through which substances can leave the body i.e. faeces, urine, milk, sweat and tears. The kidney is the main site for drug excretion and eliminates compounds made polar by the liver.

The difference between the rate of drug entry and the rate of drug exit represents the rate of drug loss across the organ. The efficiency with which an organ eliminates a compound is the clearance. It may be defined as the volume of the biological fluid that is cleared off the drug per unit time and in clinical pharmacokinetics, as body weight is an important factor it is expressed as ml/min/kg of body weight.

Shand (1978) described an easy method of calculation of systemic clearance of a drug after i.v administration of single dose of a drug, from the amount of drug reaching the systemic circulation (Dose) divided by the area under the blood concentration versus time curve (AUC)

\[ Cl_{\text{systemic}} = \frac{\text{Dose i.v}}{\text{AUC i.v}} \]
Clearance is influenced by the presence of diffusional barrier, blood flow to the organ, the degree of drug binding to blood components, the capacity of organ to eliminate the compound.

With most drugs, the rate constant $K_e$ is of first order. A drug that follows apparent first order kinetics, the rate of change of the drug in the body or the velocity of removal is proportional to the amount of drug in the body. The term plasma half-life ($t_{1/2}$) expresses the time required for any given drug concentration in plasma to decrease by one-half. Whatever may be the plasma concentration, $t_{1/2}$ remains the same for drugs with first order elimination, when the rate of elimination is directly proportional to its concentration.

By definition clearance is the velocity of removal divided by the concentration and is expressed as the half-life of the drug which is often estimated after the administration of a drug is dependent on both the values of clearance and the volume of distribution of the drug.

$$CL = K_e \cdot V_d = \frac{0.693 \cdot V_d}{t_{1/2}}$$
COMPARTMENTAL ANALYSIS

The study of compartmental analysis of a drug forms an important part of pharmacokinetics. This helps in deciding the route of administration, the appropriate dosier and the proper time course. An optimum level of the drug has to be maintained during the treatment. A lower level is inadequate to combat with the disease and a higher level may lead to unwanted side effects. In the compartmental analysis different body tissues are grouped in the form of hypothetical compartments according to the rate of distribution and elimination of the drug in such tissues.

ONE COMPARTMENT MODEL

The one compartment model is the most simple form in which the whole body is taken as a homogeneous unit. To assume the body behaves as a one compartment model does not necessarily mean that the drug concentrations in all body tissues at any given time are the same. However, a one compartment model does assume that any changes that occur in the plasma, quantitatively reflect changes occurring in the tissue drug levels. In this model drug elimination occurs from the body in a first order fashion (i.e., the rate of elimination of drug
from the body at any time is proportional to the amount of drug at that time. Half-life of the drug \( (t \frac{1}{2}) \) is calculated using the expression

\[
K_e = \frac{0.693}{t \frac{1}{2}}
\]

where \( K_e \) is the elimination rate constant defined as the rate of change of drug concentration at unit initial drug concentrations or mathematically as below:

\[
\frac{dc}{dt} = -K_eC_0
\]

where \( C_0 \) is the concentration of drug at time zero, \( dc \) is the concentration of drug at times \( t \) and \( K_e \) is the elimination rate constant. The negative sign indicates that the drug is being lost from the body.

**TWO COMPARTMENT MODEL:**

In a two compartment model the rate of decline of the drug in the plasma level is biexponential. Elimination of the drug can take place either from the central compartment, peripheral compartment or from both of these compartments. The central compartment is generally
blood and organs such as liver, kidney, lung etc. For lipid soluble drugs brain will probably be the central compartment, whereas for more polar drugs it may from a part of the peripheral compartment. In two compartment model also it is not necessary that the drug concentration in all the tissues in central compartment at any given time are the same but it is assumed that any change which occurs in plasma levels of a drug quantitatively reflects a change in other tissues of the central compartment.

In a two compartment system the expression area under the curve (AUC) which is a measure of total body load of the drug (i.e. bioavailability) is taken into consideration in calculating the drug clearance (Cl)
where $D$ is the dose given. Clearance can also be expressed in terms of the elimination rate constant:

$$Cl = Ke \cdot V_1$$

where $Ke$ is the elimination rate constant and $V_1$ is the volume of the central compartment.

Half-life of the drug can be calculated using the relationship:

$$t_{1/2} = \frac{0.693}{\beta}$$

whereas the disposition rate constant

$$\beta = \frac{1}{2} (K_{12} + K_{21} + K_{10}) - \sqrt{(K_{12} + K_{21} + K_{10})^2 - 4K_{21}K_{10}}$$

where $K_{12}$, $K_{21}$, $K_{10}$ are the rate constant as depicted in the Fig.1.1.

**THREE COMPARTMENT MODEL:**

In a three compartment model the drug follows a triexponential decline after intravenous injection. The simplest three compartment model will be that where elimination occurs from the central compartment which is reversibly connected to a "shallow" and a "deep" peripheral compartment 2 and 3 respectively.
Fig. 1.2: Schematic representation of a three compartment model.

The constants $K_{12}$, $K_{21}$, $K_{13}$ and $K_{31}$ are the apparent first order intercompartmental transfer rate constants between the 2 and central compartment and the 3 and central compartment respectively (Fig. 1.2). Similar to a two compartment model described earlier, plasma concentration of a drug at a given time and the half-life of a drug etc. are calculated taking into consideration the various rate constants.

The maintenance dose is calculated using relevant pharmacokinetic parameters with the help of the following equation:

$$D = K^1 \times M \times C_{pl} \times T \times W \times A$$

where $D$ is the dose, $K^1$ is the relative clearance,
M is the co-medication factor, $C_p$ is the desired plasma concentration, $T$ is the dosage interval, $W$ is the body weight and $A$ is the age correlation factor.

In deciding the time course, the concept of half-life is used which is a derived parameter that changes as a function of both clearance and volume of distribution. The relationship between terminal log linear half-life ($t_{1/2}$), clearance ($Cl$) and volume of distribution ($V_d$) is given by the equation:

$$t_{1/2} = \frac{0.693 V_d}{Cl}$$

**DRUG METABOLISM**

The majority of drugs undergo metabolism inside the body. This may lead to an active entity for physiological action as in the case of prodrugs or mainly to termination of action of drugs and its facile excretion from the body. There are two types of metabolism involved:

**FUNCTIONALISATION REACTION**

In this type of metabolism functionalisation occurs by following reactions:
Oxidation

A variety of substrates undergo oxidation by microsomal mixed function oxygenase (MFO) system. This is involved mainly in endogenous compound metabolism. A number of other enzymes not related to MFO are also involved in the oxidation reaction. These are alcohol dehydrogenase, aldehyde dehydrogenase, xanthine oxidase, amine oxidase, aromatases and alkylhydrazine oxidases etc. Some drugs are oxidised by a variety of flavoprotein enzymes present in the mitochondria and cytosol of the liver and other tissues e.g., drugs related to catecholamines by tyrosine hydroxylase and monoamine oxidase 6-mercaptopurine by xanthine oxidase and oxidation of alcohol or aldehyde by alcohol or aldehyde dehydrogenases.

Reduction

A number of reductive reactions are catalyzed by microsomal and non-microsomal enzymes in liver and other tissues. These reactions require NADPH but are generally inhibited by oxygen. Such compounds are azo compounds, nitro compounds, epoxides, heterocyclic ring compounds and halogenated hydrocarbons.
Azo and nitro reduction is catalyzed by cytochrome P450 (but can also be catalyzed by NADPH cytochrome-c reductase) and can involve substrates such as prontosil red and chloramphenicol. A major part of this reduction process is catalyzed by the cytochrome P450 monoxygenase and some other enzymes of the intestinal microflora in the anaerobic environment of the gut (Scheline, 1973., Gillette et al, 1966 & 1968). Epoxides are converted back to the parent hydrocarbon. Whereas some heterocyclic compounds are ring cleaved by reduction. Fluorocarbons of the halothane type are defluorinated by liver microsomes in anaerobic conditions.

**Hydrolysis**

Esters, amides, hydrazides and carbamates are readily hydrolyzed by various enzymes. Hydrolysis of esters take place in the plasma (e.g., procaine) or in liver (e.g., pathidine). Amides are hydrolysed by the plasma esterases although more slowly than the corresponding esters but are more likely hydrolysed by the liver amidases. Less common functional groups in drugs, such as hydrazide group in isoniazid or the carbamate group in previously used hypnotic, hedonal, are also hydrolysed. The hydrolysis of proteins and peptides by
enzymes are mainly found in gut secretion and are little involved in drug metabolism.

**Hydration**

Hydration can be regarded as a specialized form of hydrolysis where water is added to the compound without causing the compound to dissociate into a number of components. Epoxides are particularly prone to hydration by the enzyme, epoxide hydrolase or hydrolase. The precarcinogenic polycyclic hydrocarbon epoxides in particular undergo this reaction, which forms a transdiol.

**SYNTHETIC REACTIONS (CONJUGATION)**

This type of reactions usually represent the final step in the metabolism of lipid soluble compounds to water soluble derivatives capable of being readily excreted. The term conjugation is applied to reactions in which the drug or more usually one of its metabolites, combines with an endogenous compound. It includes the formation of glucuronides, sulphates, amino acids and mercapturic acid conjugates, acylation and O-, N-, S-methylation. Conjugation reactions are effected by transferase enzymes, which transfer the conjugating moiety from its donor coenzyme to metabolite substrate.
EXTRAHEPATIC DRUG BIOTRANSFORMATION

Although the liver is the primary site of biotransformation of most drugs, it is not the only site in many cases. Depending upon the structure of a drug, its route of administration and its distribution in tissues, biotransformation may occur at some other extrahepatic sites (Fouts and Brodie, 1956, Cooper and Brodie, 1975). Ability of drug biotransformation have been reported in kidney (Szefler and Acara, 1979), lung (Hook and Bend, 1976), intestine (Hoensch et al, 1976), skin (Ando et al, 1977) and blood (Fehske et al, 1981).

The content of cytochrome P450 and other electron transport chain components varies between organs, but the presence of these factors can act as an indicator of extrahepatic drug metabolizing ability. The order of decreasing organ concentration and activity was liver, kidney, lung, intestine, testes, adrenals, spleen, pancreas, and muscle.

Intestinal enzymes have been implicated in the biotransformation of phenobarbital (Knodell et al, 1979) prostaglandin F2α (Taylor and Sun, 1979), ethinyl estradiol (Hirai et al, 1981) flurazepam (Mahon et al, 1977).

Extrahepatic drug metabolism has been further
explored in lung (McGovern et al, 1976). Enzymes from lung have also been implicated in the metabolism of imipramine, chloropromazine (Ohmiya and Mehendale, 1979).

In kidney acetaminophen was formed to conjugate with cysteine (Fischer et al, 1981). This organ was also shown to catalyse interconversion of salicylic and salicyluric acids (Bekersky et al, 1980), 3-O-methylation of isoproterenol (Szefler and Acara, 1979) and arachidonic acid dependent conversion of 1,3-diphenylisobenzofuran to 0-dibenzoylbenzene (Zenser et al, 1979) etc.

**INTERSPECIES COMPARATIVE METABOLISM**

It is important to know species differences in evaluating the relevance of efficacy and safety data obtained in animals to man. It has been found that metabolism may differ in different species. In man glucuronide conjugates are formed predominantly and there is no evidence of the formation of epoxide (Hintze et al, 1975, Porter et al, 1975). In contrast, drug metabolism studies with methaqualone suggest that epoxidation in the tolyl moiety represents a major pathway in man but a minor one in the rat (Stilwell et al, 1975). Rhesus monkey resembles more closely to man in the overall disposition of spironolactone (Karim et al,
1976), whereas domestic pig appears to be a suitable model for oxisuran in man (Crew and Dicarlo, 1976).

An unusual species difference was reported for absorption of nadolol (Dreyfuss et al, 1978). Whereas, absorption of an oral dose of nadolol in mice, rats, hamsters, rabbits, monkeys and man differed markedly, the absorption in the dog was essentially complete.

Reduction of the 6-keto group in hydrocodone to the β-alcohol was stereoselective in various animal species, but not in man (Cone et al, 1978). In the rabbit and dog, the principal metabolites of isoxepac were the glycine and taurine conjugates, respectively, whereas in the rhesus monkey and man, isoxepac was excreted unchanged or as the glucuronide (Illing and Fromson, 1978).

Among the numerous examples on the comparative metabolism of specific drugs, one, metoclopramide metabolism, is particularly noteworthy, since eight metabolites were identified from rat, dog and human urine, but only one was common to all three species (Teng et al, 1977).

**BIOTRANSFORMATION TO ACTIVE METABOLITES**

Biotransformations may result in the formation of compounds with pharmacologic properties comparable to, or more potent than the parent compound. One such example is the antiarrhythmic drug procainamide, which
is metabolized to an active acetyl metabolite (Atkinson, Jr. et al, 1977). The conversion of prodrugs to active substances is exemplified by sulindac, a nonsteroidal anti-inflammatory agent which is converted in vivo to its active form (Duggan et al, 1977., Duggan et al, 1977).

Highly reactive metabolites can also be produced and such processes are being studied with increasing intensity, since they may be responsible for serious toxic effects of drugs (Serres et al, 1976). Formation of highly reactive metabolites has been implicated as the cause of a wide array of toxic effects, from hepatic necrosis to mutagenesis and carcinogenesis. When overdoses of acetaminophen are injected, a normal biotransformation pathway (conjugation with glutathione) is saturated and a highly reactive metabolite is formed which binds irreversibly to hepatic tissue and may result in extensive hepatic necrosis (Davis et al, 1976., Madan, 1977).

TECHNIQUES USED IN THE STUDY OF DRUG METABOLISM

The need for chemical analysis of a drug molecule, degradation products or metabolites is almost always evident. With the help of newer sophisticated and sensitive techniques, it is now possible to isolate, identify
and quantitate even traces of compounds present in the biological system.

Gas chromatography (Perrigo and Peel, 1981., Cailleux et al., 1981., Karch and Chmielewski, 1981., Kaye, 1980), is a useful separation technique whereby a vaporised sample is carried by a flowing stream of usually inert gas through a tube (column). If the column is filled with dry particles, the technique is called gas-solid chromatography. In gas-liquid chromatography, the particles or the inside walls are coated with a low volatility liquid. The use of mass spectrometry in combination with gas chromatography (GC/MS) has also been extensively used for the separation and structural elucidation of drug metabolites (Frigerio & Ghisalberti, 1977., Frigerio, 1978 and Gudzinowicz and Gudzinowicz, 1980). Use of chemical ionization and selective ion monitoring procedures are providing enhanced sensitivity and specificity along with GC/MS assays (Jenden and Cho, 1979). Liquid chromatography-Mass spectrometry (LC/MS) has been commonly used following the introduction of the thermospray interface for the structure elucidation of drug metabolites (Blake, 1987). All metabolites of teme-lastine have been identified by LC/MS in biological system from various species (Oldham et al., 1990).
High performance liquid chromatography (HPLC) is a very widely used technique in the isolation and identification of different compounds (Howie et al, 1977, Freeman, 1981). Efficiency of this technique depends upon the right choice of column and sensitivity of the detector. With the help of this technique it has become easier to identify and quantitate the metabolites formed (Shah and Jung, 1986, Aoyama and Kamata, 1990). Recently a new HPLC method has been developed for the simultaneous measurement of Prednisone and Prednisolone (Huber et al, 1990). In the case of non-volatile compounds a combination of HPLC/MS has also been successfully used (Blakley et al, 1980, McFadden, 1980).

Autoradiography is still a useful tool for studying the distribution of drug or its labelled metabolites into the biological system. (Wilking et al, 1982, Kallay et al, 1990). Recently fast atomic bombardment mass spectroscopy (FAB/MS) (Cheung et al, 1988) and tandem mass spectroscopy (Bowers et al, 1988) have been used for identification of cyclosporine metabolites in human bile, blood and urine samples.