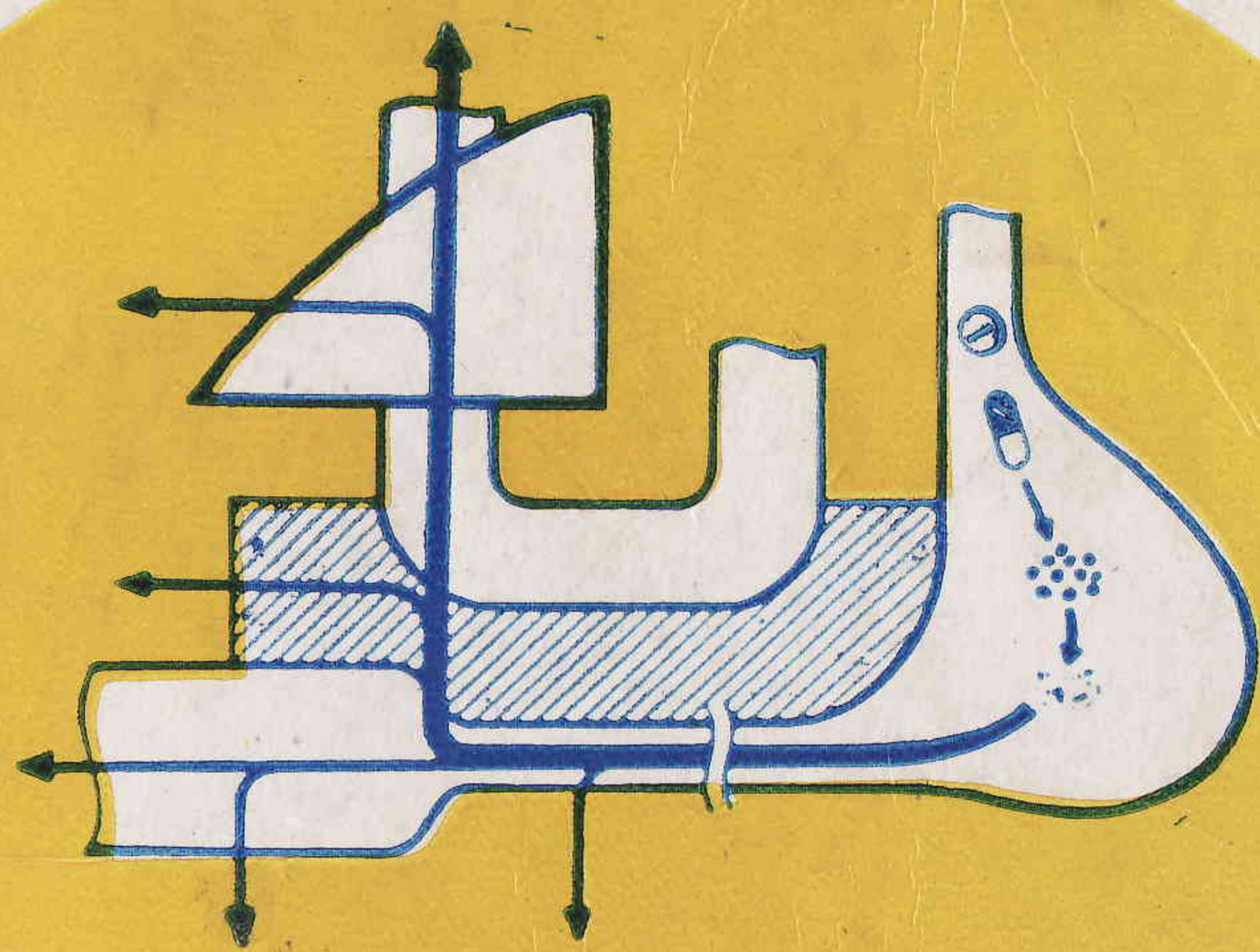
BIOPHARMACEUTICS AND PHARMACOKINETICS

A TREATISE



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Biopharmaceutics and Pharmacokinetics —A Treatise

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description of methods usually employed to enhance the bioavailability of a drug from its formulation has been included at the end of chapter 12. In addition to covering various aspects of design of dosage regimens and application of pharmacokinetic principles in clinical situations, the text contains a final chapter on *Controlled Release Medication* to familiarize the students with the principles involved in the design of innovative formulations.

We feel that a concept is more readily appreciated and understood when illustrated by a simple figure or a table. In order to exemplify the text, illustrations have been used liberally throughout the book. At the end of every chapter, a set of questions including numericals have been provided which are strategically designed to complement the text of each chapter and test the student's grasp of the text and their ability to analyze and solve problems. If this book can help to provide the reader with a clear understanding of the basic concepts of biopharmaceutics and pharmacokinetics, then we would consider our object as fulfilled.

We acknowledge the contribution of hundreds of scientists who are dedicated to improvement of drug therapy. We are also indebted to the authors of the various books and articles mentioned in bibliography which became a major source of information for writing this text. Special thanks are due to Professor W. A. Ritschel for his timely help and inspiration. We are also grateful to all those who helped in different ways during the preparation of this book especially for their support, encouragement and constructive criticism, particularly to our students for their suggestions. Further, we are appreciative of the publishers who graciously volunteered to make the publication of this book a reality.

Lastly, the authors would very much appreciate receiving critical suggestions for improvement and refinement of the text in future.

Nagpur
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Contents

Preface iii

1. Introduction 1-4

2. Absorption of Drugs 5-75
- Gastrointestinal Absorption of Drugs 6
  - Cell Membrane—Structure and Physiology 6
  - Mechanisms of drug absorption 7
  - Factors influencing drug absorption and bioavailability 16
  - Physicochemical factors affecting drug absorption 19
  - Theories of drug dissolution 20
  - Factors affecting drug dissolution and dissolution rate 25
  - pH-partition hypothesis 32
  - Dosage form factors affecting drug absorption 39
  - Patient related factors affecting drug absorption 51
- Absorption of Drugs from Non *Per Os* Extravascular Routes 63
  - Buccal/sublingual administration 64
  - Rectal administration 65
  - Topical administration 66
  - Intramuscular administration 67
  - Subcutaneous administration 68
  - Pulmonary & Intranasal administration 69
  - Intraocular & Vaginal administration 70

3. Distribution of Drugs 76-90
- Tissue Permeability of Drugs 76
  - Physicochemical properties of the drug 77
  - Physiological barriers to distribution of drugs 79
- Organ/tissue Size and Perfusion Rate 83
- Binding of Drugs and Perfusion Rate 85
- Miscellaneous Factors Affecting Drug Distribution 85
- Volume of Distribution 86

4. Protein Binding of Drugs 91-110
- Binding of Drugs to Blood Components 92
  - Plasma protein–drug binding 92
  - Binding of drug to blood cells 95
- Tissue Binding of Drugs (Tissue Localization of Drugs) 96

Factors Affecting Protein–Drug Binding 97
Drug related factors 98
Protein/tissue related factors 98
Drug interactions 99
Patient related factors 101
Significance of Protein/Tissue Binding of Drugs 103
Kinetics of Protein—Drug Binding 106

5. Biotransformation of Drugs 111-158
Drug Metabolizing Organs & Enzymes 113
Chemical Pathways of Drug Biotransformation 114
Phase I Reactions 117
Oxidative reactions 117
Reductive reactions 130
Hydrolytic reactions 134
Phase II Reactions 138
Conjugation with glucuronic acid 139
Conjugation with sulfate moieties 141
Conjugation with alpha-amino acids 142
Conjugation with glutathione & mercapturic acid formation 143
Acetylation 144
Methylation 145
Miscellaneous conjugation reactions 146
Factors Affecting Biotransformation of Drugs 147
Physicochemical properties of the drug 147
Chemical factors 148
Biological factors 150
Bioactivation and Tissue Toxicity 154

6. Prodrugs 159-177
Applications of Prodrug Design 162
Improvement of taste 162
Change of physical form of the drug 163
Reduction of GI irritation 163
Reduction of pain on injection 164
Enhancement of drug solubility and dissolution rate 164
Enhancement of chemical stability of drug 165
Enhancement of bioavailability (lipophilicity) 166
Prevention of presystemic metabolism 167
Prolongation of duration of action 167
Reduction of toxicity 168
Site-specific drug delivery (drug targeting) 169
Limitations of Prodrug Design 176

7. Excretion of Drugs 178-203
Renal Excretion of Drugs 178
Glomerular filtration 179
Active tubular secretion 180
Tubular reabsorption 181
Concept of clearance 185
Factors affecting renal excretion 187
Dose adjustment in renal failure 192
Dialysis and hemoperfusion 192
Nonrenal Routes of Drug Excretion 194
Biliary excretion of drugs–enterohepatic cycling 195
Pulmonary excretion 198
Salivary excretion 199
Mammary & Skin excretion 200
Gastrointestinal & Genital excretion 201

8. Pharmacokinetic Drug Interactions 204-211
Mechanisms of Drug Interactions 204
Interactions Affecting Absorption & Distribution of Drugs 206
Interactions Affecting Metabolism & Excretion of Drugs 207

9. Pharmacokinetics: Basic Considerations 212-229
Plasma Drug Concentration-Time profile 213
Rates, Rate Constants and Orders of Reaction 215
Zero-Order Kinetics 216
First-Order Kinetics 217
Mixed-Order Kinetics 219
Pharmacokinetic Models 220
Compartment models 221
Noncompartmental analysis 224
Physiologic models 226

10. Compartment Modeling 230-272
One-Compartment Open Model 230
Intravenous bolus administration 231
Intravenous infusion 239
Extravascular administration 244
Urinary Excretion Data 253
Multicompartment Models 259
Two-Compartment Open Model 260
Intravenous bolus administration 260
Intravenous infusion 265
Extravascular administration 266

11. Nonlinear Pharmacokinetics **273-281**
Causes of Nonlinearity 274
Michaelis-Menten Equation 275
Estimation of K_m and V_{max} 277

12. Bioavailability and Bioequivalence **282-305**
Objectives & Considerations in Bioavailability Studies 283
Measurment of Bioavailability 285
Plasma level-time studies 286
Urinary excretion studies 288
Acute pharmacologic & Therapeutic response 290
In vitro Drug Dissolution Testing Models 290
In vitro–in vivo Correlation 292
Bioequivalence Studies 294
Methods for Enhancement of Bioavailability 296

13. Applications of Pharmacokinetic Principles **306-324**
Design of Dosage Regimens 307
Individualization 315
Monitoring Drug Therapy 320

14. Drug Concentration and Pharmacologic Response **325-334**
Therapeutic Concentration Range 326
Concentration-Response Relationships 327
Onset/duration of Effect-Concentration Relationship 330
Intensity of Effect-Concentration Relationship 332

15. Controlled Release Medication **335-371**
Design of Controlled Drug Delivery Systems 337
Pharmacokinetic Principles in the Design and Fabrication of Controlled Drug Delivery Systems 342
Drug Release Patterns of Controlled Delivery Dosage Forms 343
Oral Controlled Release Systems 347
Parenteral Controlled Release Systems 357
Transdermal Drug Delivery Systems 365
Ophthalmic Drug Delivery Systems 368
Intravaginal and Intrauterine Drug Delivery Systems 369
Bioavailability Testing of Controlled Release Formulations 371

BIBLIOGRAPHY **376-378**

INDEX **379-400**

1 Introduction

Drugs, whether obtained from plant, animal or mineral sources or synthesized chemically, are rarely administered in their pure chemical form. Often, they are combined with a number of inert substances (excipients/adjuvants) and transformed into a convenient dosage form that can be administered by a suitable route. Earlier, it was believed that the therapeutic response to a drug is an attribute of its intrinsic pharmacologic activity. But today, it is very much understood that the dose-response relationship obtained after drug administration by different routes—for example, oral and parenteral, are not the same. Variations are also observed when the same drug is administered as different dosage forms or similar dosage forms produced by different manufacturers, which in turn depend upon the physicochemical properties of the drug, the excipients present in the dosage form, the method of formulation and the manner of administration. A new and separate discipline called *biopharmaceutics* has therefore been developed to account for all such factors that influence the therapeutic effectiveness of a drug.

Biopharmaceutics *is defined as the study of factors influencing the rate and amount of drug that reaches the systemic circulation and the use of this information to optimize the therapeutic efficacy of the drug products. The process of movement of drug from its site of administration to the systemic circulation is called as* **absorption**. The concentration of drug in plasma and hence the onset of action, and the intensity and duration of response depend upon the bioavailability of drug from its dosage form. **Bioavailability** *is defined as the rate and extent (amount) of drug absorption.* Any alteration in the drug's bioavailability is reflected in its pharmacologic effects. Other processes that play a role in the therapeutic activity of a drug are distribution and elimination. Together, they are known as **drug disposition**. *The movement of drug between one compartment and the other (generally blood and the extravascular tissues) is referred to as* **drug distribution**. Since the site of action is usually located in the extravascular tissues, the onset, intensity

and sometimes duration of action depend upon the distribution behavior of the drug. The magnitude (intensity) and the duration of action depend largely upon the effective concentration and the time period for which this concentration is maintained at the site of action which in turn depend upon the elimination processes. **Elimination** *is defined as the process that tends to remove the drug from the body and terminate its action.* Elimination occurs by two processes—**biotransformation** (*metabolism*), *which usually inactivates the drug*, and **excretion** *which is responsible for the exit of drug/metabolites from the body.*

In order to administer drugs optimally, knowledge is needed not only of the mechanisms of drug absorption, distribution, metabolism and excretion (*ADME*) but also of the rate (kinetics) at which they occur i.e. pharmacokinetics. **Pharmacokinetics** *is defined as the study of time course of drug ADME and their relationship with its therapeutic and toxic effects of the drug.* Simply speaking, *pharmacokinetics is the kinetics of ADME* or **KADME**. *The use of pharmacokinetic principles in optimizing the drug dosage to suit individual patient needs and achieving maximum therapeutic utility is called as* **clinical pharmacokinetics**.

Drug administration and therapy can now be conveniently divided into four phases or processes:

1. **The Pharmaceutic Process :** It is concerned with the formulation of an effective dosage form of the drug for administration by a suitable route.

2. **The Pharmacokinetic Process :** It is concerned with the ADME of drugs as elicited by the plasma drug concentration-time profile and its relationship with the dose, dosage form and frequency and route of administration. In short, it is the sum of all the processes inflicted by the body on the drug.

3. **The Pharmacodynamic Process :** It is concerned with the biochemical and physiologic effects of the drug and its mechanism of action. It is characterized by the concentration of drug at the site of action and its relation to the magnitude of effects observed. Simply speaking, *pharmacodynamics deals with what the drug does to the body in contrast to pharmacokinetics which is a study of what the body does to the drug.*

4. **The Therapeutic Process :** It is concerned with the translation of pharmacologic effect into clinical benefit.

A schematic representation of the various processes involved in the therapy with a drug is given in Fig. 1.1.

To achieve optimal therapy with a drug, the drug product must be

designed to deliver the active principle at an optimal rate and amount, depending upon the patient's needs. A knowledge of the factors affecting the bioavailability of drug helps in designing such an optimum formulation and saves many drugs that may be discarded as useless. On the

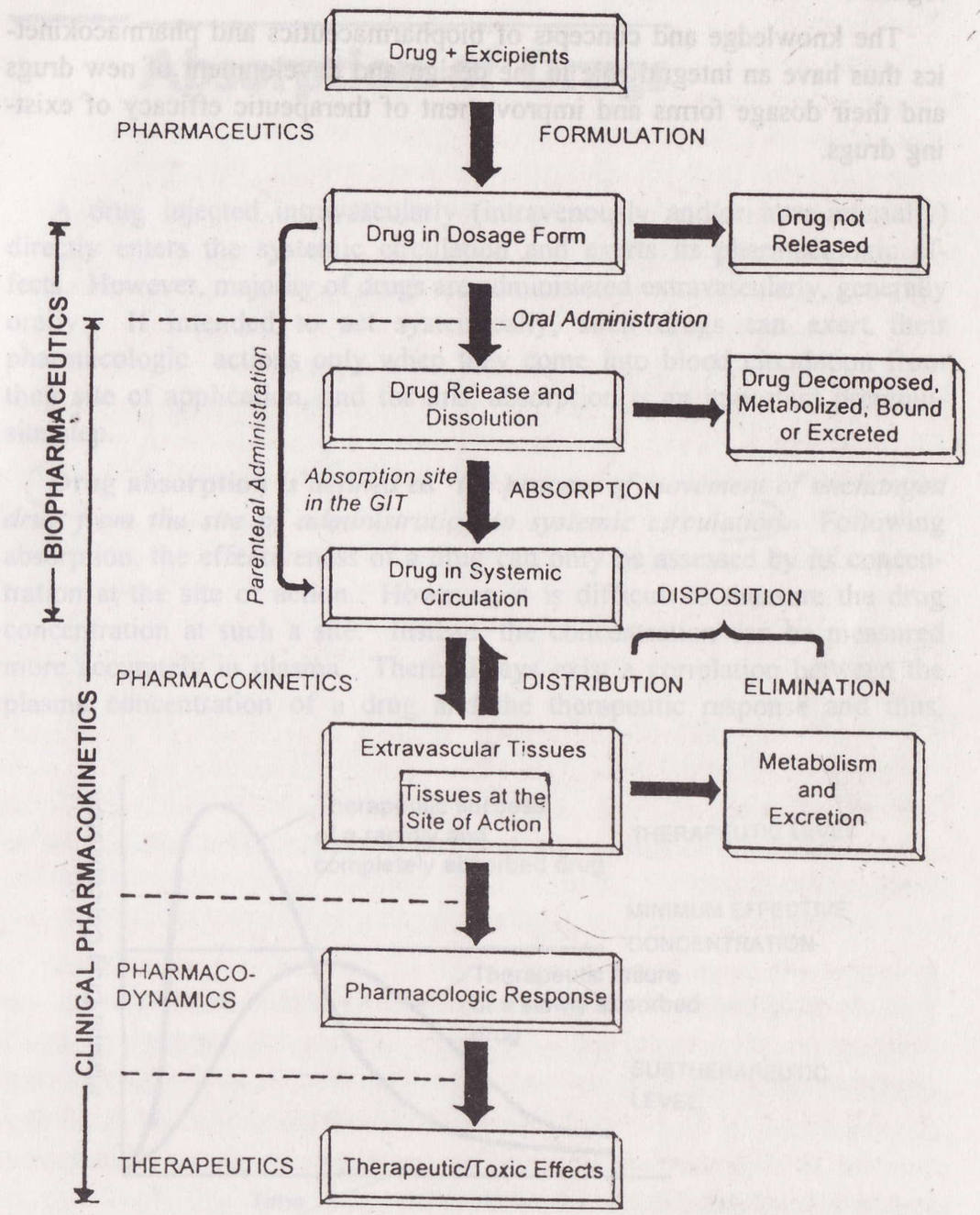

Fig. 1.1 Schematic representation of the processes involved in drug therapeutics

other hand, rational use of the drug or the therapeutic objective can only be achieved through a better understanding of pharmacokinetics (in addition to pharmacodynamics of the drug), which helps in designing a proper

dosage regimen (*the manner in which the drug should be taken*). This obviates the use of the empirical approach where a considerable experimentation is needed to arrive at the balance between the desired therapeutic and the undesired toxic effects in order to define an appropriate dosage regimen.

The knowledge and concepts of biopharmaceutics and pharmacokinetics thus have an integral role in the design and development of new drugs and their dosage forms and improvement of therapeutic efficacy of existing drugs.

2

Absorption of Drugs

A drug injected intravascularly (intravenously and/or intra-arterially) directly enters the systemic circulation and exerts its pharmacologic effects. However, majority of drugs are administered extravascularly, generally orally. If intended to act systemically, such drugs can exert their pharmacologic actions only when they come into blood circulation from their site of application, and for this, absorption is an important prerequisite step.

Drug absorption *is defined as the process of movement of unchanged drug from the site of administration to systemic circulation.* Following absorption, the effectiveness of a drug can only be assessed by its concentration at the site of action. However, it is difficult to measure the drug concentration at such a site. Instead, the concentration can be measured more accurately in plasma. There always exist a correlation between the plasma concentration of a drug and the therapeutic response and thus,

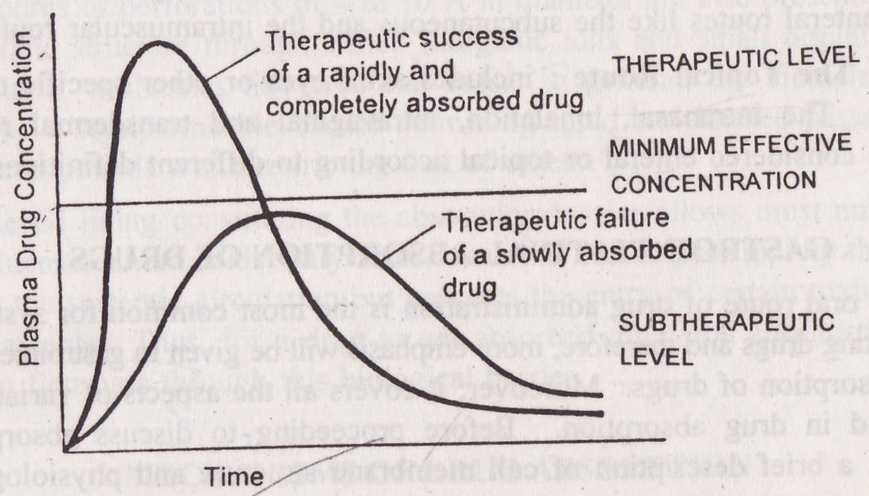

Fig. 2.1 Plots showing significance of rate and extent of absorption in drug therapy

absorption *can also be defined as the process of movement of unchanged drug from the site of administration to the site of measurement i.e. plasma.*

This definition takes into account the loss of drug that occurs after oral administration due to **presystemic metabolism** or **first-pass effect**.

Not only the magnitude of drug that comes into the systemic circulation but also the rate at which it is absorbed is important. This is clear from Fig. 2.1

A drug that is completely but slowly absorbed may fail to show therapeutic response as the plasma concentration for desired effect is never achieved. On the contrary, a rapidly absorbed drug attains the therapeutic level easily to elicit pharmacologic effect. Thus, both the rate and the extent of drug absorption are important. Such an absorption pattern has several **advantages**:

1. Lesser susceptibility of the drug for degradation or interaction due to rapid absorption
2. Higher blood levels and rapid onset of action
3. More uniform, greater and reproducible therapeutic response

Drugs that have to enter the systemic circulation to exert their effect can be administered by three major routes:

1. The Enteral Route : includes *peroral* i.e. gastrointestinal, sublingual/buccal and rectal routes. The GI route is the most common for administration of majority of drugs.

2. The Parenteral Route : includes all routes of administration through or under one or more layers of skin. While no absorption is required when the drug is administered i.v., it is necessary for extravascular parenteral routes like the subcutaneous and the intramuscular routes.

3. The Topical Route : includes skin, eyes or other specific membranes. The intranasal, inhalation, intravaginal and transdermal routes may be considered enteral or topical according to different definitions.

GASTROINTESTINAL ABSORPTION OF DRUGS

The oral route of drug administration is the most common for systemically acting drugs and therefore, more emphasis will be given to gastrointestinal (GI) absorption of drugs. Moreover, it covers all the aspects of variability observed in drug absorption. Before proceeding to discuss absorption aspects, a brief description of cell membrane structure and physiology is necessary.

Cell Membrane : Structure and Physiology

For a drug to be absorbed and distributed into organs and tissues and eliminated from the body, it must pass through one or more biological

membranes/barriers at various locations. Such a *movement of drug across the membrane* is called as **drug transport**.

The basic structure of cell membrane is shown in Fig. 2.2.

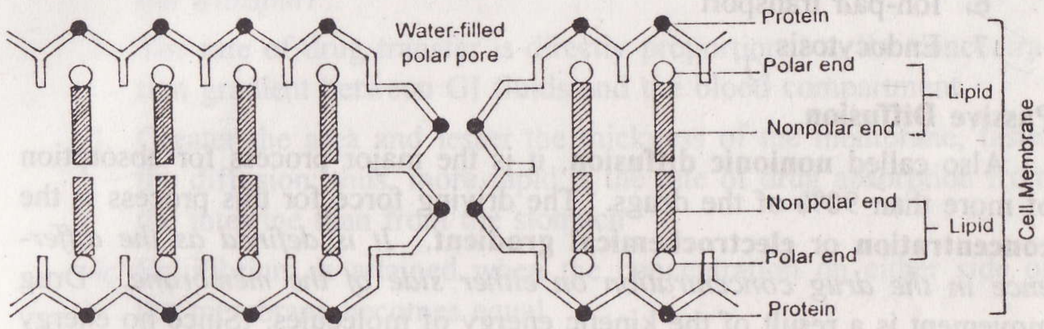

Fig. 2.2 Basic structure of functional cell membrane

The cellular membrane consists of a double layer of amphiphilic phospholipid molecules arranged in such a fashion that their hydrocarbon chains are oriented inwards to form the hydrophobic or lipophilic phase and their polar heads oriented to form the outer and inner hydrophilic boundaries of the cellular membrane that face the surrounding aqueous environment. Globular protein molecules are associated on either side of these hydrophilic boundaries and also interspersed within the membrane structure. In short, the membrane is a *mayonnaise sandwich* where a bimolecular layer of lipids is contained between two parallel monomolecular layers of proteins. The hydrophobic core of the membrane is responsible for the relative impermeability of polar molecules. Aqueous filled pores or perforations of 4 to 10 Å in diameter are also present in the membrane structure through which inorganic ions and small organic water-soluble molecules like urea can pass. In general, the biomembrane acts like a semipermeable barrier permitting rapid and limited passage of some compounds while restricting that of others.

The GI lining constituting the absorption barrier allows most nutrients like glucose, amino acids, fatty acids, vitamins, etc. to pass rapidly through it into the systemic circulation but prevents the entry of certain toxins and medicaments. Thus, for a drug to get absorbed after oral administration, it must first pass through this biological barrier.

MECHANISMS OF DRUG ABSORPTION

The principal mechanisms for transport of drug molecules across the cell membrane in order of their importance are:

1. Passive diffusion
2. Pore transport

3. Facilitated diffusion
4. Active transport
5. Ionic or electrochemical diffusion
6. Ion-pair transport
7. Endocytosis

Passive Diffusion

Also called **nonionic diffusion**, it is the major process for absorption of more than 90% of the drugs. The driving force for this process is the **concentration** or **electrochemical gradient**. *It is defined as the difference in the drug concentration on either side of the membrane.* Drug movement is a result of the kinetic energy of molecules. Since no energy source is required, the process is called as passive diffusion. During passive diffusion, the drug present in the aqueous solution at the absorption site partitions and dissolves in the lipid material of the membrane and finally leaves it by dissolving again in an aqueous medium, this time at the inside of the membrane.

Passive diffusion is best expressed by **Fick's first law of diffusion**, which states that *the drug molecules diffuse from a region of higher concentration to one of lower concentration until equilibrium is attained and that the rate of diffusion is directly proportional to the concentration gradient across the membrane.* It can be mathematically expressed by the following equation:

$$\frac{dQ}{dt} = \frac{DAK_{m/w}}{h}(C_{GIT} - C) \qquad (2.1)$$

where,

dQ/dt = rate of drug diffusion (amount/time). It also represents the rate of appearance of drug in blood

D = diffusion coefficient of the drug through the membrane (area/time)

A = surface area of the absorbing membrane for drug diffusion (area)

$K_{m/w}$ = partition coefficient of the drug between the lipoidal membrane and the aqueous GI fluids (no units)

$(C_{GIT} - C)$ = difference in the concentration of drug in the GI fluids and the plasma, called as the concentration gradient (amount/volume)

h = thickness of the membrane (length)

Based on the above equation, certain characteristics of passive diffusion can be generalized:

1. The drug moves down the concentration gradient indicating *downhill transport*
2. The rate of drug transfer is directly proportional to the concentration gradient between GI fluids and the blood compartment
3. Greater the area and lesser the thickness of the membrane, faster the diffusion; thus, more rapid is the rate of drug absorption from the intestine than from the stomach
4. Equilibrium is attained when the concentration on either side of the membrane becomes equal
5. Drugs which can exist in both ionized and unionized forms approach equilibrium primarily by the transfer of the unionized species; the rate of transfer of unionized species is 3 to 4 times the rate for ionized drugs
6. Greater the membrane/water partition coefficient of drug, faster the absorption; since the membrane is lipoidal in nature, a lipophilic drug diffuses at a faster rate by solubilizing in the lipid layer of the membrane
7. The drug diffuses rapidly when the volume of GI fluid is low; conversely, dilution of GI fluids decreases the drug concentration in these fluids (C_{GIT}) and lower the concentration gradient ($C_{GIT} - C$). This phenomena is, however, made use of in treating cases of oral overdose or poisoning.

Passive diffusion process is energy independent and nonsaturable but dependent, to a lesser extent, on the square root of the molecular size of the drug. The molecular weights of most drugs lie between 100 to 400 daltons which can be effectively absorbed passively. The diffusion generally decreases with increase in the molecular weight of the compound. However, there are exceptions—for example, cyclosporin A, a peptide of molecular weight 1200, is absorbed orally much better than any other peptide.

Initially, when the drug is ingested, $C_{GIT} >> C$ and a large concentration gradient exists thereby acting as the driving force for absorption. As equilibrium approaches, the drug diffusion should stop and consequently a large fraction of drug may remain unabsorbed. But this is not the case; once the passively absorbed drug enters blood, it is rapidly swept away and distributed into a much larger volume of body fluids and hence, the concentration of drug at the absorption site, C_{GIT}, is maintained greater

than the concentration of drug in plasma. Such a condition is called as **sink condition** for drug absorption.

Since under usual conditions of absorption, D, A, $K_{m/w}$ and h are constants, the term $DAK_{m/w}/h$ can be replaced by a combined constant P called as *permeability coefficient*. **Permeability** *refers to the ease with which a drug can penetrate or diffuse through a membrane.* Moreover, due to sink conditions, the concentration of drug in plasma C is very small in comparison to C_{GIT}. As a result, equation 2.1 may be simplified to:

$$\frac{dQ}{dt} = P\,C_{GIT} \qquad (2.2)$$

Equation 2.2 is an expression for a first-order process. Thus, passive diffusion follows first-order kinetics. Since a large concentration gradient always exist at the absorption site for passive diffusion, the rate of drug absorption is usually more rapid than the rate of elimination. Besides, dilution and distribution of the absorbed drug into a large pool of body fluids and its subsequent binding to various tissues are other reasons for elimination being slower than absorption.

Pore Transport

It is also called as **convective transport, bulk flow or filtration**. The process is important in the absorption of low molecular weight (less than 100), low molecular size (smaller than the diameter of the pore) and generally water-soluble drugs through narrow, aqueous-filled channels or pores in the membrane structure—for example, urea, water and sugars. Chain-like or linear compounds of molecular weight upto 400 daltons can be absorbed by filtration. The driving force is constituted by the hydrostatic pressure or the osmotic differences across the membrane due to which bulk flow of water alongwith small solid molecules occurs through such aqueous channels. *Water flux* that promotes such a transport is called as **solvent drag**.

Drug permeation through water-filled channels is of particular importance in renal excretion, removal of drug from the cerebrospinal fluid and entry of drugs into the liver.

Carrier-Mediated Transport

Some polar drugs cross the membrane more readily than can be predicted from their concentration gradient and partition coefficient values. This suggests presence of specialized transport mechanisms without which many essential water-soluble nutrients like monosaccharides, amino acids and vitamins will be poorly absorbed. The mechanism is thought to

involve a component of the membrane called as the *carrier* that binds reversibly or noncovalently with the solute molecules to be transported. This carrier-solute complex traverses across the membrane to the other side where it dissociates and discharges the solute molecule. The carrier then returns to its original site to complete the cycle by accepting a fresh molecule of solute. The carrier may be an enzyme or some other component of the membrane.

Important characteristics of carrier-mediated transport are:

1. The transport process is structure-specific i.e. the carriers have special affinity for and transfer a drug of specific chemical structure only; generally the carriers have special affinity for essential nutrients.
2. Since the system is structure-specific, drugs having structure similar to essential nutrients, called as *false nutrients*, are absorbed by the same carrier system. This mechanism is of particular importance in the absorption of several antineoplastic agents like 5-fluorouracil and 5-bromouracil which serve as false nutrients.
3. As the number of carriers are limited, the transport system is subject to competition between agents having similar structure.
4. Since the number of carriers is limited, the system is capacity-limited i.e. at higher drug concentration, the system becomes saturated and approaches an asymptote. It is important to note that for a drug absorbed by passive diffusion, the rate of absorption increases linearly with the concentration but in case of carrier-mediated processes, the drug absorption increases linearly with concentration until the carriers become saturated after which

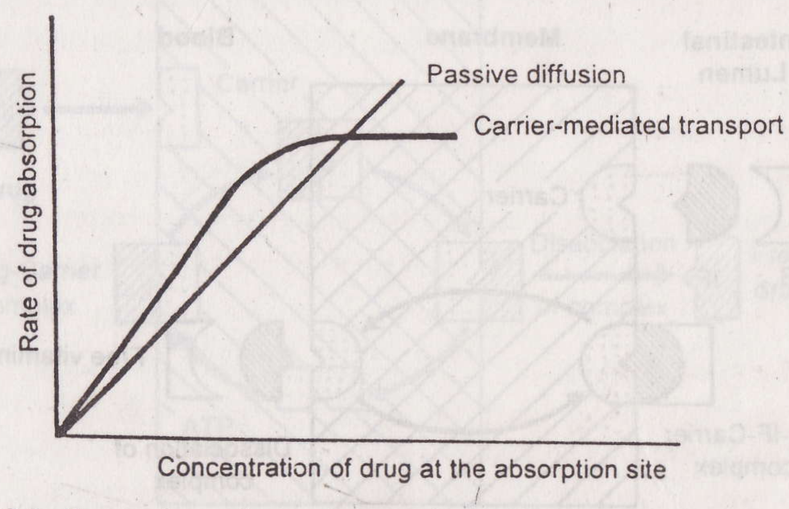

Fig. 2.3 Comparison of rate of absorption versus drug concentration plots for passive and carrier-mediated transport processes

it becomes curvilinear and approach a constant value at higher doses (*see* Fig. 2.3).

Such a capacity-limited process can be adequately described by **mixed order kinetics**, also called as **Michaelis-Menten, saturation** or **non-linear kinetics**. The process is called mixed order because it is first-order at subsaturation drug concentrations and apparent zero-order at and above saturation levels. Moreover, the capacity-limited characteristics of such a system suggest that the bioavailability of a drug absorbed by such a system decrease with increasing dose—for example, vitamins like B_1, B_2 and B_{12}. Hence, administration of a large single oral dose of such vitamins is irrational.

5. Specialized absorption or carrier-mediated absorption generally occurs from specific sites of the intestinal tract which are rich in number of carriers. Such *an area in which the carrier system is most dense* is called as **absorption window**. Drugs absorbed through such absorption windows are poor candidates for controlled release formulations.

Two types of carrier-mediated transport systems have been identified. They are—facilitated diffusion and active transport.

Facilitated Diffusion

It is a carrier-mediated transport system that operates down the concentration gradient (*downhill transport*) but at a much a faster rate than can be accounted by simple passive diffusion. The driving force is concentration gradient (hence a passive process). Since no energy expenditure is involved, the process is not inhibited by metabolic poisons that

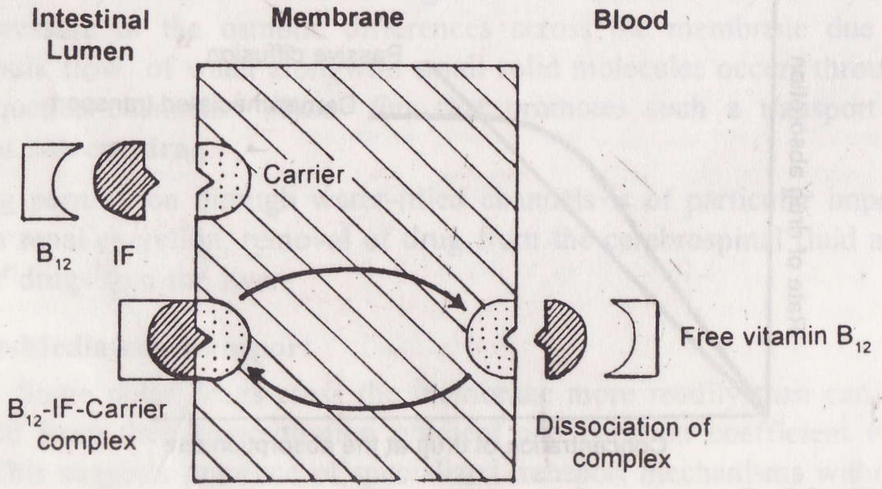

Fig. 2.4 Facilitated diffusion of vitamin B_{12}

interfere with energy production. Facilitated diffusion is of limited importance in the absorption of drugs. Examples of such a transport system include entry of glucose into RBCs and intestinal absorption of vitamins B_1 and B_2. A classic example of passive facilitated diffusion is the GI absorption of vitamin B_{12}. An intrinsic factor (IF), a glycoprotein produced by the gastric parietal cells, forms a complex with vitamin B_{12} which is then transported across the intestinal membrane by a carrier system (Fig. 2.4).

Active Transport

Active transport is a more important process than facilitated diffusion in the absorption of nutrients and drugs and differs from it in several respects:

1. The drug is transported from a region of lower to one of higher concentration i.e. against the concentration gradient or *uphill transport*, without any regard for equilibrium
2. Since the process is uphill, energy is required in the work done by the carrier
3. As the process requires expenditure of energy, it can be inhibited by metabolic poisons that interfere with energy production like fluorides, cyanide and dinitrophenol and lack of oxygen, etc.

Endogenous substances that are transported actively include sodium, potassium, calcium, iron, glucose, certain amino acids and vitamins like niacin, pyridoxin and ascorbic acid. Drugs having structural similarity to

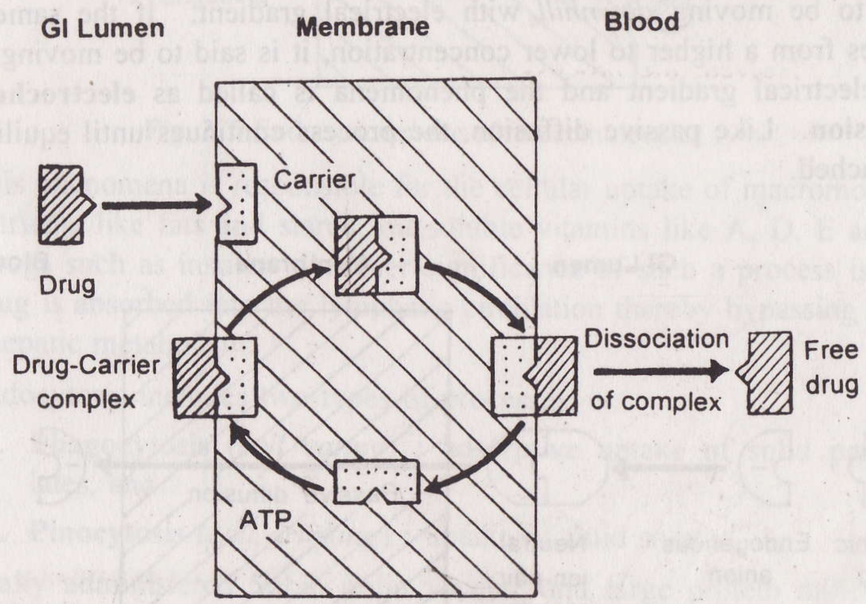

Fig. 2.5 Active absorption of a drug

such agents are absorbed actively, particularly the agents useful in cancer chemotherapy. Examples include absorption of 5-fluorouracil and 5-bromouracil via the pyrimidine transport system, absorption of methyldopa and levodopa via an L-amino acid transport system and absorption of ACE inhibitor enalapril via the small peptide carrier system. A good example of competitive inhibition of drug absorption via active transport is the impaired absorption of levodopa when ingested with meals rich in proteins. Active transport is also important in renal and biliary excretion of many drugs and their metabolites and secretion of certain acids out of the CNS (Fig. 2.5).

Ionic or Electrochemical Diffusion

The charge on the membrane influences the permeation of drugs. Molecular forms of solutes are unaffected by the membrane charge and permeate faster than ionic forms. Of the ionic forms, the anionic solute permeates faster than the cationic form. Thus, at a given pH, the rate of permeation is in the following order—*unionized molecules > anions > cations*.

The permeation of ionized drugs, particularly the cationic drugs, depend on the potential difference or electrical gradient as the driving force across the membrane. A cationic drug is repelled due to positive charge on the outside of the membrane. As a result, only those cations with a high kinetic energy penetrate the ionic barrier. However, once inside the membrane, the cations are attracted to negatively charged intracellular membrane thereby creating an electrical gradient. Such a drug is then said to be moving *downhill* with electrical gradient. If the same drug moves from a higher to lower concentration, it is said to be moving down the electrical gradient and the phenomena is called as **electrochemical diffusion**. Like passive diffusion, the process continues until equilibrium is reached.

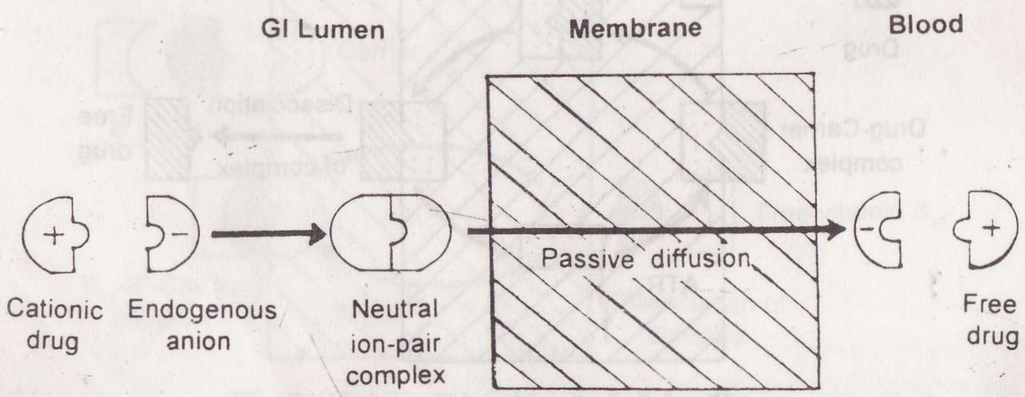

Fig. 2 6 Ion-pair transport of a cationic drug

Ion-Pair Transport

Yet another mechanism that explains the absorption of drugs like quaternary ammonium compounds and sulfonic acids, which ionize under all pH conditions, is ion-pair transport. Despite their low o/w partition coefficient values, such agents penetrate the membrane by forming reversible neutral complexes with endogenous ions of the GIT like mucin. Such neutral complexes have both the required lipophilicity as well as aqueous solubility for passive diffusion. Such a phenomena is called as **ion-pair transport** (Fig. 2.6).

Endocytosis

It is a minor transport mechanism which involves engulfing extracellular materials within a segment of the cell membrane to form a saccule or a vesicle (hence also called as **corpuscular** or **vesicular transport**) which is then pinched-off intracellularly (Fig. 2.7).

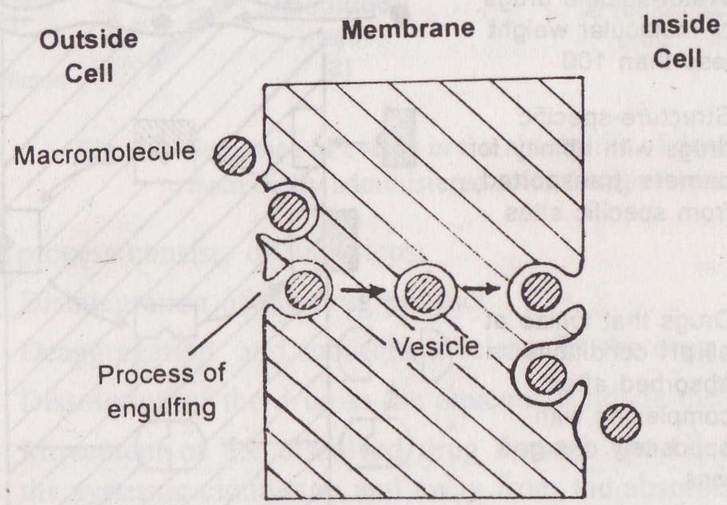

Fig. 2.7 Endocytic uptake of macromolecules

This phenomena is responsible for the cellular uptake of macromolecular nutrients like fats and starch, oil soluble vitamins like A, D, E and K and drugs such as insulin. Another significance of such a process is that the drug is absorbed into the lymphatic circulation thereby bypassing first-pass hepatic metabolism.

Endocytosis includes two types of processes:

1. **Phagocytosis** (*cell eating*) : adsorptive uptake of solid particulates, and
2. **Pinocytosis** (*cell drinking*) : uptake of fluid solute.

Orally administered Sabin polio vaccine and large protein molecules are thought to be absorbed by pinocytosis. Sometimes, an endocytic

vesicle is transferred from one extracellular compartment to another. Such a phenomenon is called as **transcytosis**.

A drug might be absorbed by more than just one mechanism—for example, cardiac glycosides are absorbed both passively as well as by active transport. The transport mechanism also depends upon the site of drug administration (*see* Table 2.8).

Absorption of drugs by various mechanisms is summarized in Fig. 2.8.

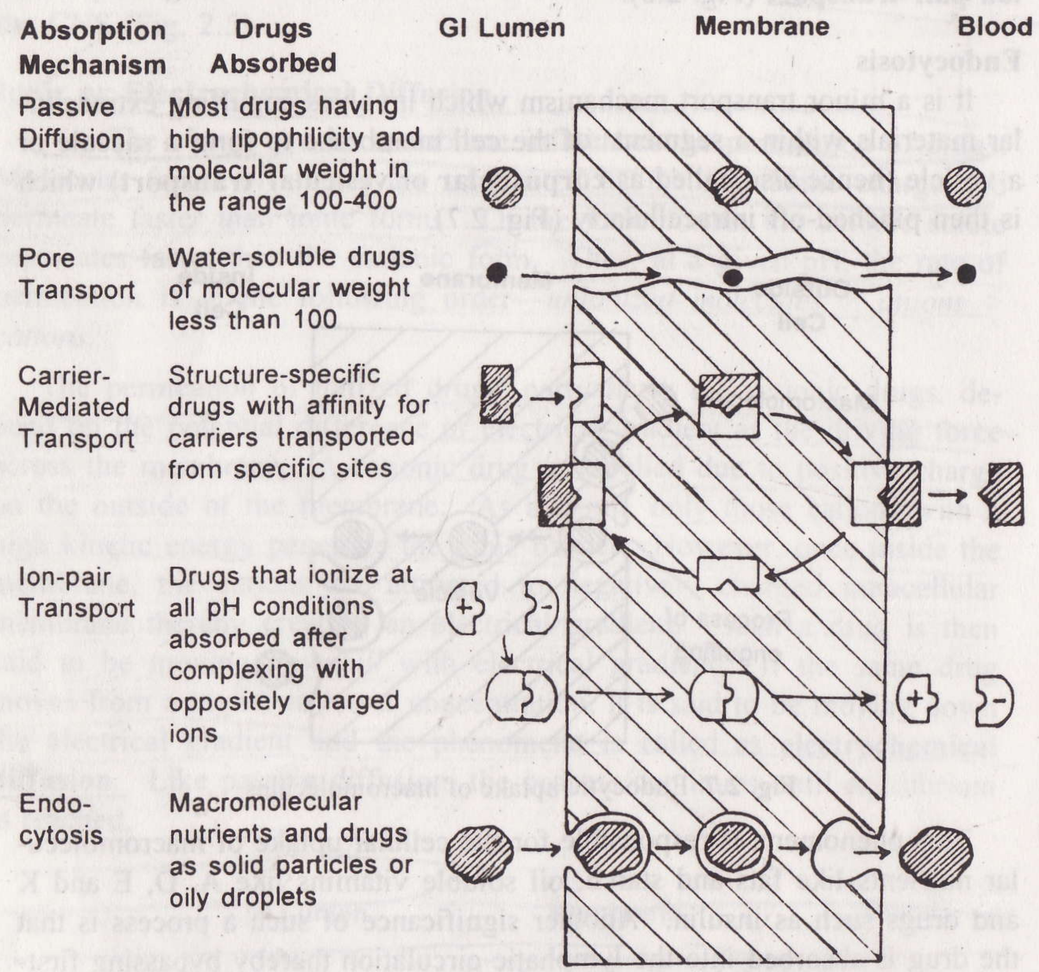

Fig. 2.8 Summary of important transport processes and drugs absorbed through them

FACTORS INFLUENCING DRUG ABSORPTION AND BIOAVAILABILITY

Biopharmaceutic Considerations in Dosage Form Design

To achieve the desired therapeutic objective, the drug product must deliver the active drug at an optimal rate and amount. By proper

biopharmaceutic design, the rate and extent of drug absorption (also called as **bioavailability**) or the systemic delivery of drug to the body can be varied from rapid and complete absorption to slow and sustained absorption depending upon the desired therapeutic objective. The chain of events that occur following administration of a solid dosage form such as a tablet or a capsule until its absorption into systemic circulation are depicted in Fig. 2.9.

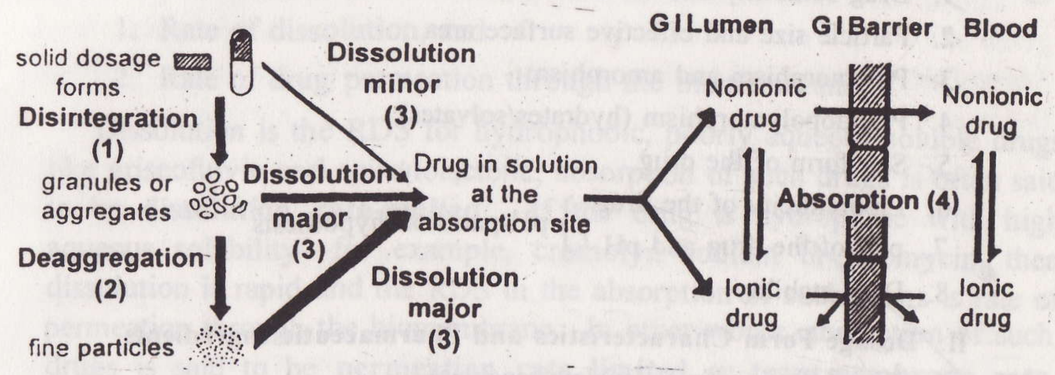

Fig. 2.9 Sequence of events in the absorption of drugs from orally administered solid dosage forms

The process consists of four steps:

1. Disintegration of the drug product
2. Deaggregation and subsequent release of the drug
3. Dissolution of the drug in the aqueous fluids at the absorption site
4. Movement of the dissolved drug through the GI membrane into the systemic circulation and away from the absorption site

As illustrated in Fig. 2.9, the drug may also dissolve before disintegration or deaggregation of the dosage form, and before or after reaching the absorption site. Unless the drug goes into solution, it cannot be absorbed into the systemic circulation.

In a series of kinetic or rate processes, *the rate at which the drug reaches the systemic circulation is determined by the slowest of the various steps involved in the sequence.* Such a step is called as the **rate-determining or rate-limiting step** (RDS). The rate and extent of drug absorption from its dosage form can be influenced by a number of factors in all these steps. The various factors that influence drug absorption (also called as **biopharmaceutic factors in the dosage form design**) can be classified as shown in Table 2.1.

TABLE 2.1

Factors influencing GI Absorption of a Drug from its Dosage Form

A. PHARMACEUTIC FACTORS : include factors relating to the physicochemical properties of the drug, and dosage form characteristics and pharmaceutic ingredients

I. **Physicochemical Properties of Drug Substances**

1. Drug solubility and dissolution rate
2. Particle size and effective surface area
3. Polymorphism and amorphism
4. Pseudopolymorphism (hydrates/solvates)
5. Salt form of the drug
6. Lipophilicity of the drug } pH-partition hypothesis
7. pK_a of the drug and pH } pH-partition hypothesis
8. Drug stability

II. **Dosage Form Characteristics and Pharmaceutic Ingredients**

1. Disintegration time (tablets/capsules)
2. Dissolution time
3. Manufacturing variables
4. Pharmaceutic ingredients (excipients/adjuvants)
5. Nature and type of dosage form
6. Product age and storage conditions

B. PATIENT RELATED FACTORS : include factors relating to the anatomic, physiologic and pathologic characteristics of the patient

1. Age
2. Gastric emptying time
3. Intestinal transit time
4. Gastrointestinal pH
5. Disease states
6. Blood flow through the GIT
7. Gastrointestinal contents:
   a. Other drugs
   b. Food
   c. Fluids
   d. Other normal GI contents
8. Presystemic metabolism by:
   a. Lumenal enzymes
   b. Gut wall enzymes
   c. Bacterial enzymes
   d. Hepatic enzymes

PHYSICOCHEMICAL FACTORS AFFECTING DRUG ABSORPTION

Drug Solubility and Dissolution Rate

Consider the events that occur following oral administration of a solid dosage form as shown in Fig. 2.9. Except in case of controlled release formulations, disintegration and deaggregation occur rapidly if it is a well formulated dosage form. Thus, the two critical slower rate-determining processes in the absorption of orally administered drugs are:

1. Rate of dissolution, and
2. Rate of drug permeation through the biomembrane.

Dissolution is the RDS for hydrophobic, poorly aqueous soluble drugs like griseofulvin and spironolactone; absorption of such drugs is often said to be **dissolution rate-limited**. If the drug is hydrophilic with high aqueous solubility—for example, cromolyn sodium or neomycin, then dissolution is rapid and the RDS in the absorption of such drugs is rate of permeation through the biomembrane. In other words, absorption of such drugs is said to be **permeation rate limited** or **transmembrane rate-limited** (Fig. 2.10).

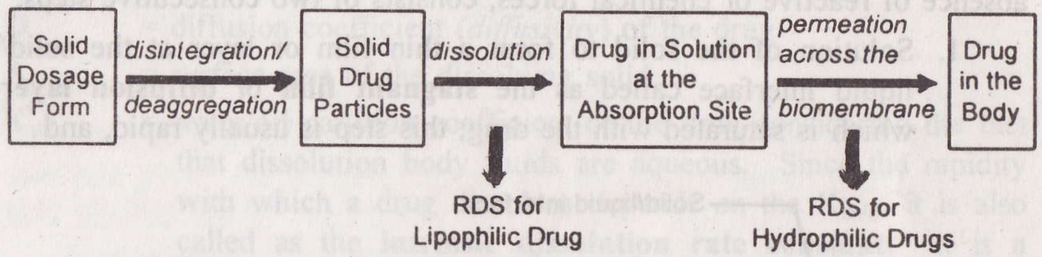

Fig. 2.10 The two rate-determining steps in the absorption of drugs from orally administered formulations

An important prerequisite for the absorption of a drug by all mechanisms except endocytosis is that it must be present in aqueous solution. This in turn depends on the drug's aqueous solubility and its dissolution rate. **Absolute or intrinsic solubility** *is defined as the maximum amount of solute dissolved in a given solvent under standard conditions of temperature, pressure and pH.* It is a static property. **Dissolution rate** *is defined as the amount of solid substance that goes into solution per unit time under standard conditions of temperature, pH and solvent composition and constant solid surface area.* It is a dynamic process. Several drugs have poor aqueous solubility to have a bearing on dissolution rate. The matter is of great concern when the solubility is less than 1 to 2 mg/ml in the pH range of 2 to 8. However, there are well known examples of drugs such as cisapride which inspite of low aqueous solubility have sufficient oral bioavailability. Two reasons can be attributed to this—one,

the rapid rate of dissolution despite low intrinsic solubility and two, the therapeutic dose of drug may be so small that the GI transit time is sufficient for adequate dissolution and absorption to occur. Thus, in contrast to absolute solubility, the dynamic process of drug dissolution is better related to drug absorption and bioavailability.

Theories of Drug Dissolution

Dissolution *is a process in which a solid substance solubilizes in a given solvent i.e. mass transfer from the solid surface to the liquid phase.* Several theories to explain drug dissolution have been proposed. Some of the important ones are:

1. Diffusion layer model/Film theory
2. Danckwert's model/Penetration or Surface renewal theory, and
3. Interfacial barrier model/Double barrier or Limited solvation theory.

Diffusion Layer Model/Film Theory

This is the simplest and the most common theory for dissolution. Here, the process of dissolution of solid particles in a liquid, in the absence of reactive or chemical forces, consists of two consecutive steps:

1. Solution of the solid to form a thin film or layer at the solid/liquid interface called as the **stagnant film** or **diffusion layer** which is saturated with the drug; this step is usually rapid, and

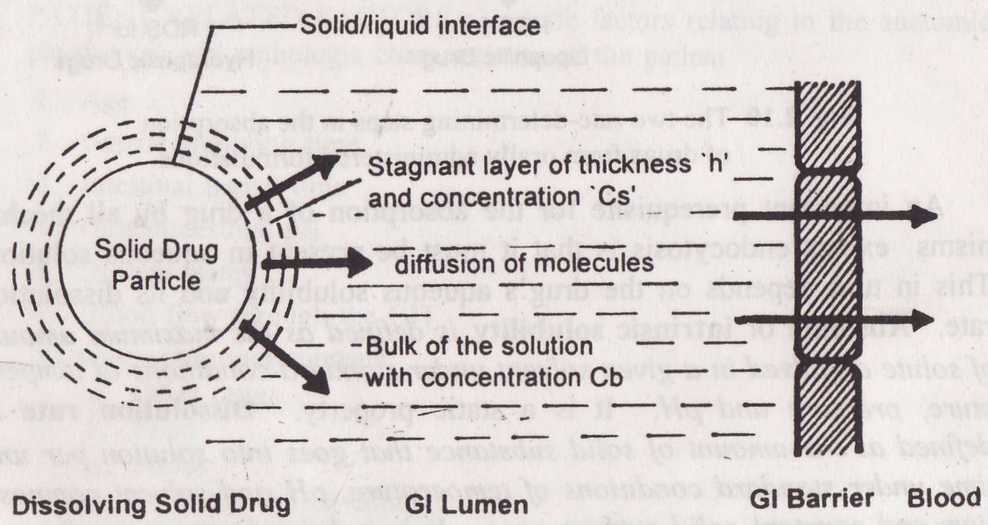

Fig. 2.11 Diffusion layer model for drug dissolution

Diffusion of the soluble solute from the stagnant layer to the bulk of the solution; this step is slower and is therefore the rate-determining step in drug dissolution. The model is depicted in

The earliest equation to explain the rate of dissolution when the process is diffusion controlled and involves no chemical reaction was given by **Noyes and Whitney**:

$$\frac{dC}{dt} = k\,(C_s - C_b) \qquad (2.3)$$

where,

dC/dt = dissolution rate of the drug,

k = dissolution rate constant (first order),

C_s = concentration of drug in the stagnant layer (also called as the **saturation or maximum drug solubility**), and

Cb = concentration of drug in the bulk of the solution at time t.

Equation 2.3 was based on Fick's second law of diffusion. Brunner incorporated Fick's first law of diffusion and modified the Noyes-Whitney's equation to:

$$\frac{dC}{dt} = \frac{DAK_{w/o}\,(C_s - C_b)}{Vh} \qquad (2.4)$$

where,

D = diffusion coefficient (*diffusivity*) of the drug

A = surface area of the dissolving solid

$K_{w/o}$ = water/oil partition coefficient of the drug considering the fact that dissolution body fluids are aqueous. Since the rapidity with which a drug dissolves depends on the $K_{w/o}$, it is also called as the **intrinsic dissolution rate constant**. It is a characteristic of drugs.

V = volume of dissolution medium.

h = thickness of the stagnant layer.

$(C_s - C_b)$ = concentration gradient for diffusion of drug.

The influence of various parameters in equation 2.4 on drug dissolution is depicted in Table 2.2.

TABLE 2.2 **Influence of Some Parameters on Dissolution Rate of Drug**

Parameters	*Symbol*	*Influence on drug dissolution*
Diffusion coefficient of drug	D	Greater the value, faster the dissolution. Diffusion decreases as the viscosity of dissolution medium increases.
Surface area of solid drug	A	Greater the surface area, faster the dissolution; can be increased by micronization of drug.

...*continued*

Parameters	*Symbol*	*Influence on drug dissolution*
Water/oil partition coefficient of drug	$K_{w/o}$	Higher the value, more the hydrophilicity and faster the dissolution in aqueous fluids.
Concentration gradient	$(C_s - C_b)$	Greater the concentration gradient, faster the diffusion and drug dissolution; can be increased by increasing drug solubility and the volume of dissolution medium.
Thickness of stagnant layer	h	More the thickness, lesser the diffusion and drug dissolution; can be decreased by increasing agitation.

Equation 2.4 represents first-order dissolution rate process, the driving force for which is the concentration gradient $(C_s - C_b)$. Under such a situation, dissolution is said to be under nonsink conditions. This is true in case of *in vitro* dissolution in a limited dissolution medium. Dissolution in such a situation slows down after sometime due to build-up in the concentration of drug in the bulk of the solution. The *in vivo* dissolution is always rapid than *in vitro* dissolution because the moment the drug dissolves, it is absorbed into the systemic circulation. As a result, $C_b = 0$, and dissolution is at its maximum. Thus, under *in vivo* conditions, there is no concentration build-up in the bulk of the solution and hence no retarding effect on the dissolution rate of the drug i.e. $C_s >> C_b$ and *sink conditions* are maintained. Under sink conditions, if the volume and surface area of solid are kept constant, then equation 2.4 reduces to:

$$\frac{dC}{dt} = K \qquad (2.5)$$

where K incorporates all the constants in equation 2.4. Equation 2.5 represents that the dissolution rate is constant under sink conditions and follows zero-order kinetics i.e. yields a linear plot (Fig. 2.12).

To obtain good *in vitro-in vivo* dissolution rate correlation, the *in vitro* dissolution must always be carried under sink conditions. This can be achieved by:

1. Bathing the dissolving solid in fresh solvent from time to time
2. Increasing the volume of dissolution fluid
3. Removing the dissolved drug by partitioning it from the aqueous phase of the dissolution fluid into an organic phase placed either above or below the dissolution fluid—for example, hexane or chloroform

4. Adding a water miscible solvent such as alcohol to the dissolution fluid, or
5. By adding selected adsorbents to remove the dissolved drug.

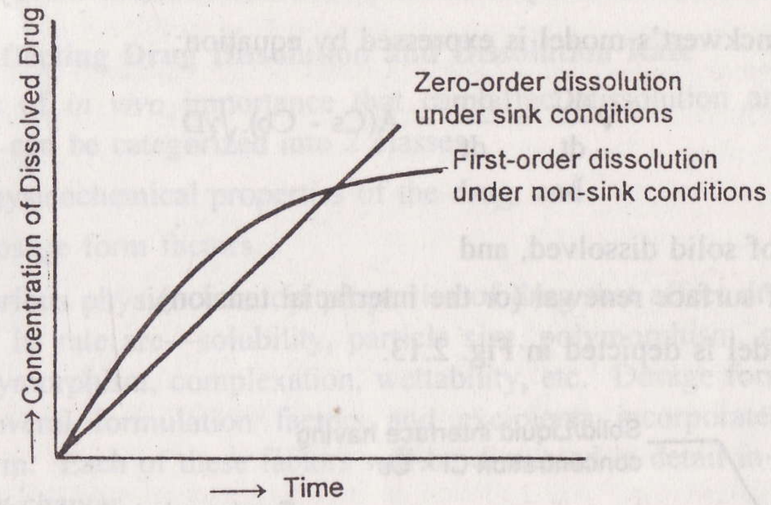

Fig. 2.12 Dissolution rate under nonsink and sink conditions.

The *in vitro* sink conditions are so maintained that C_b is always less than 10% of C_s.

The Noyes-Whitney's equation assumes that the surface area of the dissolving solid remains constant during dissolution, which is practically not possible for dissolving particles. Hence, dissolution methods that involves use of constant surface area discs are employed to determine the rate of dissolution.

To account for the particle size decrease and change in surface area accompanying dissolution, **Hixson and Crowell's cubic root law of dissolution** is used:

$$W_o^{1/3} - W^{1/3} = Kt \qquad (2.6)$$

where,

W_o = original mass of the drug

W = mass of the drug remaining to dissolve at time t

K = dissolution rate constant

Danckwert's Model (Penetration or Surface Renewal Theory)

Danckwert did not approve of the existence of a stagnant layer and suggested that turbulence in the dissolution medium exists at the solid/liquid interface. As a result, the agitated fluid consisting of macroscopic mass of eddies or packets reach the solid/liquid interface in a random fashion due to eddy currents, absorb the solute by diffusion and carry it to the bulk of the solution. Such solute containing packets are continuously

replaced with new packets of fresh solvent due to which the drug concentration at the solid/liquid interface never reaches C_s and has a lower limiting value of C_i. *Since the solvent packets are exposed to new solid surface each time, the theory is called as* **surface renewal theory**.

The Danckwert's model is expressed by equation:

$$V\frac{dC}{dt} = \frac{dm}{dt} = A(Cs - Cb).\sqrt{\gamma D} \qquad (2.7)$$

where,

m = mass of solid dissolved, and

γ = rate of surface renewal (or the interfacial tension).

The model is depicted in Fig. 2.13.

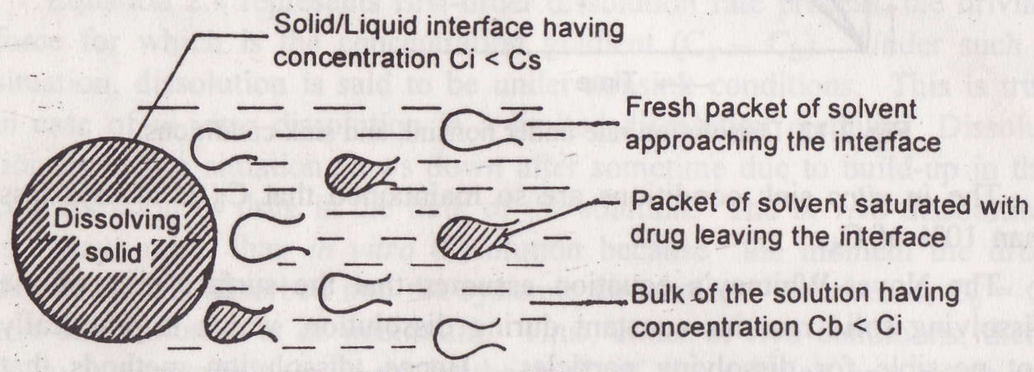

Fig. 2.13 Danckwert's model for drug dissolution

Interfacial Barrier Model (Double Barrier or Limited Solvation Theory)

The diffusion layer model and the Danckwert's model were based on two assumptions:

1. The rate-determining step that controls dissolution is the mass transport.
2. Solid-solution equilibrium is achieved at the solid/liquid interface.

According to the interfacial barrier model, an intermediate concentration can exist at the interface as a result of solvation mechanism and is a function of solubility rather than diffusion. When considering the dissolution of a crystal, each face of the crystal will have a different interfacial barrier. Such a concept is given by the following equation:

$$G = K_i\,(C_s - C_b) \qquad (2.8)$$

where,

G = dissolution rate per unit area, and

K_i = effective interfacial transport constant.

In this theory, the diffusivity D may not be independent of saturation concentration C_s. The interfacial barrier model can be extended to both diffusion layer model and the Danckwert's model (*for in vitro drug dissolution models refer chapter 12*).

Factors Affecting Drug Dissolution and Dissolution Rate

Factors of *in vivo* importance that can affect dissolution and hence absorption can be categorized into 2 classes:

1. Physicochemical properties of the drug, and
2. Dosage form factors.

The various physicochemical properties of drug that affect drug dissolution and its rate are—solubility, particle size, polymorphism, salt form, pseudopolymorphism, complexation, wettability, etc. Dosage form factors include several formulation factors and excipients incorporated in the dosage form. Each of these factors will be discussed in detail in the latter part of this chapter.

Of the various factors listed above, the factor of prime importance is drug solubility. Almost every factor that affects dissolution rate, influences the drug solubility in one way or the other. From several equations pertaining to dissolution rate, it is clear that it is directly related to drug solubility. An empirical relation which is useful to predict the dissolution rate of a drug from its solubility is:

$$R = \frac{dC}{dt} = 2.24\ C_s \qquad (2.9)$$

where R = dissolution rate of the drug.

It has been shown that a drug should have a minimum aqueous solubility of 1% to avoid bioavailability problems.

Particle Size and Effective Surface Area of the Drug

Particle size and surface area of a solid drug are inversely related to each other. Smaller the drug particle, greater the surface area. Two types of surface area of interest can be defined:

1. **Absolute surface area** *which is the total area of solid surface of any particle*, and
2. **Effective surface area** *which is the area of solid surface exposed to the dissolution medium.*

From the modified Noyes-Whitney equation 2.4, it is clear that larger the surface area, higher the dissolution rate. Since the surface area increases with decreasing particle size, a decrease in particle size, which

can be accomplished by micronization, will result in higher dissolution rates. However, it is important to note that it is not the absolute surface area but the effective surface area that is proportional to the dissolution rate. Greater the effective surface area, more intimate the contact between the solid surface and the aqueous solvent and faster the dissolution. But it is only when micronization reduces the size of particles below 0.1 microns that there is an increase in the intrinsic solubility and dissolution rate of the drug. The surface of such small particles have energy higher than the bulk of the solid resulting in an increased interaction with the solvent. This is particularly true in case of drugs which are nonhydrophobic, for example, micronization of poorly aqueous soluble drugs like griseofulvin, chloramphenicol and several salts of tetracycline results in superior dissolution rates in comparison to the simple milled form of these drugs.

Micronization has in fact enabled the formulator to decrease the dose of certain drugs because of increased absorption efficiency—for example, the griseofulvin dose was reduced to half and that of spironolactone was decreased 20 times following micronization. However, in case of hydrophobic drugs like aspirin, phenacetin and phenobarbital, micronization actually results in a decrease in the effective surface area of such powders and thus, a fall in the dissolution rate. Three reasons have been suggested for such an outcome—

1. The hydrophobic surface of the drugs adsorb air onto their surface which inhibit their wettability; such powders float on the dissolution medium.
2. The particles reaggregate to form larger particles due to their high surface free energy, which either float on the surface or settle at the bottom of the dissolution medium.
3. Extreme particle size reduction may impart surface charges that may prevent wetting; moreover electrically induced agglomeration may prevent intimate contact of the drug with the dissolution medium.

The net result of these effects is that there is a decrease in the effective surface area available to the dissolution medium and therefore a fall in the dissolution rate.

The absolute surface area of hydrophobic drugs can be converted to their effective surface area by:

1. Use of surfactant as a wetting agent that decreases the interfacial tension and displaces the adsorbed air with the solvent—for example, tween 80 increases the bioavailability of phenacetin by promoting its wettability, and

2. Adding hydrophilic diluents such as PEG, PVP, dextrose, etc. which coat the surface of hydrophobic drug particles and render them hydrophilic.

Particle size reduction and subsequent increase in the surface area and dissolution rate is not always advisable especially when the drugs are unstable and degrade in solution form (penicillin G and erythromycin), produce undesirable effects (gastric irritation caused by nitrofurantoin) or when a sustained effect is desired.

In addition to increasing the dissolution rate, the second mechanism by which a reduction in particle size improves drug dissolution is through an increase in its solubility. However, such an effect can only be achieved by reducing the particle size to a submicron level which is possible by use of one of the following specialized techniques such as formation of:

1. Molecular dispersion/solid solution where the sparingly soluble drug is molecularly trapped in the lattice of a hydrophilic agent such as cyclodextrins, or
2. Solid dispersion where such a drug is dispersed in a soluble carrier such as PVP, PEG, urea, etc.

(Refer chapter 12 for methods used in enhancing the bioavailability of drugs).

Polymorphism and Amorphism

Depending upon the internal structure, a solid can exist either in a crystalline or amorphous form (Fig. 2.14). *When a substance exists in more than one crystalline form, the different forms are designated as* **polymorphs** *and the phenomenon as* **polymorphism**. Polymorphs are of two types:

1. **Enantiotropic polymorph** *is the one which can be reversibly changed into another form by altering the temperature or pressure* e.g. sulfur, and
2. **Monotropic polymorph** *is the one which is unstable at all temperatures and pressures* e.g. glyceryl stearates.

The polymorphs differ from each other with respect to their physical properties such as solubility, melting point, density, hardness and compression characteristics. They can be prepared by crystallizing the drug from different solvents under diverse conditions. The existence of the polymorphs can be determined by using techniques such as optical crystallography, X-ray diffraction, differential scanning calorimetry, etc.

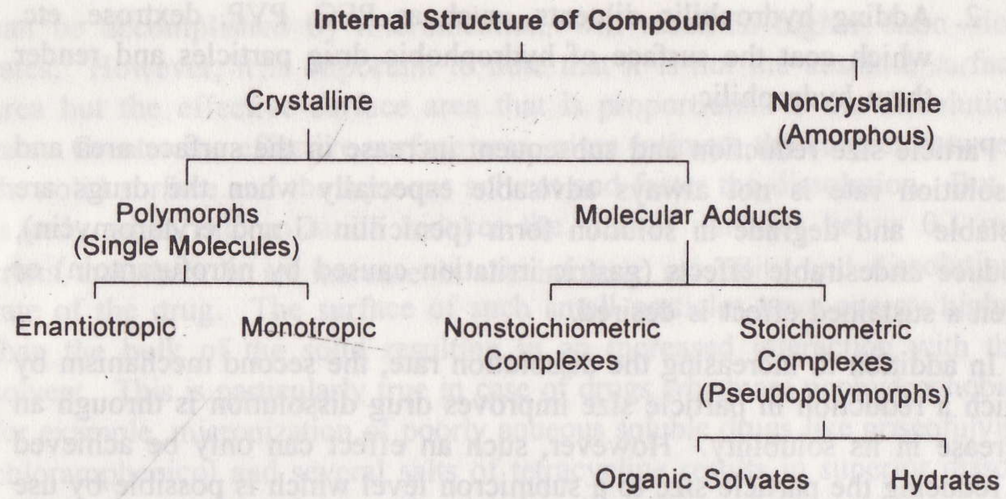

Fig. 2.14 Classification of internal structure of a compound

Depending on their relative stability, one of the several polymorphic forms will be physically more *stable* than the others. Such a stable polymorph represents the lowest energy state, has highest melting point and least aqueous solubility. The remaining polymorphs are called as *metastable* forms which represent the higher energy state, have lower melting points and higher aqueous solubilities. Because of their higher energy state, the metastable forms have a thermodynamic tendency to convert to the stable form. A metastable form cannot be called unstable because if it is kept dry, it will remain stable for years.

Since the metastable forms have greater aqueous solubility, they show better bioavailability and are therefore preferred in formulations—for example, of the three polymorphic forms of chloramphenicol palmitate -A, B and C, the B form shows best availability and the A form is virtually inactive biologically. The polymorphic form III of riboflavin is 20 times more water-soluble than the form I. Only 10% of the pharmaceuticals are present in their metastable forms. However, because of their poor thermodynamic stability, aging of dosage forms containing such metastable forms usually result in formation of less soluble, stable polymorph—for example, the more soluble crystalline form II of cortisone acetate converts to the less soluble form V in an aqueous suspension resulting in caking of solid. Such a transformation of metastable to stable form can be inhibited by dehydrating the molecule environment or by adding viscosity building macromolecules such as PVP, CMC, pectin or gelatin that prevent such a conversion by adsorbing onto the surface of the crystals.

About 40% of all organic compounds can exist in various polymorphic forms. Seventy percent of the barbiturates and 65% of sulfonamides exhibit polymorphism. Barbital, methyl paraben and sulfapyridine can

exist in as many as 6 polymorphic forms and cortisone acetate in 8 forms.

Some drugs can exist in **amorphous form** (i.e. *having no internal crystal structure*). Such drugs represent the highest energy state and can be considered as supercooled liquids. They have greater aqueous solubility than the crystalline forms because the energy required to transfer a molecule from crystal lattice is greater than that required for noncrystalline (amorphous) solid –for example, the amorphous form of novobiocin is 10 times more soluble than the crystalline form. Chloramphenicol palmitate, cortisone acetate and phenobarbital are other examples where the amorphous forms exhibit higher water solubility. Thus, the order for dissolution of different solid forms of drugs is — *amorphous > metastable > stable.*

Hydrates/Solvates (Pseudopolymorphism)

The crystalline form of a drug can either be a polymorph or a molecular adduct or both. *The stoichiometric type of adducts where the solvent molecules are incorporated in the crystal lattice of the solid are called as the* **solvates**, *and the trapped solvent as* **solvent of crystallization**. *The solvates can exist in different crystalline forms called as* **pseudopolymorphs.** *This phenomenon is called as* **pseudopolymorphism**. *When the solvent in association with the drug is water, the solvate is known as a* **hydrate**. Hydrates are most common solvate forms of drugs.

Generally, the anhydrous form of a drug has greater aqueous solubility than the hydrates. This is because the hydrates are already in interaction with water and therefore have less energy for crystal break-up in comparison to the anhydrates (thermodynamically higher energy state) for further interaction with water. The anhydrous form of theophylline and ampicillin have higher aqueous solubilities, dissolve at a faster rate and show better bioavailability in comparison to their monohydrate and trihydrate forms respectively. On the other hand, the organic (nonaqueous) solvates have greater aqueous solubility than the nonsolvates—for example, the n-pentanol solvate of fludrocortisone and succinylsulfathiazole and the chloroform solvate of griseofulvin are more water-soluble than their nonsolvated forms. Like polymorphs, the solvates too differ from each other in terms of their physical properties. In case of organic solvates, if the solvent is toxic, they are not of therapeutic use.

Salt Form of the Drug

Most drugs are either weak acids or weak bases. One of the easiest approach to enhance the solubility and dissolution rate of such drugs is to convert them into their salt forms. Generally, with weakly acidic drugs, a strong base salt is prepared such as the sodium and potassium salts of barbiturates and sulfonamides. In case of weakly basic drugs, a strong

acid salt is prepared like the hydrochloride or sulfate salts of several alkaloidal drugs.

At a given pH, the solubility of a drug, whether acidic/basic or its salt form, is a constant. The influence of salt formation on the drug solubility, rate of dissolution and absorption can be explained by considering the pH of the diffusion layer and not the pH of the bulk of the solution (refer diffusion layer theory of drug dissolution). Consider the case of a salt of a weak acid. At any given pH of the bulk of the solution, the pH of the diffusion layer (saturation solubility of the drug) of the salt form of a weak acid will be *higher* than that observable with the free acid form of the drug (can be practically observed in the laboratory). Owing to the increased pH of the diffusion layer, the solubility and dissolution rate of a weak acid in this layer is promoted, since it is a known fact that higher pH favors the dissolution of weak acids. Thus, if dissolution is faster, absorption is bound to be rapid. In case of salts of weak bases, the pH of the diffusion layer will be lower in comparison to that found with the free base form of the drug. Consequently, the solubility of a basic drug at this *lower* pH is enhanced. Thus, if:

$[H^+]_d$ = hydrogen ion concentration of the diffusion layer, and

$[H^+]_b$ = hydrogen ion concentration of the bulk of the solution, then,

for salts of weak acids, $[H^+]_d < [H^+]_b$, and

for salts of weak bases, $[H^+]_d > [H^+]_b$.

The increase and decrease in pH of the diffusion layer by the salts of weak acids and bases have been attributed to the *buffering action* of strong base cation and strong acid anion respectively.

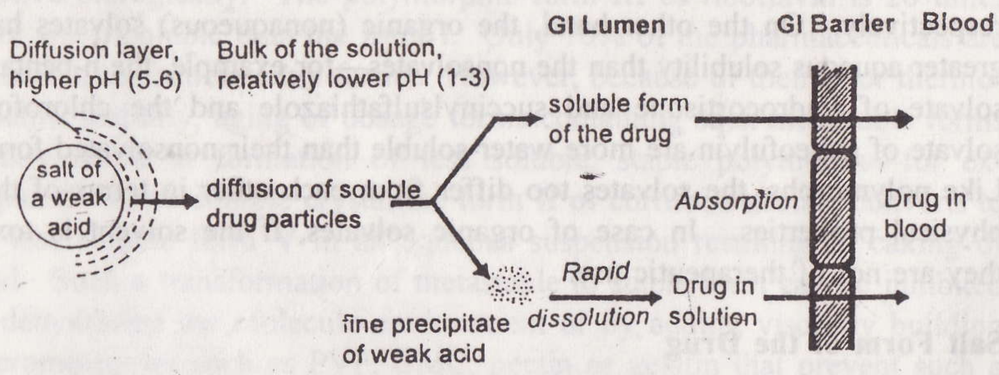

Fig. 2.15 Dissolution and absorption of an acidic drug administered in a salt form

Yet another convincing reason for enhanced solubility of salts of weak acids is the precipitation of the drug as very fine particles. When the soluble ionic form of the drug diffuses from the stagnant diffusion layer

into the bulk of the solution whose pH is low, it is transformed into its free acid form having lesser aqueous solubility at the lower pH of the bulk solution. Consequently, this free acidic form of the drug is precipitated in the form of fine particles. The resultant increase in the surface area is then responsible for the rapid dissolution and absorption in comparison to the drug administered in just the acidic form (Fig. 2.15).

The principle of *in situ salt formation* has been utilized to enhance the dissolution and absorption rate of certain drugs like aspirin and penicillin from buffered alkaline tablets. The approach is to increase the pH of the microenvironment of the drug by incorporating buffer agents and promote dissolution rate. Apart from the enhanced bioavailability, buffered aspirin tablets have two more advantages: firstly, the gastric irritation and ulcerogenic tendency of the drug is greatly reduced, and secondly, the problem with the use of sodium salt of aspirin (to enhance the solubility) which otherwise has poor hydrolytic stability, is overcome by *in situ* salt formation.

The selection of appropriate salt form for better dissolution rate is also important. It has been shown that the choline and the isopropanolamine salts of theophylline dissolve 3 to 4 times more rapidly than the ethylenediamine salt and show better bioavailability.

A factor that influences the solubility of salt forms of the drug is the *size of the counter ion.* Generally speaking, smaller the size of the counter ion, greater the solubility of salt—for example, the bioavailability of novobiocin from its sodium salt, calcium salt and free acid form was found to be in the ratio—50 : 25 : 1. Where the counter ion is very large in size and/or has poor ionic strength (as in the case of ester form of drugs), the solubility may be much lower than the free drug itself—for example, the pamoates, stearates and palmitates of weak bases have poor aqueous solubility. These forms are, however, useful in several ways such as to prolong the duration of action (steroidal salts), to overcome bad taste (chloramphenicol palmitate), to enhance GI stability (erythromycin estolate) or to decrease the side effects, local or systemic.

There are exceptions where the so called more soluble salt form of the drug showed poor bioavailability. One such study was the comparative dissolution of sodium phenobarbital and free phenobarbital from their tablets. Slower dissolution with sodium salt was observed and the reason attributed to it was that its tablet swelled but did not disintegrate and thus dissolved slowly. An identical result was obtained with hydrochloride salts of several tetracycline analogs and papaverine; better dissolution and bioavailability was observed with the free bases. The reason for poor solubility and dissolution rate was the suppression action of the common ion effect.

Drug pK_a and Lipophilicity and GI pH—pH Partition Hypothesis

The pH partition theory (Brodie et al.) explains in simple terms, the process of drug absorption from the GIT and its distribution across all biologic membranes. *The theory states that for drug compounds of molecular weight greater than 100, which are primarily transported across the biomembrane by passive diffusion, the process of absorption is governed by:*

1. The dissociation constant (pK_a) of the drug.
2. The lipid solubility of the unionized drug (a function of drug $K_{o/w}$).
3. The pH at the absorption site.

Since most drugs are weak electrolytes (weak acids or weak bases), their degree of ionization depends upon the pH of the biological fluid. If the pH on either side on the membrane is different, then the compartment whose pH favors greater ionization of the drug will contain greater amount of drug, and only the unionized or undissociated fraction of drug, if sufficiently lipid soluble, can permeate the membrane passively until the concentration of unionized drug on either side of the membrane becomes equal i.e. until equilibrium is attained.

The above statement of the hypothesis was based on the *assumptions* that:

1. The GIT is a simple lipoidal barrier to the transport of drug.
2. Larger the fraction of unionized drug, faster the absorption.
3. Greater the lipophilicity ($K_{o/w}$) of the unionized drug, better the absorption.

Drug pK_a and Gastrointestinal pH

The amount of drug that exists in unionized form is a function of dissociation constant (pK_a) of the drug and pH of the fluid at the absorption site.

It is customary to express the dissociation constants of both acidic and basic drugs by pK_a values. The lower the pK_a of an acidic drug, stronger the acid i.e. greater the proportion of ionized form at a particular pH. The higher the pK_a of a basic drug, the stronger the base. Thus, from the knowledge of pK_a of the drug and pH at the absorption site (or biological fluid), the relative amount of ionized and unionized drug in solution at a particular pH and the percent of drug ionized at this pH can be determined by *Henderson-Hasselbach equations*:

for weak acids,

$$pH = pK_a + \log \frac{\text{ionized drug concentration}}{\text{unionized drug concentration}} \quad (2.10)$$

$$\% \text{ Drug Ionized} = \frac{10^{pH - pKa}}{1 + 10^{pH - pKa}} \times 100 \quad (2.11)$$

for weak bases,

$$pH = pK_a + \log \frac{\text{unionized drug concentration}}{\text{ionized drug concentration}} \quad (2.12)$$

$$\% \text{ Drug Ionized} = \frac{10^{pKa - pH}}{1 + 10^{pKa - pH}} \times 100 \quad (2.13)$$

When the concentration of ionized and unionized drug becomes equal, the second term of equations 2.10 and 2.12 reduces to zero (since log 1 = zero), and thus $pH = pK_a$. The pK_a is a characteristic of the drug.

If there is a membrane barrier that separates the aqueous solutions of different pH such as the GIT and the plasma, then the theoretical ratio R of drug concentration on either side of the membrane can be given by equations derived by *Shore et al*:

for weak acids,

$$R_a = \frac{C_{GIT}}{C_{plasma}} = \frac{1 + 10^{pHGIT - pKa}}{1 + 10^{pHplasma - pKa}} \quad (2.14)$$

for weak bases,

$$R_b = \frac{C_{GIT}}{C_{plasma}} = \frac{1 + 10^{pKa - pHGIT}}{1 + 10^{pKa - pHplasma}} \quad (2.15)$$

If one considers the pH range in the GIT from 1 to 8, that of the stomach from 1 to 3 and of the intestine (from duodenum to colon) 5 to 8, then certain *generalizations* regarding ionization and absorption of drugs can be made, as predicted from the pH-partition hypothesis:

For Weak Acids:

1. Very weak acids ($pK_a > 8$) such as phenytoin, ethosuximide and several barbiturates are essentially unionized at all pH values and therefore their absorption is rapid and independent of GI pH.
2. Acids in the pK_a range 2.5 to 7.5 are greatly affected by changes in pH and therefore their absorption is pH-dependent; e.g. several NSAIDs like aspirin, ibuprofen, phenylbutazone, and a number of penicillin analogs. Such drugs are better absorbed from acidic conditions of stomach ($pH < pK_a$) where they largely exist in unionized form.

3. Stronger acids with $pK_a < 2.5$ such as cromolyn sodium are ionized in the entire pH range of GIT and therefore remain poorly absorbed.

For Basic Drugs:

1. Very weak bases ($pK_a < 5.0$) such as caffeine, theophylline and a number of benzodiazepines like diazepam, oxazepam and nitrazepam are essentially unionized at all pH values and therefore their absorption is rapid and pH-independent.
2. Bases in the pK_a range 5 to 11.0 are greatly affected by changes in pH and hence their absorption is pH-dependent; e.g. several morphine analogs, chloroquine, imipramine and amitriptyline. Such drugs are better absorbed from the relatively alkaline conditions of the intestine where they largely exist in unionized form.
3. Stronger bases with $pK_a > 11.0$ like mecamylamine and guanethidine are ionized in the entire pH range of GIT and therefore poorly absorbed.

A summary of above discussion is given in Table 2.3.

TABLE 2.3

Influence of drug pK_a and GI pH on Drug Absorption

Drugs	*pK_a*	*pH/site of absorption*
Very weak acids ($pK_a > 8.0$)		
Pentobarbital	8.1	Unionized at all pH values;
Hexobarbital	8.2	absorbed along the entire length of GIT
Phenytoin	8.3	
Ethosuximide	9.3	
Moderately weak acids (pK_a 2.5 to 7.5)		
Cloxacillin	2.7	Unionized in gastric pH and
Aspirin	3.5	ionized in intestinal pH; better
Ibuprofen	4.4	absorbed from stomach
Phenylbutazone	4.5	
Stronger acids ($pK_a < 2.5$)		
Disodium cromoglycate	2.0	Ionized at all pH values; poorly absorbed from GIT.
Very weak bases ($pK_a < 5.0$)		
Theophylline	0.7	Unionized at all pH values;
Caffeine	0.8	absorbed along the entire length of GIT.
Oxazepam	1.7	
Diazepam	3.7	

...continued

Drugs	*pK_a*	*pH/site of absorption*
Moderately weak bases (pK_a 5 to 11.0)		
Reserpine	6.6	Ionized at gastric pH,
Heroin	7.8	relatively unionized at intestinal pH;
Codeine	8.2	better absorbed from intestine.
Amitriptyline	9.4	
Stronger bases ($pK_a > 11.0$)		
Mecamylamine	11.2	Ionized at all pH values;
Guanethidine	11.7	poorly absorbed from GIT.

By using equations from 2.10 to 2.15, one can calculate the relative amounts of unionized (absorbable) and ionized (unabsorbable) forms of the drug and predict the extent of absorption at a given pH of GIT. An example of this is illustrated in Fig. 2.16.

Drug	Stomach pH = 1.5	← Membrane Barrier → Plasma pH = 7.4	Intestine pH = 5.0
Weak Acid e.g. Ibuprofen pKa = 4.4	[HA] = 100 ⇌ [A⁻] = 0.13	[HA] = 100 ⇌ [A⁻] = 100,000	[HA] = 100 ⇌ [A⁻] = 398.1
	[Total] = 100.13	[Total] = 100,100	[Total] = 498.1
Weak Base e.g. Nitrazepam pKa = 3.2	[BOH] = 100 ⇌ [B⁺] = 5012	[BOH] = 100 ⇌ [B⁺] = 0.006	[BOH] = 100 ⇌ [B⁺] = 1.6
	[Total] = 5112	[Total] = 100.006	[Total] = 101.6

Fig. 2.16 Influence of pH on ionization of drug. [HA] and [BOH] are concentration of unionized acid and base, and [A⁻] and [B⁺] are concentration of ionized acid and base respectively

Lipophilicity and Drug Absorption

As mentioned earlier, it is the pK_a of a drug that determines the degree of ionization at a particular pH and that only the unionized drug, if sufficiently lipid soluble, is absorbed into the systemic circulation. Thus, even if the drug exists in the unionized form, it will be poorly absorbed if it has poor lipid solubility (or low $K_{o/w}$). Ideally, for optimum absorption, a drug should have sufficient aqueous solubility to dissolve in the fluids at the absorption site and lipid solubility ($K_{o/w}$) high enough to facilitate the partitioning of the drug in the lipoidal biomembrane and into the systemic circulation. In other words, *a perfect* **hydrophilic -lipophilic balance (HLB)** *should be there in the structure of the drug for optimum bioavailability.*

The lipid solubility of a drug is determined from its oil/water partition coefficient ($K_{o/w}$) value. This value is a measure of the degree of distribution of drug between one of the several organic, water immiscible, lipophilic solvents such as n-octanol, chloroform, n-heptane, etc. and an aqueous phase (water or a suitable buffer). In general, the octanol/pH 7.4 buffer partition coefficient value in the range of 1 to 2 of a drug is sufficient for passive absorption across lipoidal membranes. A direct correlation between a drug's $K_{o/w}$ and extent of absorption is illustrated in Table 2.4.

TABLE 2.4

Comparison between Intestinal Absorption of Some Drugs through the Rat Intestine and $K_{o/w}$ of the Ionized Form of the Drugs

Drugs	$K_{heptane/water}$	*% Absorbed*
Rapid rate of absorption		
Phenylbutazone	100.0	54
Thiopental	3.3	67
Benzoic acid	0.19	54
Salicylic acid	0.12	60
Moderate rate of absorption		
Aspirin	0.03	21
Theophylline	0.02	30
Theobromine	< 0.002	22
Sulfanilamide	< 0.002	24
Slow rate of absorption		
Barbituric acid	< 0.002	5
Sulfaguanidine	< 0.002	2

Source: Schanker, *J. Med. Pharm.*, **2**, 343 (1960).

In yet another study by Schanker on a series of barbituric acid derivatives having same pK_a, the percent absorbed increased with an increase in the partition coefficient of the drug. Thus, to enhance the bioavailability of a drug, not only its dissolution rate but also its rate of permeability should be considered. Rate of dissolution can be increased by altering the physical properties such as particle size or crystalline structure but its permeability can only be promoted by modification of chemical structure (*see* chapter 6 on prodrugs).

Limitations of pH-Partition Hypothesis

The pH-partition hypothesis over-simplified the otherwise complicated process of drug absorption and therefore has its own limitations. Some of the deviations from the theory are:

1. Presence of virtual membrane pH
2. Absorption of ionized drug
3. Influence of GI surface area and residence time of drug
4. Presence of aqueous unstirred diffusion layer

1. Presence of Virtual Membrane pH : The pH-partition hypothesis suggested that only the unionized drug at a given GI lumen pH is absorbed. An S-shaped curve, called as the **pH-absorption curve** denoting the dissociation of drug, is obtained when pH is plotted versus rate of drug absorption (Fig. 2.17).

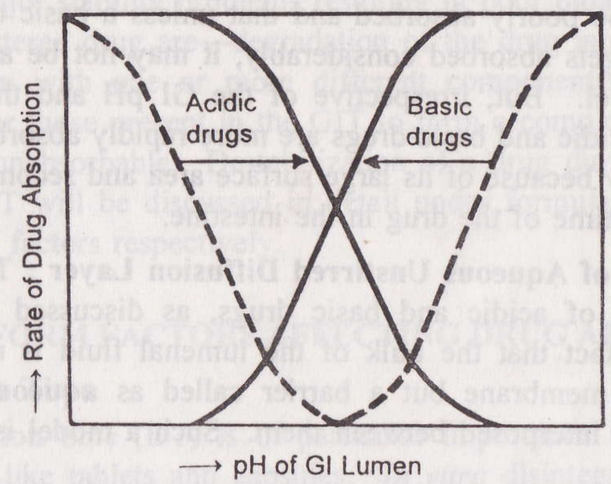

Fig. 2.17 pH-absorption curve for acidic and basic drugs. Dotted lines indicate curves predicted by pH-partition hypothesis and bold lines indicate the practical curves.

However, differences in the extent of absorption of salicylic acid has been observed at a given GI pH than that predicted by pH-partition hypothesis. The experimental pH-absorption curves are less steep and shift to the left (lower pH values) for a basic drug and to the right (higher pH values) for an acidic drug. This led to the suggestion that a **virtual pH**, also called as the **microclimate pH**, different from the lumenal pH exists at the membrane surface. This virtual membrane pH actually determines the extent of drug ionization and thus, drug absorption.

2. Absorption of Ionized Drugs : An important assumption of the theory was that only unionized form of the drug is absorbed and permeation of the ionized drug is negligible since its rate of absorption is 3 to 4 times less than that of unionized drug. This is true to a large extent as ionized drugs have low lipid solubility and relatively poor permeability. However, the pH-absorption curve shift suggested that ionized forms of some drugs also get absorbed to a considerable extent. If such drugs have

a large lipophilic group in their structure, despite their ionization, they will be absorbed passively—for example, morphinan derivatives. Other mechanisms are also involved in the absorption of ionized drugs such as active transport, ion-pair transport and convective flow.

3. Influence of GI Surface Area and Residence Time of Drug : According to the pH-partition theory, acidic drugs are best absorbed from stomach (acidic pH) and basic drugs from intestine (alkaline pH) in which conditions they are unionized to a large extent. This could be true under conditions where the surface area of stomach and intestine are same. It could also mean that once an acidic drug reaches the intestine, the remaining fraction will be poorly absorbed and that unless a basic drug reaches the intestine and gets absorbed considerably, it may not be able to attain its therapeutic level. But, irrespective of the GI pH and the degree of ionization, both acidic and basic drugs are more rapidly absorbed from the intestine, primarily because of its large surface area and secondly, because of long residence time of the drug in the intestine.

4. Presence of Aqueous Unstirred Diffusion Layer : The **pH-shift** in the absorption of acidic and basic drugs, as discussed earlier, also accounts for the fact that the bulk of the lumenal fluid is not in direct contact with the membrane but a barrier called as **aqueous unstirred diffusion layer** is interposed between them. Such a model is depicted in Fig. 2.18.

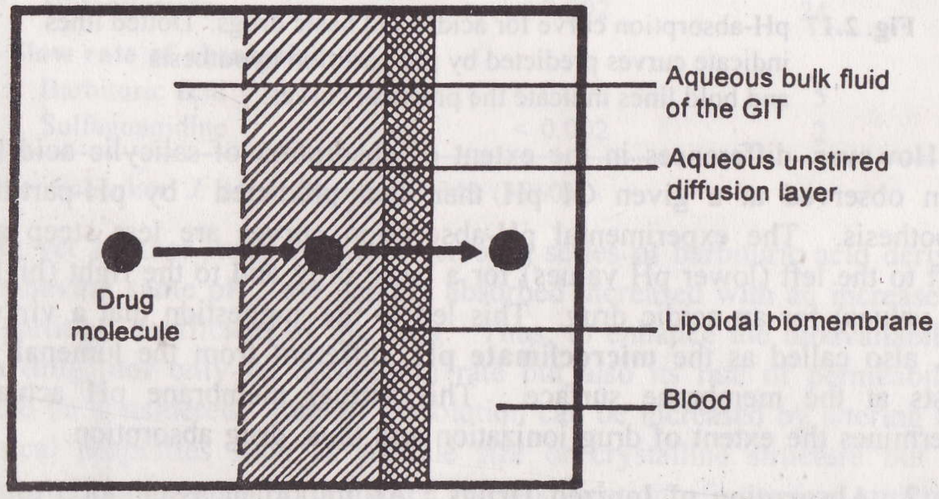

Fig. 2.18 Presence of aqueous unstirred diffusion layer on the membrane surface

Such a layer has a real thickness and is a barrier to absorption of drugs. In the original pH-partition theory, the rate-limiting step in the absorption of drugs was the partitioning in the lipid barrier. With the incorporation of unstirred aqueous diffusion layer, a drug must diffuse first through this aqueous barrier and then through the lipoidal barrier.

Thus, drugs having large partition coefficient can rapidly penetrate the lipid membrane but diffusion through unstirred water layer is the rate-limiting step in their absorption. This applies in particular to high molecular weight fatty acids and bile acids.

Despite its limitations, the pH-partition theory is still useful in the basic understanding of drug absorption and movement of drug between various body compartments.

Drug Stability

A drug for oral use may destabilize either during its shelf-life or in the GIT. Two major stability problems resulting in poor bioavailability of an orally administered drug are—degradation of the drug into inactive form, and interaction with one or more different component(s) either of the dosage form or those present in the GIT to form a complex that is poorly soluble or is unabsorbable. Destabilization of a drug during its shelf-life and in the GIT will be discussed in detail under formulation factors and patient related factors respectively.

DOSAGE FORM FACTORS AFFECTING DRUG ABSORPTION

Disintegration Time

Disintegration time (DT) is of particular importance in case of solid dosage forms like tablets and capsules. *In vitro* disintegration test is by no means a guarantee of drug's bioavailability because if the disintegrated drug particles do not dissolve, absorption is not possible. However, if a solid dosage form does not conform to the DT, it portends bioavailability problems because the subsequent process of dissolution will be much slower and absorption may be insufficient. Coated tablets, especially sugar coated ones have long DT. Rapid disintegration is thus important in the therapeutic success of a solid dosage form. DT of a tablet is directly related to the amount of binder present and the compression force (hardness) of a tablet. A harder tablet with large amount of binder has a long DT. Disintegration can be aided by incorporating disintegrants in suitable amounts during formulation.

After disintegration of a solid dosage form into granules, the granules must deaggregate into fine particles as dissolution from such tiny particles is faster than that from granules.

Manufacturing/Processing Variables

Drug dissolution is the single most important factor in the absorption of drugs, especially from the most widely used conventional solid dosage forms, tablets and capsules. The dosage form related factors that influ-

ence dissolution and hence absorption of a drug from such formulations are:

1. Excipients (formulation ingredients apart from the active principles), and
2. Manufacturing processes.

The influence of excipients such as binders, lubricants, disintegrants, etc. on drug dissolution will be discussed in the subsequent section of this chapter.

Several manufacturing processes influence drug dissolution from solid dosage forms. Processes of such importance in the manufacture of tablets are:

1. Method of granulation, and
2. Compression force.

The processing factor of importance in the manufacture of capsules that can influence its dissolution is the intensity of packing of capsule contents.

Method of Granulation : The wet granulation process is the most conventional technique in the manufacture of tablets and was once thought to yield tablets that dissolve faster than those made by other granulation methods. The limitations of this method include—(i) formation of crystal bridge by the presence of liquid, (ii) the liquid may act as a medium for affecting chemical reactions such as hydrolysis and (iii) the drying step may harm the thermolabile drugs. The method also involves a large number of steps each of which can influence drug dissolution—method and duration of blending, method, time and temperature of drying, etc. The method of direct compression has been utilized to yield tablets that dissolve at a faster rate. One of the more recent methods that has resulted in superior product is **agglomerative phase of communition (APOC)**. The process involves grinding of drugs in a ball mill for time long enough to affect spontaneous agglomeration. The tablets so produced were stronger and showed rapid rate of dissolution in comparison to tablets made by other methods. The reason attributed to it was an increase in the internal surface area of the granules prepared by APOC method.

Compression Force : The compression force employed in tableting process influence density, porosity, hardness, disintegration time and dissolution of tablets. The curve obtained by plotting compression force versus rate of dissolution can take one of the 4 possible shapes shown in Fig. 2.19.

On the one hand, higher compression force increases the density and hardness of tablet, decreases porosity and hence penetrability of the solvent into the tablet, retards wettability by forming a firmer and more effective sealing layer by the lubricant, and in many cases, promotes tighter bonding between the particles, all of which result in slowing of the

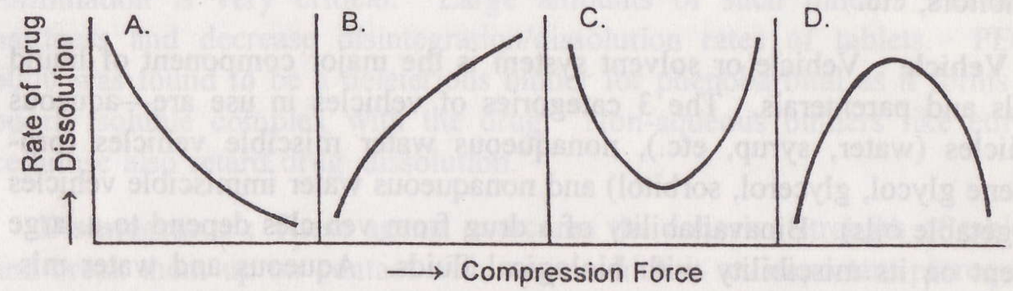

Fig. 2.19 Influence of compression force on dissolution rate of tablets

dissolution rate of tablets (curve A of Fig. 2.19). On the other hand, higher compression forces cause deformation, crushing or fracture of drug particles into smaller ones or convert a spherical granule into a disc shaped particle with a large increase in the effective surface area. This results in an increase in the dissolution rate of the tablet (curve B of Fig. 2.19). A combination of both the curves A and B is also possible as shown in curves C and D. In short, the influence of compression force on the dissolution rate is difficult to predict and a thorough study on each formulation should be made to ensure better dissolution and bioavailability.

Intensity of Packing of Capsule Contents : Like the compression force for tablets, packing density in case of capsule dosage form can either inhibit or promote dissolution. Diffusion of GI fluids into the tightly filled capsules creates a high pressure within the capsule resulting in rapid bursting and dissolution of contents. Opposite is also possible. It has been shown that capsules with finer particles and intense packing have poor drug release and dissolution rate due to a decrease in pore size of the compact and poor penetrability by the GI fluids.

Pharmaceutic Ingredients/Excipients (Formulation factors)

A drug is rarely administered in its original form. Almost always, a convenient dosage form to be administered by a suitable route is prepared. Such a formulation contains a number of **excipients** (*non-drug components of a formulation*). Excipients are added to ensure acceptability, physicochemical stability during the shelf-life, uniformity of composition and dosage, and optimum bioavailability and functionability of the drug product. Despite their inertness and utility in the dosage form, excipients can influence absorption of drugs. The more the number of excipients in a dosage form, the more complex it is and greater the potential for absorption and bioavailability problems. Commonly used excipients in

various dosage forms are vehicles, diluents (fillers), binders and granulating agents, disintegrants, lubricants, coatings, suspending agents, emulsifiers, surfactants, buffers, complexing agents, colorants, sweeteners, crystal growth inhibitors, etc.

Vehicle : Vehicle or solvent system is the major component of liquid orals and parenterals. The 3 categories of vehicles in use are—aqueous vehicles (water, syrup, etc.), nonaqueous water miscible vehicles (propylene glycol, glycerol, sorbitol) and nonaqueous water immiscible vehicles (vegetable oils). Bioavailability of a drug from vehicles depend to a large extent on its miscibility with biological fluids. Aqueous and water miscible vehicles are miscible with the body fluids and drugs from them are rapidly absorbed. Quite often, a drug is more soluble in water miscible vehicles like propylene glycol (serving as a *co-solvent*) and show better bioavailability. Sometimes dilution of such vehicles with the body fluids results in precipitation of drug as fine particles which, however, dissolve rapidly. Solubilizers such as tween 80 are sometimes used to promote solubility of a drug in aqueous vehicles. In case of water immiscible vehicles, the rate of drug absorption depends upon its partitioning from the oil phase to the aqueous body fluids, which could be a rate-limiting step. Viscosity of the vehicles is another factor in the absorption of drugs. Diffusion into the bulk of GI fluids and thus absorption of a drug from a viscous vehicle may be slower.

Diluents (Fillers) : Diluents are commonly added to tablet (and capsule) formulations if the required dose is inadequate to produce the necessary bulk. A diluent may be organic or inorganic. Among organic diluents, carbohydrates are very widely used—for example, starch, lactose, microcrystalline cellulose, etc. These hydrophilic powders are very useful in promoting the dissolution of poorly water-soluble, hydrophobic drugs like spironolactone and triamterene by forming a coat onto the hydrophobic surface of drug particles and rendering them hydrophilic. Among the inorganic diluents, dicalcium phosphate (DCP) is most common. One classic example of drug-diluent interaction resulting in poor bioavailability is that of tetracycline and DCP. The cause is formation of divalent calcium-tetracycline complex which is poorly soluble and thus, unabsorbable.

Binders and Granulating Agents : These materials are used to hold powders together to form granules or promote cohesive compacts for directly compressible materials and to ensure that the tablet remains intact after compression. Popular binders include polymeric materials (natural, semisynthetic and synthetic) like starch, cellulose derivatives, acacia, PVP, etc. Others include gelatin and sugar solution. In general, like fillers, the

hydrophilic (aqueous) binders show better dissolution profile with poorly wettable drugs like phenacetin by imparting hydrophilic properties to the granule surface. However, the proportion of strong binders in the tablet formulation is very critical. Large amounts of such binders increase hardness and decrease disintegration/dissolution rates of tablets. PEG 6000 was found to be a deleterious binder for phenobarbital as it forms a poorly soluble complex with the drug. Non-aqueous binders like ethyl cellulose also retard drug dissolution.

Disintegrants : These agents overcome the cohesive strength of tablet and break them up on contact with water which is an important prerequisite to tablet dissolution. Almost all the disintegrants are hydrophilic in nature. A decrease in the amount of disintegrant can significantly lower bioavailability. Adsorbing disintegrants like bentonite and veegum should be avoided with low dose drugs like digoxin, alkaloids and steroids since a large amount of dose is permanently adsorbed and only a fraction is available for absorption. Microcrystalline cellulose is a very good disintegran (and a binder too) but at high compression forces, it may retard drug dissolution.

Lubricants/Antifrictional Agents : These agents are added to tablet formulations to aid flow of granules, to reduce interparticle friction and sticking or adhesion of particles to dies and punches. The commonly used lubricants are hydrophobic in nature (several metallic stearates and waxes) and known to inhibit wettability, penetration of water into tablet and their disintegration and dissolution. This is because the disintegrant gets coated with the lubricant if blended simultaneously which however can be prevented by adding the lubricant in the final stage. The best alternative is use of soluble lubricants like SLS and carbowaxes which promote drug dissolution.

Coatings : In general, the deleterious effect of various coatings on drug dissolution from a tablet dosage form is in the following order: *enteric coat > sugar coat > nonenteric film coat.* The dissolution profile of certain coating materials change on aging; e.g. shellac coated tablets, on prolonged storage, dissolve more slowly in the intestine. This can, however, be prevented by incorporating little PVP in the coating formulation.

Suspending Agents/Viscosity Imparters : Popular suspending agents are hydrophilic polymers like vegetable gums (acacia, tragacanth, etc.), semisynthetic gums (CMC, MC) and synthetic gums which primarily stabilize the solid drug particles by reducing their rate of settling through an increase in the viscosity of the medium. These agents and some

sugars are also used as viscosity imparters to affect palatability and pourability of solution dosage forms. Such agents can influence drug absorption in several ways. The macromolecular gums often form unabsorbable complexes with drugs—for example, sodium CMC forms a poorly soluble complex with amphetamine. An increase in viscosity by these agents acts as a mechanical barrier to the diffusion of drug from the dosage form into the bulk of GI fluids and from GI fluids to the mucosal lining by forming a viscid layer on the GI mucosa. They also retard the GI transit of drugs.

Surfactants : Surfactants are widely used in formulations as wetting agents, solubilizers, emulsifiers, etc. Their influence on drug absorption is very complex. They may enhance or retard drug absorption either by interacting with the drug or the membrane or both. Mechanisms involved in the increased absorption of drug by use of surfactants include:

1. Promotion of wetting (through increase in effective surface area) and dissolution of drugs e.g. tween 80 with phenacetin
2. Better membrane contact of the drug for absorption
3. Enhanced membrane permeability of the drug

The beneficial effects of surfactants have been observed at pre-critical micelle concentration levels. However, physiologic surfactants like the bile salts (anionic) and lysolecithin (nonionic) promote absorption of hydrophobic drugs like steroids, oil soluble vitamins and griseofulvin by their micellar solubilizing property.

Decreased absorption of drug in the presence of surfactants has been suggested to be due to:

1. Formation of unabsorbable drug-micelle complex at surfactant concentrations above critical micelle concentration
2. Laxative action induced by a large surfactant concentration

Buffers : Buffers are sometimes useful in creating the right atmosphere for drug dissolution as was observed for buffered aspirin tablets. However, certain buffer systems containing potassium cations inhibit the drug absorption as seen with vitamin B_2 and sulfanilamide. The reason attributed to it was the uptake of fluids by the intestinal epithelial cells due to which the effective drug concentration in the tissue is reduced and the absorption rate is decreased. Such an inhibitory effect of the various buffer cations on the drug transfer rate is in the following order: $K^+ > NH_4^+ > Li^+ > Na^+ > TRIS^+$. Hence, the buffer system for a salt of a drug should contain the same cation as the drug salt and introduce no additional cations.

Complexing Agents : Complex formation has been used to alter the physicochemical and biopharmaceutical properties of a drug. A complexed drug may have altered stability, solubility, molecular size, partition coefficient and diffusion coefficient. Basically, such complexes are pharmacologically inert and must dissociate either at the absorption site or following absorption into the systemic circulation. Several examples where complexation has been used to enhance drug bioavailability are:

1. Enhanced dissolution through formation of a soluble complex e.g. ergotamine tartarate-caffeine complex and hydroquinone-digoxin complex,
2. Enhanced lipophilicity for better membrane permeability e.g. caffeine-PABA complex, and
3. Enhanced membrane permeability e.g. enhanced GI absorption of heparin (normally not absorbed from the GIT) in presence of EDTA which chelates calcium and magnesium ions of the membrane.

Complexation can be deleterious to drug absorption due to formation of poorly soluble or poorly absorbable complex e.g. complexation of tetracycline with divalent and trivalent cations like calcium (milk, antacids), iron (hematinics), magnesium (antacids) and aluminium (antacids). Reasons for poor bioavailability of some complexes are failure to dissociate at the absorption site and large molecular size of the complex that cannot diffuse through the cell membrane—for example, drug-protein complex.

Colorants : Even a very low concentration of water-soluble dye can have an inhibitory effect on dissolution rate of several crystalline drugs. The dye molecules get adsorbed onto the crystal faces and inhibit drug dissolution—for example, brilliant blue retards dissolution of sulfathiazole. Dyes have also been found to inhibit micellar solubilization effect of bile acids which may impair the absorption of hydrophobic drugs like steroids. Cationic dyes are more reactive than the anionic ones due to their greater power for adsorption on primary particles.

Crystal Growth Inhibitors : In addition to maintaining the initial physical properties of a drug in suspension, crystal growth inhibitors like PVP and PEG inhibit conversion of a high energy metastable polymorph into stable, less soluble polymorph.

Nature and Type of Dosage Form

Apart from the proper selection of drug, clinical success often depends to a great extent on the proper selection of dosage form of that drug. For a given drug, a 2 to 5 fold or perhaps more difference could be observed

in the oral bioavailability of a drug depending upon the nature and type of dosage form. Such a difference is due to the relative rate at which a particular dosage form releases the drug to the biological fluids and the membrane. The relative rate at which a drug from a dosage form is presented to the body depends upon the complexity of dosage form. The more complex a dosage form, greater the number of rate-limiting steps and greater the potential for bioavailability problems.

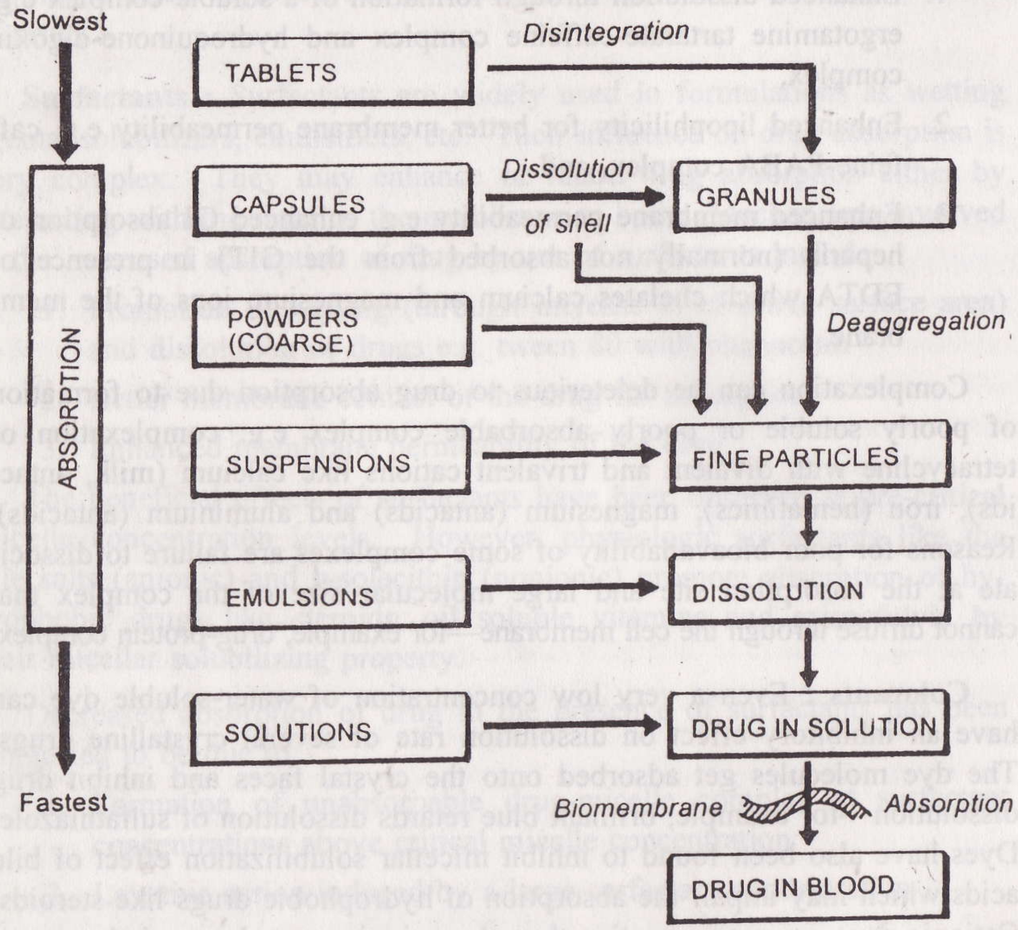

Fig. 2.20 Course of events that occur following oral administration of various dosage forms

The rate at which a particular dosage form releases the drug following administration is given in Fig. 2.20.

As a general rule, the bioavailability of a drug from various dosage forms decreases in the following order : *Solutions > Emulsions > Suspensions > Capsules > Tablets > Coated Tablets > Enteric Coated Tablets > Sustained Release Products*. Thus, absorption of a drug from solution is fastest with least potential for bioavailability problems whereas absorption from a sustained release product is slowest with greatest bioavailability risk.

Several factors, especially the excipients which influence bioavailability of a drug from its dosage form, have been discussed earlier.

Solutions : A drug in a solution (syrups, elixirs, etc.) is most rapidly absorbed since the major rate-limiting step, drug dissolution, is absent. Factors that influence bioavailability of a drug from solution dosage form include—the nature of solvent (aqueous, water miscible, etc.), viscosity, surfactants, solubilizers, stabilizers, etc. Quite often, dilution of a drug in solution with GI fluids results in precipitation of drug as fine particles which generally dissolve rapidly. Factors that limit the formulation of a drug in solution form include stability, solubility, taste, cost of the product, etc.

Emulsions : Emulsion dosage forms have been found to be superior to suspensions in administering poorly aqueous soluble lipophilic drugs. It was observed with indoxole (an NSAID) that when it is dissolved in a vegetable oil and emulsified in water, absorption increases 3 fold over its aqueous suspension. Emulsion dosage form present a large surface area of oil to the GIT for absorption of a drug. Scientists have claimed that a drug administered in oily vehicle (emulsified and solubilized in the GIT by bile salts to form mixed micelles) can direct the distribution of drug directly into the lymphatic system thereby avoiding the hepatic portal vein and first-pass metabolism.

Suspensions : The major rate-limiting step in the absorption of a drug from suspension dosage form is drug dissolution which is generally rapid due to the large surface area of the particles. Important factors in the bioavailability of a drug from suspensions include particle size, polymorphism, wetting agents, viscosity of the medium, suspending agents, etc.

Powders : Though powders are superior to tablets and capsules, they are not in use nowadays due to handling and palatability problems. Major factors to be considered in the absorption of a drug from powders are particle size, polymorphism, wettability, etc.

Capsules : Powders and granules are popularly administered in hard gelatin capsules whereas viscous fluids and oils in soft elastic shells. Factors of importance in case of hard gelatin capsules include drug particle size, density, polymorphism, intensity of packing and influence of diluents and excipients. Hydrophilic diluents like lactose improve wettability, deaggregation and dispersion of poorly aqueous soluble drugs whereas inhibitory effect is observed with hydrophobic lubricants like magnesium stearate. A hydrophobic drug with a fine particle size in capsule results in a decrease in porosity of powder bed and thus, decreased penetrability by

the solvent with the result that clumping of particle occurs. This can be overcome by incorporating a large amount of hydrophilic diluent (upto 50%), a small amount of wetting agent cum lubricant such as SLS (upto 1%) and/or by wet granulation to convert an impermeable powder bed to the one having good permeability. Other factors of importance include possible interaction between the drug and the diluent (e.g. tetracycline-DCP) and between drug and gelatin shell. The influence of capsule processing factors on drug dissolution and bioavailability have already been discussed.

Soft elastic capsules as such dissolve faster than hard gelatin capsules and tablets and show better drug availability from oily solutions, emulsions or suspensions of medicaments (especially hydrophobic drugs). One of the best examples of this is the faster dissolution of indoxole (equivalent to that of an emulsion dosage form) when formulated as softgel in comparison to hard gelatin capsule and aqueous suspension. Such poorly soluble drugs can be dissolved in PEG or other suitable solvent with the aid of surfactants and encapsulated without difficulty. Softgels are thus of particular use where the drug dose is low, drug is lipophilic or when oily or lipid based medicaments are to be administered. A problem with softgels is the high water content of the shell (above 20%). This moisture migrates into the shell content and crystallization of drug occurs during the drying stage resulting in altered drug dissolution characteristics.

Tablets : Compressed tablets are the most widely used convenience and cost effective dosage forms. A schematic representation of disintegration, deaggregation, dissolution and absorption of a drug from a tablet dosage form is shown in Fig. 2.21.

The bioavailability problems with tablets arise from the reduction in the effective surface area due to granulation and subsequent compression into a dosage form. Since dissolution is most rapid from primary drug particles due to their large surface area, disintegration of a tablet into granules and subsequent deaggregation of granules into fine particles is very important. A number of formulation and processing factors influencing these steps and also the physicochemical properties of drug substance that influence bioavailability have already been discussed in the earlier sections of this chapter.

Coated Tablets : In addition to factors that influence drug release from compressed tablets, the coating acts as yet another barrier which must first dissolve or disrupt to give way to disintegration and dissolution of tablet. Of the two types of coatings, the film coat, which is thin, dissolves rapidly and does not significantly affect drug absorption. The

sugar coat which though soluble, is generally tough and takes longer to dissolve. The sealing coat which is generally of shellac, is most deleterious. Press coated tablets may be superior to sugar coated tablets in such cases.

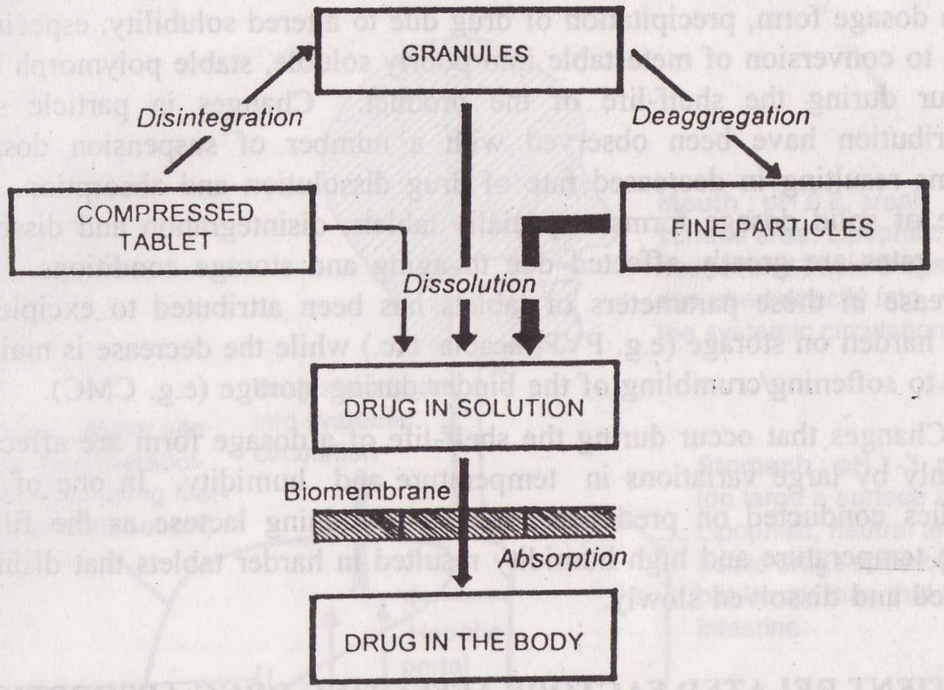

Fig. 2.21 Sequence of events in the absorption of a drug from tablet dosage form

Enteric Coated Tablets : Enteric coated tablets have great potential in creating bioavailability problems because the coat dissolves only in the alkaline pH of the intestine and it may take as long as 2 to 4 hours for such a tablet to empty from the stomach into the intestine depending upon the meals and the GI motility. Hence, the pharmacologic response may eventually be delayed by as much as 6 to 8 hours. The problem of gastric emptying can, however, be overcome by enteric coating the granules or pellets and presenting them in a capsule or compressing into a tablet. The thickness of enteric coat is yet another determinant factor in drug dissolution, increasing thickness being more problematic. Aging of the dosage form also affects drug release, especially with shellac. In one of the studies, shellac coated tablets of salicylic acid stored for 2 years showed a 60% decrease in the peak plasma level.

Sustained Release Products : Drug release from such products is most unpredictable, the problems ranging from dose dumping to unsatisfactory or no drug release at all. However, with the development of newer innovations and technologies, it is becoming increasingly reliable and the results reproducible with little intersubject variations.

Product Age and Storage Conditions

A number of changes, especially in the physicochemical properties of a drug in dosage form, can result due to aging and alterations in storage conditions which can adversely affect bioavailability. With solution dosage form, precipitation of drug due to altered solubility, especially due to conversion of metastable into poorly soluble, stable polymorph can occur during the shelf-life of the product. Changes in particle size distribution have been observed with a number of suspension dosage forms resulting in decreased rate of drug dissolution and absorption. In case of solid dosage forms, especially tablets, disintegration and dissolution rates are greatly affected due to aging and storage conditions. An increase in these parameters of tablets has been attributed to excipients that harden on storage (e.g. PVP, acacia, etc.) while the decrease is mainly due to softening/crumbling of the binder during storage (e.g. CMC).

Changes that occur during the shelf-life of a dosage form are affected mainly by large variations in temperature and humidity. In one of the studies conducted on prednisone tablets containing lactose as the filler, high temperature and high humidity resulted in harder tablets that disintegrated and dissolved slowly.

PATIENT RELATED FACTORS AFFECTING DRUG ABSORPTION

Before dealing with the patient related factors influencing bioavailability of a drug, the anatomy and physiology of the gastrointestinal tract will be discussed briefly.

Gastrointestinal tract

The gastrointestinal tract (GIT) comprises of a number of components, their primary function being secretion, digestion and absorption. The mean length of the entire GIT is 450 cm. The major functional components of the GIT are stomach, small intestine (duodenum, jejunum and ileum) and large intestine (colon) which grossly differ from each other in terms of anatomy, function, secretions and pH (Fig. 2.22 and Table 2.5).

The entire length of the GI mucosa from stomach to large intestine is lined by a thin layer of mucopolysaccharides (mucus/mucin) which normally acts as an impermeable barrier to the particulates such as bacteria, cells or food particles.

Stomach : The stomach is a bag like structure having a smooth mucosa and thus small surface area. Its acidic pH, due to secretion of HCl, favors absorption of acidic drugs if they are soluble in the gastric fluids since they are unionized to a large extent in such a pH. The gastric

pH aids dissolution of basic drugs due to salt formation and subsequent ionization which are therefore absorbed to a lesser extent from stomach because of the same reason.

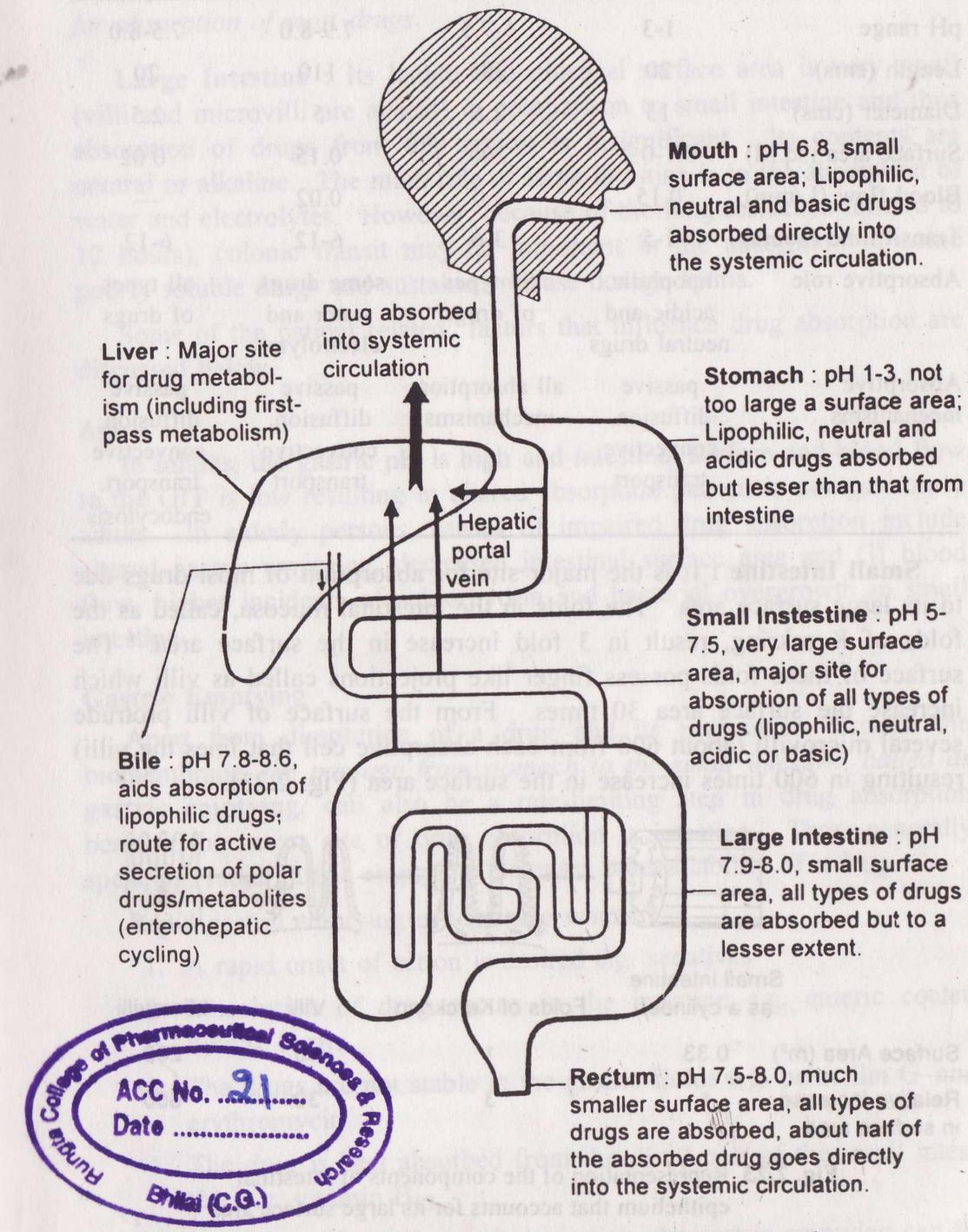

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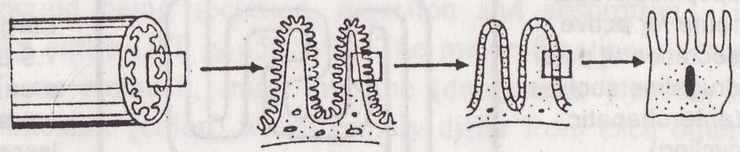
Fig. 2.22 Schematic representation of the GIT and different sites of drug absorption

TABLE 2.5 **Anatomical and Functional Differences Between the Important Regions of the GIT**

	Stomach	*Small Intestine*	*Large Intestine*	*Rectum*
pH range	1-3	5-7.5	7.9-8.0	7.5-8.0
Length (cms)	20	285	110	20
Diameter (cms)	15	2.5	5	2.5
Surface area (sq.M)	0.1-0.2	200	0.15	0.02
Blood flow (L/min)	0.15	1.0	0.02	—
Transit time (hours)	1-5	3-6	6-12	6-12
Absorptive role	lipophilic, acidic and neutral drugs	all types of drugs	some drugs, water and electrolytes	all types of drugs
Absorptive mechanisms	passive diffusion, convective transport	all absorption mechanisms	passive diffusion, convective transport	passive diffusion, convective transport, endocytosis

Small Intestine : It is the major site for absorption of most drugs due to its large surface area. The folds in the intestinal mucosa, called as the folds of Kerckring, result in 3 fold increase in the surface area. The surface of these folds possess finger like projections called as villi which increase the surface area 30 times. From the surface of villi protrude several microvilli (about 600 from each absorptive cell that lines the villi) resulting in 600 times increase in the surface area (Fig. 2.23).



	Small intestine as a cylinder	Folds of Kerckring	Villi	Microvilli
Surface Area (m^2)	0.33	1	10	200
Relative increase in surface area	1	3	30	600

Fig. 2.23 Representation of the components of intestinal epithelium that accounts for its large surface area

All these, combined with the great length of small intestine (approximately 285 cms), result in more than 200 square meters of surface which

is several times that of stomach. The blood flow to the small intestine is 6 to 10 times that of stomach. Moreover, the pH range of 5 to 7.5 is most favorable for most drugs to remain unionized. The peristaltic movement of intestine is slow, transit time is long, and permeability is high. Thus, a contribution of all the above factors make *intestine the best site for absorption of most drugs.*

Large Intestine : Its length and mucosal surface area is very small (villi and microvilli are absent) in comparison to small intestine and thus absorption of drugs from this region is insignificant. Its contents are neutral or alkaline. The main role of large intestine is in the absorption of water and electrolytes. However, because of the long residence time (6 to 12 hours), colonic transit may be important in the absorption of some poorly soluble drugs and sustained release dosage forms.

Some of the patient related factors that influence drug absorption are discussed below.

Age

In infants, the gastric pH is high and intestinal surface and blood flow to the GIT is low resulting in altered absorption pattern in comparison to adults. In elderly persons, causes of impaired drug absorption include altered gastric emptying, decreased intestinal surface area and GI blood flow, higher incidents of achlorhydria and bacterial overgrowth in small intestine.

Gastric Emptying

Apart from dissolution of a drug and its permeation through the biomembrane, *the passage from stomach to the small intestine, called as* **gastric emptying**, can also be a rate-limiting step in drug absorption because the major site of drug absorption is intestine. Thus, generally speaking, rapid gastric emptying increases bioavailability of a drug.

Rapid gastric emptying is advisable where:

1. A rapid onset of action is desired e.g. sedatives
2. Dissolution of drug occurs in the intestine e.g. enteric coated dosage forms
3. The drugs are not stable in the gastric fluids e.g. penicillin G and erythromycin
4. The drug is best absorbed from the distal part of the small intestine e.g. vitamin B_{12}

For better drug dissolution and absorption, the gastric emptying can be promoted by taking the drug on empty stomach. Since gastric emptying

is altered by several factors due to which large intersubject variations are observed, all biopharmaceutic studies that require the drug to be taken orally, are performed in volunteers on empty stomach.

Gastric emptying of a drug is delayed by co-administering food because unless the gastric contents are fluid enough or the size of the solid particles is reduced below 2 mm, its passage through the pylorus into the intestine is not possible. Delay in gastric emptying is recommended in particular where:

1. The food promotes drug dissolution and absorption e.g. griseofulvin
2. Disintegration and dissolution of dosage form is promoted by gastric fluids
3. The drugs dissolve slowly e.g. griseofulvin
4. The drugs irritate the gastric mucosa e.g. aspirin, phenylbutazone and nitrofurantoin
5. The drugs are absorbed from the proximal part of the small intestine and prolonged drug-absorption site contact is desired e.g. vitamin B_2 and vitamin C

Gastric emptying is a first-order process. Several parameters are used to quantify gastric emptying:

1. **Gastric emptying rate** *is the speed at which the stomach contents empty into the intestine.*
2. **Gastric emptying time** *is the time required for the gastric contents to empty into the small intestine.* Longer the gastric emptying time, lesser the gastric emptying rate.
3. **Gastric emptying $t_{1/2}$** *is the time taken for half the stomach contents to empty.*

In vivo gastric emptying of a drug (so also the disintegration of dosage form and drug release) can be studied by using radio-opaque contrast materials like barium sulfate or tagging the drug with a radioisotope and scanning the stomach at regular intervals of time.

A large number of factors influence gastric emptying as discussed below.

1. **Volume of meal :** Larger the bulk of the meals, longer the gastric emptying time. However, an initial rapid rate of emptying is observed with a large meal volume and an initial lag phase in emptying of a small volume meal. Since gastric emptying is a first-order process, a plot of log of volume of contents remaining in the stomach versus time yields a straight line.

2. **Composition of meal :** Predictably, the rate of gastric emptying for various food materials is in the following order: *carbohydrates > proteins > fats*. Fats promote secretion of bile which too has an inhibitory effect on gastric emptying. Delayed gastric emptying as observed with fatty meals, is beneficial for the absorption of poorly soluble drugs like griseofulvin.
3. **Physical state and viscosity of meal :** Liquid meals take less than an hour to empty whereas a solid meal may take as long as 6 to 7 hours. Viscous materials empty at a slow rate in comparison to less viscous materials.
4. **Temperature of the meal :** High or low temperature of the ingested fluid (in comparison to body temperature) reduce the gastric emptying rate.
5. **Gastrointestinal pH :** Gastric emptying is retarded at low stomach pH and promoted at higher or alkaline pH. Chemicals that affect gastrointestinal pH also alter gastric emptying. The inhibitory effect of various acids on gastric emptying decreases with increase in molecular weight and is in the following order: *HCl > acetic > lactic > tartaric > citric*. With alkaline solutions, a low base concentration (1% $NaHCO_3$) increases the gastric emptying rate more than the one of higher concentration (5%).
6. **Electrolytes and osmotic pressure :** Water, isotonic solutions, and solutions of low salt concentration empty the stomach rapidly whereas a higher electrolyte concentration decreases gastric emptying rate.
7. **Body posture :** Gastric emptying is favored while standing and by lying on the right side since the normal curvature of the stomach provides a *downhill* path whereas lying on the left side or in supine position retards it.
8. **Emotional state :** Stress and anxiety promote gastric motility whereas depression retards it.
9. **Exercise :** Vigorous physical training retards gastric emptying.
10. **Disease states :** Diseases like gastroenteritis, gastric ulcer, pyloric stenosis, diabetes and hypothyroidism retard gastric emptying. Partial or total gastrectomy, duodenal ulcer and hyperthyroidism promote gastric emptying rate.
11. **Drugs :** Drugs that retard gastric emptying include poorly soluble antacids (aluminium hydroxide), anticholinergics (atropine, propantheline), narcotic analgesics (morphine) and tricyclic anti-

depressants (imipramine, amitriptyline). Metoclopramide, domperidone and cisapride (antiemetics) stimulate gastric emptying.

The passage of drug through the esophagus, called as **esophageal transit**, is important in persons who swallow the solid dosage form lying down in supine position or with little or no water. In such cases, the dosage form remains lodged in the esophagus and disintegrates slowly which may result in delayed absorption or local damage to the mucosa from drugs like NSAIDs.

Intestinal Transit

Since small intestine is the major site for absorption of most drugs, long intestinal transit time is desirable for complete drug absorption (*see* Table 2.6).

TABLE 2.6

Transit Time for Contents from Different Regions of Intestine

Intestinal region	*Transit time*
Duodenum	5 minutes
Jejunum	2 hours
Ileum	3 to 6 hours
Cecum	0.5 to 1 hour
Colon	6 to 12 hours

The residence time depends upon the intestinal motility or contractions. The *mixing movement* of the intestine that occurs due to peristaltic contractions promote drug absorption, firstly, by increasing the drug-intestinal membrane contact, and secondly, by enhancing drug dissolution especially of poorly soluble drugs, through induced agitation.

Delayed intestinal transit is desirable for:

1. Drugs that dissolve or release slowly from their dosage form (sustained release products)
2. Drugs that dissolve only in the intestine (enteric coated formulations)
3. Drugs absorbed from specific sites in the intestine (several B vitamins)

Intestinal transit time is relatively short in comparison to the gastric emptying time and as the contents move down the intestine into the colon, its viscosity gradually increases due to absorption of water and electrolytes which limits the design of sustained release products of drugs having short biological half-lives.

Like gastric emptying, intestinal transit is influenced by several factors like food, drugs and diseases. Food, decreased digestive secretions and pregnancy retard intestinal transit whereas diarrhea promotes it. Drugs like metoclopramide that promote gastric emptying and intestinal transit enhance absorption of rapidly soluble drugs. Laxatives also promote the rate of intestinal transit. Drugs such as anticholinergics that retard gastric and intestinal transit promote absorption of poorly soluble drugs—for example, propantheline shows a 100%, 50% and 30% rise in the absorption of vitamin B_2, nitrofurantoin and hydrochlorothiazide respectively.

Gastrointestinal pH

A tremendous 10^7 fold difference in the hydrogen ion concentration is observed between the gastric and colon fluids. The GI pH generally increases gradually as one moves down the stomach to the colon and rectum (*see* Fig. 2.22). GI fluid pH influence drug absorption in several ways:

1. Disintegration : The disintegration of some dosage forms is pH sensitive. With enteric coated formulations, the coat dissolves only in the intestine followed by disintegration of the tablet.

2. Dissolution : A large number of drugs are either weak acids or weak bases whose solubility is greatly affected by pH. A pH that favors formation of salt of the drug enhances the dissolution of that drug. Since drug dissolution is one of the important rate-determining steps in drug absorption, GI pH is of great significance in the oral bioavailability of drugs. Weakly acidic drugs dissolve rapidly in the alkaline pH of the intestine whereas basic drugs dissolve in the acidic pH of the stomach. Since the primary site for absorption of most drugs is small intestine, the poorly water-soluble basic drugs must first dissolve in the acidic pH of stomach before moving into the intestine.

3. Absorption : Depending upon the drug pK_a and whether its an acidic or a basic drug, the GI pH influences drug absorption by determining the amount of drug that would exist in the unionized form at the site of absorption. This topic has already been dealt with in sufficient details under pH-partition hypothesis.

4. Stability : GI pH also influences the chemical stability of drugs. The acidic stomach pH is known to affect degradation of penicillin G and erythromycin. This can be overcome by preparing prodrugs of such drugs that do not degrade or dissolve in acidic pH e.g. carindacillin and erythromycin estolate. With basic drugs, formation of insoluble drug hydroxide in the alkaline pH of the intestine has been observed.

Disease States

Several disease states can influence the rate and extent of drug absorption. The 3 major classes of disease states that can influence the bioavailability of a drug are:

1. Gastrointestinal diseases,
2. Cardiovascular diseases, and
3. Hepatic diseases.

1. Gastrointestinal diseases : A number of pathologic conditions of the GIT can influence changes in drug absorption pattern, namely:

(a) *Altered GI motility* : (discussed earlier)

(b) *Gastrointestinal diseases and infections*: The influence of achlorhydria (decreased gastric acid secretion and increased stomach pH) on gastric emptying and drug absorption, especially that of acidic drugs (decreased absorption, e.g. aspirin) has been studied. Two of the intestinal disorders related with *malabsorption syndrome* that influence drug availability are *celiac disease* (characterized by destruction of villi and microvilli) and *Crohn's disease*. Abnormalities associated with celiac disease include increased gastric emptying rate and GI permeability, altered intestinal drug metabolism, steatorrhea (impaired secretion of bile thus affecting absorption of lipophilic drugs and vitamins) and reduced enterohepatic cycling of bile salts, all of which can significantly impair drug absorption. Conditions associated with Crohn's disease that can alter absorption pattern are altered gut wall microbial flora, decreased gut surface area and intestinal transit rate. Malabsorption is also induced by drugs such as antineoplastics and alcohol which increase permeability of agents not normally absorbed. GI infections like shigellosis, gastroenteritis, cholera and food poisoning also result in malabsorption. Colonic diseases such as colitis, amebiasis and constipation can also alter drug absorption.

(c) *Gastrointestinal surgery* : Gastrectomy can result in drug dumping in the intestine, osmotic diarrhea and reduced intestinal transit time. Intestinal surgery also influences drug absorption for predictable reasons.

2. Cardiovascular diseases : Several changes associated with congestive cardiac failure influence bioavailability of a drug viz. edema of the intestine, decreased blood flow to the GIT and gastric emptying rate and altered GI pH, secretions and microbial flora.

3. Hepatic diseases : Disorders such as hepatic cirrhosis influence bioavailability mainly of drugs that undergo considerable first-pass hepatic metabolism e.g. propranolol.

Blood Flow to the GIT

The GIT is extensively supplied by blood capillary network and the lymphatic system. The absorbed drug can thus be taken up by the blood or the lymph. Since the blood flow rate to the GIT (splanchnic circulation) is 500 to 1000 times (28% of cardiac output) more than the lymph flow, most drugs reach the systemic circulation via blood whereas only a few drugs, especially low molecular weight, lipid soluble compounds are removed by lymphatic system.

The high perfusion rate of GIT ensures that once the drug has crossed the membrane, it is rapidly removed from the absorption site thus maintaining the *sink conditions* and concentration gradient for continued drug absorption.

For drugs that have high permeation rates, e.g. highly lipid soluble drugs or drugs absorbed through pores, the GI perfusion rate could be a rate-limiting step in the absorption, e.g. tritiated water. This is not so in the case of drugs having poor permeability coefficient, e.g. ribitol. Blood flow is also important for actively absorbed drugs since oxygen and energy is required for transportation.

Food influences blood flow to the GIT. The perfusion rate increases after meals and persists for few hours but drug absorption is not influenced significantly.

Gastrointestinal Contents

A number of GI contents can influence drug absorption as discussed below:

1. Food-drug interactions : Presence of food may either delay, reduce, increase or may not affect drug absorption (Table 2.7).

TABLE 2.7 **Influence of Food on Drug Absorption**

Delayed	*Decreased*	*Increased*	*Unaffected*
Aspirin	Penicillins	Griseofulvin	Methyldopa
Paracetamol	Erythromycin	Nitrofurantoin	Propylthiouracil
Diclofenac	Ethanol	Diazepam	Sulfasomidine
Nitrofurantoin	Tetracyclines	Actively absorbed water soluble vitamins	
Digoxin	Levodopa		
	Iron		

Food-drug interactions may be due to the influence of food on physiologic functions (alterations in the GI emptying rate, GI fluid secretions, pH, blood flow and absorptive processes) and/or a consequence of physicochemical interaction with the drug (alteration in drug dissolution profile, complexation and adsorption).

As a general rule, *drugs are better absorbed under fasting conditions and presence of food retards or prevents it.* Food does not significantly influence absorption of a drug taken half an hour or more before meals and two hours or more after meals. Delayed or decreased drug absorption by the food could be due to one or more of the several mechanisms:

(a) Delayed gastric emptying, affecting drugs unstable in the stomach e.g. penicillins, or preventing the transit of enteric coated tablets into the intestine which may be as long as 6 to 8 hours

(b) Formation of a poorly soluble, unabsorbable complex e.g. tetracycline-calcium

(c) Increased viscosity due to food thereby preventing drug dissolution and/or diffusion towards the absorption site

Increased drug absorption following a meal could be due to one or more of the under mentioned reasons:

(a) Increased time for dissolution of a poorly soluble drug

(b) Enhanced solubility due to GI secretions like bile

(c) Prolonged residence time and absorption site contact of the drug e.g. water-soluble vitamins

(d) Increased lymphatic absorption e.g. acitretin

The specific meal components also have an influence on drug absorption. Meals high in fat aid solubilization of poorly aqueous soluble drugs e.g. isotretinoin. Food high in proteins inhibit absorption of levodopa. An interesting example of influence of high protein meal is that of increased oral availability of propranolol to which two reasons were attributed—firstly, such a meal increases the hepatic blood flow due to which the drug can bypass first-pass hepatic metabolism (propranolol is a drug with high hepatic extraction), and secondly, it promotes blood flow to the GIT thus aiding drug absorption.

2. Fluid volume : Administration of a drug with large fluid volume results in better dissolution, rapid gastric emptying and enhanced absorption—for example, erythromycin is better absorbed when taken with a glass of water under fasting condition than when taken with meals.

3. Interaction of drug with normal GI constituents : The GIT contains a number of normal constituents such as mucin, bile salts and

enzymes which influence drug absorption. Mucin, a protective mucopolysaccharide that lines the GI mucosa, interacts with streptomycin and certain quaternary ammonium compounds and retards their absorption. It also acts as a barrier to diffusion of drugs. The bile salts aid solubilization and absorption of lipid soluble drugs like griseofulvin and vitamins A, D, E and K on one hand and on the other, decreases absorption of neomycin and kanamycin by forming water insoluble complexes.

The influence of GI enzymes on absorption will be discussed later.

4. Drug-Drug interactions in the GIT : Like food-drug interactions, drug-drug interactions can either be physicochemical or physiological.

(a) *Physicochemical drug-drug interactions* can be due to—

Adsorption : antidiarrheal preparations containing adsorbents like attapulgite or kaolin-pectin retard/prevent absorption of a number of drugs co-administered with them e.g. promazine and lincomycin.

Complexation : antacids and/or mineral substitutes containing heavy metals such as aluminium, calcium, iron, magnesium or zinc retard absorption of tetracyclines through formation of unabsorbable complexes. The anion exchange resins, cholestyramine and colestipol, bind cholesterol metabolites, bile salts and a number of drugs in the intestine and prevent their absorption.

pH change : basic drugs dissolve in gastric pH; co-administration of such drugs, e.g. tetracycline with antacids such as sodium bicarbonate results in elevation of stomach pH and hence decreases dissolution rate or causes precipitation of drug.

(b) *Physiologic drug-drug interactions* can be due to following mechanisms—

Decreased GI transit : anticholinergics such as propantheline retard GI motility and promote absorption of drugs like ranitidine and digoxin, whereas delay absorption of paracetamol and sulfamethoxazole.

Increased gastric emptying : metoclopramide promotes GI motility and enhances absorption of tetracycline, pivampicillin and levodopa.

Altered GI metabolism : antibiotics inhibit bacterial metabolism of drugs, e.g. erythromycin enhances efficacy of digoxin by this mechanism. Co-administration of antibiotics with oral contraceptives like ethinyl estradiol decreases the efficacy of latter by decreasing enterohepatic cycling of steroid conjugates which otherwise are hydrolyzed by gut bacteria after biliary excretion.

Presystemic Metabolism/First-Pass Effects

For a drug administered orally, the 2 main reasons for its decreased bioavailability are:

a. Decreased absorption, and

b. First-pass/presystemic metabolism.

Before a drug reaches blood circulation, it has to pass for the first time through organs of elimination namely the GIT and the liver. *The loss of drug through biotransformation by such eliminating organs during its passage to systemic circulation is called as* **first-pass** or **presystemic metabolism**. The diminished drug concentration or rarely, complete absence of the drug in plasma after oral administration is indicative of first-pass effects. The 4 primary systems which affect presystemic metabolism of a drug are (Fig. 2.24):

1. Lumenal enzymes,
2. Gut wall enzymes/mucosal enzymes,
3. Bacterial enzymes, and
4. Hepatic enzymes.

1. Lumenal enzymes : These are the enzymes present in the gut fluids and include enzymes from intestinal and pancreatic secretions. The latter contains hydrolases which hydrolyze ester drugs like chloramphenicol palmitate into active chloramphenicol, and peptidases which split amide linkages and inactivate protein/polypeptide drugs. Thus, one of the approaches to effect oral absorption of peptides is to deliver them to colon which lack peptidases.

2. Gut wall enzymes : Also called as **mucosal enzymes**, they are present in stomach, intestine and colon. Alcohol dehydrogenase (ADH) is an enzyme of stomach mucosa that inactivates ethanol. Intestinal mucosa contains both phase I and phase II (predominant) enzymes, e.g. sulfation of ethinyl estradiol and isoprenaline. The colonic mucosa also contain both phase I and phase II enzymes. However, it is only the enzymes of the proximal small intestine that are most active.

3. Bacterial enzymes : The GI microflora is scantily present in stomach and small intestine and is rich in colon. Hence, most orally administered drugs remain unaffected by them. The colonic microbes generally render a drug more active or toxic on biotransformation—for example sulfasalazine, a drug used in ulcerative colitis, is hydrolyzed to sulfapyridine and 5-amino salicylic acid by the microbial enzymes of the colon. An important role of intestinal microflora is that in enterohepatic cycling. Their enzymes hydrolyze the conjugates of drugs actively secreted

via bile such as glucuronides of digoxin and oral contraceptives. The free drugs are reabsorbed into the systemic circulation.

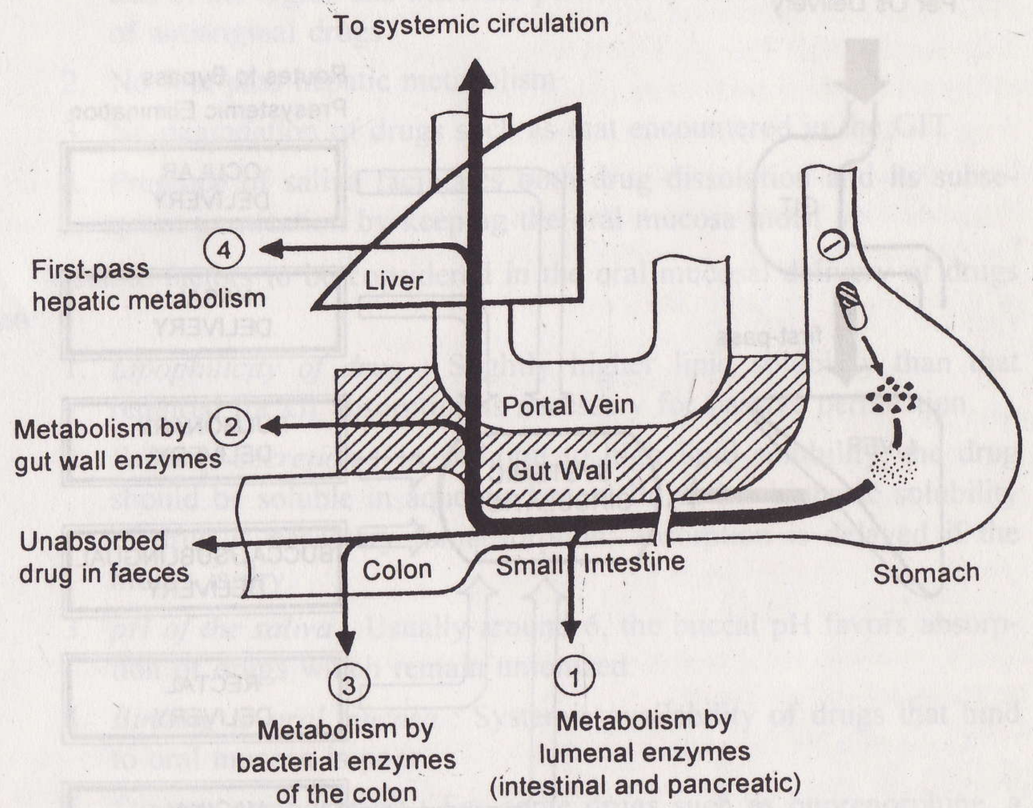


Fig. 2.24 Different sites of presystemic metabolism

4. Hepatic enzymes : Several drugs undergo first-pass hepatic metabolism, the highly extracted ones being isoprenaline, propranolol, alprenolol, pentoxifylline, nitroglycerine, diltiazem, nifedipine, lidocaine, morphine, etc.

ABSORPTION OF DRUGS FROM NON PER OS EXTRAVASCULAR ROUTES

Drug absorption from all nonoral extravascular sites is governed by the same factors that influence absorption from GIT viz. the physicochemical properties of drug, formulation factors, and anatomic, physiologic and pathologic characteristics of the patient. This is so because the barrier to transport of drugs into the systemic circulation from all such sites is a lipoidal membrane similar to the GI barrier and the major mechanism in the absorption is passive diffusion. One of the major advantages of administering drugs by noninvasive transmucosal routes such as nasal, buccal, rectal, etc. is that greater systemic availability is

attainable for drugs normally subjected to extensive presystemic elimination due to GI degradation and/or hepatic metabolism (Fig. 2.25). Moreover,

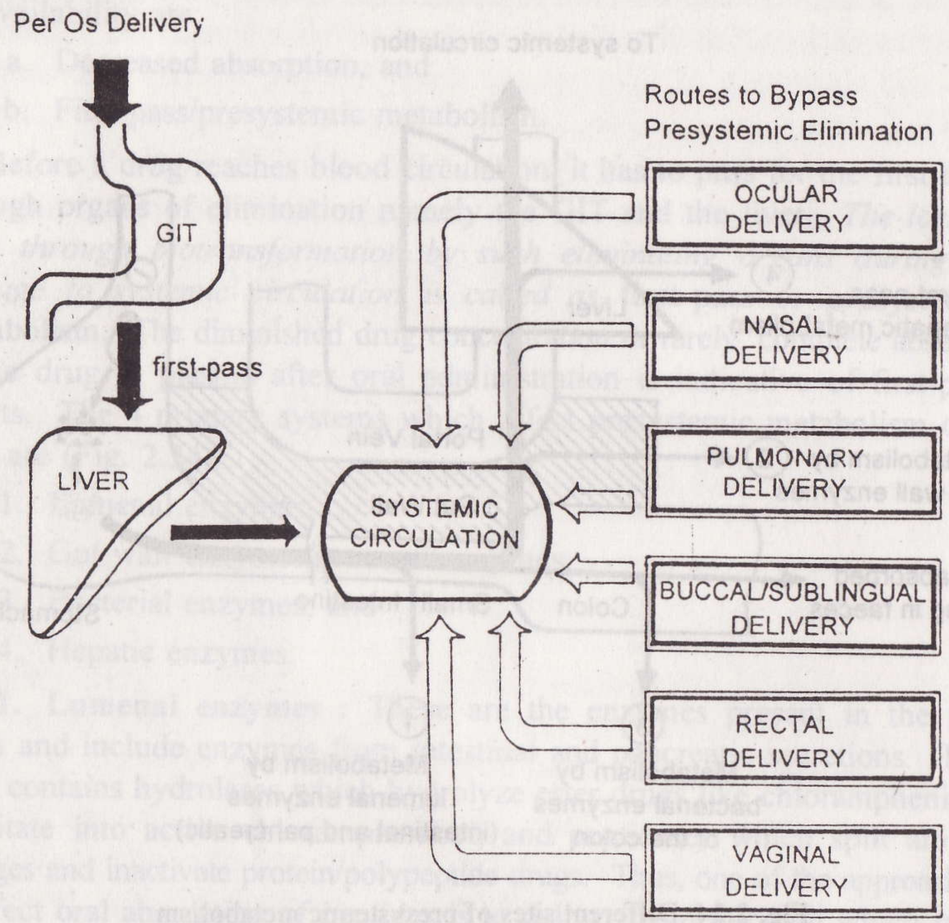


Fig. 2.25 Various transmucosal noninvasive routes of drug administration to bypass presystemic elimination in GIT/liver

peptide and protein drugs can also be delivered by such routes. Some of the more important biopharmaceutic and pharmacokinetic principles that must be considered for nonoral absorption will be discussed here.

Buccal/Sublingual Administration

The two sites for oral mucosal delivery of drugs are:

1. **Sublingual route** : *The drug is placed under the tongue and allowed to dissolve*
2. **Buccal route** : *The medicament is placed between the cheek and the gum*

The barrier to drug absorption from these routes is the epithelium of oral mucosa. Passive diffusion is the major mechanism for absorption of most drugs; nutrients may be absorbed by carrier-mediated processes.

Some of the advantages of these routes are:

1. Rapid absorption and higher blood levels due to high vascularization of the region and therefore particularly useful for administration of antianginal drugs
2. No first-pass hepatic metabolism
3. No degradation of drugs such as that encountered in the GIT
4. Presence of saliva facilitates both drug dissolution and its subsequent permeation by keeping the oral mucosa moist

Notable factors to be considered in the oral mucosal delivery of drugs are:

1. *Lipophilicity of drug* : Slightly higher lipid solubility than that required for GI absorption is necessary for passive permeation.
2. *Salivary secretion* : In addition to high lipid solubility, the drug should be soluble in aqueous buccal fluids i.e. biphasic solubility of drug is necessary for absorption; absorption is delayed if the mouth is dry.
3. *pH of the saliva* : Usually around 6, the buccal pH favors absorption of drugs which remain unionized.
4. *Binding to oral mucosa* : Systemic availability of drugs that bind to oral mucosa is poor.
5. *Storage compartment* : For some drugs such as buprenorphine, a storage compartment in the buccal mucosa appears to exist which is responsible for the slow absorption of drugs.
6. *Thickness of oral epithelium* : Sublingual absorption is faster than buccal since the epithelium of former region is thinner and immersed in a larger volume of saliva.

Factors that limit drug administration by these routes are: limited mucosal surface area (thus only a small dose can be administered), concern for taste of the medicament and discomfort (since the region is highly innervated).

Examples of drugs administered by oral mucosal route include antianginals like nitrites and nitrates, antihypertensives like nifedipine, analgesics like morphine and bronchodilators like fenoterol. Certain steroids like estradiol and peptides like oxytocin can also be administered. Apart from tablets, the drugs may be administered as a buccal spray especially to children.

Rectal Administration

Despite its diminished popularity, the rectal route of drug administration is still an important route for children and old patients. The drugs

may be administered as solutions (microenemas) or suppositories. Absorption is more rapid from solutions than from suppositories but is more variable in comparison to oral route. Irritating suppository bases such as PEG promotes defecation and drug loss. Presence of fecal matter retards drug absorption. Though highly vascularized, absorption is slower because of limited surface area. The pH of rectal fluids (around 8) also influences drug absorption according to pH-partition hypothesis. Absorption of drugs from the lower half of rectum bypasses presystemic hepatic metabolism. Drugs administered by this route include aspirin, paracetamol, theophylline, few barbiturates, etc.

Topical Administration

Excluding the respiratory tract's contact with the inhaled air, the skin is virtually the sole human surface directly interfacing the body with the external environment. It is the largest organ of the body weighing approximately 2 Kg and 2 meter square in area and receives about 1/3rd of total blood circulating through the body. Though tolerant to many chemicals, topically contacted xenobiotics can evoke both local and systemic effects. *When topically applied drugs are meant to exert their effects systemically, the mode of administration is called as* **percutaneous or transdermal delivery**.

Anatomically, the skin is made of 3 distinct layers—the epidermis, the dermis and the subcutaneous fat tissue. Epidermis is the nonvascular, multilayered outer region of the skin. The dermis or true skin is a highly vascular region; drugs permeating to this region are taken up into the systemic circulation and sink conditions are maintained.

The principal barrier to the entry of xenobiotics is the most superficial layer of epidermis called as **stratum corneum**. It is composed of dead, keratinized, metabolically inactive horny cells that act as the major rate-limiting barrier to passive diffusion of drugs. In order to act either locally or systemically, a topically applied drug may diffuse through the skin by hair follicles, sweat glands or sebaceous glands but permeation through the multiple lipid bilayers of stratum corneum is the dominant pathway though the rate is very slow. Several factors influence passive percutaneous absorption of drugs:

1. *Thickness of stratum corneum* : absorption is very slow from regions such as foot and palm where the skin has thickened stratum corneum.
2. *Presence of hair follicles* : absorption is rapid from regions where numerous hair follicles exist e.g. scalp.

3. *Trauma*—cuts, rashes, inflammation, mild burns or other conditions in which the stratum corneum is destroyed, promote drug absorption.
4. *Hydration of skin* : soaking the skin in water or occluding it by using emollients, plastic film or dressing, promote hydration of skin and drug absorption.
5. *Environment humidity and temperature* : higher humidity and temperature increase both the rate of hydration as well as local blood flow and hence drug absorption.
6. *Age* : gross histological changes take place as the skin ages. Aged skin is more prone to allergic and irritant effects of topically contacted chemicals as a result of hardening of blood vessels. Infants absorb drug through skin as efficiently as adults. Their ratio of surface area to body weight is 3 times that of adults; hence, systemic toxicity of topically applied drugs is of particular concern in infants.
7. *Grooming* : the frequency and vigor with which one bathes and the type of soap that is used also contribute to variability in drug absorption.
8. *Exposure to chemicals* : occupational exposure to solvents can accelerate shedding of epidermal cells and enhance drug absorption.
9. *Vehicle or base* : the vehicle in which the drug is incorporated influences drug absorption; the one in which the drug is dissolved rather than dispersed promotes absorption.
10. *Permeation enhancers* : incorporation of certain chemicals such as DMSO, propylene glycol, azone, etc. in the topical formulations aid drug penetration.
11. *Chronic use of certain drugs* : long term use of cortisol or keratolytics like salicylic acid results in enhanced drug penetration.

Drugs that are administered percutaneously include nitroglycerine, lidocaine, betamethasone, estradiol, testosterone, etc. The route is particularly useful for drugs with low oral availability and short duration of action; the effect of the latter category of drugs is prolonged because percutaneous absorption is a slow process.

Intramuscular Administration

Absorption of drugs from i.m. sites is relatively rapid but much slower in comparison to i.v. injections. Factors that determine rate of drug absorption from i.m. sites are:

1. *Vascularity of the injection site* : the decreasing order of blood flow rate to muscular tissues in which drugs are usually injected is: *arm (deltoid) > thigh (vastus lateralis) > buttocks (gluteus maximus)*. Since blood flow rate is often the rate-limiting step in absorption of drugs from i.m. sites, most rapid absorption is from deltoid muscles and slowest from gluteal region. The absorption rate decreases in circulatory disorders such as hypotension.
2. *Lipid solubility and ionization of drug* : highly lipophilic drugs are absorbed rapidly by passive diffusion whereas hydrophilic and ionized drugs are slowly absorbed through capillary pores.
3. *Molecular size of the drug* : small molecules and ions gain direct access into capillaries through pores whereas macromolecules are taken up by the lymphatic system.
4. *Volume of injection and drug concentration* : a drug in concentrated injection and large volume is absorbed faster than when given in dilute form and small volume.
5. *pH, composition and viscosity of injection vehicle* : a solution of drug in acidic or alkaline pH (e.g. phenytoin, pH 12) or in a nonaqueous solvent such as propylene glycol or alcohol (e.g. digoxin) when injected intramuscularly result in precipitation of drug at the injection site followed by slow and prolonged absorption. Viscous vehicles such as vegetable oils also slow drug absorption. The principle can however be utilized to control rate of drug delivery.

Subcutaneous Administration

All factors that influence i.m. drug absorption are also applicable to absorption from subcutaneous site. Generally, absorption of drugs from a s.c. site is slower than that from i.m. sites due to poor perfusion, but this fact is of particular importance for the administration of drugs for which a rapid response is not desired and for drugs that degrade when taken orally e.g. insulin and sodium heparin. The rate of absorption of a drug from subcutaneous site can be increased in 2 ways:

1. *Enhancing blood flow to the injection site* : by massage, application of heat, co-administration of vasodilators locally, or by exercise, and
2. *Increasing the drug-tissue contact area* : by co-administering the enzyme hyaluronidase that breaks down the connective tissue and permits spreading of drug solution over a wide area.

Absorption can be slowed down by causing vasoconstriction through

local cooling or co-injection of a vasoconstrictor like adrenaline or by immobilization of limb. Because of relatively slow drug absorption from s.c. tissues, the region is very popular for controlled release medication like implants.

Pulmonary Administration

In principle, all drugs intended for systemic effects can be administered by inhalation since the large surface area of the alveoli, high permeability of the alveolar epithelium and rich perfusion permit extremely rapid absorption just like exchange of gases between the blood and the inspired air. However, the route has been limited for administering drugs that affect pulmonary system such as bronchodilators (salbutamol), anti-inflammatory steroids (beclomethasone) and antiallergics (cromolyn). Lipid soluble drugs are rapidly absorbed by passive diffusion and polar drugs by pore transport. Absorption of drugs whose ionization is pH sensitive is dependent upon pH of pulmonary fluids. The drugs are generally administered by inhalation either as gases (volatile/gaseous anesthetics) or aerosol. In the latter case, drug delivery to lungs is largely dependent upon the particle size of the aerosolized droplets—particles larger than 10 microns impact on the mouth, throat or upper respiratory tract mucosa and do not reach the pulmonary tree whereas very small particles (0.6 microns) from which drug absorption is rapid, penetrate rapidly but are susceptible to easy exhalation. Sometimes, the patients inability to inhale a sufficient amount of drug limits drug delivery to lungs.

Intranasal Administration

The nasal route is becoming increasingly popular for systemic delivery especially of some peptide and protein drugs. Drug absorption from nasal mucosa is as rapid as observed after parenteral administration because of its rich vasculature and high permeability. The route is otherwise used for drugs to treat local symptoms like nasal congestion, rhinitis, etc.

Two mechanisms for drug transport across the nasal mucosa have been suggested—a faster rate that is dependent upon drug lipophilicity, and a slower rate which is dependent upon drug molecular weight. In case of lipophilic drugs, rapid absorption by diffusion is observed upto 400 daltons and satisfactory absorption upto 1000 daltons. By use of permeability enhancers such as surfactants, even a drug with molecular weight of 6000 daltons shows reasonable bioavailability. For polar compounds primarily absorbed by pore transport, an upper threshold of 200 daltons is the limiting factor. Other factors that may influence nasal permeation of drugs include pH of nasal secretions (5.5 to 6.5) and its viscosity, and pathological conditions such as common cold and rhinitis. Drugs known

to influence cleansing function of nasal cilia should not be administered by this route.

Intraocular Administration

Topical application of drugs to the eyes is mainly meant for local effects such as mydriasis, miosis, anesthesia or treatment of infections, glaucoma, etc. Sterile aqueous solutions of drugs are widely used ophthalmic formulations and administered in the conjunctival *cul-de-sac*. The barrier to intraocular penetration of drugs is the cornea which posseses both hydrophilic and lipophilic characteristics. Thus, for optimum intraocular permeation, drugs should possess biphasic solubility. The pH of lacrimal fluid influences absorption of weak electrolytes such as pilocarpine. On the other hand, pH of the formulation influences lacrimal output—higher pH decreases tear flow and promotes drug absorption whereas lower pH solutions increase lacrimation and subsequent drug loss due to drainage. Rate of blinking also influences drainage loss. The volume of fluid instilled into the eyes also affect bioavailability and effectiveness of the drug. Normally, the human eye can hold around 10 μl of fluid; hence, instillation of small volume of drug solution in concentrated form increases its effectiveness than when administered in large volume in dilute form. Viscosity imparters in the formulation increase bioavailability by prolonging drug's contact time with the eye. Oily solutions, ointments and gels show sustained drug action for the same reason. Sometimes systemic absorption of a drug with low therapeutic index such as timolol may precipitate undesirable toxic effects. Systemic entry of drugs occur by way of absorption into lacrimal duct which drains lacrimal fluid into the nasal cavity and finally into the GIT. This can be prevented by simple eyelid closure or nasolacrimal occlusion by pressing the finger tip to the inside corner of the eye after drug instillation.

Vaginal Administration

Drugs meant for intravaginal application are generally intended to act locally in the treatment of bacterial or fungal infections or prevent conception. The route is now used for systemic delivery of contraceptives and other steroids, without the disadvantage of first-pass metabolism. Controlled delivery and termination of drug action when desired, is possible with this route. Factors that may influence drug absorption from intravaginal site include pH of lumen fluids (4 to 5), vaginal secretions and the microorganisms present in the vaginal lumen which may metabolize the drug.

A summary of mechanisms and drugs absorbed from various noninvasive routes (other than the GI) is given in Table 2.8.

TABLE 2.8

Absorption of Drugs from Non *per os* Extravascular Routes

Route	*Absorption Mechanism(s)*	*Drugs Delivered*
Buccal/ Sublingual	passive diffusion, carrier-mediated transport	nitrites and nitrates antianginals, nifedipine, morphine, fenoterol, etc.
Rectal	passive diffusion	aspirin, paracetamol, theophylline, few barbiturates, etc.
Transdermal	passive diffusion	nitroglycerine, lidocaine, scopolamine, testosterone, betamethasone, etc.
Intramuscular	passive diffusion, endocytosis, pore transport	phenytoin, digoxin, several steroids and antibiotics and many other drugs.
Subcutaneous	passive diffusion	insulin, heparin, C.R. implants.
Inhalation	passive diffusion, pore transport	salbuterol, cromolyn, beclomethasone.
Intranasal	passive diffusion, pore transport	phenylpropanolamine, antihistamines.
Intraocular	passive diffusion	atropine, pilocarpine, adrenaline, antibiotics, etc.
Vaginal	passive diffusion	steroidal drugs and contraceptives, metronidazole, etc.

QUESTIONS

1. Define drug absorption. What are the various routes from which absorption of a drug is necessary for pharmacologic action?
2. Why are both rapidity and completeness of drug absorption important? What is their significance in drug therapy?
3. What is the major mechanism for absorption of most drugs? What is the driving force for such a process?
4. What do you understand by sink conditions? How is it maintained and responsible for complete passive absorption of drugs from the GIT?
5. List the characteristics of passive diffusion of drugs.
6. Why is the absorption rate of a sufficiently water-soluble but lipophilic drug always greater than its rate of elimination?
7. What are the characteristics of specialized transport systems? How can the kinetics of such processes by described?
8. It is always advisable to administer B vitamins in small multiple doses rather than as a single large dose. Why?

9. Discuss the similarities and differences between passive and facilitated diffusion.
10. Why is active transport not a predominant mechanism for absorption of drugs? What could be the reason for active absorption of several antineoplastics or nutrient analogs?
11. How are ionic/ionizable drugs absorbed?
12. What is the only absorption mechanism for which aqueous solution of a drug is not a prerequisite? What is the significance of such a transport process?
13. Suggest the likely mechanism for oral absorption of following agents: lithium carbonate, ibuprofen, cyanocobalamin, methotrexate, quaternary ammonium compounds and insulin.
14. Enlist and illustrate the steps involved in the absorption of a drug from orally administered solid dosage forms.
15. Define the rate-determining step. What are the two major RDS' in the absorption of orally administered drugs? Based on the solubility profile, to which drugs they apply?
16. Classify and enumerate the biopharmaceutic factors influencing bioavailability of a drug from its dosage form.
17. Justify the statement—dissolution rate is better related to drug absorption and bioavailability than solubility.
18. Name the various theories that explain drug dissolution.
19. Using Noyes-Whitney's equation, discuss the diffusion layer theory and the variables that influence drug dissolution.
20. Why is *in vivo* drug dissolution always faster than *in vitro* dissolution? What conditions should be simulated in order to obtain a good relationship?
21. What assumptions are made in diffusion layer and Danckwert's models?
22. What is the difference between absolute and effective surface area? How can the latter of a hydrophobic drug be increased?
23. Micronization of hydrophobic drugs actually results in reduction in effective surface area and dissolution rate. Why?
24. For which drugs an increase in surface area by micronization is not advisable?
25. Classify the internal structure of a solid compound and define: polymorphism, amorphism and pseudopolymorphism.
26. How will you account for the rapid dissolution of amorphs, metastable polymorphs and organic solvates in comparison to their respective counterparts?
27. Why is the pH of the diffusion layer high and low respectively for salts of weak acids and weak bases in comparison to that observed with free forms of the drugs?
28. Give two reasons for higher solubility and better dissolution of salt forms of the drug in comparison to their free acidic or basic forms.

29. Buffered aspirin tablets are more suitable than sodium salt form of aspirin. Why?
30. What is the influence of the size of counter ion on solubility of salt forms of the drugs?
31. State the pH-partition hypothesis briefly. On what assumptions this statement is based?
32. Based on pH-partition theory, predict the degree of ionization and absorption of very weak, weak and strong acidic and basic drugs from stomach and intestine.
33. For optimum absorption, a drug should have biphasic solubility or perfect HLB in its structure. Explain.
34. Discuss the limitations and significance of pH-partition hypothesis.
35. Why is disintegration test not considered a guarantee of a drug's bioavailability from its solid dosage form?
36. The influence of compression force on drug dissolution and absorption from tablets is unpredictable. Explain.
37. Discuss briefly the influence of pharmaceutical excipients on drug bioavailability.
38. How do the surfactants promote bioavailability of a poorly water-soluble drug? What is their influence when used in higher concentrations?
39. Quote examples of complexation used to enhance bioavailability of a drug.
40. How does the nature and type of dosage form influence drug absorption?
41. List the orally administered dosage forms in order of decreasing bioavailability.
42. What are the anatomical and physiological reasons for differences in the rate and extent of absorption of a drug from various regions of the GIT?
43. Why are all types of drugs, whether acidic, basic or neutral, better absorbed from small intestine?
44. For which drugs rapid GE is desirable and when should it be slow?
45. Discuss briefly the factors affecting GE of drugs.
46. What are the possible reasons for delayed and for enhanced absorption of a drug after meals? Why biopharmaceutic studies that require the drug to be taken orally, be performed in volunteers on empty stomach?
47. Delayed intestinal transit is sometimes desirable. Why?
48. What are the consequences of various disease states on oral bioavailability of a drug?
49. What are the various mechanisms for drug-drug interactions in the GIT?
50. What are the different sites of presystemic metabolism of orally administered drugs?
51. How can the metabolic role of colonic microflora be utilized for drug targeting to large intestine?
52. What are the advantages of administering drugs by non *per os* noninvasive transmucosal routes? Name such routes.

53. Discuss the factors in the absorption of drugs from various non *per os* transmucosal and transdermal routes.

54. Transdermal delivery as well as most of the transmucosal routes other than the GI are limited for systemic administration of low dose drugs only. Explain.

55. How can the absorption of drugs from subcutaneous sites be promoted?

56. What factors limit drug administration by pulmonary route? Why is this route restricted for administration of drugs affecting pulmonary function?

57. Name the physicochemical and biological factors that limit drug administration by oral route.

58. Given below in the Table are 3 basic drugs together with some of their physicochemical and biological properties:

Properties	*Diazepam*	*Loratidine*	*Guanethidine*
Molecular weight	285.0	383.0	296.0
pK_a	3.7	5.0	11.7
Aqueous solubility	slight	insoluble	high
$K_{o/w}$	high	27.0	low
% Presystemic metabolism	nil	90.0	40.0

a. Based on the aqueous solubility and $K_{o/w}$, what could be the rate-limiting step in the passive absorption of drugs?

b. Determine the per cent drug ionization in stomach pH 3.0 and intestine pH 5.0.

Answer : Diazepam : 83% and 4.8%, Loratidine : 99% and 50% and Guanethidine : 100% and 100%.

c. Based on pH-partition hypothesis, from which site the drugs will be best absorbed? Which drug will be absorbed along the entire length of the GIT?

d. Assuming that only the unionized drug is absorbed, what per cent of drug will be absorbed from the intestine at pH 7.4?

Answer : Diazepam : 100%, Loratidine : 99.6% and Guanethidine : 0%.

e. Determine the relative amount of drug present in the intestine at pH 5.0 and plasma at pH 7.4.

Answer : Diazepam—1:1, Loratidine—2:1 and Guanethidine—500:1.

f. Delayed GI transit and food intake will be beneficial to absorption of which drug?

g. From the above results and from presystemic metabolism data, a change in the route of administration is advisable for which drug(s)?

h. If guanethidine shows an oral availability of 20%, what could be the possible mechanism for its absorption ?

3

Distribution of Drugs

After entry into the systemic circulation, either by intravascular injection or by absorption from any of the various extravascular sites, the drug is subjected to a number of processes called as **disposition processes** *that tend to lower its plasma concentration.* The two major drug disposition processes are:

1. **Distribution** *which involves reversible transfer of a drug between compartments*, and

2. **Elimination** *which causes irreversible loss of drug from the body.* Elimination is further divided into two processes viz. **biotransformation** (metabolism) and **excretion**.

The interrelationship between drug distribution, biotransformation and excretion and the drug in plasma is shown in Fig. 3.1.

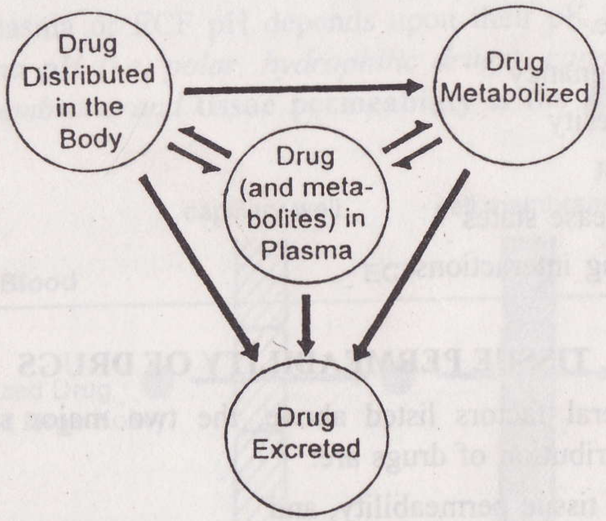


Fig. 3.1 Interrelationship between different processes of drug disposition

As stated above, **distribution** *is defined as the reversible transfer of a drug between one compartment and another.* Since the process is carried out by the circulation of blood, one of the compartments is always the

blood or the plasma and the other represents extravascular fluids and other body tissues. In other words, **distribution** *is reversible transfer of a drug between the blood and the extravascular fluids and tissues.* Distribution is a passive process, for which, the driving force is concentration gradient between the blood and the extravascular tissues. The process occurs by diffusion of free drug only until equilibrium is achieved. As the pharmacologic action of a drug depends upon its concentration at the site of action, distribution plays a significant role in the onset, intensity and sometimes duration of drug action.

Distribution of a drug is not uniform throughout the body because different tissues receive the drug from plasma at different rates and to different extents. Differences in drug distribution among the tissues essentially arise as result of a number of factors as enumerated below:

1. Tissue permeability of the drug:
   a. Physicochemical properties of the drug like molecular size, pK_a and o/w partition coefficient
   b. Physiological barriers to diffusion of drugs
2. Organ/tissue size and perfusion rate
3. Binding of drugs to tissue components:
   a. Binding of drugs to blood components
   b. Binding of drugs to extravascular tissue proteins
4. Miscellaneous factors:
   a. Age
   b. Pregnancy
   c. Obesity
   d. Diet
   e. Disease states
   f. Drug interactions

TISSUE PERMEABILITY OF DRUGS

Of the several factors listed above, the two major rate-determining steps in the distribution of drugs are:

1. Rate of tissue permeability, and
2. Rate of blood perfusion.

If the blood flow to the entire body tissues were rapid and uniform, differences in the degree of distribution between tissues will be indicative of differences in the tissue penetrability of the drug and the process will

be *tissue permeability rate-limited.* The tissue permeability of a drug depends upon the physicochemical properties of the drug as well as the physiologic barriers that restrict diffusion of drug into tissues.

Physicochemical Properties of the Drug

Important physicochemical properties that influence drug distribution are molecular size, degree of ionization and partition coefficient.

Almost all drugs having molecular weight less than 500 to 600 daltons easily cross the capillary membrane to diffuse into the extracellular interstitial fluids. However, penetration of drugs from the extracellular fluid into the cells is a function of molecular size, ionization constant and lipophilicity of the drug. Only small, water-soluble molecules and ions of size below 50 daltons enter the cell through aqueous filled channels whereas those of larger size are restricted unless a specialized transport system exists for them.

The degree of ionization of a drug is an important determinant in its tissue penetrability. The pH of the blood and the extravascular fluid also play a role in the ionization and diffusion of drugs into cells. A drug that remains unionized at these pH values can permeate the cells relatively more rapidly. Since the blood and the ECF pH normally remains constant at 7.4, they do not have much of an influence on drug diffusion unless altered in conditions such as systemic acidosis or alkalosis.

Most drugs are either weak acids or weak bases and their degree of ionization at plasma or ECF pH depends upon their pK_a. *All drugs that ionize at plasma pH (i.e. polar, hydrophilic drugs), cannot penetrate the lipoidal cell membrane and* **tissue permeability is the rate-limiting step**

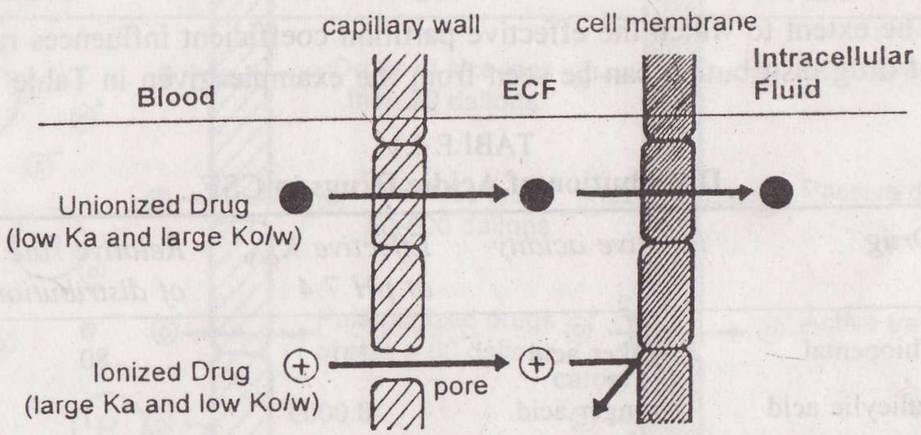


Fig. 3.2 Permeation of unionized and ionized drugs across the capillary and the cell membrane

in the distribution of such drugs. Only unionized drugs which are generally lipophilic, rapidly cross the cell membrane. Among the drugs that have same o/w partition coefficient but differ in the extent of ionization at blood pH, the one that ionizes to a lesser extent will have greater penetrability than that which ionizes to a larger extent; for example, pentobarbital and salicylic acid have almost the same $K_{o/w}$ but the former is more unionized at blood pH and therefore distributes rapidly. The influence of drug pK_a and $K_{o/w}$ on distribution is illustrated by the example that thiopental, a nonpolar, lipophilic drug, largely unionized at plasma pH, readily diffuses into the brain whereas penicillins which are polar, water-soluble and ionized at plasma pH do not cross the blood-brain barrier (Fig. 3.2).

Since the extent to which a drug exists in unionized form governs the distribution pattern, situations that result in alteration of blood pH affect such a pattern; for example, acidosis (metabolic or respiratory) results in decreased ionization of acidic drugs and thus increased intracellular drug concentration and pharmacologic action. Opposite is the influence of alkalosis. Sodium bicarbonate induced alkalosis is sometimes useful in the treatment of barbiturate (and other acidic drugs) poisoning to drive the drug out and prevent further entry into the CNS and promote their urinary excretion by favoring ionization. Converse is true for basic drugs; acidosis favors extracellular whereas alkalosis, intracellular distribution.

In case of polar drugs where permeability is the rate-limiting step in the distribution, the driving force is the **effective partition coefficient** *of drug.* It is calculated by the following formula:

$$\text{Effective } K_{o/w} = \text{Fraction unionized at pH 7.4} \times K_{o/w} \text{ of unionized drug} \quad (3.1)$$

The extent to which the effective partition coefficient influences rapidity of drug distribution can be seen from the example given in Table 3.1.

TABLE 3.1
Distribution of Acidic Drugs in CSF

Drug	*Relative acidity*	*Effective $K_{o/w}$ at pH 7.4*	*Relative rate of distribution*
Thiopental	weaker acid	2.0	80
Salicylic acid	stronger acid	0.0005	1

Thus, thiopental distributes in CSF at a rate 80 times faster than salicylic acid.

Physiologic Barriers to Distribution of Drugs

A membrane (or a barrier) with special structural features can be a permeability restriction to distribution of drugs to some tissues. Some of the important simple and specialized physiologic barriers are:

1. Simple capillary endothelial barrier
2. Simple cell membrane barrier
3. Blood-brain barrier
4. Cerebrospinal fluid barrier
5. Placental barrier
6. Blood-testis barrier

The Simple Capillary Endothelial Barrier : The membrane of capillaries that supply blood to most tissues is, practically speaking, not a barrier to moieties which we call drugs. Thus, all drugs, ionized or unionized, with a molecular size less than 600 daltons, diffuse through the capillary endothelium and into the interstitial fluid. Only drugs bound to the blood components are restricted because of the large molecular size of the complex.

The Simple Cell Membrane Barrier : Once a drug diffuses from the capillary wall into the extracellular fluid, its further entry into cells of most tissues is limited by its permeability through the membrane that lines such cells. Such a simple cell membrane is similar to the lipoidal barrier in the GI absorption of drugs (*discussed in chapter 2*).

The physicochemical properties that influence permeation of drugs across such a barrier are summarized in Fig. 3.3.

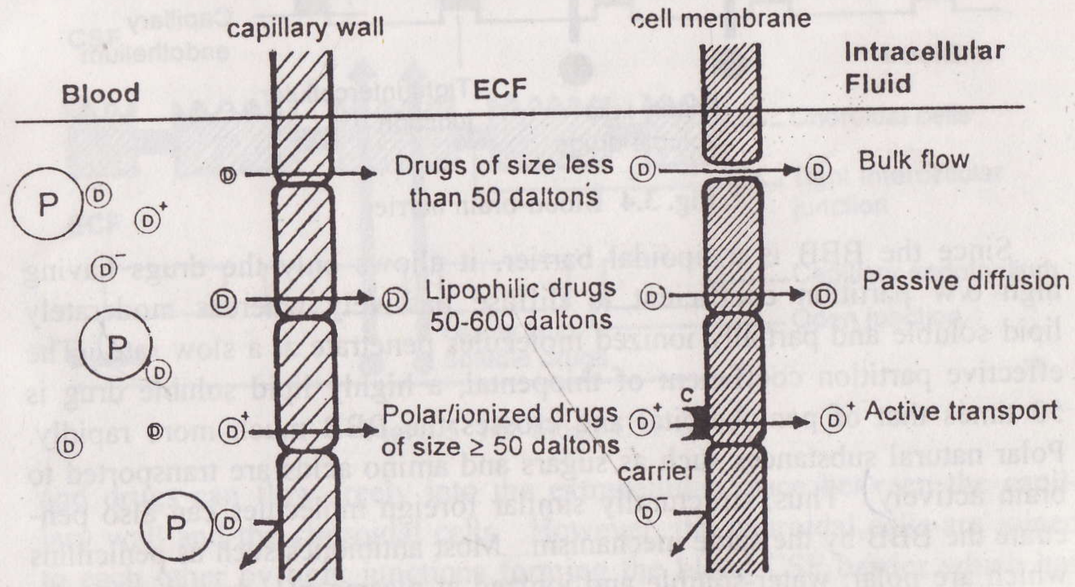


Fig. 3.3 Plasma membrane barrier and drug diffusion across it

Blood-Brain Barrier (BBB) : Unlike the capillaries found in other parts of the body, the capillaries in the brain are highly specialized and much less permeable to water-soluble drugs. *The brain capillaries consist of endothelial cells which are joined to one another by continuous tight intercellular junctions comprising what is called as the* **blood-brain barrier** (Fig. 3.4). Moreover, the presence of special cells called as *astrocytes*, which are the elements of the supporting tissue found at the base of endothelial membrane, form a solid envelope around the brain capillaries. As a result, the intercellular passage is blocked and for a drug to gain access from the capillary circulation into the brain, it has to pass through the cells rather than between them. (However, there are specific sites in the brain where the BBB does not exist, namely, the trigger area and the median hypothalamic eminence. Moreover, drugs administered intranasally may diffuse directly into the CNS because of the continuity between submucosal areas of the nose and the subarachnoid space of the olfactory lobe).

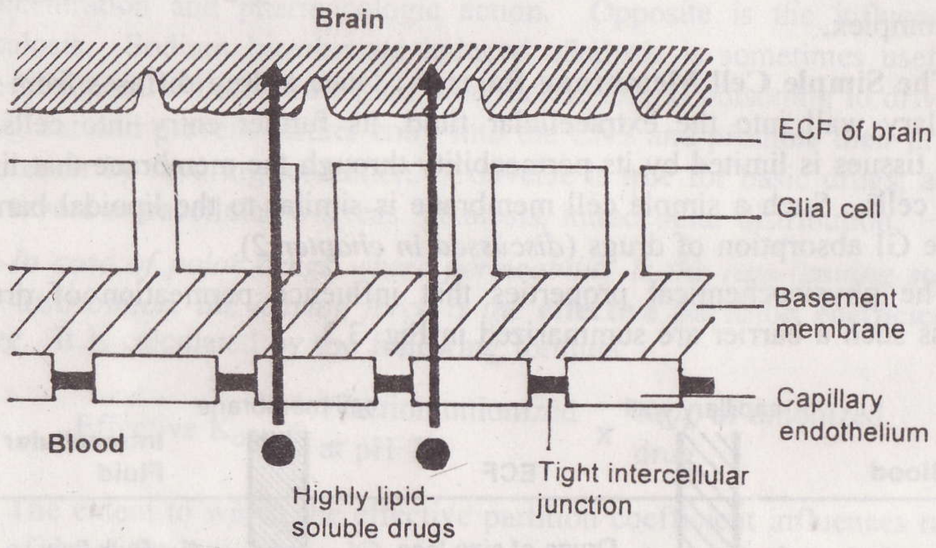


Fig. 3.4 Blood-brain barrier

Since the BBB is a lipoidal barrier, it allows only the drugs having high o/w partition coefficient to diffuse passively whereas moderately lipid soluble and partially ionized molecules penetrate at a slow rate. The effective partition coefficient of thiopental, a highly lipid soluble drug is 50 times that of pentobarbital and crosses the BBB much more rapidly. Polar natural substances such as sugars and amino acids are transported to brain actively. Thus, structurally similar foreign molecules can also penetrate the BBB by the same mechanism. Most antibiotics such as penicillins which are polar, water-soluble and ionized at plasma pH, do not cross the BBB under normal circumstances.

The selective permeability of lipid soluble moieties through the BBB makes appropriate choice of a drug to treat CNS disorders an essential part of therapy; for example, Parkinsonism, a disease characterized by depletion of dopamine in the brain, cannot be treated by administration of dopamine as it does not cross the BBB. Hence, levodopa, which can penetrate the CNS where it is metabolized to dopamine, is used in its treatment. Targeting of polar drugs to brain in certain conditions such as tumor had always been a problem. Three different approaches have been utilized successfully to promote crossing the BBB by drugs:

i. Use of permeation enhancers such as dimethyl sulfoxide (DMSO)

ii. Osmotic disruption of the BBB by infusing internal carotid artery with mannitol

iii. Use of *dihydropyridine redox system* as drug carriers to the brain

In the latter case, the lipid soluble dihydropyridine is linked as a carrier to the polar drug to form a prodrug that readily crosses the BBB. In the brain, the CNS enzymes oxidize the dihydropyridine moiety to the polar pyridinium ion form that cannot diffuse back out of the brain. As a result, the drug gets trapped in the CNS. Such a redox system has been used to deliver steroidal drugs to the brain (*see chapter 6 on Prodrugs*).

Blood-Cerebrospinal Fluid Barrier : The cerebrospinal fluid (CSF) is formed mainly by the choroid plexus of the lateral, third and fourth ventricles and is similar in composition to the ECF of brain. The capillary endothelium that lines the choroid plexus have open junctions or gaps

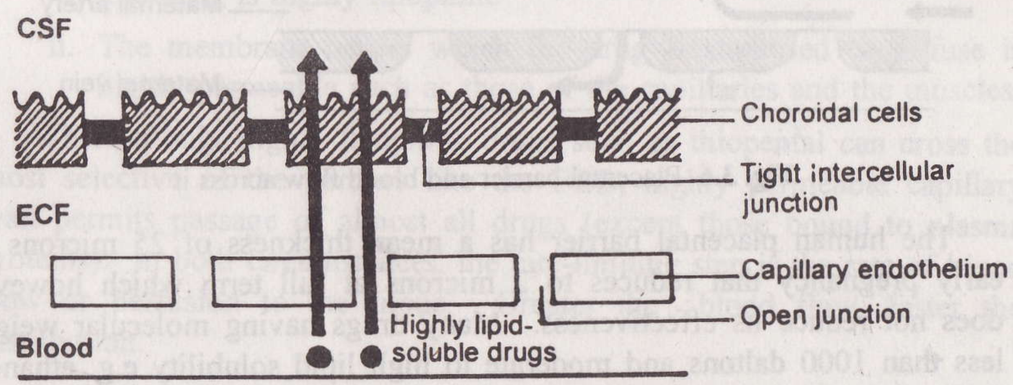


Fig. 3.5 The blood-CSF barrier

and drugs can flow freely into the extracellular space between the capillary wall and the choroidal cells. However, the choroidal cells are joined to each other by tight junctions forming the blood-CSF barrier which has permeability characteristics similar to that of the BBB (Fig. 3.5).

As in the case of BBB, only highly lipid soluble drugs can cross the blood-CSF barrier with relative ease whereas moderately lipid soluble and partially ionized drugs permeate slowly. A drug that enters the CSF slowly cannot achieve a high concentration as the bulk flow of CSF continuously removes the drug. For any given drug, its concentration in the brain will always be higher than in the CSF.

Although the mechanisms for diffusion of drugs into the CNS and CSF are similar, the degree of uptake may vary significantly. In some cases, CSF drug concentration may be higher than its cerebral concentration e.g. sulfamethoxazole and trimethoprim, and vice versa in other cases, e.g. certain β-blockers.

Placental Barrier : The maternal and the fetal blood vessels are separated by a number of tissue layers made of fetal trophoblast basement membrane and the endothelium which together constitute the placental barrier. The flow of blood in the maternal and the fetal blood vessels is shown in Fig. 3.6.

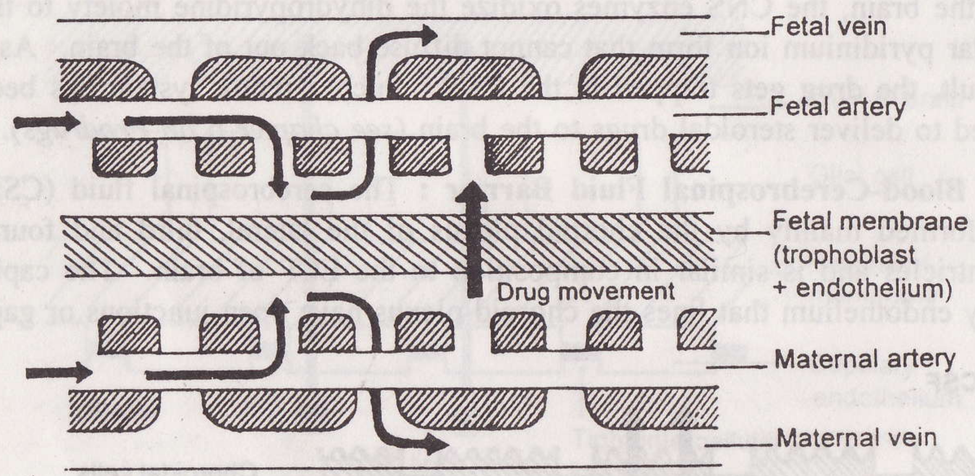


Fig. 3.6 Placental barrier and blood flow across it

The human placental barrier has a mean thickness of 25 microns in early pregnancy that reduces to 2 microns at full term which however does not reduce its effectiveness. Many drugs having molecular weight less than 1000 daltons and moderate to high lipid solubility e.g. ethanol, sulfonamides, barbiturates, gaseous anesthetics, steroids, narcotic analgesics, anticonvulsants and some antibiotics, cross the barrier by simple diffusion quite rapidly. This shows that the placental barrier is not as effective a barrier as BBB. Nutrients essential for the fetal growth are transported by carrier-mediated processes. Immunoglobulins are transported by endocytosis.

Drugs are particularly dangerous to the fetus during 2 stages—

i. In the first trimester when the fetal organs develop; it is during this stage that most drugs show their teratogenic effects (congenital defects) e.g. thalidomide, phenytoin, isotretinoin, testosterone, methotrexate, etc.

ii. In the latter stages of pregnancy when drugs are known to affect physiologic functions, e.g. respiratory depression by morphine.

It is, therefore, always better to restrict all drugs during pregnancy because of the uncertainty of their hazardous effects.

Blood-Testis Barrier : This barrier is located not at the capillary endothelium level but at sertoli-sertoli cell junction. It is the tight junctions between the neighboring sertoli cells that act as the blood-testis barrier. This barrier restricts the passage of drugs to spermatocytes and spermatids.

ORGAN/TISSUE SIZE AND PERFUSION RATE

As discussed until now, distribution is **permeability rate-limited** in the following cases:

i. When the drug under consideration is ionic, polar or water soluble

ii. Where the highly selective physiologic barriers restrict the diffusion of such drugs to the inside of the cell

In contrast, distribution will be **perfusion rate-limited** when:

i. The drug is highly lipophilic

ii. The membrane across which the drug is supposed to diffuse is highly permeable such as those of the capillaries and the muscles.

Whereas only highly lipophilic drugs such as thiopental can cross the most selective of the barriers like the BBB, highly permeable capillary wall permits passage of almost all drugs (except those bound to plasma proteins). In both circumstances, the rate-limiting step is the rate of blood flow or perfusion to the tissue. Greater the blood flow, faster the distribution.

Perfusion rate *is defined as the volume of blood that flows per unit time per unit volume of the tissue.* It is expressed in ml/min/ml of the tissue. The perfusion rate of various tissues are given in Table 3.2.

In Table 3.2, the various tissues are listed in decreasing order of their perfusion rate which indicates the rapidity with which the drug will be distributed to the tissues. Highly perfused tissues such as lungs, kidneys,

adrenal, liver, heart and brain are rapidly equilibrated with lipid soluble drugs.

TABLE 3.2

Relative Volume of Different Organs, Blood Flow and Perfusion Rate under Basal Conditions Assuming the Total Body Volume to be 70 liters

Organ/tissue	*% of Body Volume*	*Blood Flow (ml/min)*	*% of Cardiac Output*	*Perfusion Rate (ml/min/ml)*
I. **Highly Perfused**				
1. Lungs	0.7	5000	100.0	10.2
2. Kidneys	0.4	1250	25.0	4.5
3. Adrenals	0.03	25	0.5	1.2
4. Liver	2.3	1350	27.0	0.8
5. Heart	0.5	200	4.0	0.6
6. Brain	2.0	700	14.0	0.5
II. **Moderately Perfused**				
7. Muscles	42.0	1000	20.0	0.034
8. Skin	15.0	350	7.0	0.033
III. **Poorly Perfused**				
9. Fat (adipose)	10.0	200	4.0	0.03
10. Bone (skeleton)	16.0	250	5.0	0.02

If $K_{t/b}$ is the tissue/blood partition coefficient of drug then the first-order distribution rate constant, K_t, is given by following equation:

$$K_t = \frac{\text{Perfusion Rate}}{K_{t/b}} \qquad (3.2)$$

The tissue distribution half-life is given by equation:

$$\text{Distribution half-life} = \frac{0.693}{K_t} = \frac{0.693\ K_{t/b}}{\text{Perfusion Rate}} \qquad (3.3)$$

The extent to which a drug is distributed in a particular tissue or organ depends upon the size of the tissue (i.e. tissue volume) and the tissue/blood partition coefficient of the drug. Consider the classic example of thiopental. This lipophilic drug has a high tissue/blood partition coefficient towards the brain and still higher for adipose tissue. Since the brain (site of action) is a highly perfused organ, following i.v. injection, thiopental readily diffuses into the brain showing a rapid onset of action. Adipose tissue being poorly perfused, takes longer to get distributed with the same drug. But as the concentration of thiopental in the adipose proceeds

towards equilibrium, the drug rapidly diffuses out of the brain and localizes in the adipose tissue whose volume is more than 5 times that of brain and has greater affinity for the drug. The result is rapid termination of action of thiopental due to such a tissue redistribution.

BINDING OF DRUGS TO TISSUE COMPONENTS

A drug in the body can bind to several components such as the plasma proteins, blood cells and hemoglobin (i.e. blood components) and extravascular proteins and other tissues. This topic is dealt comprehensively in *chapter 4 on Protein Binding of Drugs*.

MISCELLANEOUS FACTORS AFFECTING DRUG DISTRIBUTION

Age

Differences in distribution pattern of a drug in different age groups are mainly due to differences in—

a. *Total body water* (both intracellular and extracellular) — is much greater in infants

b. *Fat content* — is also higher in infants and elderly

c. *Skeletal muscles* — are lesser in infants and in elderly

d. *Organ composition* — the BBB is poorly developed in infants, the myelin content is low and cerebral blood flow is high, hence greater penetration of drugs in the brain

e. *Plasma protein content* — low albumin content in both infants and in elderly

Pregnancy

During pregnancy, the growth of uterus, placenta and fetus increases the volume available for distribution of drugs. The fetus represents a separate compartment in which a drug can distribute. The plasma and the ECF volume also increase but there is a fall in albumin content.

Obesity

In obese persons, the high adipose tissue content can take up a large fraction of lipophilic drugs despite the fact that perfusion through it is low. The high fatty acid levels in obese persons alter the binding characteristics of acidic drugs.

Diet

A diet high in fats will increase the free fatty acid levels in circulation thereby affecting binding of acidic drugs such as NSAIDs to albumin.

Disease States

A number of mechanisms may be involved in the alteration of drug distribution characteristics in disease states:

a. Altered albumin and other drug-binding protein concentration

b. Altered or reduced perfusion to organs or tissues

c. Altered tissue pH

An interesting example of altered permeability of the physiologic barriers is that of BBB. In meningitis and encephalitis, the BBB becomes more permeable and thus polar antibiotics such as penicillin G and ampicillin which do not normally cross it, gain access to the brain. In a patient suffering from CCF, the perfusion rate to the entire body decreases affecting distribution of all drugs.

Drug Interactions

Drug interactions that affect distribution are mainly due to differences in plasma protein or tissue binding of drugs. This topic is discussed under the same heading in *chapter 4*.

VOLUME OF DISTRIBUTION

A drug in circulation distributes to various organs and tissues. When the process of distribution is complete (at distribution equilibrium), different organs and tissues contain varying concentrations of drug which can be determined by the volume of tissues in which the drug is present. Since different tissues have different concentrations of drug, the volume of distribution cannot have a true physiologic meaning. However, there exists a constant relationship between the concentration of drug in plasma, C, and the amount of drug in the body, X.

$$X \propto C$$

$$\text{or} \qquad X = V_d\, C \qquad (3.4)$$

where V_d = proportionality constant having the unit of volume and popularly called as **apparent volume of distribution**. *It is defined as the hypothetical volume of body fluid into which a drug is dissolved or distributed.* It is called as **apparent volume** because all parts of the body equilibrated with the drug do not have equal concentration.

Thus, from equation 3.4, V_d is given by the ratio:

$$\text{Apparent Volume of Distribution} = \frac{\text{Amount of Drug in the Body}}{\text{Plasma Drug Concentration}}$$

$$\text{or} \qquad V_d = \frac{X}{C} \qquad (3.5)$$

The apparent volume of distribution bears no direct relationship with the real volume of distribution.

The **real volume of distribution** has direct physiologic meaning and is related to the body water. The body water is made up of 3 distinct compartments as shown in the Table 3.3.

TABLE 3.3
Fluid Compartments of a 70 Kg Adult

Body Fluid	*Volume (liters)*	*% of Body Weight*	*% of TBW*
1. Vascular fluid/blood (Plasma)	6 (3)	9 (4.5)	15 (7.5)
2. Extracellular fluid (excluding plasma)	12	17	28
3. Intracellular fluid (excluding blood cells)	24	34	57
Total Body Water (TBW)	42	60	100

The volume of each of these real physiologic compartments can be determined by use of specific *tracers* or *markers* (Table 3.4). The **plasma volume** can be determined by use of substances of high molecular weight or substances that are totally bound to plasma albumin, for e.g. high molecular weight dyes such as Evans blue, indocyanine green and I-131 albumin. When given i.v., these remain confined to the plasma. The total blood volume can also be determined if the hematocrit is known. The **extracellular fluid (ECF) volume** can be determined by substances that easily penetrates the capillary membrane and rapidly distribute throughout the ECF but do not cross the cell membranes, for e.g. the Na^+, Cl^-, Br^-, SCN^- and SO_4^{2-} ions and inulin, mannitol and raffinose. However, none of these substances are completely kept out of the cells. The ECF volume, excluding plasma is approximately 15 liters. The **total body water (TBW) volume** can be determined by use of substances that distribute equally in all water compartments of the body (both intra- and extracellular), for e.g. heavy water (D_2O), tritiated water (HTO) and lipid soluble substances such as antipyrine. The **intracellular fluid volume** is determined as the difference between the TBW and ECF volume. The intracellular fluid volume including those of blood cells is approximately 27 liters.

TABLE 3.4

Markers Used to Measure the Volume of Real Physiologic Compartments

Physiologic Fluid Compartment	*Markers Used*	*Approximate Volume (liters)*
Plasma	Evans blue, indocyanine green, I-131 albumin	3
Erythrocytes	Cr-51	2
Extracellular fluid	Nonmetabolizable saccharides like raffinose, inulin, mannitol and radioisotopes of selected ions : Na^+, Cl^-, Br^-, SO_4^{2-}	15
Total body water	D_2O, HTO, antipyrine	42

Since the tracers are not bound or negligibly bound to plasma or tissue proteins, their apparent volume of distribution is same as their true volume of distribution. The situation is different with most drugs which bind to plasma proteins or extravascular tissues or both. Certain *generalizations* can be made regarding the apparent volume of distribution of such drugs:

1. Drugs which bind selectively to plasma proteins or other blood components, e.g. warfarin (i.e. those that are less bound to extravascular tissues), have apparent volume of distribution smaller than their true volume of distribution. The V_d of such drugs lies between blood volume and TBW volume (i.e. between 6 to 42 liters); for example, warfarin has a V_d of about 10 liters.

2. Drugs which bind selectively to extravascular tissues, e.g. chloroquine (i.e. those that are less bound to blood components), have apparent volume of distribution larger than their real volume of distribution. The V_d of such drugs is always greater than 42 liters or TBW volume; for example, chloroquine has a V_d of approximately 15,000 liters. Such drugs leave the body slowly and are generally more toxic than drugs that do not distribute deeply into body tissues.

Thus, factors that produce an alteration in binding of drug to blood components, result in an *increase in V_d* and those that influence drug binding to extravascular components result in a *decrease in V_d*. Other factors that may influence V_d are changes in tissue perfusion and permeability, changes in the physicochemical characteristics of the drug e.g. ionization, changes in the body weight and age and several disease states.

Apparent volume of distribution is expressed in liters and sometimes in liters/Kg body weight. The V_d of various drugs range from as low as 3 liters (plasma volume) to as high as 40,000 liters (much above the total body size). Many drugs have V_d greater than 30 liters. The V_d is a characteristic of each drug under normal conditions and is altered under conditions that affect distribution pattern of the drug.

QUESTIONS

1. Define—(a) disposition, and (b) distribution of drugs. What is the major mechanism for distribution of drugs and what is its driving force?
2. Unless distribution occurs, the drug may not elicit pharmacologic response. Explain.
3. Why is distribution of a drug not uniform throughout the body? List the factors influencing drug distribution.
4. What are the two major rate-limiting steps in the distribution of drugs? Under what circumstances are they applicable?
5. Which physicochemical properties of the drug limit its distribution?
6. Phenobarbital and salicylic acid have almost the same $K_{o/w}$ but the former shows extensive distribution. Why?
7. What is the influence of change in plasma pH on distribution pattern of a drug? Based on pK_a values, which drugs are most affected and which will be least affected by a change in plasma pH?
8. What parameter is considered to be the driving force for distribution of polar drugs?
9. Why cannot the capillary endothelium be considered a barrier to distribution of unbound drugs? What would be the consequence or fate of drugs had it been a selective barrier?
10. Name the specialized barriers to distribution of drugs.
11. Describe the anatomy and physiology of blood brain barrier. What characteristics of a drug are necessary to penetrate such a barrier?
12. How do nutrients which are generally polar, make their way into the brain?
13. Polar drugs such as penicillin normally do not cross BBB but do so in meningitis. Explain.
14. Name the three approaches by which a polar drug can be targeted to brain.
15. Drugs that penetrate the CNS slowly may never achieve adequate therapeutic brain concentrations. Why?
16. Why is the placental barrier not as effective as BBB?
17. In which periods drugs are particularly harmful to fetus in pregnant women?
18. How are body tissues classified on the basis of perfusion rate?
19. Thiopental is a highly lipophilic, centrally acting drug. By which route should it be administered for rapid onset of action? Why? What is the reason for its rapid termination of action?

20. Which are the factors responsible for the differences in drug distribution in persons of different age groups?

21. What are the various mechanisms involved in the alteration of drug distribution characteristics in disease states?

22. Define apparent volume of distribution. Why cannot the volume of distribution of a drug have a true physiologic meaning?

23. What are the various physiologic fluid compartments of the body? What are their volumes and how are they estimated?

24. The tracers used to determine the volume of body fluids have V_d same as their true volume. Why?

25. How are the binding characteristics of a drug related to its V_d? Explain why some drugs have V_d value larger than TBW volume?

26. It is better to express V_d in liters/Kg body weight. Why?

27. Can a drug have two or more V_d values. Explain why?

28. The tissue/blood partition coefficient values of a drug and tissue perfusion rates are given in table below:

Tissues	$K_{t/b}$	*Perfusion Rate*	K_t	*Distribution* $t_{1/2}$
Liver	1	0.8		
Brain	4	0.5		
Muscle	8	0.035		
Fat	40	0.03		

Determine the rate constants for distribution and distribution half-lives.

Answer : K_t values (per minute) : liver - 0.8, brain - 0.125, muscle - 0.0044 and fat - 0.00075; distribution $t_{1/2}$ values (in minutes): liver - 0.86, brain - 5.54, muscle - 158.4 and fat - 924.

29. The V_d of fluoxetine is 3000 liters. Calculate —

a. The amount of drug in the body when the plasma concentration is 1ng/ml.

Answer : 3 mg.

b. The plasma concentration when the amount of drug in the body is 2 mg.

Answer : 0.67 ng/ml.

c. The % of drug that is present in plasma.

Answer : 0.1%.

30. The V_d of three drugs—A, B and C are 12, 42 and 400 liters respectively.

a. Determine the % drug present outside plasma.

Answer : drug A - 75%, drug B - 93% and drug C - 99.3%.

b. Which drug is most extensively distributed in e.v. tissues?

c. If binding of drugs is negligible, which of the three can be used as markers and for which fluid compartments?

4 Protein Binding of Drugs

A drug in the body can interact with several tissue components of which the two major categories are blood and extravascular tissues. The interacting molecules are generally the macromolecules such as proteins, DNA or adipose. The proteins are particularly responsible for such an interaction. *The phenomena of complex formation with proteins is called as* **protein binding of drugs**. The importance of such a binding derives from the fact that the bound drug is both pharmacokinetically as well as pharmacodynamically inert i.e. a protein bound drug is neither metabolized nor excreted nor is it pharmacologically active. A bound drug is also restricted since it remains confined to a particular tissue for which it has greater affinity. Moreover, such a bound drug, because of its enormous size, cannot undergo membrane transport and thus its half-life is increased.

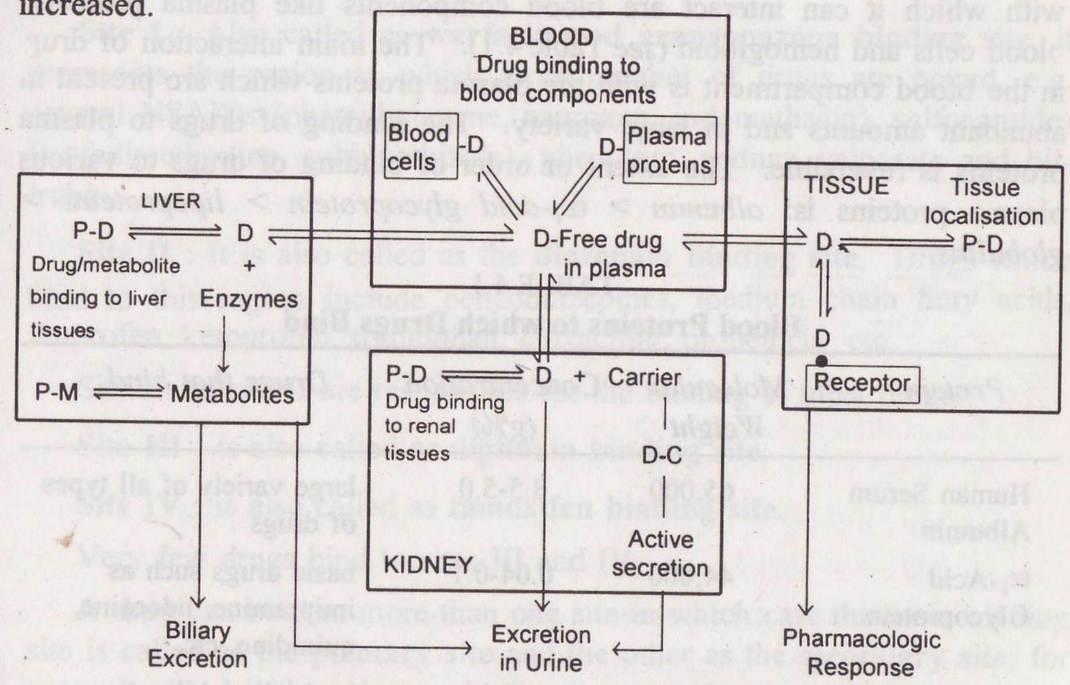


Fig. 4.1 Protein-drug binding: Binding of drugs to various tissue components and its influence on disposition and clinical response. Note that only the unbound drug moves reversibly between the compartments.

Binding of drugs generally involves weak chemical bonds such as hydrogen bonds, hydrophobic bonds, ionic bonds or *van der Waal's* forces and, therefore, is a reversible process. Irreversible drug binding, though rare, arises as a result of covalent binding and is often a reason for the carcinogenicity or tissue toxicity of the drug; for example, covalent binding of chloroform and paracetamol metabolites to liver results in hepatotoxicity.

Binding of drugs falls into 2 classes:

1. Binding of drugs to blood components like—
   a. Plasma proteins
   b. Blood cells
2. Binding of drugs to extravascular tissue proteins, fats, bones, etc.

The influence of binding on drug disposition and clinical response is shown in Fig. 4.1.

Of all types of binding, the plasma protein-drug binding is the most significant and most widely studied.

BINDING OF DRUGS TO BLOOD COMPONENTS

Plasma Protein-Drug Binding

Following entry of a drug into the systemic circulation, the first thing with which it can interact are blood components like plasma proteins, blood cells and hemoglobin (*see* Table 4.1). The main interaction of drug in the blood compartment is with the plasma proteins which are present in abundant amounts and in large variety. The binding of drugs to plasma proteins is reversible. The extent or order of binding of drugs to various plasma proteins is: *albumin* > *α_1-acid glycoprotein* > *lipoproteins* > *globulins*.

TABLE 4.1
Blood Proteins to which Drugs Bind

Protein	*Molecular Weight*	*Concentration (g%)*	*Drugs that bind*
Human Serum Albumin	65,000	3.5-5.0	large variety of all types of drugs
α_1-Acid Glycoprotein	44,000	0.04-0.1	basic drugs such as imipramine, lidocaine, quinidine, etc.
Lipoproteins	200,000 to 3,400,000	variable	basic, lipophilic drugs like chlorpromazine

...*continued*

Protein	*Molecular Weight*	*Concentration (g%)*	*Drugs that bind*
α_1-Globulin	59,000	0.003-0.007	steroids like corticosterone, and thyroxine and cyanocobalamin
α_2-Globulin	1,34,000	0.015-0.06	vitamins A, D, E and K and cupric ions
Hemoglobin	64,500	11-16	phenytoin, pentobarbital, and phenothiazines

Binding of Drugs to Human Serum Albumin

The human serum albumin (HSA), having a molecular weight of 65,000, is the most abundant plasma protein (59% of total plasma and 3.5 to 5.0 g%) with a large drug binding capacity. The therapeutic doses of most drugs are relatively much smaller and their plasma concentration do not normally reach equimolar concentration with HSA. The HSA can bind several compounds having varied structures. Both endogenous compounds such as fatty acids, bilirubin and tryptophan as well as drugs bind to HSA. A large variety of drugs ranging from weak acids, neutral compounds to weak bases bind to HSA. Four different sites on HSA have been identified for drug-binding (Fig. 4.2). They are:

Site I : Also called as **warfarin and azapropazone binding site**, it represents the region to which large number of drugs are bound, e.g. several NSAIDs (phenylbutazone, naproxen, indomethacin), sulfonamides (sulfadimethoxine, sulfamethizole), phenytoin, sodium valproate and bilirubin.

Site II : It is also called as the **diazepam binding site**. Drugs which bind to this region include benzodiazepines, medium chain fatty acids, ibuprofen, ketoprofen, tryptophan, cloxacillin, probenicid, etc.

Site I and site II are responsible for the binding of most drugs.

Site III : is also called as **digitoxin binding site**.

Site IV : is also called as **tamoxifen binding site**.

Very few drugs bind to sites III and IV.

A drug can bind to more than one site in which case the main binding site is called as the **primary site** and the other as the **secondary site**; for example, site I is the primary site for dicoumarol and site II the secondary site. Groups of drugs that bind to the same site, compete with each other for binding, but drugs that bind to one site do not competitively inhibit

binding of drugs to other sites. However, they may either promote or retard binding of a drug to another site by energetic coupling mechanisms.

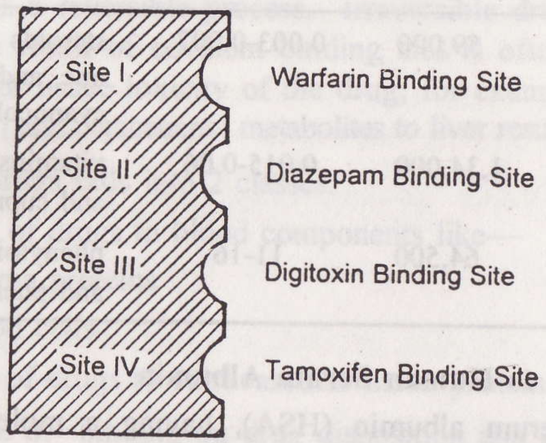


Fig. 4.2 Four major drug binding sites on human serum albumin

Binding of Drugs to α_1-Acid Glycoprotein (α_1-AGP or AAG)

Also called as the **orosomucoid**, it has a molecular weight of 44,000 and a plasma concentration range of 0.04 to 0.1 g%. It binds to a number of basic drugs like imipramine, amitriptyline, nortriptyline, lidocaine, propranolol, quinidine and disopyramide.

Binding of Drugs to Lipoproteins

Binding of drugs to HSA and AAG involve hydrophobic bonds. Since only lipophilic drugs can undergo hydrophobic bonding, lipoproteins can also bind to such drugs because of their high lipid content. However, the plasma concentration of lipoproteins is much less in comparison to HSA and AAG.

A drug that binds to lipoproteins does so by dissolving in the lipid core of the protein and thus its capacity to bind depends upon its lipid content. The molecular weight of lipoproteins vary from 2 lakhs to 34 lakhs depending on their chemical composition. They are classified on the basis of their density.

The 4 classes of lipoproteins are:

1. Chylomicrons
2. Very low density lipoproteins (VLDL)
3. Low density lipoproteins (LDL) (predominant in humans)
4. High density lipoproteins (HDL)

The lipid core of these macromolecules consists of triglycerides and cholesteryl esters and the outside is made of apoproteins (free cholesterol

and proteins). Predictably, VLDL is rich in triglycerides and HDL is rich in apoproteins.

The binding of drugs to lipoproteins is noncompetitive. A number of acidic (diclofenac), neutral (cyclosporin A) and basic drugs (chlorpromazine) bind to lipoproteins. Basic, lipophilic drugs have relatively more affinity. Lipoprotein binding becomes significant in cases of drugs that predominantly bind to them, and secondly, when levels of HSA and AAG in plasma are decreased.

Binding of Drugs to Globulins

Several plasma globulins have been identified and are labeled as α_1-, α_2-, β_1-, β_2- and γ-globulins.

1. **α_1-globulin** : also called as **transcortin** or CBG (corticosteroid binding globulin), it binds a number of steroidal drugs such as cortisone and prednisone. It also binds to thyroxine and cyanocobalamin.

2. **α_2-globulin** : also called as **ceruloplasmin**, it binds vitamins A, D, E and K and cupric ions.

3. **β_1-globulin** : also called as **transferrin**, it binds to ferrous ions.

4. **β_2-globulin** : binds to carotinoids .

5. **γ-globulin** : binds specifically to antigens.

Binding of Drugs to Blood Cells

More than 40% of the blood comprises of blood cells of which the major cell component is the RBC. The RBCs constitute 95% of the total blood cells. Thus, significant RBC drug binding is possible. The red cell is 500 times in diameter as the major plasma protein binding component, albumin. The RBC comprises of 3 components each of which can bind to drugs:

1. **Hemoglobin** : It has a molecular weight of 64,500 (almost equal to that of HSA) but is 7 to 8 times the concentration of albumin in blood. Drugs like phenytoin, pentobarbital and phenothiazines bind to hemoglobin.

2. **Carbonic Anhydrase** : Drugs known to bind to it are acetazolamide and chlorthalidone (i.e. carbonic anhydrase inhibitors).

3. **Cell Membrane** : Imipramine and chlorpromazine are reported to bind with the RBC membrane.

It has been shown that the rate and extent of entry into RBC is more for lipophilic drugs, e.g. phenytoin. Hydrophilic drugs like ampicillin do not enter RBC.

TISSUE BINDING OF DRUGS (TISSUE LOCALIZATION OF DRUGS)

The body tissues, apart from HSA, comprise 40% of the body weight which is 100 times that of HSA. Hence, tissue-drug binding is much more significant than thought to be.

A drug can bind to one or more of the several tissue components. Tissue-drug binding is important in distribution from two viewpoints: firstly, it increases the apparent volume of distribution of drugs in contrast to plasma protein binding which decreases it; this is because the parameter is related to the ratio of amount of drug in the body to the plasma concentration of free drug and the latter is decreased under conditions of extensive tissue binding of drugs, and secondly, tissue-drug binding results in localization of a drug at a specific site in the body (with a subsequent increase in biological half-life). This is more so because a number of drugs bind *irreversibly* with the tissues (contrast to plasma protein-drug binding); for example, oxidation products of paracetamol, phenacetin, chloroform, carbon tetrachloride and bromobenzene bind covalently to hepatic tissues.

Factors influencing localization of drugs in tissues include lipophilicity and structural features of the drug, perfusion rate, pH differences, etc. Extensive tissue-drug binding suggests that a tissue can act as the *storage site* for drugs. Drugs that bind to both tissue and plasma components result in competition between drug binding sites.

For majority of drugs that bind to extravascular tissues, the order of binding is: *liver* > *kidney* > *lung* > *muscle*. Several examples of extravascular tissue-drug binding are:

1. Liver : As stated earlier, epoxides of a number of halogenated hydrocarbons and paracetamol bind irreversibly to liver tissues resulting in hepatotoxicity.

2. Lungs : Basic drugs like imipramine, chlorpromazine and antihistamines accumulate in lungs.

3. Kidneys : Metallothionin, a protein present in kidneys, binds to heavy metals such as lead, mercury, and cadmium and results in their renal accumulation and toxicity.

4. Skin : Chloroquine and phenothiazines accumulate in skin by interacting with melanin.

5. Eyes : The retinal pigments of the eye also contain melanin. Binding of chloroquine and phenothiazines to it is responsible for retinopathy.

6. Hairs : Arsenicals, chloroquine and phenothiazines are reported to deposit in hair shafts.

7. Bones : Tetracycline is a well known example of a drug that binds to bones and teeth. Administration of this antibiotic to infants or children during odontogenesis results in permanent brown-yellow discoloration of teeth. Lead is known to replace calcium from bones and cause their brittleness.

8. Fats : Lipophilic drugs such as thiopental and the pesticide DDT accumulate in adipose tissues by partitioning into it. However, high o/w partition coefficient is not the only criteria for adipose distribution of drugs since several highly lipophilic (more than thiopental) basic drugs like imipramine and chlorpromazine are not localized in fats. The poor perfusion of adipose could be the reason for such an ambiguity. Reports have stated that adipose localization of drugs is a result of binding competition between adipose and non-adipose tissues (lean tissues like muscles, skin and viscera) and not partitioning.

9. Nucleic Acids : Molecular components of cells such as DNA interact strongly with drugs like chloroquine and quinacrine resulting in distortion of its double helical structure.

FACTORS AFFECTING PROTEIN-DRUG BINDING

Factors affecting protein-drug binding can be broadly categorized as—

1. Factors relating to the drug
   a. Physicochemical characteristics of the drug
   b. Concentration of drug in the body
   c. Affinity of a drug for a particular binding component
2. Factors relating to the protein and other binding components
   a. Physicochemical characteristics of the protein or binding agent
   b. Concentration of protein or binding component
   c. Number of binding sites on the binding agent
3. Drug interactions
   a. Competition between drugs for the binding site (displacement interactions)
   b. Competition between drugs and normal body constituents
   c. Allosteric changes in protein molecule
4. Patient related factors
   a. Age
   b. Intersubject variations
   c. Disease states

DRUG RELATED FACTORS

Physicochemical Characteristics of the Drug

As mentioned earlier, protein binding is directly related to the lipophilicity of drug. An increase in lipophilicity increases the extent of binding; for example, the slow absorption of cloxacillin in comparison to ampicillin after i.m. injection is attributed to its higher lipophilicity and larger (95%) binding to proteins while the latter is less lipophilic and just 20% bound to proteins. Highly lipophilic drugs such as thiopental tend to localize in adipose tissues. Anionic or acidic drugs such as penicillins and sulfonamides bind more to HSA whereas cationic or basic drugs such as imipramine and alprenolol bind to AAG. Neutral, unionized drugs bind more to lipoproteins.

Concentration of Drug in the Body

The extent of protein-drug binding can change with both changes in drug as well as protein concentration. The concentration of drugs that bind to HSA does not have much of an influence as the therapeutic concentration of any drug is insufficient to saturate it. However, therapeutic concentration of lidocaine can saturate AAG with which it binds as the concentration of AAG is much less in comparison to that of HSA in blood.

Drug-Protein/Tissue Affinity

Lidocaine has greater affinity for AAG than for HSA. Digoxin has more affinity for proteins of cardiac muscles than those of skeletal muscles or plasma. Iophenoxic acid, a radioopaque medium, has so great an affinity for plasma proteins that it has a half-life of 2½ years.

PROTEIN/TISSUE RELATED FACTORS

Physicochemical Properties of Protein/Binding Component

Lipoproteins and adipose tissue tend to bind lipophilic drugs by dissolving them in their lipid core. The physiologic pH determines the presence of active anionic and cationic groups on the albumin molecules to bind a variety of drugs.

Concentration of Protein/Binding Component

Among the plasma proteins, binding predominantly occurs with albumin as it is present in a higher concentration in comparison to other plasma proteins. The amount of several proteins and tissue components available for binding, changes during disease states. This effect will be discussed in the subsequent sections.

Number of Binding Sites on the Protein

Albumin has a large number of binding sites as compared to other proteins and is a high capacity binding component. Several drugs are capable of binding at more than one site on albumin, e.g. fluocloxacillin, flurbiprofen, ketoprofen, tamoxifen and dicoumarol bind to both primary and secondary sites on albumin. Indomethacin is known to bind to 3 different sites. AAG is a protein with limited binding capacity because of its low concentration and low molecular size. Though pure AAG has only one binding site for lidocaine, in presence of HSA, two binding sites have been reported which was suggested to be due to direct interaction between HSA and AAG.

DRUG INTERACTIONS

Competition Between Drugs for the Binding Sites (Displacement Interactions)

When two or more drugs can bind to the same site, competition between them for interaction with the binding site results. If one of the drugs (drug A) is bound to such a site, then administration of another drug (drug B) having affinity for the same site results in displacement of drug A from its binding site. Such a *drug-drug interaction for the common binding site* is called as **displacement interaction**. The drug A here is called as the **displaced drug** and drug B as the **displacer**. Warfarin and phenylbutazone have same degree of affinity for HSA. Administration of phenylbutazone to a patient on warfarin therapy results in displacement of latter from its binding site. The free warfarin may cause adverse hemorrhagic reactions which may be lethal. Phenylbutazone is also known to displace sulfonamides from their HSA binding sites. Displacement interactions can result in unexpected rise in free concentration of the displaced drug which may enhance clinical response or toxicity. Even a drug metabolite can affect displacement interaction.

Clinically significant interactions will result when:

I. *The displaced drug* (e.g. warfarin) —
   a. is more than 95% bound
   b. has a small volume of distribution (less than 0.15 L/Kg)
   c. shows a rapid onset of therapeutic or adverse effects
   d. has a narrow therapeutic index

II. *The displacer drug* (e.g. phenylbutazone) —
   a. has a high degree of affinity as the drug to be displaced
   b. competes for the same binding sites

c. the drug/protein concentration ratio is high (above 0.10), and
d. shows a rapid and large increase in plasma drug concentration.

It will be worthwhile to mention here that, both the concentration of the displacer drug and its affinity for the binding site with respect to that of the drug to be displaced, will determine the extent to which displacement will occur.

For a drug that is 95% bound, a displacement of just 5% of the bound drug results in a 100% rise in free drug concentration. If the displaced drug has a small volume of distribution, it remains confined to the blood compartment and shows serious toxic responses. On the contrary, if such a drug has a large V_d, it redistributes into a large volume of body fluids and clinical effects may be negligible or insignificant. The increase in free drug concentration following displacement also makes it more available for elimination by the liver and the kidneys (Fig. 4.3). If the drug is easily metabolizable or excretable, its displacement results in significant reduction in elimination half-life.

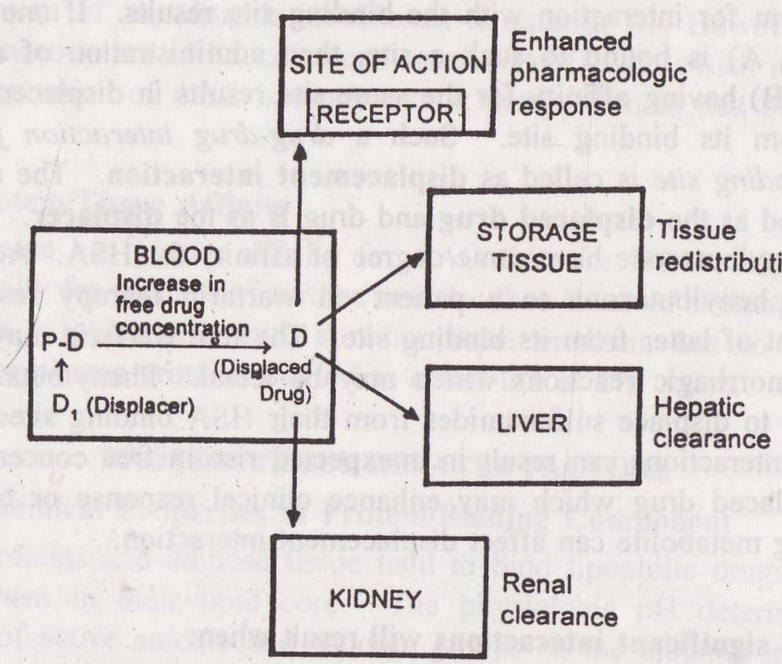


Fig. 4.3 Fate of a drug after displacement interaction

Displacement also becomes insignificant with the use of more selective, potent, low dose drugs.

Competition Between Drugs and Normal Body Constituents

Among the various normal body constituents, the free fatty acids are known to interact with a number of drugs that bind primarily to HSA. The free fatty acid level is increased in several physiologic (fasting), pathologic (diabetes, myocardial infarction, alcohol abstinence) and pharmacologically induced conditions (after heparin and caffeine administration). The fatty acids, which also bind to albumin, influence binding of several benzodiazepines and propranolol (decreased binding) and warfarin (increased binding). Bilirubin binding to HSA can be impaired by certain drugs and is of great concern in neonates whose BBB and bilirubin metabolizing capacity are not very efficient. Acidic drugs such as sodium salicylate, sodium benzoate and sulfonamides displace bilirubin from its albumin binding site. The free bilirubin is not conjugated by the liver of the neonates and thus crosses the BBB and precipitates the condition called as **kernicterus** (characterized by degeneration of brain and mental retardation).

Allosteric Changes in Protein Molecule

This is yet another mechanism by which drugs can affect protein binding interactions. The process involves alteration of the protein structure by the drug or its metabolite thereby modifying its binding capacity. The agent that produces such an effect is called as **allosteric effector**, e.g. aspirin acetylates the lysine fraction of albumin thereby modifying its capacity to bind NSAIDs like phenylbutazone (increased affinity) and flufenamic acid (decreased affinity).

PATIENT RELATED FACTORS

Age

Modification in protein-drug binding as influenced by age of the patient is mainly due to differences in the protein content in various age groups.

i. *Neonates* : Albumin content is low in newborn; as a result, the unbound concentration of drug that primarily bind to albumin, for example phenytoin and diazepam, is increased.

ii. *Young infants* : An interesting example of differences in protein-drug binding in infants is that of digoxin. Infants suffering from congestive cardiac failure are given a digitalizing dose 4 to 6 times the adult dose on body weight basis. This is contrary to one's belief that infants should be given low doses considering their poorly developed drug eliminating system. The reason attributed for use of a large digoxin dose is greater binding of the drug in infants (the other reason is abnormally large renal clearance of digoxin in infants).

iii. *Elderly* : In old age, the albumin content is lowered and free concentration of drugs that bind primarily to it is increased. Old age is also characterized by an increase in the levels of AAG and thus decreased

free concentration is observed for drugs that bind to it. The situation is complex and difficult to generalize for drugs that bind to both HSA and AAG, e.g lidocaine and propranolol.

Intersubject Variations

Intersubject variability in drug binding as studied with few drugs showed that the difference is small and no more than two fold. These differences have been attributed to genetic and environmental factors.

Disease States

Several pathologic conditions are associated with alteration in protein content. Since albumin is the major drug binding protein, hypoalbuminemia can severely impair protein-drug binding. Hypoalbuminemia is caused by several conditions like aging, CCF, trauma, burns, inflammatory states, renal and hepatic disorders, pregnancy, surgery, cancer, etc. Almost every serious chronic illness is characterized by decreased albumin content. Some of the diseases that modify protein-drug binding are depicted in Table 4.2. Hyperlipoproteinemia, caused by hypothyroidism, obstructive liver disease, alcoholism, etc., affects binding of lipophilic drugs.

TABLE 4.2 **Influence of Disease States on Protein-Drug Binding**

Disease	*Influence on Plasma Protein*	*Influence on Protein-Drug Binding*
1. Renal failure (uremia)	decreased albumin content	decreased binding of acidic drugs; neutral and basic drugs unaffected.
2. Hepatic failure	decreased albumin synthesis	decreased binding of acidic drugs; binding of basic drugs is normal or reduced depending on AAG levels.
3. Inflammatory states (trauma, surgery, burns, infections, etc.)	increased AAG levels	increased binding of basic drugs; neutral and acidic drugs unaffected.

Putting in a nutshell, all factors, especially drug interactions and patient related factors that affect protein or tissue binding of drugs, influence:

1. *Pharmacokinetics of drugs* : A decrease in plasma protein—drug binding i.e. an increase in unbound drug concentration, favors tissue redistribution and/or clearance of drugs from the body (enhanced biotransformation and excretion).
2. *Pharmacodynamics of drugs* : An increase in concentration of free or unbound drug results in increased intensity of action (therapeutic/toxic).

SIGNIFICANCE OF PROTEIN/TISSUE BINDING OF DRUGS

Absorption

The absorption equilibrium is attained by transfer of free drug from the site of administration into the systemic circulation and when the concentration in these two compartments become equal. Following equilibrium, the process may stop. However, binding of the absorbed drug to plasma proteins decreases free drug concentration and disturbs such an equilibrium. Thus, sink conditions and the concentration gradient are re-established which now act as the driving force for further absorption. This is particularly useful in case of ionized drugs which are transported with difficulty.

Systemic Solubility of Drugs

Water insoluble drugs, neutral endogenous macromolecules such as heparin and several steroids and oil soluble vitamins are circulated and distributed to tissues by binding especially to lipoproteins which act as a vehicle for such hydrophobic compounds.

Distribution

Plasma protein binding restricts the entry of drugs that have specific affinity for certain tissues. This prevents accumulation of a large fraction of drug in such tissues and thus, subsequent toxic reactions. Plasma protein-drug binding thus favors uniform distribution of drugs throughout the body by its buffer function (maintains equilibrium between the free and the bound drug). A protein bound drug in particular does not cross the BBB, the placental barrier and the glomerulus.

Tissue Binding, Apparent Volume of Distribution and Drug Storage

A drug that is extensively bound to blood components remains confined to blood. Such a drug has a small volume of distribution. A drug that shows extravascular tissue binding has a large volume of distribution. A tissue or blood component that has great affinity for a particular drug acts as a *depot* or *storage site* for that drug; for example, RBC is a storage site for the lipophilic compound tetrahydrocannabinol.

The relationship between tissue-drug binding and apparent volume of distribution can be established as follows:

$$V_d = \frac{\text{Amount of drug in the body}}{\text{Plasma drug concentration}} = \frac{X}{C} \qquad (4.1)$$

$$\text{or, the amount of drug in the body,} \quad X = V_d\, C \qquad (4.2)$$

Similarly, we can write,

$$\text{Amount of drug in plasma} = V_p\ C \tag{4.3}$$

and,

$$\text{Amount of drug in extravascular tissues} = V_t\ C \tag{4.4}$$

The total amount of drug in the body is the sum of amount of drug in plasma and the amount of drug in extravascular tissues. Thus,

$$V_d\ C = V_p\ C + V_t\ C_t \tag{4.5}$$

where V_d = apparent volume of distribution of drug
V_p = volume of plasma
V_t = volume of extravascular tissues
C_t = tissue drug concentration

Dividing equation 4.5 with C we get:

$$V_d = V_p + V_t.C_t/C \tag{4.6}$$

The fraction of drug unbound (f_u) in plasma is given as:

$$f_u = \frac{\text{Concentration of Unbound Drug in Plasma}}{\text{Total Plasma Drug Concentration}} = \frac{C_u}{C} \tag{4.7}$$

Similarly, fraction of drug unbound to tissues is:

$$f_{ut} = \frac{C_{ut}}{C_t} \tag{4.8}$$

Assuming that at distribution equilibrium, the unbound or free drug concentration in plasma equals that in extravascular tissues i.e. $C_u = C_{ut}$, equations 4.7 and 4.8 can be combined to give:

$$\frac{C_t}{C} = \frac{f_u}{f_{ut}} \tag{4.9}$$

Substitution of equation 4.9 in 4.6 yields:

$$V_d = V_p + \frac{V_t.f_u}{f_{ut}} \tag{4.10}$$

From equation 4.10 it is clear that greater the unbound or free concentration of drug in plasma, larger its V_d.

Elimination

Only the unbound or free drug is capable of being eliminated. This is because the drug-protein complex cannot penetrate into the metabolizing organ (liver). The large molecular size of the complex also prevents it

from getting filtered through the glomerulus. Thus, drugs which are more than 95% bound are eliminated slowly i.e. they have long elimination half-lives; for example, tetracycline, which is only 65% bound, has an elimination half-life of 8.5 hours in comparison to 15.1 hours of doxycycline which is 93% bound to plasma proteins. However, penicillins have short elimination half-lives despite being extensively bound to plasma proteins. This is because rapid equilibration occurs between the free and the bound drug and the free drug is equally rapidly excreted by active secretion in renal tubules.

Displacement Interactions and Toxicity

As stated earlier, displacement interactions are significant in case of drugs which are more than 95% bound. This is explained from the example given in Table 4.3. A displacement of just 1% of a 99% bound drug results in doubling of the free drug concentration i.e. a 100% rise. For a drug that is bound to a lesser extent e.g. 90%, displacement of 1% results in only a 10% rise in free drug concentration which may be insignificant clinically.

TABLE 4.3 **Influence of Percent Binding and Displacement on Change in Free Concentration of Drugs**

		Drug A	*Drug B*
% drug before displacement			
	bound	99	90
	free	1	10
% drug after displacement			
	bound	98	89
	free	2	11
% increase in free drug concentration		100	10

Kernicterus in infants is an example of a disorder caused by displacement of bilirubin from albumin binding sites by the NSAIDs. Another example discussed earlier was that of interaction between warfarin and phenylbutazone. Yet another example of displacement is that of digoxin with quinidine. Digoxin represents a drug with a large volume of distribution (i.e. shows extensive extravascular tissue binding). Since displacement interactions may precipitate toxicity of displaced drug, a reduction in its dose may be called for. This may become necessary for a drug having a small V_d such as warfarin since displacement can result in a large increase in free drug concentration in plasma. With a drug of large V_d such as digoxin, even a substantial increase in the degree of displacement of drug in plasma may not effect a large increase in free drug concentration and

dose adjustment may not be required. This is for two reasons—one, only a small fraction of such a drug is present in plasma whereas most of it is localized in extravascular tissues, and secondly, following displacement, the free drug, because of its large V_d, redistributes in a large pool of extravascular tissues. The extent to which the free plasma drug concentration of drugs with different V_d values will change when displaced, can be computed from Equation 4.10.

Diagnosis

The chlorine atom of chloroquine when replaced with radiolabeled I-131 can be used to visualize melanomas of the eye since chloroquine has a tendency to interact with the melanin of eyes. The thyroid gland has great affinity for iodine containing compounds, hence any disorder of the same can be detected by tagging such a compound with a radioisotope of iodine.

Therapy and Drug Targeting

The binding of drugs to lipoproteins can be used for site-specific delivery of hydrophilic moieties. This is particularly useful in cancer therapies since certain tumor cells have greater affinity for LDL than normal tissues. Thus, binding of a suitable antineoplastic to it can be used as a therapeutic tool. HDL are similarly transported more to adrenal and testes. An example of site-specific drug delivery in cancer treatment is that of estramustine. Estradiol binds selectively and strongly to prostrate and thus prostrate cancer can be treated by attaching nitrogen mustard to estradiol for targeting of prostrate glands. Drug targeting prevents normal cells from getting destroyed.

KINETICS OF PROTEIN-DRUG BINDING

If P represents proteins and D the drug, then applying **law of mass action** to reversible protein-drug binding, we can write:

$$P + D \underset{K_d}{\overset{K_a}{\rightleftharpoons}} PD \qquad (4.11)$$

At equilibrium,

$$K_a = \frac{[PD]}{[P]\,[D]} \qquad (4.12)$$

$$[PD] = K_a\,[P]\,[D] \qquad (4.13)$$

where [P] = concentration of free protein
[D] = concentration of free drug
[PD] = concentration of protein-drug complex
K_a = association rate constant
K_d = dissociation rate constant

$K_a > K_d$ indicates forward reaction i.e. protein-drug binding is favored. If P_T is the total concentration of protein present, bound and unbound, then:

$$P_T = [PD] + [P] \qquad (4.14)$$

If r is the number of moles of drug bound to total moles of protein, then,

$$r = \frac{[PD]}{[P_T]} = \frac{[PD]}{[PD] + [P]} \qquad (4.15)$$

Substituting the value of [PD] from equation 4.13 in equation 4.15 we get:

$$r = \frac{K_a\,[P]\,[D]}{K_a\,[P]\,[D] + [P]} = \frac{K_a\,[D]}{K_a\,[D] + 1} \qquad (4.16)$$

Equation 4.16 holds when there is only one binding site on the protein and the protein-drug complex is a 1:1 complex. If more than one or N number of binding sites are available per mole of the protein then:

$$r = \frac{N\,K_a\,[D]}{K_a\,[D] + 1} \qquad (4.17)$$

The value of association constant, K_a and the number of binding sites N can be obtained by plotting equation 4.17 in three different ways as shown below.

1. **Direct Plot** is made by plotting r versus [D] as shown in Fig. 4.4.

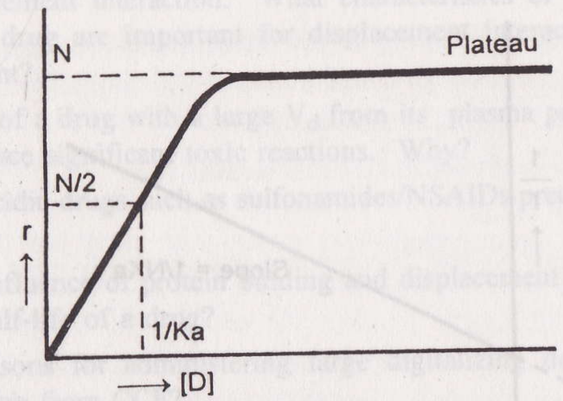


Fig. 4.4 Direct plot of r versus [D]. Note that when all the binding sites are occupied by the drug, the protein is saturated and plateau is reached. At the plateau, r = N. When r = N/2, [D] = $1/K_a$.

2. Scatchard Plot is made by transforming equation 4.17 into a linear form. Thus,

$$r = \frac{N K_a [D]}{K_a [D] + 1} \quad (4.17)$$

$$r + r K_a [D] = N K_a [D]$$

$$r = N K_a [D] - r K_a [D]$$

Therefore,

$$\frac{r}{[D]} = N K_a - r K_a \quad (4.18)$$

A plot of r/[D] versus r yields a straight line (Fig. 4.5). Slope of the line = $-K_a$, y-intercept = NK_a and x-intercept = N.

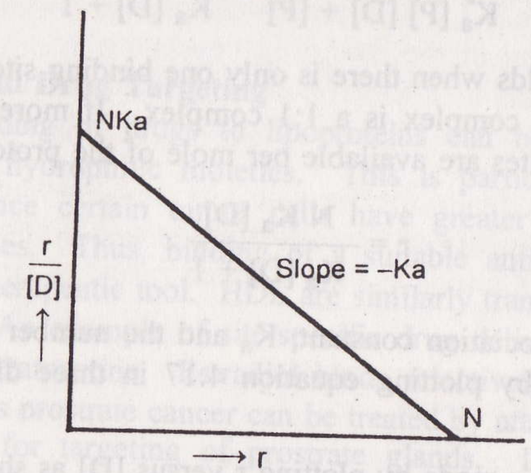


Fig. 4.5 Scatchard plot for protein-drug binding

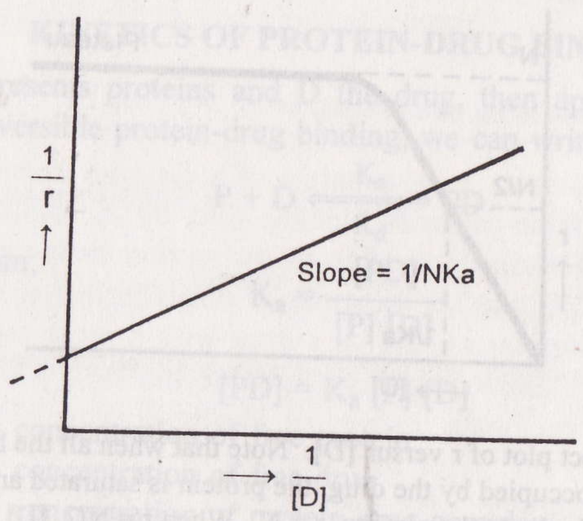


Fig. 4.6 Double reciprocal plot for protein-drug binding

3. Double Reciprocal Plot (Lineweaver-Burk Plot) : The reciprocal of equation 4.17 yields:

$$\frac{1}{r} = \frac{1}{N K_a [D]} + \frac{1}{N} \quad (4.19)$$

A plot of 1/r versus 1/[D] yields a straight line with slope $1/NK_a$ and y-intercept 1/N (Fig. 4.6).

QUESTIONS

1. A protein bound drug is both pharmacokinetically as well as pharmacodynamically inert. Explain.
2. When is drug-binding considered irreversible? What could be the consequence of such an interaction?
3. Classify the body components to which drugs normally bind.
4. Why does binding of a drug to plasma proteins occur to a large extent in comparison to binding to other tissue components?
5. Why is HSA considered a versatile protein for drug binding?
6. With examples, name the various drug binding sites on HSA.
7. Binding of drugs to erythrocytes could be as significant as binding to HSA. Explain.
8. From distribution viewpoint, what is the significance of tissue-drug binding?
9. Though imipramine and chlorpromazine are more lipophilic than thiopental, they do not localize in fats. Why?
10. List the factors influencing protein binding of drugs.
11. Define displacement interaction. What characteristics of the displacer and the displaced drug are important for displacement interactions to be clinically significant?
12. Displacement of a drug with a large V_d from its plasma protein binding site may not produce significant toxic reactions. Why?
13. How do the acidic drugs such as sulfonamides/NSAIDs precipitate kernicterus in neonates?
14. What is the influence of protein binding and displacement interaction on the elimination half-life of a drug?
15. Give two reasons for administering large digitalizing dose of digoxin to infants suffering from CCF?
16. What is the influence of various disease states on plasma protein level and drug-binding?
17. How would the plasma protein-drug binding influence sink conditions and absorption of a drug from the GIT?

18. Renal excretion of penicillins is unaffected by protein-drug binding. Why?

19. Give examples where binding of an agent to a specific tissue can be used for diagnostic purpose.

20. How can the principle of binding be used for drug targeting?

21. Derive the relationship showing that greater the free concentration of drug in plasma, larger its V_d.

22. Warfarin has a V_d of 8 liters in a 70 Kg man and is 90% bound to plasma proteins. Given that the plasma volume is 3 liters and tissue volume 39 liters, determine -

a. the fraction of drug unbound to tissues.
Answer : 0.78.

b. the % increase in free plasma drug concentration if 10% of bound warfarin is displaced by phenylbutazone.
Answer : 90%.

c. the new V_d after the displacement assuming that the tissue binding is unaffected.
Answer : 12.5 liters.

d. from the answer of question (a), suggest whether the displacement will be clinically significant or not.

23. For a drug showing protein binding, the two points on the Scatchard plot of r versus r/[D] are: (0.6, 1.2×10^4) and (1.2, 0.9×10^4).

a. Determine the association constant.
Answer : $K_a = 0.5 \times 10^4$.

b. How many binding sites are present on the protein molecule?
Answer : N = 3.

c. If the points of Scatchard plot are transformed onto the Lineweaver-Burk plot (1/r versus 1/[D]), what will be the slope of the line?
Answer : Slope = $1/NK_a = 0.67 \times 10^{-4}$

d. Compute the x-axis intercept of Lineweaver-Burk plot.
Answer : 1/N = 0.33.

e. Are the values of K_a and N computed from both the plots same?

5

Biotransformation of Drugs

The onset of pharmacologic response depends upon two pharmacokinetic processes—drug absorption, and drug distribution (since most sites of action are in the extravascular tissues). The duration and intensity of action depend upon the rate of drug removal from the body/site of action or simply speaking, on the rate of elimination and tissue redistribution of drug. **Elimination** is the major process for removal of a drug from the body and termination of its action. *It is defined as the irreversible loss of drug from the body.* Elimination occurs by two processes viz. biotransformation and excretion.

Biotransformation *of drugs is defined as the conversion from one chemical form to another.* The term is used synonymously with **metabolism**. The chemical changes are usually affected enzymically in the body and thus, the definition excludes chemical instability of a drug within the body; for e.g. conversion of penicillin to penicilloic acid by the bacterial penicillinase and mammalian enzymes is metabolism but its degradation by the stomach acid to penicillenic acid is chemical instability.

All chemical substances that are not nutrients for the body and enter the body through, ingestion, inhalation or absorption are called as **xenobiotics** (Greek: *xenos* = *foreign*) or **exogenous compounds**. Drugs are also xenobiotics which enter the body by virtue of their lipophilicity. It is interesting to note that for effective absorption, a drug needs to be sufficiently lipid soluble but it is this same physicochemical property that enables it to bypass excretion. This is because only water-soluble agents undergo renal excretion (major route for exit of drugs from the body) whereas lipid soluble substances are passively reabsorbed from the renal tubules into the blood after glomerular filtration. Thus, if such a phenomenon continues, drugs would accumulate in the body and precipitate toxic reactions. However, to prevent such a consequence, the body is armed with the *metabolic system* which transforms the water insoluble, lipophilic, nonpolar drugs into polar and water-soluble products that can be easily excreted by the kidneys and are poorly reabsorbed; for instance, hippuric

acid, the metabolite of benzoic acid, is 2.5 times more water-soluble. *Drug biotransformation is thus a* **detoxification process**. However, exceptions are there when biotransformation leads to products with decreased water solubility. The N-acetyl derivatives of sulfonamides are less water-soluble than the parent drug and thus have a tendency to cause crystalluria.

Biotransformation :

normally results in *pharmacologic inactivation* of drugs, i.e. it results in formation of metabolites with little or no pharmacologic activity; e.g. conversion of phenytoin to p-hydroxy phenytoin,

occasionally yields metabolites with equal activity; e.g. conversion of phenylbutazone to oxyphenbutazone, and

rarely leads to *toxicologic activation* of drugs, i.e. it results in formation of metabolites with high tissue reactivity; e.g. conversion of paracetamol to reactive metabolites that cause hepatic necrosis.

Inactive drugs (prodrugs) also depend upon biotransformation for activation, the process being called as *pharmacologic activation*; e.g. conversion of enalapril to enalaprilat. A change in pharmacologic activity of the drug on metabolism has also been observed (*see* Table 5.1.).

TABLE 5.1 **Metabolites and Relative Activity of Drugs**

Drugs	*Metabolites*
Pharmacologic Inactivation	
Active	*Inactive*
Amphetamine	Phenylacetone
Phenobarbital	Hydroxyphenobarbital
Phenytoin	p-Hydroxy phenytoin
Salicylic acid	Salicyluric acid
No Change in Pharmacologic Activity	
Active	*Active*
Amitriptyline	Nortriptyline
Imipramine	Desipramine
Codeine	Morphine
Phenylbutazone	Oxyphenbutazone
Diazepam	Temazepam
Digitoxin	Digoxin
Toxicologic Activation	
Active	*Reactive Intermediates*
Isoniazid	Tissue acylating intermediate
Paracetamol	Imidoquinone of N-hydroxylated metabolite

...*continued*

Drugs	*Metabolites*
Pharmacologic Activation	
Inactive (Prodrugs)	*Active*
Aspirin	Salicylic acid
Phenacetin	Paracetamol
Sulfasalazine	Mesalamine and Sulfapyridine
Pivampicillin	Ampicillin
Enalapril	Enalaprilat
Chloramphenicol palmitate	Chloramphenicol
Change in Pharmacologic Activity	
Iproniazid (antidepressant)	Isoniazid (antitubercular)
Diazepam (tranquilizer)	Oxazepam (anticonvulsant)

In comparison with xenobiotics, the natural endogenous substances such as neurotransmitters (dopamine, GABA, epinephrine, norepinephrine, etc.), steroids (testosterone, progesterone, cortisol, etc.) and insulin which are also used as therapeutic agents, are inactivated rapidly because of the body's well developed system for metabolizing such agents. These substances are therefore called as **soft drugs**. Such soft drugs do not precipitate unexpected toxicity when used in concentrations close to their normal levels.

Drug Metabolizing Organs

Liver is the primary site for metabolism of almost all drugs (and other xenobiotics) because of its relative richness in possessing a large variety of enzymes in large amounts. *Metabolism by organs other than liver (called as* **extrahepatic metabolism**) is of minor importance since lower level of drug metabolizing enzymes are present in such tissues. The decreasing order of drug metabolizing ability of various organs is: *liver > lungs > kidneys > intestine > placenta > adrenals > skin*. Brain, testes, muscles, spleen, etc. also metabolize drugs to a small extent.

Drug Metabolizing Enzymes

The enzymes that biotransform xenobiotics differ from those that metabolize food materials. They are versatile and nonspecific in metabolizing a large number of drugs. The enzymes are broadly divided into 2 categories: **microsomal** and **non-microsomal**. The microsomal enzymes catalyze a majority of drug biotransformation reactions. The microsomes are basically artifacts which resulted when attempts were first made to isolate endoplasmic reticulum of the liver homogenate. These vesicular fragments or microsomes are derived from rough endoplasmic reticulum

(rough due to the presence of RNA rich ribosomes on the membrane surface whose function is protein synthesis) which shed their ribosomes to become smooth surfaced. The large variety of microsomal enzymes catalyze a number of oxidative, reductive and hydrolytic and glucuronidation reactions.

Some *important characteristics of microsomal enzyme system* are:

1. The intact nature of lipoidal membrane bound enzyme of the microsomes is essential for its selectivity towards lipid-soluble substrates.
2. A number of lipid-soluble substrates (xenobiotics in general) can interact nonspecifically with the microsomal enzymes; natural endogenous substances which are generally water-soluble do not interact.
3. The lipid soluble substrate is biotransformed into a water-soluble metabolite by the microsomal enzymes which can be readily excreted.

The **nonmicrosomal enzymes** include those that are present in soluble form in the cytoplasm and those attached to the mitochondria but not to endoplasmic reticulum. These are also nonspecific enzymes that catalyze few oxidative reactions, a number of reductive and hydrolytic reactions and conjugation reactions other than glucuronidation. It is interesting to note that, in contrast to microsomal enzymes, the nonmicrosomal enzymes, especially the soluble enzymes, act on relatively water-soluble xenobiotics (as well as endogenous compounds), e.g. oxidases, peroxidases, dehydrogenases, esterases, etc.

CHEMICAL PATHWAYS OF DRUG BIOTRANSFORMATION

R.T.Williams divided the pathways of drug metabolism reactions into two general categories: phase I and phase II reactions.

Phase I Reactions

These reactions generally precede phase II reactions and include oxidative, reductive and hydrolytic reactions. By way of these reactions, a polar functional group is either *introduced* or *unmasked* if already present on the otherwise lipid soluble substrate, e.g. -OH, -COOH, $-NH_2$ and -SH. Thus, *phase I reactions are also called as* **functionalization reactions**. These transformations are also called as **asynthetic reactions**, opposite to the synthetic phase II reactions. The resulting product of phase I reaction is susceptible to phase II reactions.

Phase II Reactions

These reactions generally involve covalent attachment of small polar endogenous molecules such as glucuronic acid, sulfate, glycine, etc. to either unchanged drugs or phase I products having suitable functional groups viz. -OH, -COOH, $-NH_2$ and -SH and form highly water-soluble *conjugates* which are readily excretable by the kidneys (or bile). Thus, these reactions are called as **conjugation reactions**. Since the outcome of such processes are generally products with increased molecular size (and altered physicochemical properties), they are also called as **synthetic reactions**. Quite often, a phase I reaction may not yield a metabolite that is sufficiently hydrophilic or pharmacologically inert but conjugation reactions generally result in products with total loss of pharmacologic activity and high polarity. Hence, *phase II reactions are better known as* **true detoxification reactions**. Since these reactions generally involve transfer of moieties to the substrate to be conjugated, the enzymes responsible are called as *transferases*.

The biotransformation of drug metabolites, particularly the glutathione conjugates which are excreted via bile in the gut, by the intestinal microflora, is considered by few researchers as **phase III reactions**.

Quite commonly, the biotransformation reactions proceed *sequentially* and the combination of several phase I and phase II reactions yield a range of metabolites (Fig. 5.1).

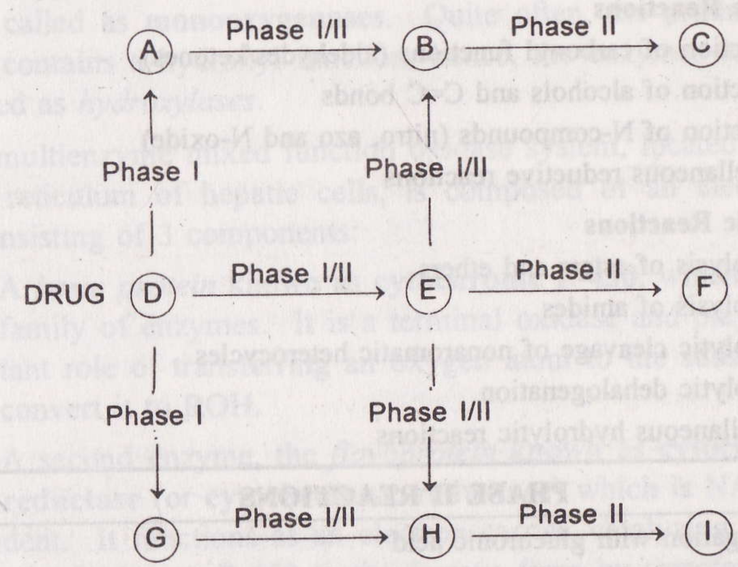


Fig. 5.1 Sequence of phase I and phase II reactions yielding a range of products

The various phase I and phase II reactions are listed in Table 5.2

TABLE 5.2—Chemical Pathways of Drug Biotransformation—(M) and (N) Indicate Reactions Catalyzed by Microsomal and Nonmicrosomal Enzymes

PHASE I REACTIONS	
A. Oxidative Reactions	
1. Oxidation of aromatic carbon atoms	(M)
2. Oxidation of olefins (C=C bonds)	(M)
3. Oxidation of benzylic, allylic carbon atoms and carbon atoms alpha to carbonyl and imines	(M)
4. Oxidation of aliphatic carbon atoms	(M)
5. Oxidation of alicyclic carbon atoms	(M)
6. Oxidation of carbon-heteroatom systems:	
a. Carbon-Nitrogen systems (aliphatic and aromatic amines)	
i. N-Dealkylation	(M)
ii. Oxidative deamination	(M),(N)
iii. N-Oxide formation	(M)
iv. N-Hydroxylation	(M)
b. Carbon-Sulfur systems:	
i. S-Dealkylation	(M)
ii. Desulfuration	(M)
iii. S-oxidation	(M)
c. Carbon-Oxygen systems (O-dealkylation)	(M)
7. Oxidation of alcohol, carbonyl and acid functions	(M),(N)
8. Miscellaneous oxidative reactions	
B. Reductive Reactions	
1. Reduction of carbonyl functions (aldehydes/ketones)	(M),(N)
2. Reduction of alcohols and C=C bonds	(M)
3. Reduction of N-compounds (nitro, azo and N-oxide)	(M),(N)
4. Miscellaneous reductive reactions	
C. Hydrolytic Reactions	
1. Hydrolysis of esters and ethers	(M),(N)
2. Hydrolysis of amides	(M),(N)
3. Hydrolytic cleavage of nonaromatic heterocycles	(M),(N)
4. Hydrolytic dehalogenation	
5. Miscellaneous hydrolytic reactions	
PHASE II REACTIONS	
1. Conjugation with glucuronic acid	(M)
2. Conjugation with sulfate moieties	(N)
3. Conjugation with alpha amino acids	(N)
4. Conjugation with glutathione and mercapturic acid formation	(N)
5. Acetylation reactions	(N)
6. Methylation reactions	(N)
7. Miscellaneous conjugation reactions	(N)

PHASE I REACTIONS

OXIDATIVE REACTIONS

Oxidative reactions are the most important and most common metabolic reactions. Almost all drugs that undergo phase I biotransformation undergo oxidation at some stage or the other. A simple reason for oxidation being a predominant reaction is that energy in animals is primarily derived by oxidative combustion of organic molecules containing carbon and hydrogen atoms.

Oxidative reactions increase hydrophilicity of xenobiotics by introducing polar functional groups such as—OH. Such a polar metabolite can thus rapidly undergo phase II reaction or is excretable by the kidneys.

Oxidation of xenobiotics is nonspecifically catalyzed by a number of enzymes located in the microsomes. Such enzymes require both *molecular oxygen* (O_2) and the reducing agent *NADPH* to effect reaction. They are therefore referred to as the **mixed function oxidases**. The overall stoichiometry of this reaction involving the substrate RH which yields the product ROH, is given by the following equation:

$$RH + O_2 + NADPH + H^+ \longrightarrow ROH + H_2O + NADP^+$$

where NADPH = reduced nicotinamide adenine dinucleotide phosphate.

Since only one oxygen atom from the molecular oxygen (dioxygen or O_2) is incorporated in the product formed, the mixed function oxidases are also called as **monooxygenases**. Quite often, the product of such a reaction contains a hydroxyl function, hence, the enzymes are sometimes also called as *hydroxylases*.

The multienzyme mixed function oxidase system, located in the endoplasmic reticulum of hepatic cells, is composed of an electron transfer chain consisting of 3 components:

1. A *heme protein* known as **cytochrome P-450**, which is actually a family of enzymes. It is a terminal oxidase and plays the important role of transferring an oxygen atom to the substrate RH and convert it to ROH.
2. A second enzyme, the *flavoprotein* known as **cytochrome P-450 reductase** (or **cytochrome c reductase**) which is NADPH dependent. It functions as an electron carrier, catalyzing the reduction of cytochrome P-450 to the ferrous form by transferring an electron from NADPH.
3. A *heat stable lipid component* known as **phosphatidylcholine**. Its function is to facilitate electron transfer from NADPH to cytochrome P-450.

Magnesium ions are also required for maximal activity of mixed function oxidases.

The most important component of mixed function oxidases is the cytochrome P-450 since it binds to substrate and activates oxygen. The reduced form of this enzyme (Fe^{++}) binds with carbon monoxide to form a complex that shows maximum absorption at 450 nm, hence the name. The mechanism of cytochrome P-450 catalyzed metabolism of xenobiotics is depicted in the redox cycle in Fig. 5.2.

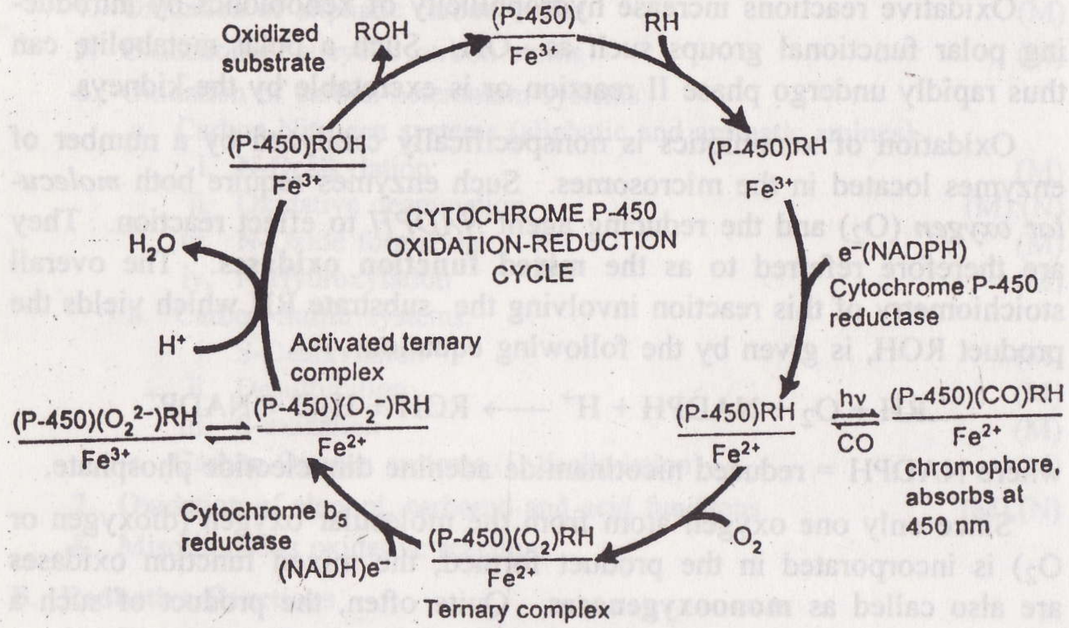


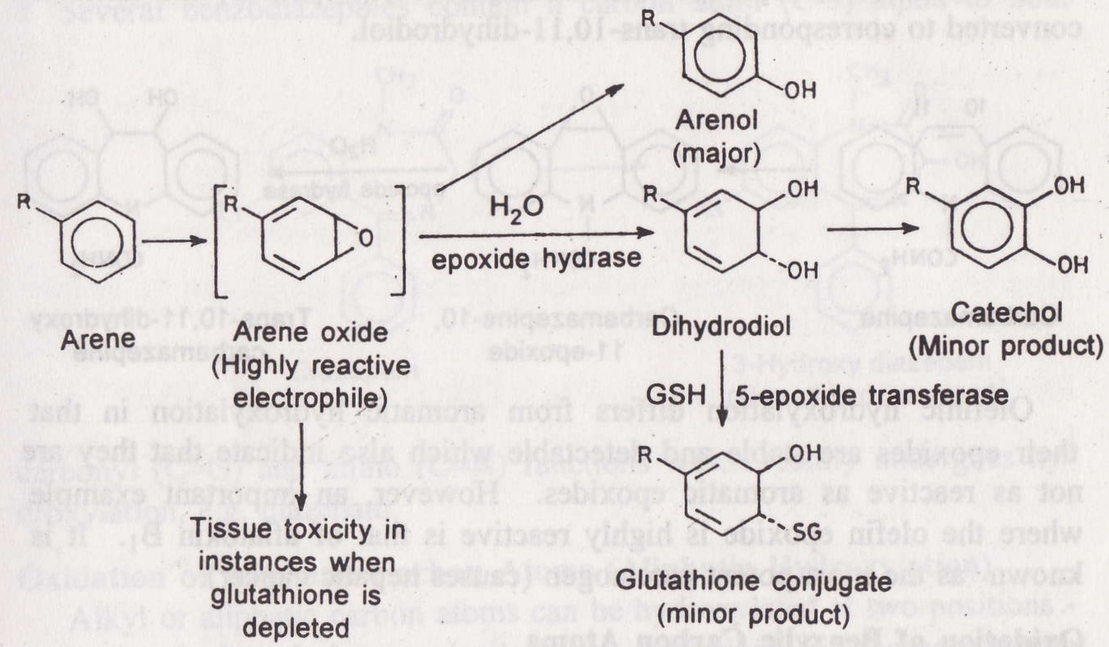
Fig. 5.2 Cytochrome P-450 oxidation-reduction cycle

The various *steps in the oxidation of xenobiotics* are:

1. Binding of the substrate (RH) to the oxidized form of the cytochrome P-450 (Fe^{+++}) to form a complex.
2. A one electron transfer from NADPH to the complex by cytochrome P-450 reductase to form reduced (Fe^{++}) P-450 substrate complex; this step is considered as the rate-limiting step in the overall oxidation of xenobiotics.
3. The reduced enzyme-substrate complex combines with a molecule of oxygen to form a *ternary complex*.
4. The ternary complex combines with a second electron supplied by NADH in presence of enzyme cytochrome b_5 reductase to form a ternary *activated oxygen P-450 substrate complex*.
5. One atom of oxygen from the activated oxygen complex is transferred to the substrate to yield the oxidized product and the other atom forms water. The free oxidized form of cytochrome P-450 is now ready to attach to yet another molecule of substrate.

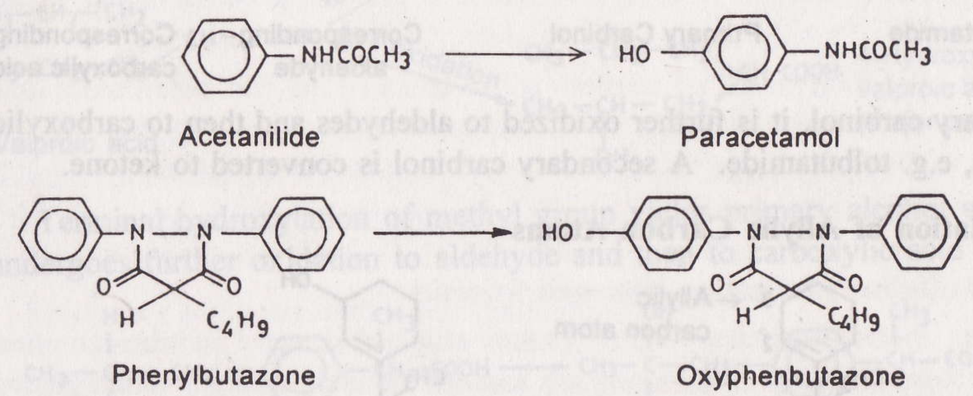
Oxidation of Aromatic Carbon Atoms (Aromatic Hydroxylation)

This reaction proceeds via formation of a reactive intermediate arene oxide (epoxide) which in most cases undergoes rearrangement to yield arenols and in some cases catechols and glutathione conjugates.



The arene oxide intermediate is highly reactive and known to be carcinogenic or cytotoxic in some instances, e.g. epoxides of bromobenzene and benzo(a)pyrene.

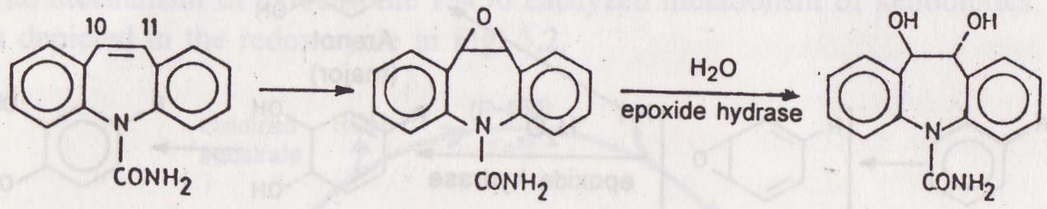
Monosubstituted benzene derivatives can be hydroxylated at ortho-, meta- or para-positions but para-hydroxylated product is most common, e.g. conversion of acetanilide to paracetamol, and phenylbutazone to oxyphenbutazone.



Such a reaction is favored if the substituent is an activating group (electron rich) like the amino group. Deactivating or electron withdrawing groups such as carboxyl and sulfonamide retard or prevent aromatic hydroxylation, e.g. probenicid.

Oxidation of Olefins

Oxidation of nonaromatic carbon-carbon double bonds is analogous to aromatic hydroxylation i.e. it proceeds via formation of epoxides to yield 1,2-dihydrodiols. A better known example of olefinic oxidation is conversion of carbamazepine to carbamazepine-10,11-epoxide; the latter is converted to corresponding trans-10,11-dihydrodiol.

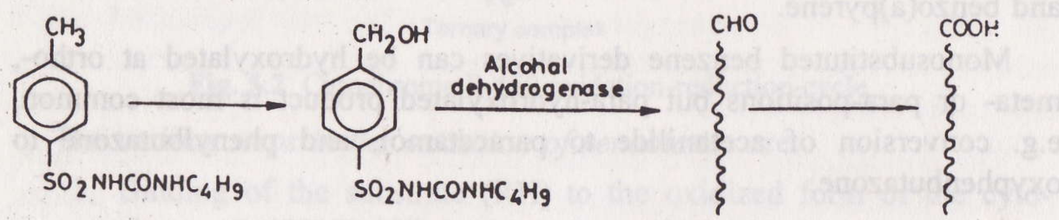


Carbamazepine — Carbamazepine-10, 11-epoxide — Trans-10,11-dihydroxy carbamazepine

Olefinic hydroxylation differs from aromatic hydroxylation in that their epoxides are stable and detectable which also indicate that they are not as reactive as aromatic epoxides. However, an important example where the olefin epoxide is highly reactive is that of aflatoxin B_1. It is known as the most potent carcinogen (causes hepatic cancer).

Oxidation of Benzylic Carbon Atoms

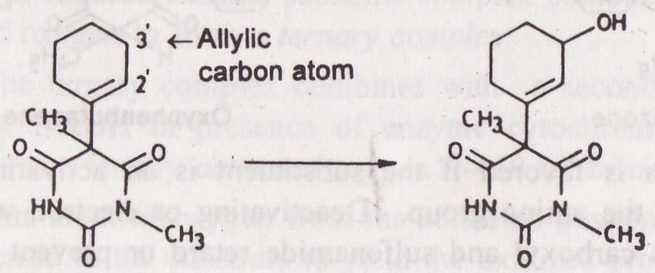
Carbon atoms attached directly to the aromatic rings (benzylic carbon atoms) are hydroxylated to corresponding carbinols. If the product is a



Tolbutamide — Primary Carbinol — Corresponding aldehyde — Corresponding carboxylic acid

primary carbinol, it is further oxidized to aldehydes and then to carboxylic acids, e.g. tolbutamide. A secondary carbinol is converted to ketone.

Oxidation of Allylic Carbon Atoms

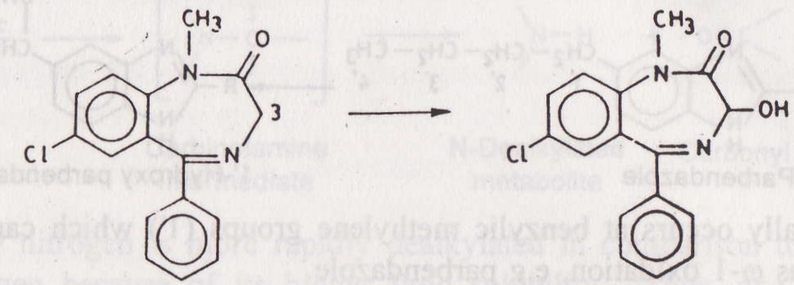


Hexobarbital — 3'-Hydroxy hexobarbital

Carbon atoms adjacent to olefinic double bonds (are allylic carbon atoms) also undergo hydroxylation in a manner similar to benzylic carbons, e.g. hydroxylation of hexobarbital to 3'-hydroxy hexobarbital.

Oxidation of Carbon Atoms Alpha to Carbonyls and Imines

Several benzodiazepines contain a carbon atom (C-3) alpha to both

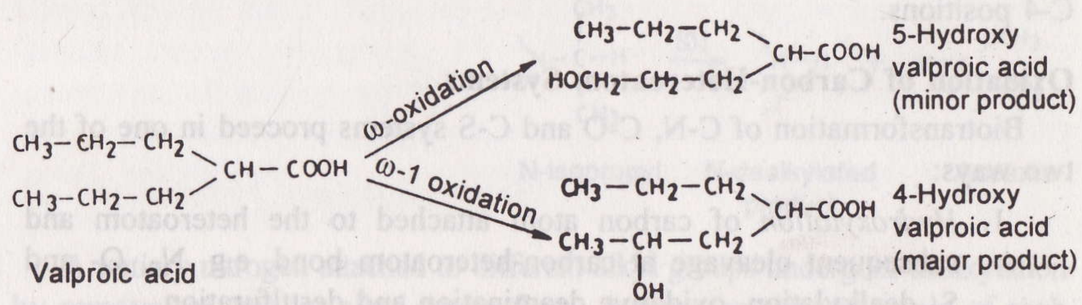


Diazepam — 3-Hydroxy diazepam (N-methyl oxazepam)

carbonyl (C=O) and imino (C=N) functions which readily undergoes hydroxylation, e.g. diazepam.

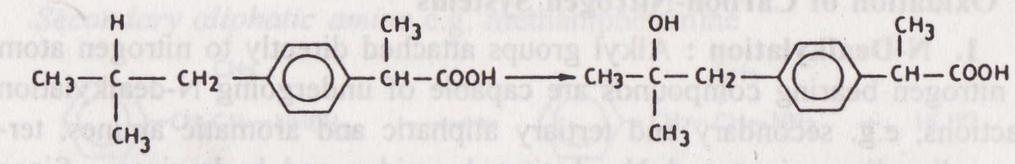
Oxidation of Aliphatic Carbon Atoms (Aliphatic Hydroxylation)

Alkyl or aliphatic carbon atoms can be hydroxylated at two positions - at the terminal methyl group (called as **ω-oxidation**) and the penultimate carbon atom (called as **ω-1 oxidation**) of which the latter accounts for the major product, e.g valproic acid. Hydroxylation at other carbon atoms in long chain compounds is less common.



Valproic acid

Terminal hydroxylation of methyl group yields primary alcohol which undergoes further oxidation to aldehyde and then to carboxylic acid quite

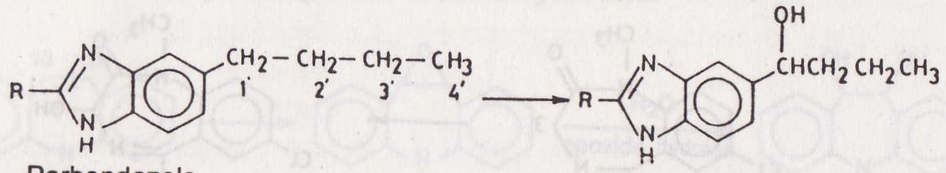


Ibuprofen — Tertiary alcohol metabolite

rapidly. Penultimate carbon atom can be secondary or tertiary of which the latter type is also equally reactive, e.g. ibuprofen.

The ω-1 oxidation of secondary and tertiary penultimate carbons yield corresponding alcohols. The secondary alcohol products seldom undergo further oxidation to ketones as the latter are less hydrophilic. Further oxidation of tertiary alcohol products is improbable.

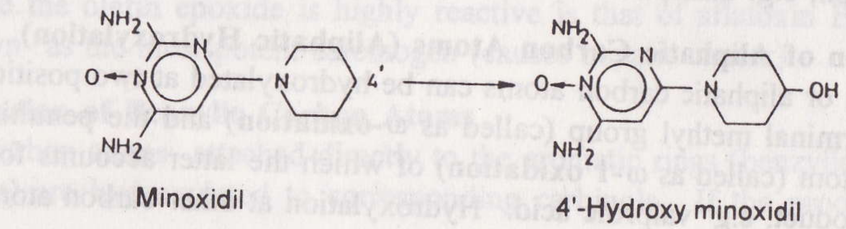
Hydroxylation of aliphatic side chains attached to an aromatic ring



generally occurs at benzylic methylene groups (1') which can be considered as ω-1 oxidation, e.g parbendazole.

Oxidation of Alicyclic Carbon Atoms (Alicyclic Hydroxylation)

Cyclohexane (alicyclic) and piperidine (nonaromatic heterocycle) rings are commonly found in a number of molecules, e.g. acetohexamide and



minoxidil respectively. Such rings are generally hydroxylated at C-3 or C-4 positions.

Oxidation of Carbon-Heteroatom Systems

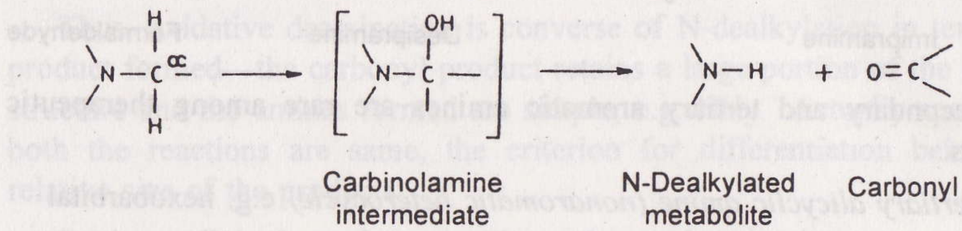
Biotransformation of C-N, C-O and C-S systems proceed in one of the two ways:

1. *Hydroxylation* of carbon atom attached to the heteroatom and subsequent cleavage at carbon-heteroatom bond, e.g. N-, O- and S- dealkylation, oxidative deamination and desulfuration.
2. *Oxidation* of the heteroatom itself, e.g. N- and S-oxidation.

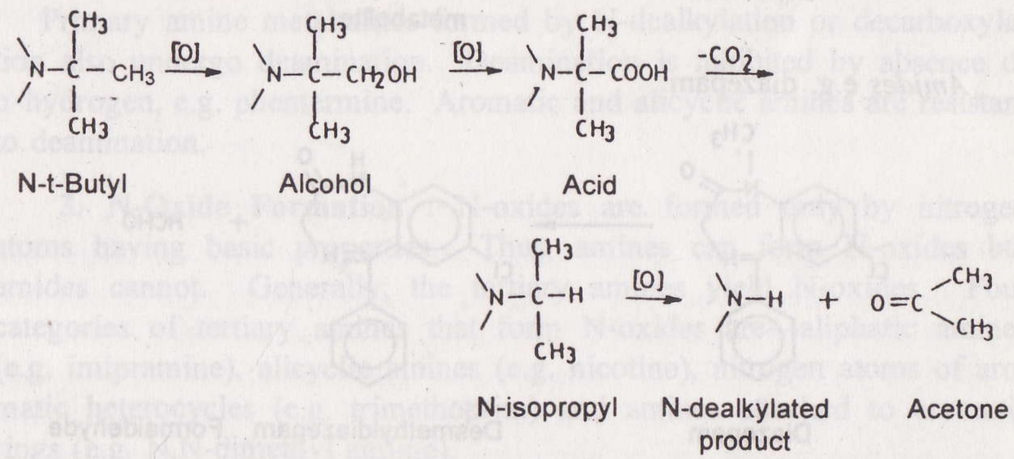
Oxidation of Carbon-Nitrogen Systems

1. N-Dealkylation : Alkyl groups attached directly to nitrogen atom in nitrogen bearing compounds are capable of undergoing N-dealkylation reactions, e.g. secondary and tertiary aliphatic and aromatic amines, tertiary alicyclic amines and N-substituted amides and hydrazines. Since N-dealkylation of amines yield amines and amides yield amides, the reaction is said to undergo without any change in the state of oxidation.

It is however the removed alkyl group that is oxidized. Mechanism of N-dealkylation involve oxidation of α-carbon to generate an intermediate *carbinolamine* which rearranges by cleavage of C-N bond to yield the N-dealkylated product and the corresponding carbonyl of the alkyl group (a primary alkyl is transformed to aldehyde and a secondary alkyl to ketone).

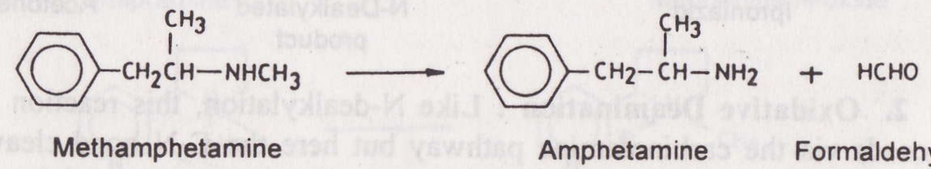


A tertiary nitrogen is more rapidly dealkylated in comparison to secondary nitrogen because of its higher lipid solubility. Thus, one alkyl from a tertiary nitrogen compound is removed rapidly and the second one slowly. Small alkyl groups like methyl, ethyl, n-propyl, isopropyl, etc. are removed rapidly. N-dealkylation of t-butyl group is not possible because the α-carbon cannot be hydroxylated. Such groups are, however, removed via initial hydroxylation of one of the methyl groups to alcohol.

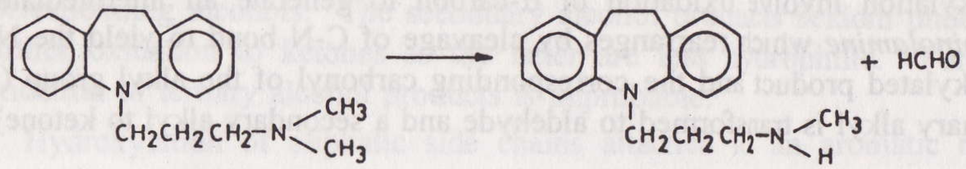


A tertiary nitrogen attached to different alkyl groups undergoes dealkylation by removal of smaller alkyl group first. A representative example of each of the chemical classes of compounds capable of undergoing N-dealkylation is given below.

Secondary aliphatic amine e.g. methamphetamine

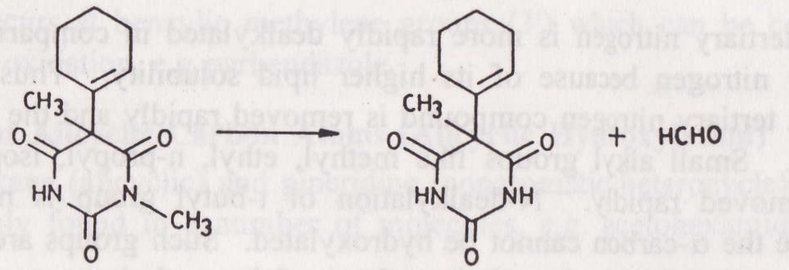


Tertiary aliphatic amine e.g. imipramine

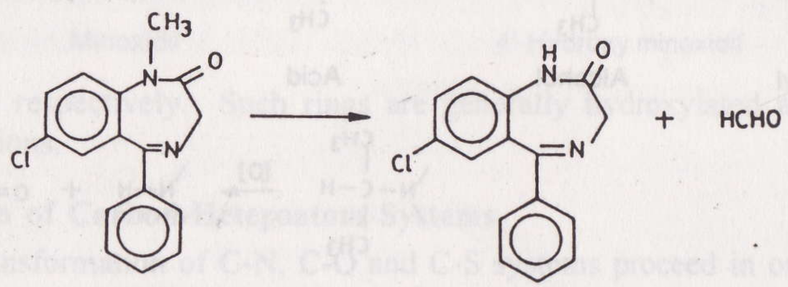


Secondary and tertiary aromatic amines are rare among therapeutic agents.

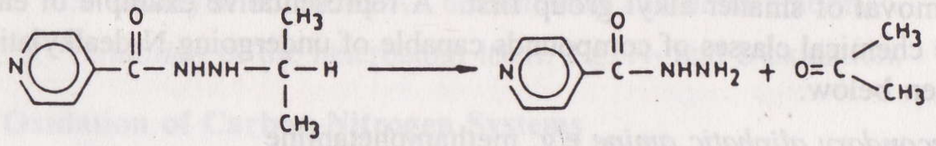
Tertiary alicyclic amine (nonaromatic heterocycle) e.g. hexobarbital



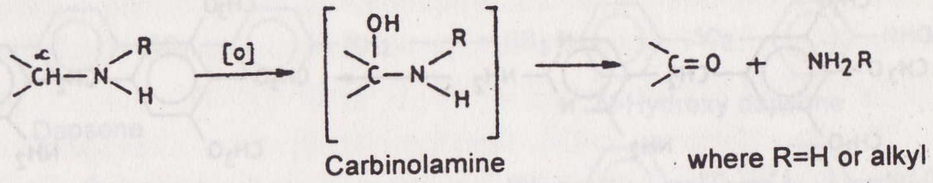
Amides e.g. diazepam



Hydrazines e.g. iproniazid

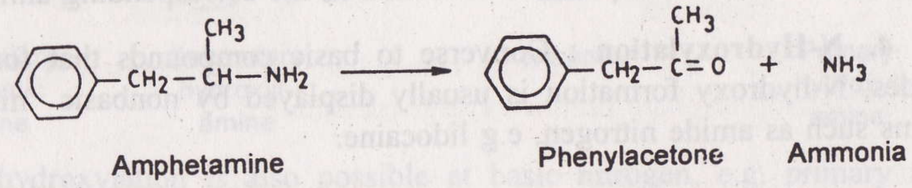


2. Oxidative Deamination : Like N-dealkylation, this reaction also proceeds via the carbinolamine pathway but here the C-N bond cleavage occurs at the bond that links amino group to the larger portion of the drug molecule.



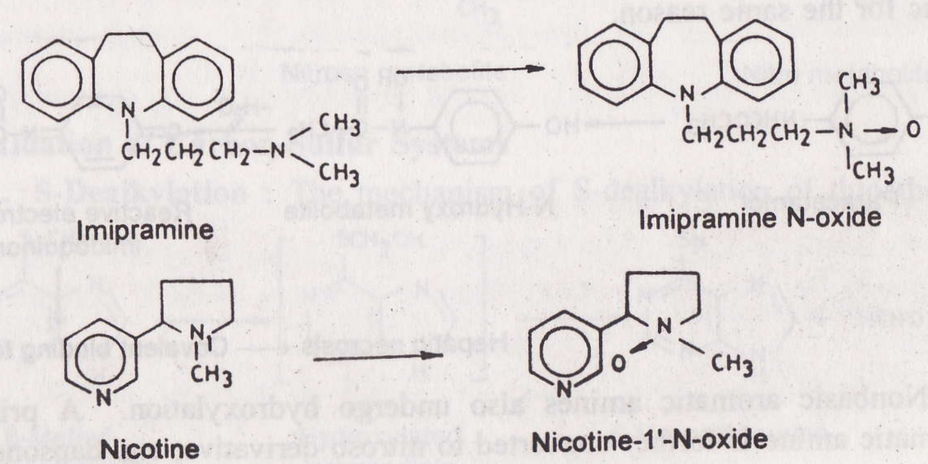
Thus, oxidative deamination is converse of N-dealkylation in terms of product formed—the carbonyl product retains a large portion of the parent structure and the amines formed are simple, e.g. NH_3. Actually speaking, both the reactions are same, the criterion for differentiation being the relative size of the products.

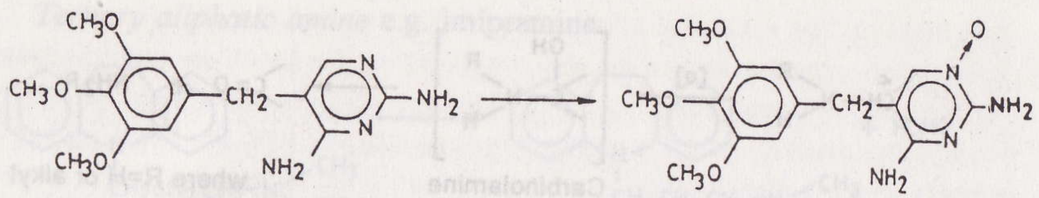
Primary aliphatic amines readily undergo deamination, e.g. amphetamine, while secondary and tertiary amines are deaminated only when bulky groups are attached to nitrogen, e.g. propranolol.



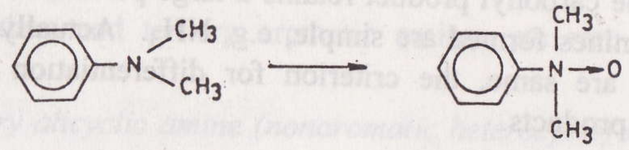
Primary amine metabolites formed by N-dealkylation or decarboxylation also undergo deamination. Deamination is inhibited by absence of α-hydrogen, e.g. phentermine. Aromatic and alicyclic amines are resistant to deamination.

3. N-Oxide Formation : N-oxides are formed only by nitrogen atoms having basic properties. Thus, amines can form N-oxides but amides cannot. Generally, the tertiary amines yield N-oxides. Four categories of tertiary amines that form N-oxides are—aliphatic amines (e.g. imipramine), alicyclic amines (e.g. nicotine), nitrogen atoms of aromatic heterocycles (e.g. trimethoprim) and amines attached to aromatic rings (e.g. N,N-dimethyl aniline).





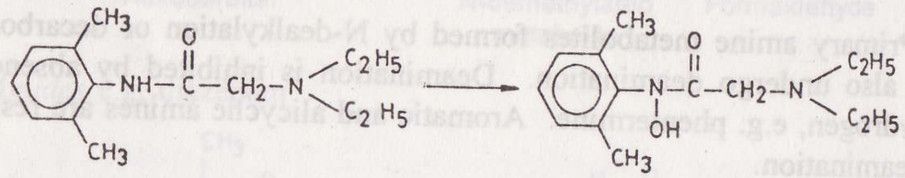
Trimethoprim — Trimethoprim-1-N-oxide



N,N-Dimethyl aniline — N-Oxide metabolite

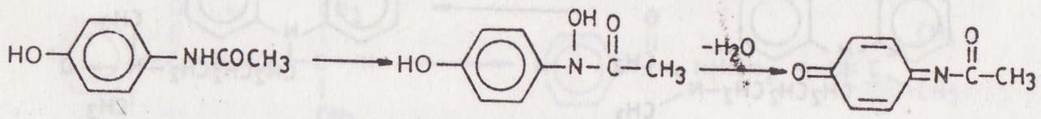
The N-oxide products are highly water-soluble and excreted in urine. They are however susceptible to reduction to the corresponding amine.

4. N-Hydroxylation : Converse to basic compounds that form N-oxides, N-hydroxy formation is usually displayed by nonbasic nitrogen atoms such as amide nitrogen, e.g lidocaine.



Lidocaine — N-Hydroxy lidocaine

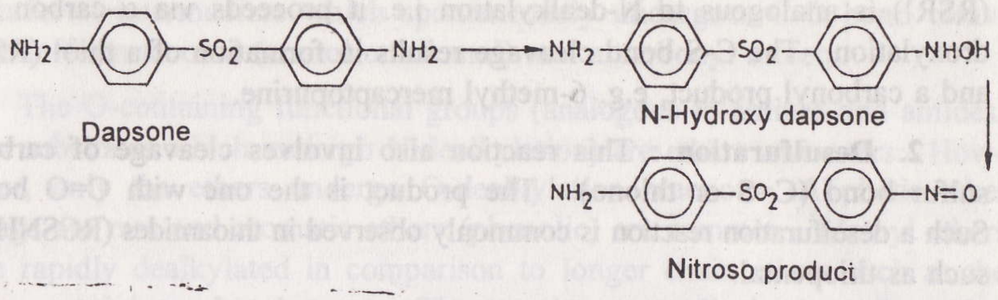
N-Hydroxylation of amides often leads to generation of chemically reactive intermediates capable of binding covalently with macromolecules, e.g paracetamol. Paracetamol is safe in therapeutic doses since its reactive metabolite imidoquinone is neutralized by glutathione; however, in high doses, the glutathione level becomes insufficient and significant covalent tissue binding thus occurs resulting in hepatotoxicity. Phenacetin is also toxic for the same reason.



Paracetamol — N-Hydroxy metabolite — Reactive electrophilic imidoquinone

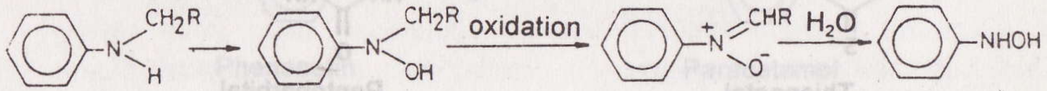
Hepatic necrosis ⟵ Covalent binding to liver

Nonbasic aromatic amines also undergo hydroxylation. A primary aromatic amine is further converted to nitroso derivative, e.g. dapsone.



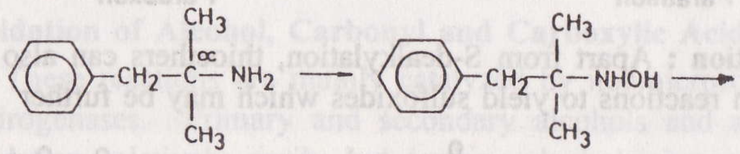
Dapsone — N-Hydroxy dapsone — Nitroso produci

The N-hydroxy dapsone can oxidize ferrous form of hemoglobin to ferric form and cause methemoglobinemia. A secondary aromatic amine yields a nitrone subsequent to formation of secondary hydroxylamine which is further hydrated to primary hydroxylamine.

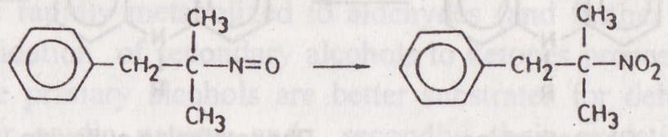


Secondary aromatic amine — Secondary hydroxyl-amine — Nitrone — Primary hydroxyl-amine

N-hydroxylation is also possible at basic nitrogen, e.g. primary and secondary amines. Phentermine, a primary aliphatic amine, cannot undergo deamination as the α-carbon does not contain hydrogen and the drug is therefore N-hydroxylated.



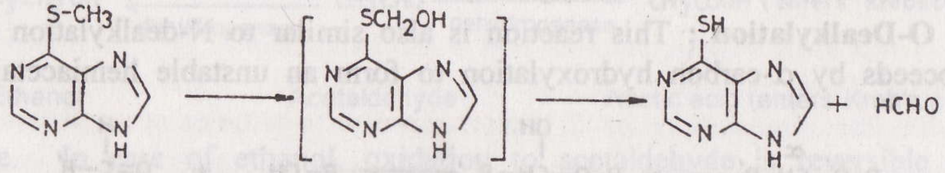
Phentermine — N-Hydroxy phentermine



Nitroso metabolite — Nitro metabolite

Oxidation of Carbon-Sulfur Systems

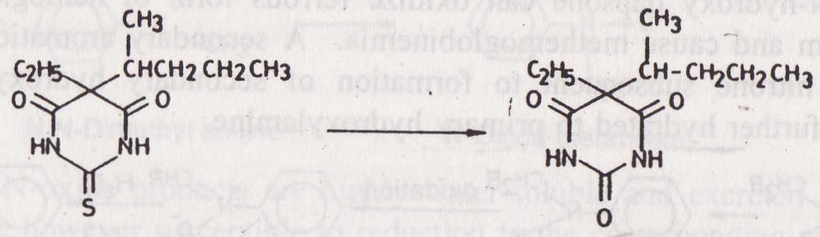
1. S-Dealkylation : The mechanism of S-dealkylation of thioethers



6-Methyl mercaptopurine — Hydroxylated intermediate — 6-Mercaptopurine + HCHO

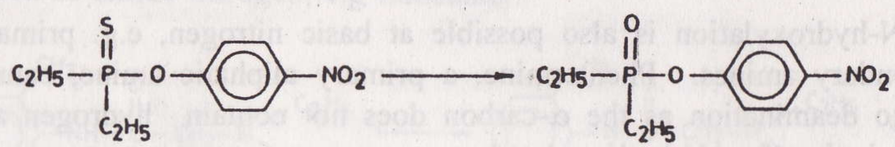
(RSR') is analogous to N-dealkylation i.e. it proceeds via α-carbon hydroxylation. The C-S bond cleavage results in formation of a thiol (RSH) and a carbonyl product, e.g. 6-methyl mercaptopurine.

2. Desulfuration : This reaction also involves cleavage of carbon-sulfur bond (C=S or thiono). The product is the one with C=O bond. Such a desulfuration reaction is commonly observed in thioamides (RCSNHR') such as thiopental.



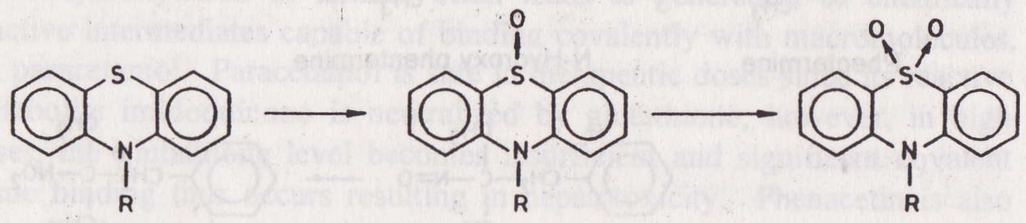
Thiopental Pentobarbital

Desulfuration also occurs with compounds containing P=S bonds such as the organophosphate pesticides, e.g. parathion.



Parathion Paraoxon

3. S-Oxidation : Apart from S-dealkylation, thioethers can also undergo S-oxidation reactions to yield sulfoxides which may be further

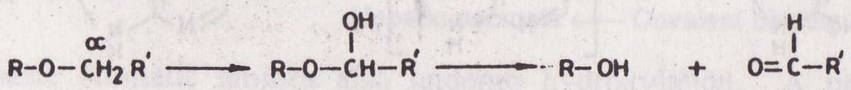


Phenothiazine Phenothiazine sulfoxide Phenothiazine sulfone

oxidized to sulfones (RSO_2R). Several phenothiazines, e.g. chlorpromazine, undergo S-oxidation.

Oxidation of Carbon-Oxygen Systems

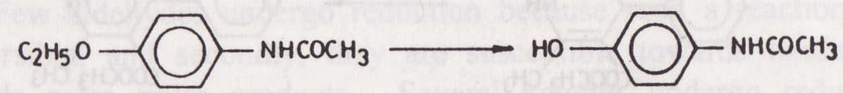
O-Dealkylation : This reaction is also similar to N-dealkylation and proceeds by α-carbon hydroxylation to form an unstable hemiacetal or



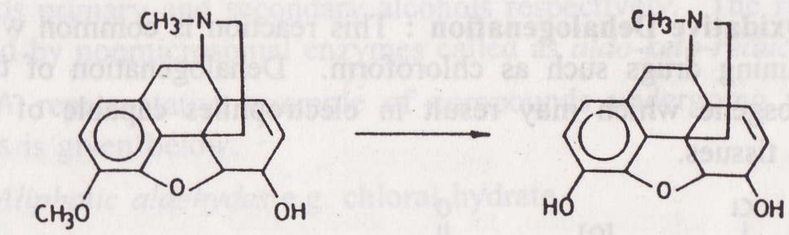
Ether Hemiacetal/hemiketal Alcohol Aldehyde/ketone

hemiketal intermediate which spontaneously undergoes C-O bond cleavage to form alcohol (arenol or alkanol) and a carbonyl moiety.

The O-containing functional groups (analogous to amines and amides, the substrates which undergo N-dealkylation) are ethers and esters. However, only the ethers undergo O-dealkylation reaction. Aliphatic ether drugs are rare and aromatic ethers (phenolic) are common. Methyl ethers are rapidly dealkylated in comparison to longer chain ethers such as the one containing n-butyl group. The reaction generally leads to formation of active metabolites, e.g. phenacetin to paracetamol, and codeine to morphine.



Phenacetin Paracetamol

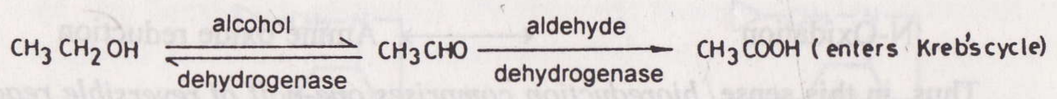


Codeine Morphine

Oxidation of Alcohol, Carbonyl and Carboxylic Acid

These reactions are mainly catalyzed by nonmicrosomal enzymes, dehydrogenases. Primary and secondary alcohols and aldehydes undergo oxidation relatively easily but tertiary alcohols, ketones and carboxylic acids are resistant as such a reaction involves cleavage of C-C bonds.

Primary alcohols are rapidly metabolized to aldehydes (and further to carboxylic acids) but oxidation of secondary alcohols to ketones proceeds slowly. This is because primary alcohols are better substrates for dehydrogenases due to their acidic nature, and secondly, their oxidation products, carboxylic acids, are more water-soluble than ketones. Since primary alcohols are inactivated rapidly, drugs bearing such groups are



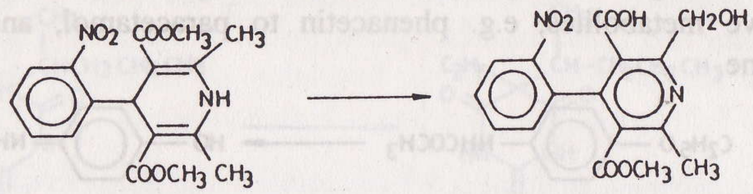
Ethanol Acetaldehyde Acetic acid (enters Kreb's cycle)

rare. In case of ethanol, oxidation to acetaldehyde is reversible and further oxidation of the latter to acetic acid is very rapid since acetaldehyde is highly toxic and should not accumulate in the body. Compounds

with two primary alcohol functions are oxidized stepwise and not simultaneously. With secondary alcohols, the rate of oxidation increases with an increase in alkyl chain length. Compounds with both primary and secondary alcohol groups are oxidized preferentially at the primary group.

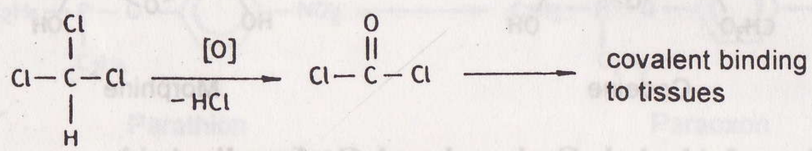
Miscellaneous Oxidative Reactions

a. Oxidative Aromatization/Dehydrogenation : An example of metabolic aromatization of drugs is nifedipine.



Nifedipine Pyridine metabolite

b. Oxidative Dehalogenation : This reaction is common with halogen containing drugs such as chloroform. Dehalogenation of this drug yields phosgene which may result in electrophiles capable of covalent binding to tissues.



Chloroform Phosgene

Oxidative ring cleavage, oxidation of arenols to quinones, etc. are other oxidative reactions.

REDUCTIVE REACTIONS

Bioreductions are also capable of generating polar functional groups such as hydroxy and amino which can undergo further biotransformation or conjugation. A number of reductive reactions are exact opposite of oxidation. For example:

Alcohol dehydrogenation ⟷ Carbonyl reduction

N-Oxidation ⟷ Amine oxide reduction

Thus, in this sense, *bioreduction comprises one-half of reversible reactions*. Such reactions may be catalyzed by the same enzyme (**true reversible reaction**) or by different enzymes (**apparent reversible reaction**). Since reversible reactions usually lead to conversion of inactive metabolites into active drug, they may result in delay of drug removal from the body and hence prolongation of action.

Reduction of Carbonyls (Aldehydes and Ketones)

Depending on their reactivity towards bioreduction, carbonyls can be divided into 3 categories:

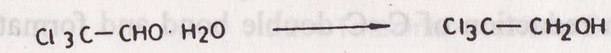
1. The aliphatic aldehydes and ketones.
2. The aromatic aldehydes and ketones.
3. The esters, acids and amides.

The order of reactivity of these categories of drugs in undergoing reduction is 1 > 2 > 3 i.e. aliphatic aldehydes and ketones undergo extensive bioreduction whereas esters, acids and amides are least reactive.

Few aldehydes undergo reduction because such a reaction is usually reversible, and secondly, they are susceptible towards oxidation which yields more polar products. Several ketones undergo reduction as it results in more polar metabolites. Reduction of aldehydes and ketones yields primary and secondary alcohols respectively. The reaction is catalyzed by nonmicrosomal enzymes called as *aldo-keto-reductases*.

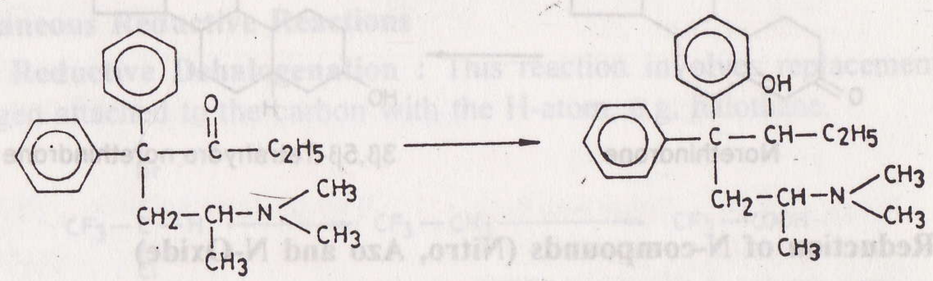
A representative example of compounds undergoing reductive reactions is given below.

Aliphatic aldehydes e.g. chloral hydrate



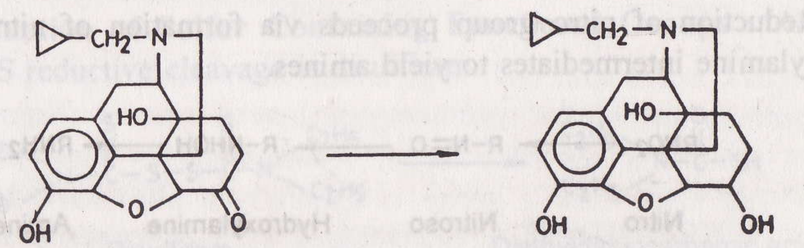
Chloral hydrate Trichloroethanol

Aliphatic ketones e.g. methadone



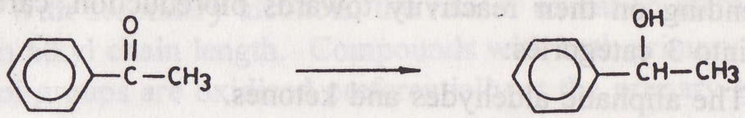
Methadone Methadol

Alicyclic ketone e.g. naltrexone



Naltrexone Isomorphine derivative

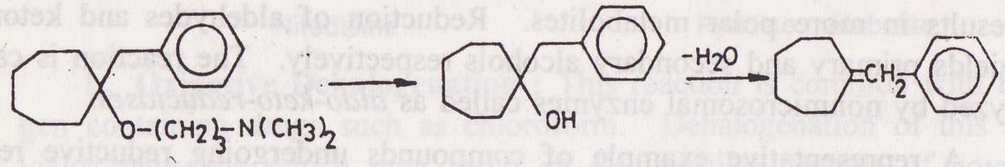
Aromatic ketone e.g. acetophenone



Acetophenone Methyl phenyl carbinol

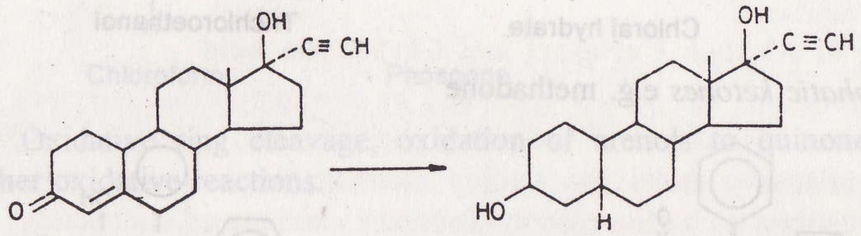
Reduction of Alcohols and Carbon-Carbon Double Bonds

These two reductions are considered together because the groups are interconvertible by simple addition or loss of a water molecule. Before an alcohol is reduced, it is dehydrated to C=C bond, e.g. bencyclane (antispasmodic).



Bencyclane Bencyclanol Benzylidine cycloheptane

Reduction of norethindrone, an α,β-unsaturated carbonyl compound, results in both reduction of C=C double bond and formation of alcohol.



Norethindrone 3β,5β-Tetrahydro norethindrone

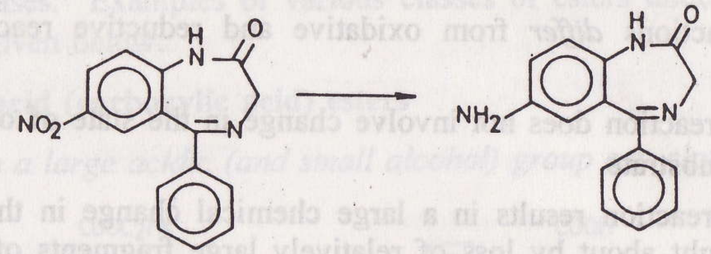
Reduction of N-compounds (Nitro, Azo and N-Oxide)

The N-containing functional groups that commonly undergo bioreduction are nitro, azo and N-oxide. It is important to note that such a reaction is *reverse of oxidation.*

Reduction of nitro group proceeds via formation of nitroso and hydroxylamine intermediates to yield amines.

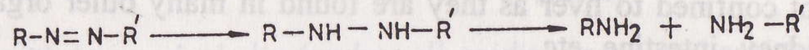
Nitro Nitroso Hydroxylamine Amine

For example, reduction of nitrazepam.



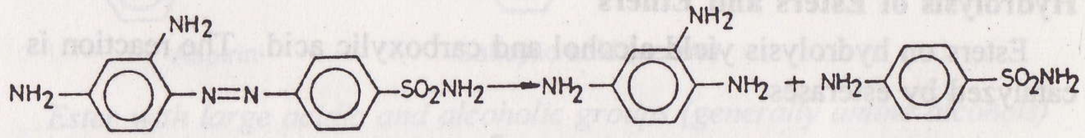
Nitrazepam 7-Aminometabolite

Reduction of azo compounds yield primary amines via formation of hydrazo intermediate (-NH-NH-) which undergoes cleavage at N-N bond.



Azo Hydrazo Amines

An important example of azo reduction is prontosil. It is reduced to active sulfanilamide.

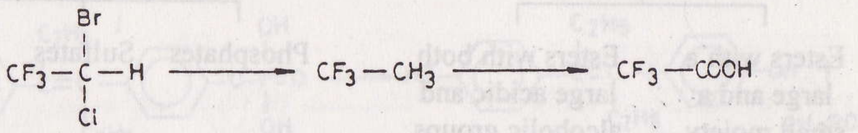


Prontosil 1,2,4-Triaminobenzene Sulfanilamide

Aliphatic and aromatic tertiary amine N-oxides are reduced to the corresponding amines, e.g. imipramine N-oxide to imipramine.

Miscellaneous Reductive Reactions

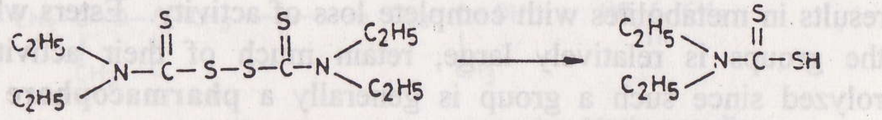
1. Reductive Dehalogenation : This reaction involves replacement of halogen attached to the carbon with the H-atom, e.g. halothane.



Halothane 1,1,1-Trifluoroethane Trifluoroacetic acid

The C-F bond is resistant to reduction.

2. Reduction of Sulfur Containing Functional Groups : An example of S-S reductive cleavage is disulfiram.



Disulfiram Diethyldithiocarbamic acid

Sulfoxides are sometimes reduced to sulfides, e.g. sulindac.

HYDROLYTIC REACTIONS

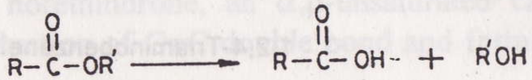
These reactions *differ* from oxidative and reductive reactions in 3 respects:

1. The reaction does not involve change in the state of oxidation of the substrate.
2. The reaction results in a large chemical change in the substrate brought about by loss of relatively large fragments of the molecule.
3. The hydrolytic enzymes that metabolize xenobiotics are the ones that also act on endogenous substrates; moreover, their activity is not confined to liver as they are found in many other organs like kidney, intestine, etc.

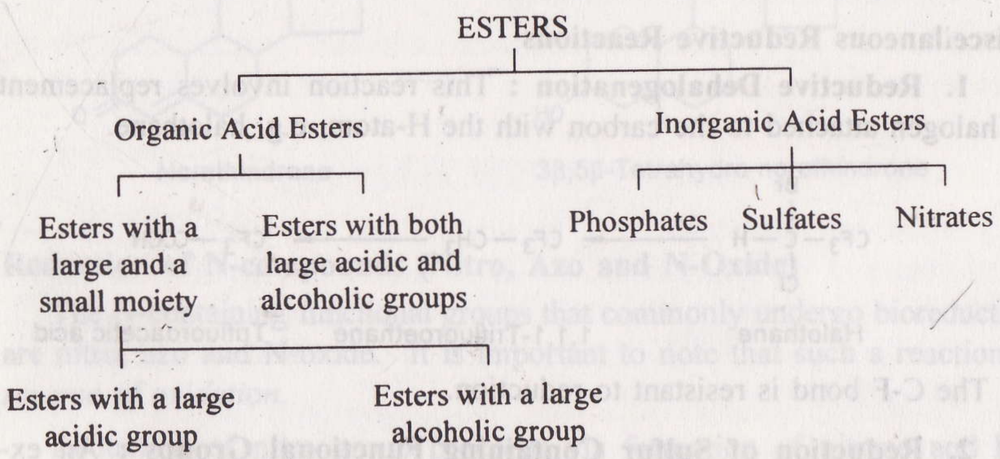
A number of functional groups are hydrolyzed viz. esters, ethers, amides, hydrazides, etc.

Hydrolysis of Esters and Ethers

Esters on hydrolysis yield alcohol and carboxylic acid. The reaction is catalyzed by esterases.



The ester substrates undergoing hydrolysis can be classified as under:

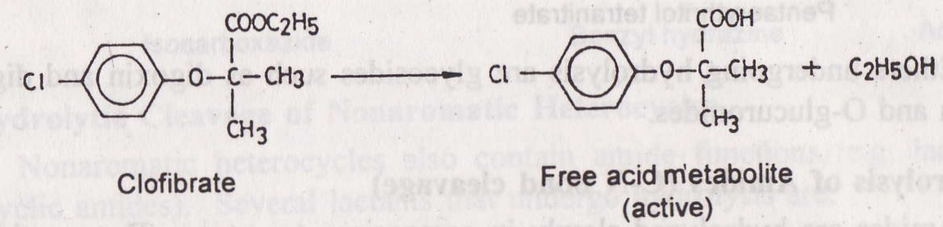


Organic esters with both large acidic and alcoholic groups on hydrolysis results in metabolites with complete loss of activity. Esters where one of the groups is relatively large, retain much of their activity when hydrolyzed since such a group is generally a **pharmacophore** (*having pharmacologic activity*). In many cases, such esters are prodrugs which rely on hydrolysis for their transformation into active form, e.g. chloramphenicol palmitate.

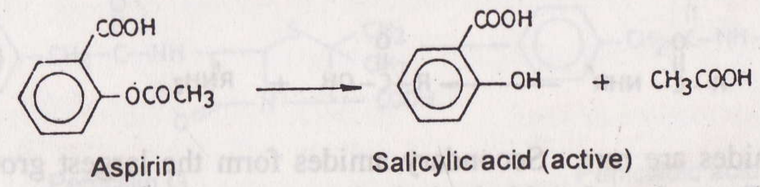
Aromatic esters are hydrolyzed by arylesterases and aliphatic esters by carboxylesterases. Examples of various classes of esters undergoing hydrolysis are given below.

Organic acid (carboxylic acid) esters

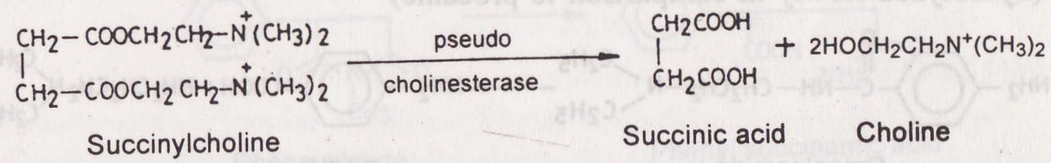
Ester with a large acidic (and small alcohol) group e.g. clofibrate



Ester with large alcoholic (and small acidic) group e.g. aspirin.

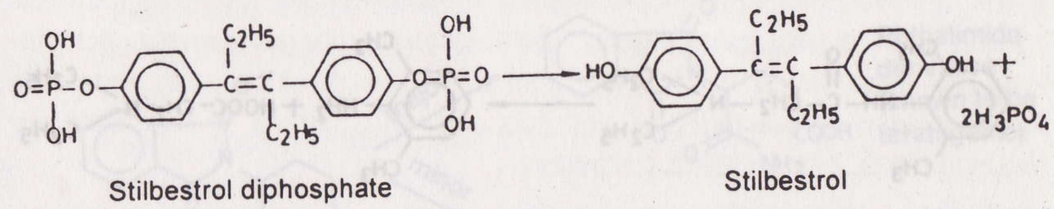


Ester with large acidic and alcoholic groups (generally amine alcohols) e.g. succinylcholine.

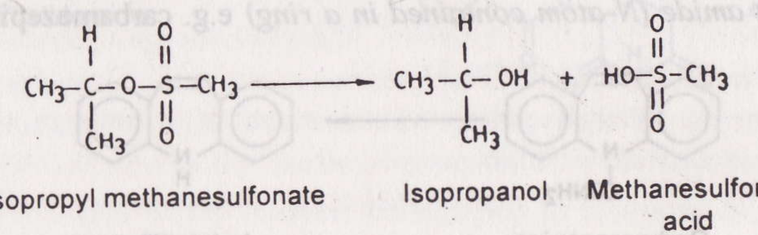


Inorganic Acid Esters

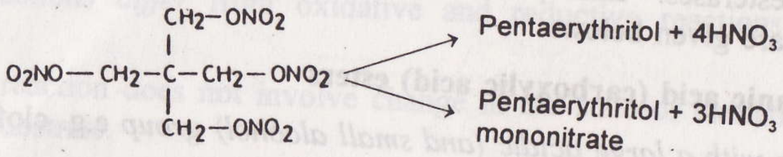
Phosphates e.g. stilbestrol diphosphate



Sulfates e.g. isopropyl methanesulfonate



Nitrates e.g. pentaerythritol tetranitrate

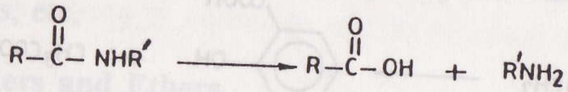


Pentaerythritol tetranitrate

Ethers undergoing hydrolysis are glycosides such as digoxin and digitoxin and O-glucuronides.

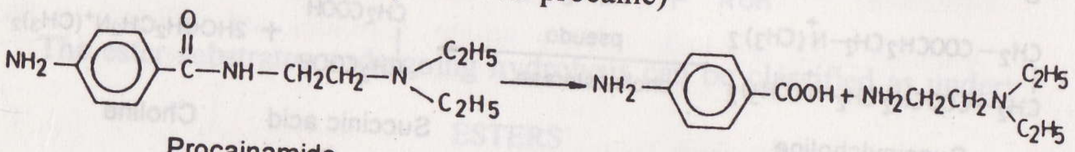
Hydrolysis of Amides (C-N bond cleavage)

Amides are hydrolyzed slowly in comparison to esters. The reaction, catalyzed by *amidases*, involves C-N cleavage to yield carboxylic acid and amine.



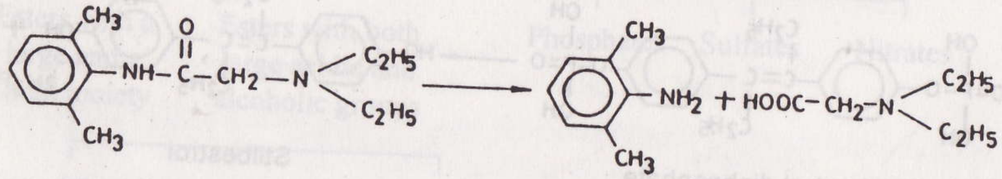
Primary amides are rare. Secondary amides form the largest group of amide drugs. Examples of amide hydrolysis are given below.

Secondary amide with aliphatic substituent on N-atom e.g. procainamide (hydrolyzed slowly in comparison to procaine)



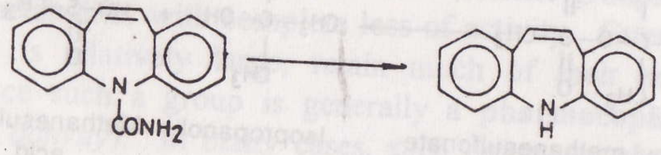
Procainamide PABA

Secondary amide with aromatic substituent on N-atom (anilides) e.g. lidocaine



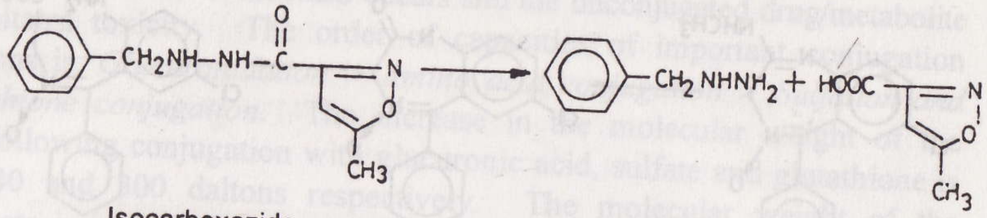
Lidocaine 2,6-Xylidine N,N-Diethylglycine

Tertiary amide (N-atom contained in a ring) e.g. carbamazepine



Carbamazepine Iminostilbene

Hydrazides are also a class of amides e.g. isocarboxazide

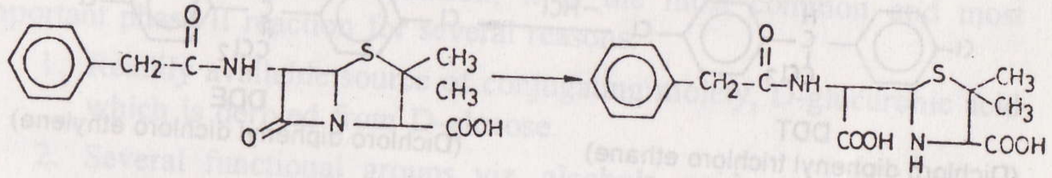


Isocarboxazide Benzyl hydrazine Acid

Hydrolytic Cleavage of Nonaromatic Heterocycles

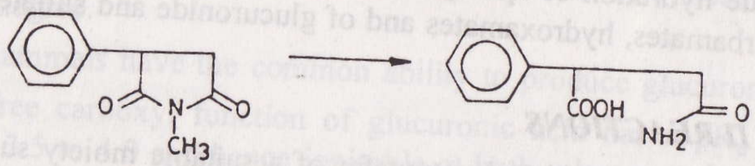
Nonaromatic heterocycles also contain amide functions, e.g. lactams (cyclic amides). Several lactams that undergo hydrolysis are:

1. *Four-membered lactams (ß-lactam)* e.g. penicillins



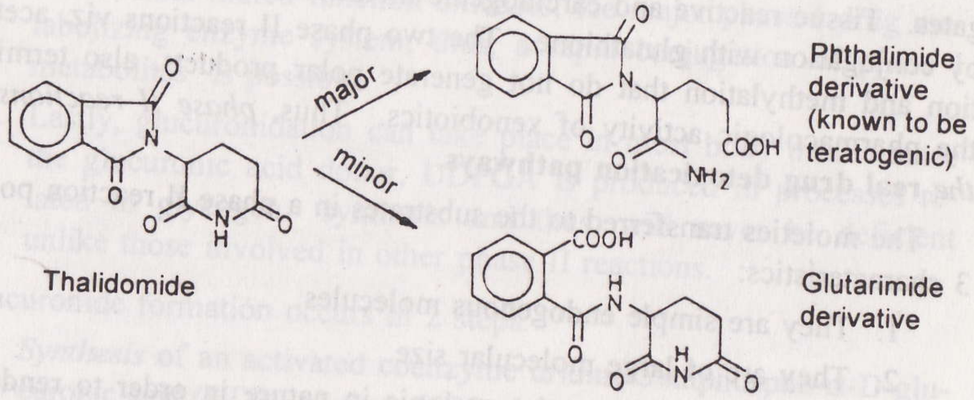
Penicillin G Penicilloic acid metabolite

2. *Five-membered lactams* e.g. succinimides



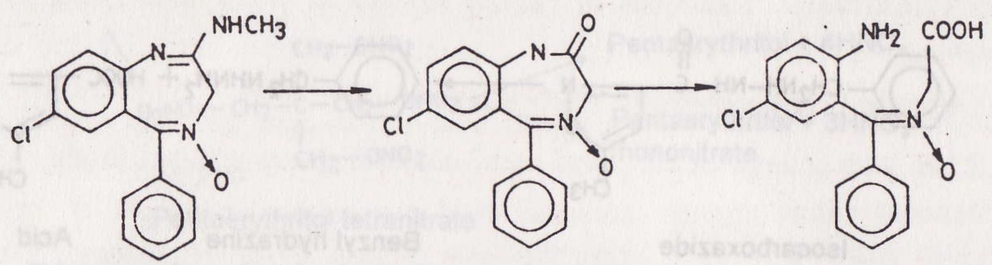
Phensuximide Phenyl succinamic acid

3. *Six-membered lactams* e.g. thalidomide



Thalidomide

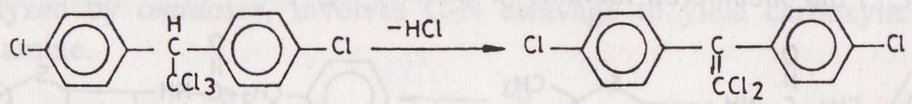
4. *Seven-membered lactams* e.g. chlordiazepoxide



Chlordiazepoxide Lactam metabolite Open-lactam metabolite

Hydrolytic Dehalogenation

Chlorine atoms attached to aliphatic carbons are dehalogenated easily, e.g. DDT.



DDT (Dichloro diphenyl trichloro ethane) DDE (Dichloro diphenyl dichloro ethylene)

Miscellaneous Hydrolytic Reactions

Include hydration of epoxides and arene oxides, hydrolysis of sulfonyl ureas, carbamates, hydroxamates and of glucuronide and sulfate conjugates.

PHASE II REACTIONS

Phase II reactions involve transfer of a suitable moiety such as glucuronic acid, sulfate, glycine, etc. in presence of enzyme transferase to drugs or metabolites of phase I reactions having suitable functional groups to form highly polar, readily excretable and pharmacologically inert conjugates. Tissue reactive and carcinogenic metabolites are rendered harmless by conjugation with glutathione. The two phase II reactions viz. acetylation and methylation that do not generate polar products, also terminate the pharmacologic activity of xenobiotics. Thus, *phase II reactions are the* **real drug detoxication pathways**.

The moieties transferred to the substrates in a phase II reaction possess 3 characteristics:

1. They are simple endogenous molecules.
2. They are of large molecular size.
3. They are strongly polar or ionic in nature in order to render the substrate water-soluble.

An important aspect of conjugation reaction is that they are capacity-limited due to the availability of endogenous conjugating molecules. Thus,

when doses of drugs are higher than normal levels of conjugating molecules, saturation of metabolism occurs and the unconjugated drug/metabolite precipitates toxicity. The order of capacities of important conjugation reactions is: *Glucuronidation > Amino acid conjugation > Sulfation and Glutathione conjugation.* The increase in the molecular weight of the drug following conjugation with glucuronic acid, sulfate and glutathione is 176, 80 and 300 daltons respectively. The molecular weight of the conjugate is important in dictating its route of excretion. High molecular weight conjugates are excreted predominantly in bile while the low molecular weight ones are excreted in urine.

CONJUGATION WITH GLUCURONIC ACID

Also called as **glucuronidation**, it is the most common and most important phase II reaction for several reasons:

1. Readily available source of conjugating moiety, D-glucuronic acid which is derived from D-glucose.
2. Several functional groups viz. alcohols, acids, amines, etc. can combine easily with D-glucuronic acid.
3. Quantitatively, conjugation with D-glucuronic acid occurs to a high degree.
4. All mammals have the common ability to produce glucuronides,
5. The free carboxyl function of glucuronic acid has a pK_a in the range 3.5 to 4.0 and hence ionisable at both plasma and urine pH thereby greatly increasing the water solubility of the conjugated substrate.
6. The glucuronidation enzymes are in close association with the microsomal mixed function oxidases, the major phase I drug metabolizing enzyme system; thus, a rapid conjugation of phase I metabolites is possible.
7. Lastly, glucuronidation can take place in most body tissues since the glucuronic acid donor, UDPGA is produced in processes related to glycogen synthesis and thus, will never be deficient unlike those involved in other phase II reactions.

Glucuronide formation occurs in 2 steps:

1. *Synthesis* of an activated coenzyme uridine-5'-diphospho-α-D-glucuronic acid (UDPGA) from UDP-glucose (UDPG). The coenzyme UDPGA acts as the donor of glucuronic acid. UDPG is synthesized by interaction of α-D-glucose-1-phosphate with uridine triphosphate (UTP).

2. *Transfer* of the glucuronyl moiety from UDPGA to the substrate RXH in presence of enzyme UDP-glucuronyl transferase to form the conjugate. In this step, the α-configuration of glucuronic acid undergoes *inversion* and thus, the resulting product is **β-D-glucuronide** (also called as **glucosiduronic acid** or **glucopyranosiduronic acid** conjugate).

The steps involved in glucuronide synthesis are depicted below:

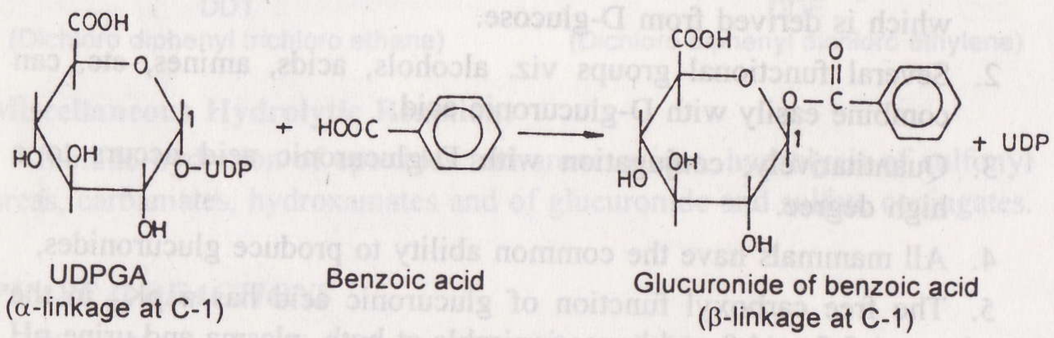
$$\alpha\text{-D-glucose-1-phosphate} + \text{UTP} \xrightarrow{\text{pyrophosphorylase}} \text{UDPG} + \text{PPi}$$

$$\text{UDPG} + 2\text{NAD}^+ + \text{H}_2\text{O} \xrightarrow{\text{UDPG-dehydrogenase}} \text{UDPGA} + 2\text{NADH} + 2\text{H}^+$$

$$\text{UDPGA} + \text{RXH} \xrightarrow{\text{UDP-glucuronyl transferase}} \text{RX-glucuronic acid} + \text{UDP}$$

where X = O, COO, NH or S.

An example of glucuronidation of benzoic acid is shown below.



A large number of functional groups are capable of forming oxygen, nitrogen and sulfur glucuronides. Carbon glucuronides have also been detected in a few cases.

Oxygen or O-Glucuronides

Xenobiotics with hydroxyl and/or carboxyl functions form O-glucuronides.

1. **Hydroxyl Compounds :** These form *ether glucuronides*. Several examples of such compounds are given below.

Aliphatic alcohols	chloramphenicol, trichloroethanol
Alicyclic alcohols	hydroxylated hexobarbital
Arenols (phenols)	morphine, paracetamol
Benzylic alcohols	methyl phenyl carbinol
Enols	4-hydroxy coumarin
N-hydroxyl amines	N-hydroxy dapsone
N-hydroxyl amides	N-hydroxy-2-acetyl aminofluorine

2. **Carboxyl Compounds :** These form *ester glucuronides*

Aryl acids	salicylic acid
Arylalkyl acids	fenoprofen

Nitrogen or N-Glucuronides

Xenobiotics with amine, amide and sulfonamide functions form N-glucuronides.

Aliphatic 2° amines	desipramine
Aliphatic 3° amines	tripelennamine
Nonaromatic 3° heterocyclic amines	cyproheptadiene
Amides	meprobamate
Sulfonamides	sulfadimethoxine

Sulfur or S-Glucuronides

Thiols (SH) form *thioether glucuronides* e.g. thiophenol.

Carbon or C-Glucuronides

Xenobiotics with nucleophilic carbon atoms such as phenylbutazone form C-glucuronides.

Certain endogenous compounds such as steroids, bilirubin, catechols and thyroxine also form glucuronides.

CONJUGATION WITH SULFATE MOIETIES

Sulfation is similar to glucuronidation but it is catalyzed by nonmicrosomal enzymes and occurs less commonly as the moiety that transfers sulfate to the substrate is easily depleted. Thus, this process is saturable and predominates at low substrate concentration whereas glucuronidation is dominant at high concentration.

Like glucuronidation, sulfation also occurs in 2 steps:

1. *Synthesis* of an activated coenzyme 3'-phosphoadenosine-5'-phosphosulfate (PAPS) which acts as a donor of sulfate to the substrate. This also occurs in two steps—an initial interaction between the sulfate and the adenosine triphosphate (ATP) to yield adenosine-5'-phosphosulfate (APS) followed by the activation of latter to PAPS.
2. *Transfer* of sulfate group from PAPS to the substrate RXH in presence of enzyme sulfotransferase (sulfokinase) and subsequent liberation of 3'-phosphoadenosine-5'-phosphate (PAP).

The steps are summarized in the equations below:

$$ATP + SO_4^{2-} \xrightarrow{\text{ATP-sulfurylase/}Mg^{++}} APS + PPi$$

$$APS + ATP \xrightarrow{\text{APS-phosphokinase/}Mg^{++}} PAPS + ADP$$

$$PAPS + RXH \xrightarrow{\text{sulfotransferase}} RX\text{-}SO_3 + PAP$$

where X = O, NH

Functional groups capable of forming sulfate conjugates include phenols, alcohols, arylamines, N-hydroxylamines and N-hydroxyamides. The reaction product is a sulfate ester, also called as *ethereal sulfate*.

Examples of compounds undergoing sulfation are:

Phenols	paracetamol, salbutamol
Alcohols	aliphatics C-1 to C-5
Arylamines	aniline

Sulfoconjugates can be tissue reactive, e.g. the O-sulfate conjugate of N-hydroxy phenacetin covalently binds to hepatic and renal tissues.

Endogenous substances can also undergo sulfation, e.g. steroids, biologic amines, etc.

CONJUGATION WITH ALPHA AMINO ACIDS

This reaction also occurs to a limited extent because of limited availability of amino acids. The reaction occurs in two steps:

1. *Activation* of carboxylic acid drug substrate with ATP and coenzyme A (CoA) to form an acyl CoA intermediate. Thus, the reaction is a contrast of glucuronidation and sulfation where the donor coenzyme is activated and not the substrate.
2. *Acylation* of the α-amino acid by the acyl CoA in presence of enzyme N-acyl transferase.

The reaction is summarized below.

$$RCOOH + ATP \xrightarrow{\text{acyl synthetase}} RCOAMP + H_2O + PPi$$

$$RCOAMP + CoASH \xrightarrow{\text{acyl CoA transferase}} RCOSCoA + AMP$$

$$RCOSCoA + H_2N\text{-}R'\text{-}COOH \xrightarrow{\text{N-acyl transferase}} RCONH\text{-}R'\text{-}COOH + CoASH$$

where R' = $-CH_2-$ (if glycine) or $>CH-CH_2-CH_2-CONH_2$ (if glutamine)

Conjugation occurs commonly with glycine. Glutamine conjugation occurs to a lesser extent. Conjugation with other amino acids like aspartic acid, serine and taurine is still uncommon. The substrate is generally an acid (aromatic in particular) and the reaction product is an amide.

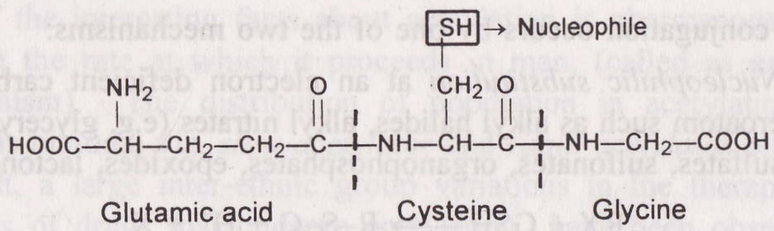
Examples of drugs forming glycine or glutamine conjugates are:

Aliphatic acids	isopropoxyacetic acid
Alicyclic acids	cholic acid
Aryl acids	salicylic acid
Arylacetic acids	phenylacetic acid
Heterocyclic aryl acids	nicotinic acid

Amino acid conjugation occurs extensively in the liver mitochondria and thus can be used to estimate *hepatic function*. The diagnostic marker used is benzoic acid which on conjugation with glycine yields hippuric acid. Hippuric acid is rapidly excreted in urine. Thus, the rate and extent of urinary excretion of hippuric acid following oral or i.v. administration of benzoic acid indicates functioning of liver. A decreased output indicates hepatic disorder.

CONJUGATION WITH GLUTATHIONE AND MERCAPTURIC ACID FORMATION

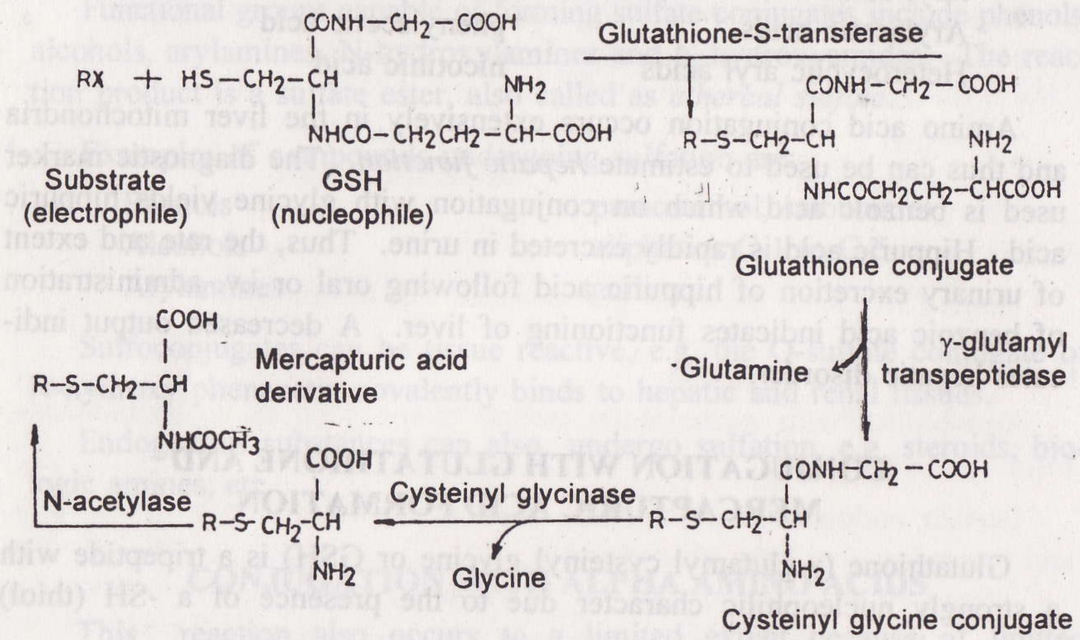
Glutathione (γ-glutamyl cysteinyl glycine or GSH) is a tripeptide with a strongly nucleophilic character due to the presence of a -SH (thiol) group in its structure.



Thus, it has great affinity for electrophilic substrates, a number of which are potentially toxic compounds. It is important to note that a highly electrophilic metabolite has a tendency to react with tissue nucleophilic groups such as -OH, $-NH_2$ and -SH and precipitate toxicities such as tissue necrosis, carcinogenesis, mutagenesis, teratogenesis, etc. Conjugation with glutathione protects the tissue from such reactive moieties and thus the reaction is an important detoxication route.

GSH conjugation differs from other conjugation reactions in that the process does not require initial activation of the coenzyme or the substrate since the GSH, which is a nucleophile itself, is highly reactive towards an electrophilic substrate. The interaction between the substrate and the GSH

is catalyzed by enzyme glutathione-S-transferase to form S-substituted glutathione conjugate. This conjugate is not excreted as such in the urine but undergoes cleavage, first by the enzyme γ-glutamyl transpeptidase to release the free glutamyl residue and cysteinyl glycine derivative; the latter is cleaved by enzyme cysteinyl glycinase to produce free glycine and the cysteine conjugate. Finally, N-acetylation of this conjugate by the enzyme N-acetylase yields S-substituted-N-acetyl cysteine products called as **mercapturic acids**.



GSH conjugation occurs by one of the two mechanisms:

1. *Nucleophilic substitution* at an electron deficient carbon or heteroatom such as alkyl halides, alkyl nitrates (e.g. glyceryl trinitrate), sulfates, sulfonates, organophosphates, epoxides, lactones, etc.

$$RX + GSH \longrightarrow R\text{–}S\text{–}G + H^+ + X^-$$

2. *Nucleophilic addition* at the electron deficient double bond such as the α,β-unsaturated carbonyl compounds, e.g. ethacrynic acid.

$$R\text{–}CH{=}CH\text{–}R' + GSH \longrightarrow RCH\text{–}S\text{–}G\text{–}CH_2\text{–}R'$$

where R = electron withdrawing group.

The hepatotoxicity from paracetamol overdose is due to depletion of GSH.

ACETYLATION

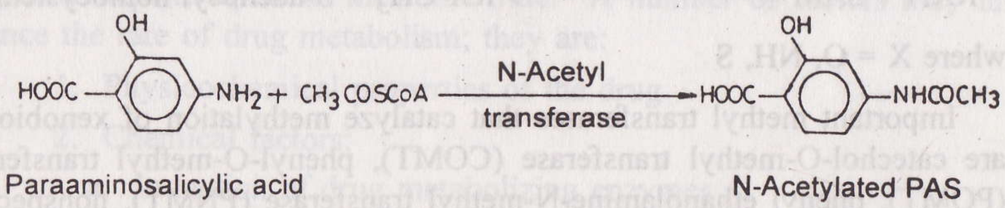
This reaction is basically an acylation reaction and thus similar to conjugation with α-amino acids. The analogy also lies in the fact that

both reactions yield amide products. Acetylation however differs from α-amino acid conjugation in that the substrates are exogenous amines (and not carboxylic acids) and the acylating agent is endogenous acetyl CoA ($CH_3COSCoA$). The general sequence of reaction is similar to that for α-amino acid conjugation. The enzyme involved is the nonmicrosomal N-acetyl transferase.

Acetylation is an important metabolic pathway for drugs containing primary amino groups. Alcohols (e.g. choline) and thiols (e.g. CoASH) also undergo acetylation but only the endogenous ones.

Examples of drugs undergoing acetylation are :

Primary aliphatic amines	histamine, mescaline
Primary aromatic amines	procainamide, PAS, PABA, dapsone
Sulfonamides	sulfanilamide, sulfapyridine
Hydrazines/hydrazides	hydralazine, isoniazid, phenelzine



Acetylation may sometimes lead to toxic products, e.g. acetyl derivatives of some sulfonamides (cause renal toxicity due to decreased water solubility of the metabolites formed) and reactive arylacetamides.

One of the interesting facts about acetylation is pharmacogenetic differences in the rate at which it proceeds in man, (called as **acetylation polymorphism**). The distribution of population in acetylating certain substrates is bimodal viz. *slow acetylator* and *rapid acetylator* phenotypes. As a result, a large inter-ethnic group variations in the therapeutic and toxic levels of drugs that undergo acetylation have been observed, e.g. isoniazid.

METHYLATION

This reaction *differs* from general characteristics of phase II reactions in several ways:

1. The metabolites formed are not polar or water-soluble.
2. The metabolites, in a number of instances, have equal or greater pharmacologic activity than the parent drug, e.g. morphine formed from normorphine.
3. The reaction is of lesser importance in metabolism of xenobiotics. It is more important in the biosynthesis (e.g. adrenaline, melato-

nin) and inactivation of endogenous amines (e.g. noradrenaline, serotonin, histamine).

Methylation can be considered as intermediate of phase I and phase II reactions. It can be called as a phase I reaction as it is reverse of demethylation reaction and can be classed as a phase II reaction because of its mechanism.

Methylation of substrates proceeds in two steps:

1. *Synthesis* of an activated coenzyme S-adenosyl methionine (SAM), the donor of methyl group, from L-methionine and ATP.
2. *Transfer* of the methyl group from SAM to the substrate in presence of nonmicrosomal enzyme methyl transferase.

$$\text{L-Methionine} + \text{ATP} \xrightarrow{\text{methionine adenosyl transferase}} \text{SAM} + \text{PPi} + \text{Pi}$$

$$\text{RXH} + \text{SAM} \xrightarrow{\text{methyl transferase}} \text{RX-CH}_3 + \text{S-adenosyl homocysteine}$$

where X = O, NH, S

Important methyl transferases that catalyze methylation of xenobiotics are catechol-O-methyl transferase (COMT), phenyl-O-methyl transferase (POMT), phenyl ethanolamine-N-methyl transferase (PNMT), nonspecific transferases, etc.

Examples of substrates undergoing methylation are:

O-Methylation

Phenols	morphine
Catechols	α-methyl dopa, L-DOPA, isoprenaline

N-Methylation

Primary aliphatic amines	norephedrine
Secondary alicyclic amines	normorphine
Aromatic heterocycles	nicotine, histamine

S-Methylation

Thiols	propylthiouracil, 6-mercaptopurine

MISCELLANEOUS CONJUGATION REACTIONS

Some of the rare conjugation reactions are mentioned below.

Conjugation of Cyanide

The toxicity of cyanide ion is due to its ability to arrest enzymes involved in cellular respiration and convert hemoglobin to cyanomethemoglobin which lacks the ability to transport oxygen to tissues. Conjugation of cyanide ion involves transfer of sulfur atom from thiosulfate to the

cyanide ion in presence of enzyme rhodanese to form inactive thiocyanate.

$$S_2O_3^{2-} + CN^- \xrightarrow{\text{rhodanese}} SCN^- + SO_3^{2-}$$

Conjugation with Ribose

Endogenous purine and pyrimidine bases conjugate with ribose to form nucleotides.

Conjugation with Taurine

Taurine, a β-amino sulfonic acid, conjugates the endogenous bile acids to produce components of bile.

FACTORS AFFECTING BIOTRANSFORMATION OF DRUGS

The therapeutic efficacy, toxicity and biological half-life of a drug greatly depend upon its metabolic rate. A number of factors may influence the rate of drug metabolism; they are:

1. Physicochemical properties of the drug
2. Chemical factors:
   a. Induction of drug metabolizing enzymes
   b. Inhibition of drug metabolizing enzymes
   c. Environmental chemicals
3. Biological factors:
   a. Species differences
   b. Strain differences
   c. Sex differences
   d. Age
   e. Diet
   f. Altered physiologic factors:
      i. Pregnancy
      ii. Hormonal imbalance
      iii. Disease states
   g. Temporal factors:
      i. Circadian rhythm
      ii. Circannual rhythm

PHYSICOCHEMICAL PROPERTIES OF THE DRUG

Just as the absorption and distribution of a drug are influenced by its physicochemical properties, so is its interaction with the drug metabolizing enzymes. Molecular size and shape, pK_a, acidity/basicity, lipophilicity

and steric and electronic characteristics of a drug influence its interaction with the active sites of enzymes and the biotransformation processes to which it is subjected. However, such an interrelationship is not clearly understood.

CHEMICAL FACTORS

Induction of Drug Metabolizing Enzymes

The phenomenon of increased drug metabolizing ability of the enzymes (especially of microsomal monooxygenase system) by several drugs and chemicals is called as **enzyme induction** *and the agents which bring about such an effect are known as* **inducers**. Most inducers are in general lipophilic compounds with long elimination half-lives.

Two categories of inducers have been defined:

1. **Phenobarbital type inducers :** includes several drugs and pesticides which increase the rate of metabolism of a large number of drugs.
2. **Polycyclic hydrocarbon type inducers :** such as 3-methyl cholanthrene and cigarette smoke which stimulate the metabolic rate of few drugs.

Some drugs such as carbamazepine, meprobamate, cyclophosphamide, rifampicin, etc. stimulate their own metabolism, the phenomenon being called as **auto-induction** or **self-induction**.

Mechanisms involved in enzyme induction may be increased enzyme synthesis, decreased rate of enzyme degradation, enzyme stabilization or enzyme activation.

TABLE 5.3
Inducers of Drug Metabolizing Enzyme System and Drugs Commonly Affected by them

Inducers	*Drugs with Enhanced Metabolism*
Barbiturates	Coumarins, Phenytoin, Cortisol, Testosterone, Oral Contraceptives
Alcohol	Pentobarbital, Coumarins, Phenytoin
Phenytoin	Cortisol, Coumarins, Oral Contraceptives, Tolbutamide
Rifampicin	Coumarins, Oral Contraceptives, Tolbutamide, Rifampicin
Cigarette Smoke	Nicotine, Amino Azo Dyes

Enzyme induction results in decreased pharmacologic activity of most drugs, increased activity where the metabolites are active and altered physiologic status due to enhanced metabolism of endogenous compounds such as sex hormones. Some examples of inducers and drugs affected by them are given in Table 5.3.

Inhibition of Drug Metabolizing Enzymes

A decrease in the drug metabolizing ability of an enzyme is called as **enzyme inhibition**. The process of inhibition may be direct or indirect.

1. **Direct Inhibition :** may result from interaction at the enzymic site, the net outcome being a change in enzyme activity. Direct enzyme inhibition may be competitive or noncompetitive.

   a. *Competitive Inhibition* : results when structurally similar compounds compete for the same site on an enzyme. Such an inhibition due to substrate competition is *reversible* and can be overcome by high concentration of one of the substrates, e.g. methacholine inhibits metabolism of acetyl choline by competing with it for cholinesterase.

   b. *Noncompetitive Inhibition* : arises when a structurally unrelated agent interacts with the enzyme and prevents the metabolism of drugs. Since the interaction is not structure- specific, metals like lead, mercury and arsenic and organophosphorus insecticides inhibit the enzymes noncompetitively. Isoniazid inhibits the metabolism of phenytoin by the same mechanism.

   Direct inhibition also results *when the metabolic product competes with the substrate for the same enzyme* (*called as* **product inhibition**). Certain specific inhibitors such as xanthine oxidase inhibitors (e.g. allopurinol) and MAO inhibitors (e.g. phenelzine) also inhibit the enzyme activity directly.

2. **Indirect Inhibition :** is brought about by one of the two mechanisms:

   a. *Repression* : is defined as the decrease in enzyme content. It may be due to a fall in the rate of enzyme synthesis as affected by ethionine, puromycin and actinomycin D or because of rise in the rate of enzyme degradation such as by carbon tetrachloride, carbon disulfide, disulfiram, etc.

   b. *Altered Physiology* : due to nutritional deficiency or hormonal imbalance.

Direct enzyme inhibition is usually rapid (a single dose of inhibitor may be sufficient) and therefore more important than enzyme induction.

Enzyme inhibition generally results in prolonged pharmacologic action of a drug. Some examples of inhibitors and drugs affected by them are given in Table 5.4.

TABLE 5.4

Enzyme Inhibitors and Drugs Affected by them

Inhibitors	*Drugs with Decreased Metabolism*
MAO Inhibitors	Barbiturates, Tyramine
Coumarins	Phenytoin
Allopurinol	6-Mercaptopurine
PAS	Phenytoin, Hexobarbital

Environmental Chemicals

Several environmental agents influence the drug metabolizing ability of enzymes. Halogenated pesticides such as DDT and polycyclic aromatic hydrocarbons contained in cigarette smoke have enzyme induction effect. Organophosphate insecticides and heavy metals such as mercury, tin, nickel, cobalt and arsenic inhibit drug metabolizing ability of enzymes.

Other environmental factors that may influence drug metabolism are temperature, altitude, pressure, atmosphere, etc.

BIOLOGICAL FACTORS

Species Differences

Screening of new therapeutic molecules to ascertain their activity and toxicity requires study in several laboratory animal species. Differences in drug response due to species differences are taken into account while extrapolating the data to man.

Species differences have been observed in both phase I and phase II reactions. In phase I reactions, both qualitative and quantitative variations in the enzyme and their activity have been observed. An example of this is the metabolism of amphetamine and ephedrine. In men and rabbit, these drugs are predominantly metabolized by oxidative deamination whereas in rats the aromatic oxidation is the major route. In phase II reactions, the variations are mainly qualitative and characterized either by the presence of, or complete lack of certain conjugating enzymes; for example, in pigs, the phenol is excreted mainly as glucuronide whereas its sulfate conjugate dominates in cats. Certain birds utilize ornithine for conjugating aromatic acids instead of glycine.

Strain Differences/Pharmacogenetics

Enzymes influencing metabolic reactions are under the genetic control. Just as the differences in drug metabolizing ability between different species are attributed to genetics, so also are the differences observed between strains of the same animal species. *A study of intersubject variability in drug response (due to differences in, for example, rate of biotransformation) is called as* **pharmacogenetics**. The intersubject variations in drug biotransformation may either be monogenically or polygenically controlled. A *polygenic control* has been observed in studies in twins. In identical twins (monozygotic), very little or no difference in the metabolism of phenylbutazone, dicoumarol and antipyrine was detected but large variations were apparent in fraternal twins (dizygotic; twins developed from two different eggs) for the same drugs.

Differences observed in the metabolism of a drug among different races are called as **ethnic variations**. Such a variation may be monomorphic or polymorphic. *When a unimodal frequency distribution is observed in the entire population, the variations are called as* **continuous** or **monomorphic**; for example, the entire human race acetylate PABA and PAS to only a small extent. *A polymodal distribution is indicative of* **discontinuous variation (polymorphism)**. An example of polymorphism is the acetylation of isoniazid (INH) in humans. A bimodal population distribution was observed comprising of slow acetylator or inactivator phenotypes (metabolize INH slowly) and rapid acetylator or inactivator phenotypes (metabolize INH rapidly) (*see* Table 5.5.).

TABLE 5.5

Ethnic Variations in the N-Acetylation of Isoniazid

Ethnic Group	*% Slow Acetylators*	*% Rapid Acetylators*
Whites (USA and Canada)	45	55
Blacks (USA)	48	52
Latin Americans	67	33
American Indians	79	21
Japanese	87	13
Eskimos	95	05

Source : Kalow. W.: *Pharmacogenetics: Heredity and the Response to Drugs.* Saunders. Philadelphia. 1962.

Approximately equal percent of slow and rapid acetylators are found among whites and blacks whereas the slow acetylators dominate Japanese and Eskimo populations. Dose adjustments are therefore necessary in the

latter groups since high levels of INH may cause peripheral neuritis. Other drugs known to exhibit pharmacogenetic differences in metabolism are debrisoquine, succinyl choline, phenytoin, dapsone and sulfadimidine.

Sex Differences

Sex related differences in the rate of metabolism can be attributed to regulation of such processes by sex hormones since variations among male and female are generally observed following puberty. Such sex differences are widely studied in rats; the male rats have greater drug metabolizing capacity. In humans, women metabolize benzodiazepines slowly than men and several studies show that women on contraceptive pills metabolize a number of drugs at a slow rate.

Age

Differences in the drug metabolic rate in different age groups is mainly due to variations in the enzyme content, enzyme activity and hemodynamics. In neonates (upto 2 months), the microsomal enzyme system is not fully developed and many drugs are biotransformed slowly; for example, caffeine has a half-life of 4 days in neonates in comparison to 4 hours in adults. A major portion of this drug is excreted unchanged in urine by the neonates. Conjugation with sulfate is well developed (paracetamol is excreted mainly as sulfate) but glucuronidation occurs to a very small extent. As a result, hyperbilirubinemia precipitates kernicterus and chloramphenicol leads to cyanosis or Gray baby syndrome in new born. Similarly, sulfonamides cause renal toxicity and paracetamol causes hepatotoxicity. Infants (between 2 months and one year) show almost a similar profile as neonates in metabolizing drugs with improvement in the capacity as age advances and enzyme activity increases. Children (between one year and 12 years) and older infants metabolize several drugs much more rapidly than adults as the rate of metabolism reaches a maximum somewhere between 6 months and 12 years of age. As a result, they require large mg/Kg doses in comparison to adults; for example, the theophylline half-life in children is two-third of that in adults. In very elderly persons, the liver size is reduced, the microsomal enzyme activity is decreased and hepatic blood flow also declines as a result of reduced cardiac output all of which contribute to decreased metabolism of drugs. Drug conjugation however remains unaffected.

Diet

The enzyme content and activity is altered by a number of dietary components. In general, low protein diet decreases and high protein diet increases the drug metabolizing ability. This is because the enzyme

synthesis is promoted by protein diet which also raises the level of amino acids for conjugation with drugs. The protein-carbohydrate ratio in the diet is also important; a high ratio increases the microsomal mixed function oxidase activity. Fat free diet depresses cytochrome P-450 levels since phospholipids, which are important components of microsomes, become deficient. Dietary deficiency of vitamins (e.g. vitamin A, B_2, B_3, C and E) and minerals such as Fe, Ca, Mg, Cu and Zn retard the metabolic activity of enzymes. Starvation results in decreased amount of glucuronides formed than under normal conditions. Malnutrition in women results in enhanced metabolism of sex hormones. Alcohol ingestion results in a short term decrease followed by an increase in the enzyme activity.

Altered Physiologic Factors

Pregnancy : Studies in animals have shown that the maternal drug metabolizing ability (of both phase I and phase II reactions) is reduced during the later stages of pregnancy. This was suggested as due to high levels of steroid hormones in circulation during pregnancy. In women, the metabolism of promazine and pethidine is reduced during pregnancy or when receiving oral contraceptives. Higher rate of hepatic metabolism of anticonvulsants during pregnancy is thought to be due to induction of drug metabolizing enzymes by the circulating progesterone.

Hormonal Imbalance : The influence of sex hormones on drug metabolism has already been discussed. The effect of other hormones is equally complex. Higher levels of one hormone may inhibit the activity of few enzymes while inducing that of others. Adrenolectomy, thyroidectomy and alloxan induced diabetes in animals showed an impairment in the enzyme activity with a subsequent fall in the rate of metabolism. A similar effect was observed with pituitary growth hormone. Stress related changes in ACTH levels also influence drug biotransformation.

Disease States : As liver is the primary site for metabolism of most drugs, all pathologic conditions associated with it result in enhanced half-lives of almost all drugs. Thus, a reduction in hepatic drug metabolizing ability is apparent in conditions such as hepatic carcinoma, hepatitis cirrhosis, obstructive jaundice, etc. Biotransformations such as glycine conjugation of salicylates, oxidation of vitamin D and hydrolysis of procaine which occur in kidney, are impaired in renal diseases. Congestive cardiac failure and myocardial infarction which result in a decrease in the blood flow to the liver, impair metabolism of drugs having high hepatic extraction ratio e.g. propranolol and lidocaine. In diabetes, glucuronidation is reduced due to decreased availability of UDPGA.

Temporal Factors

Circadian Rhythm : *Diurnal variations or variations in the enzyme activity with light cycle is called as* **circadian rhythm** *in drug metabolism.* It has been observed that the enzyme activity is maximum during early morning (6 to 9 a.m.) and minimum in late afternoon (2 to 5 p.m.) which was suggested to correspond with the high and low serum levels of corticosterone (the serum corticosterone level is dependent upon the light-dark sequence of the day). Clinical variation in therapeutic effect of a drug at different times of the day is therefore apparent. *The study of variations in drug response as influenced by time is called as* **chronopharmacology**. *Time dependent change in drug kinetics is known as* **chronokinetics**. Drugs such as aminopyrine, hexobarbital and imipramine showed diurnal variations in rats. The half-life of metyrapone was shown to be 2.5 times longer during the night than in the day, in rats.

BIOACTIVATION AND TISSUE TOXICITY

Formation of highly reactive metabolites (from relatively inert chemical compounds) which interact with the tissues to precipitate one or more of the several forms of toxicities such as carcinogenesis and teratogenesis is called as **bioactivation** or **toxicological activation**. The reactive, chemically unstable species, capable of toxication, are broadly divided into two categories—electrophiles and free radicals (Fig. 5.3.).

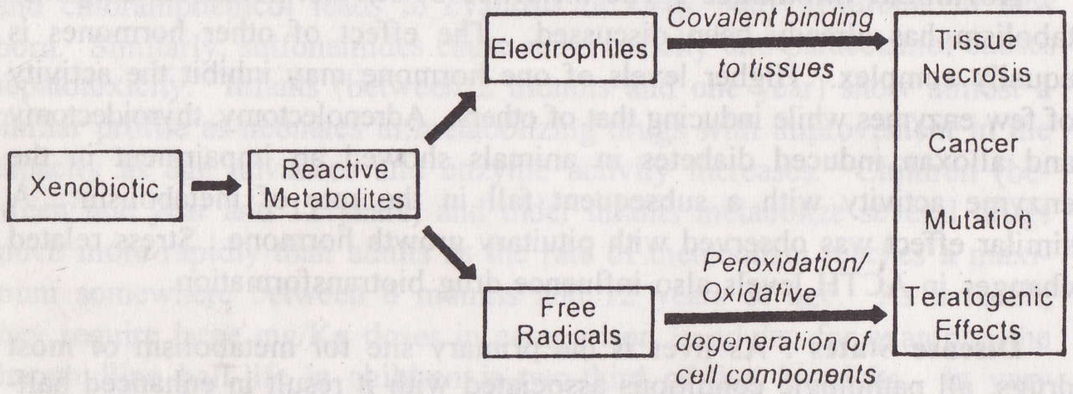
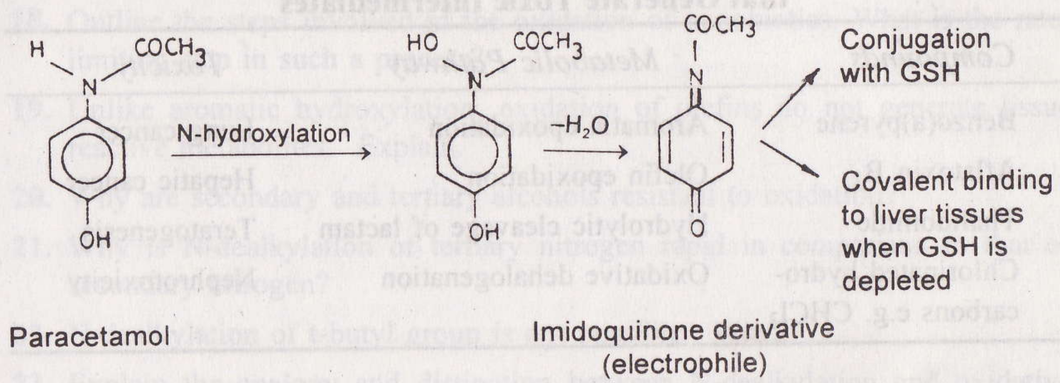


Fig. 5.3 Mechanisms of tissue toxicity by bioactivation of drugs

Electrophiles *are species deficient in electron pair.* The enzyme system through which they are generated is cytochrome P-450. Carbon, nitrogen or sulfur-containing compounds can be metabolically activated to yield electrophiles. Important electrophiles are epoxides (e.g. epoxide of benzo(a)pyrene present in cigarette smoke which causes cancer), hydroxylamines, nitroso and azoxy derivatives, nitrenium ions and elemental sulfur. The mechanism by which electrophiles precipitate toxicity is through

covalent binding to nucleophilic tissue components such as macromolecules (proteins, nucleic acids and lipids) or low molecular weight cellular constituents. Covalent binding to DNA is responsible for carcinogenicity and tumor formation. The body's defence against electrophiles is their inactivation by conjugation with glutathione, the most abundant cellular nucleophile with -SH group. An example of tissue toxicity due to electrophiles is hepatotoxicity of paracetamol metabolites.



Free Radicals : *are species containing an odd number of electrons.* They may be positively charged (*cation radical*), negatively charged (*anion radical*) or neutral (*neutral radical*).

$R.^+$	$R.^-$	R.
Cation Radical	Anion Radical	Neutral Radical

Free radicals are generally formed via NADPH cytochrome P-450 reductase or other flavin containing reductases. Xenobiotics that on metabolic activation yield free radicals are quinones, arylamines, nitroaryls and carbon tetrachloride. Endogenous compounds such as epinephrine and DOPA can also generate free radicals. Most free radicals are organic. They produce toxicity by peroxidation of cellular components. An important class of free radicals is inorganic free radicals such as hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-). These oxidative moieties can cause tremendous tissue damage leading to mutations or cancer. The potential toxicity of free radicals is far greater than that of the electrophiles. Cellular defence mechanisms against free radicals include control imposed by membrane structure, neutralization by glutathione, control exerted by nonenzymatic antioxidant scavengers such as vitamins A, E and C and enzymatic inactivation of oxygen derived free radicals.

Generation of reactive metabolites is indicated by modification in enzyme activities, formation of glutathione conjugates (or mercapturic acids) and depletion in tissue levels of glutathione. Since the availability of glutathione in the body determine the threshold for toxic response,

thiols (e.g. N-acetyl cysteine) can be used to treat poisoning by drugs such as paracetamol that yield reactive metabolites.

Table 5.6 lists some of the compounds whose metabolites are tissue reactive.

TABLE 5.6
Compounds and their Metabolic Reaction that Generate Toxic Intermediates

Compounds	*Metabolic Pathway*	*Toxicity*
Benzo(a)pyrene	Aromatic epoxidation	Lung cancer
Aflatoxin B_1	Olefin epoxidation	Hepatic cancer
Thalidomide	Hydrolytic cleavage of lactam	Teratogenesis
Chlorinated hydrocarbons e.g. $CHCl_3$	Oxidative dehalogenation	Nephrotoxicity

QUESTIONS

1. Name and define the pharmacokinetic processes involved in the termination of drug action.
2. Why are drugs referred to as xenobiotics?
3. How does biotransformation of a drug differ from its chemical instability?
4. What will happen if a lipophilic drug that is absorbed into the systemic circulation is not metabolized?
5. What is the major function of metabolic reactions? What would be the consequence if biotransformation of a drug leads to generation of a less soluble metabolite?
6. What are soft drugs? Why are they considered safe (w.r.t. the metabolites formed) and have short half-lives?
7. What are the various sites of drug metabolism in the body? Why is liver considered the major site for such a process?
8. Depending upon the relative activity of the metabolites formed, quote with examples the various end products of biotransformation processes.
9. On what category of drugs do the microsomal and nonmicrosomal (soluble) enzymes act?
10. What are the characteristics of microsomal enzymes?
11. Classify the chemical pathways of drug metabolism.
12. Which metabolic reactions are considered phase III reactions?
13. Why are phase I reactions called as functionalization reactions?

14. Phase II reactions are called as true detoxication reactions. Explain.
15. Why oxidative reactions are predominant in comparison to other phase II reactions?
16. Explain why the oxidative enzymes are called by different names - mixed function oxidases, monooxygenases and hydroxylases.
17. How was the name cytochrome P-450 derived? Why is it considered to be the most important component of mixed function oxidases?
18. Outline the steps involved in the oxidation of xenobiotics. What is the rate-limiting step in such a process?
19. Unlike aromatic hydroxylation, oxidation of olefins do not generate tissue reactive metabolites. Explain.
20. Why are secondary and tertiary alcohols resistant to oxidation?
21. Why is N-dealkylation of tertiary nitrogen rapid in comparison to that of secondary nitrogen?
22. N-dealkylation of t-butyl group is not possible. Why?
23. Explain the analogy and distinction between N-dealkylation and oxidative deamination reactions.
24. What is the reason for hepatotoxicity of paracetamol, an otherwise safe drug, when consumed in large doses? Why is it that the tissue at the greatest risk for toxicity is liver when a tissue reactive metabolite is generated?
25. Cite examples of O-dealkylation reactions that yield active metabolites.
26. Why is it that drugs containing primary alcohol groups are rare?
27. Justify the statement - bioreductions are one-half of reversible reactions. Explain why such reactions may prolong the drug action.
28. In what respects the hydrolytic reactions differ from oxidative and reductive reactions?
29. Why does hydrolysis of esters with one large and one small moiety leads to metabolites that retain much of their activity?
30. List the characteristics of moieties transferred to the substrate in conjugation reactions. Why are such reactions capacity-limited?
31. Explain why glucuronidation is the commonest and most important of all phase II reactions.
32. What is the similarity between glucuronidation, sulfation and methylation reactions? How does conjugation with amino acids differ from them?
33. What aspect of conjugation with amino acids can be put to diagnostic use?
34. Why is it that glucuronidation can take place in most body tissues?
35. The type of conjugation reaction a drug/metabolite undergoes determines its route of excretion. Comment.

36. What is the special significance of glutathione conjugation in comparison to other phase II reactions? Why does such a reaction not require initial activation of coenzyme or the substrate?

37. Glutathione conjugates are not detectable in urine. Why?

38. How does acetylation reaction differ from conjugation with amino acids? What class of drugs undergo such a reaction?

39. In what respects methylation reaction differs from conventional phase II reactions? What is the biochemical importance of such a reaction?

40. How is cyanide inactivated in the body?

41. What are the possible mechanisms of enzyme induction and enzyme inhibition by xenobiotics? What are their consequences? Why is enzyme inhibition considered more dangerous than enzyme induction?

42. What is the interesting phenomenon observed with acetylation reactions in human race?

43. Define pharmacogenetics. What is the major cause of intersubject variability in drug response?

44. A drug eliminated primarily by hepatic biotransformation shows greater intersubject variability than those eliminated by urinary excretion. Explain.

45. To what factors are the sex related differences in drug metabolism attributed?

46. Why do children require large mg/Kg doses of some drugs in comparison to adults?

47. Neonates are at greater risk from drug intoxication than infants and children. Explain?

48. How does diet influence drug metabolism?

49. Define chronokinetics. What factors govern the diurnal variations in drug metabolism?

50. Define toxicologic activation. Classify tissue reactive metabolites and explain how they are generated.

51. What mechanisms are involved in carcinogenesis or tissue toxicity with electrophiles and free radicals?

52. What is the biochemical indication of generation of tissue reactive metabolites?

53. What is the body's defence in inactivating potential carcinogens? How can dietary habits prevent tissue toxicity?

54. Outline metabolic pathways, with structures, for following drugs:
    a. Diazepam
    b. Ibuprofen
    c. Lidocaine
    d. Hexobarbital

6

Prodrugs

Almost all drugs possess some undesirable physicochemical and biological properties. Their therapeutic efficacy can be improved by minimizing or eliminating the undesirable properties while retaining the desirable ones. This can be achieved through biological, physical or chemical means. The **biological approach** is to alter the route of administration, which may or may not be acceptable to the patient. The **physical approach** is to modify the design of the dosage form such as controlled delivery of drugs. The third and the best approach in enhancing drug selectivity while minimizing toxicity is the **chemical approach**.

The three chemical means of optimizing the drug therapeutics are:

1. Design and development of new drugs with desirable features; this approach, however, requires screening of thousands of chemicals for biological activity of which only one may become a clinically useful drug.

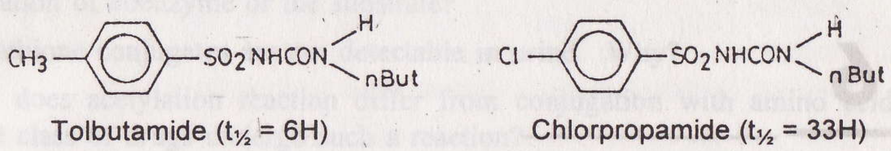
2. Design of hard and soft drugs (which are basically analogs of existing drugs) with desirable characteristics.

3. Design of prodrugs.

In the latter two approaches, the aim of the design is to develop moieties having predictable biotransformation or excretion in contrast to conventional new drug development methods.

A **hard drug** *is one which is resistant to biotransformation and, therefore, has a long biological half-life.* Such a drug will be eliminated by the body through excretion in unchanged form only. Design of hard drugs involve **metabolic stabilization** of the existing drug molecules by replacing functional groups susceptible to biotransformation with the stable ones. Apart from enhanced duration of action and less wastage of active moiety, such a hard drug also avoids generation of potentially harmful metabolites, decreases tendency for drug-drug interactions and limits intersubject variations. An example of this approach is chlorpropamide

(not really a true hard drug since it is metabolized, though slowly), an analog of tolbutamide with p-methyl replaced with a chloro group.



Nevertheless, hard drugs are not without drawbacks; too long half-lives and a potential risk of accumulation are their limitations.

In contrast to metabolic stabilization involved in hard drug design, **metabolic switching** or **metabolic promotion** concept, which involves introduction in a pharmacophore, a functional group of predictable metabolic reactivity, is used in soft drug and prodrug design.

A **soft drug** *is a biologically active compound that is biotransformed in vivo in a rapid and predictable manner into non-toxic moieties.* Such an agent has therefore a very short duration of action. Several natural endogenous agents such as insulin and adrenaline, which are also used as therapeutic compounds, are soft drugs. Design of synthetic soft drugs involves introduction of a group or a bond susceptible to rapid metabolic action; for example, replacement of a part of the alkyl side chain of the drug with an ester group that can be readily hydrolyzed *in vivo*. An important advantage of soft drugs is formation of relatively inert metabolites.

A **prodrug** *is a chemically modified inert drug precursor which upon biotransformation liberates the pharmacologically active parent compound.* Thus, in contrast to soft drugs, prodrugs are inactive per se and biotransformed in a predictable manner into active metabolites. A prodrug is also called as **pro-agent**, **bioreversible derivative** or **latentiated drug**. *The design approach is also referred to as* **drug latentiation**.

Depending upon the constitution, lipophilicity, method of bioactivation and the catalyst involved in bioactivation, prodrugs are classified into two categories: carrier-linked prodrugs and bioprecursors.

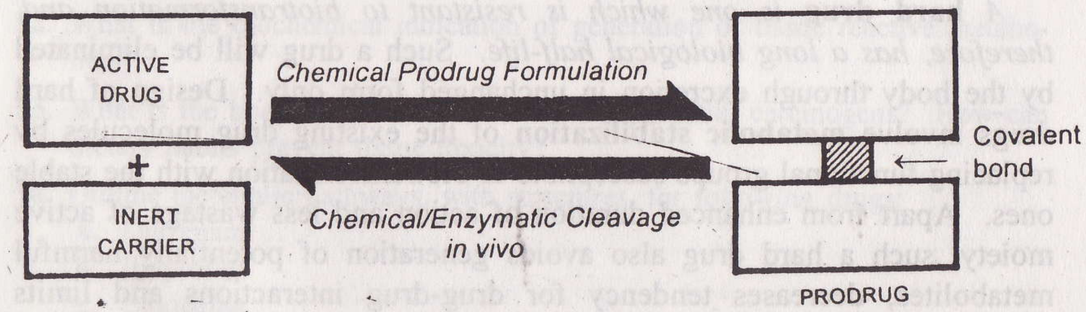
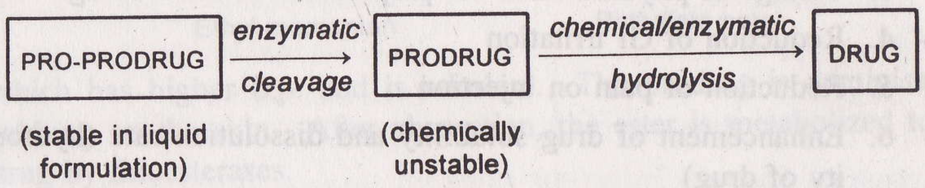


Fig. 6.1 Carrier type prodrug : formulation and drug release

Carrier-linked prodrugs (or **simple prodrugs**) *are the ones where the active drug is covalently linked to an inert* **carrier** *or* **transport moiety**. They are generally esters or amides. Such prodrugs have greatly modified lipophilicity due to the attached carrier. The active drug is released by hydrolytic cleavage, either chemically or enzymically (Fig. 6.1).

Bioprecursors or **metabolic precursors** *are inert molecules obtained by chemical modification of the active drug but do not contain a carrier.* Such a moiety has almost the same lipophilicity as the parent drug and is bioactivated generally by redox biotransformation, only enzymatically; for example, arylacetic acid NSAID such as fenbufen from aroyl propionic acid precursors. **Mixed type prodrugs** are also possible.

In some cases of carrier type prodrugs which are required to be formulated as ophthalmic, parenteral or oral liquid preparations, the conversion to active drug is chemically (nonenzymatically) triggered by a change in pH thereby creating stability problems. This can be overcome by use of **double prodrug**, **pro-prodrug** or **cascade latentiation** concept. Here, the prodrug is further derivatized in a fashion such that only enzymatic conversion to prodrug is possible before the latter can cleave to release the active drug—for example, diesters of pilocarpic acid.



In contrast to simple prodrugs where the carrier used is biologically inert, there are instances where the prodrug comprises of two pharmacologically active agents coupled together to form a single molecule such that each acts as the carrier for the other. Such *prodrugs of two active compounds are called as* **mutual prodrugs** e.g. benorylate is a mutual prodrug of NSAIDs aspirin and paracetamol.

In the subsequent discussion, the general term **prodrug** will be used for all categories of such agents to avoid confusion.

One of the earliest prodrugs to be discovered accidentally was prontosil which is converted to the antibacterial agent sulfanilamide in the body. Nowadays, prodrugs are intentionally designed to achieve specific goals. Some of the earlier developed prodrugs are aspirin and hexamine. Aspirin is a prodrug of salicylic acid designed to decrease GI irritation. Hexamine or methenamine is a prodrug which when excreted in urine is converted to formaldehyde in the acidic urine pH and acts as urinary tract antibacterial.

An **ideal prodrug** should possess following properties:

1. It should not have intrinsic pharmacologic activity.
2. It should rapidly transform, chemically or enzymatically, into the active form where desired.
3. The metabolic fragments, apart from the active drug, should be nontoxic.

A *drug candidate for chemical modification* into a prodrug is generally a therapeutic agent having a specific disadvantage which can be overcome through such an approach. *Since the shortcomings or insufficiencies are corrected through chemical conversion of the active drug into an acceptable form, the principle of* **prodrug design** *is also called as* **chemical formulation**.

The various applications of this approach are:

I. Pharmaceutical Applications : The undesirable organoleptic properties and the physicochemical problems associated with drug formulations can be resolved

1. Improvement of taste
2. Improvement of odor
3. Change of physical form for preparation of solid dosage forms
4. Reduction of GI irritation
5. Reduction of pain on injection
6. Enhancement of drug solubility and dissolution rate (hydrophilicity of drug)
7. Enhancement of chemical stability of drug

II. Pharmacokinetic Applications:

1. Enhancement of bioavailability (lipophilicity)
2. Prevention of presystemic metabolism
3. Prolongation of duration of action
4. Reduction of toxicity
5. Site-specific drug delivery (drug targeting)

Improvement of Taste

One of the reasons for poor patient compliance, particularly in case of children, is the bitterness, acidity or causticity of the drug. Two approaches can be utilized to overcome the bad taste of drug. The first is reduction of drug solubility in saliva and the other is to lower the affinity of drug towards taste receptors, thus making the bitterness or causticity

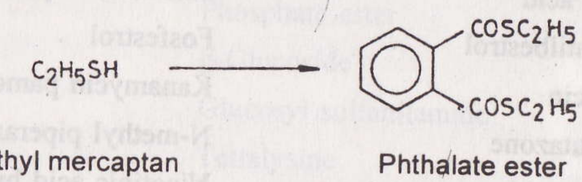
imperceptible. Examples of drugs with improved taste are given in Table 6.1.

TABLE 6.1
Prodrugs with Improved Taste

Parent Drug	*Prodrug with Improved Taste*
Chloramphenicol	Palmitate ester
Clindamycin	Palmitate ester
Sulfisoxazole	Acetyl ester
Triamcinolone	Diacetate ester

Improvement of Odor

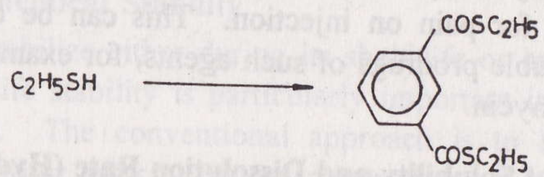
The odor of a compound depends upon its vapor pressure (and hence boiling point); a liquid with high vapor pressure (and low b.p.) will have a strong odor. Ethyl mercaptan (ethanethiol) is one such drug which is a foul smelling liquid of b.p. 35°. The drug, useful in the treatment of leprosy, is converted into its phthalate ester, diethyldithio-isophthalate

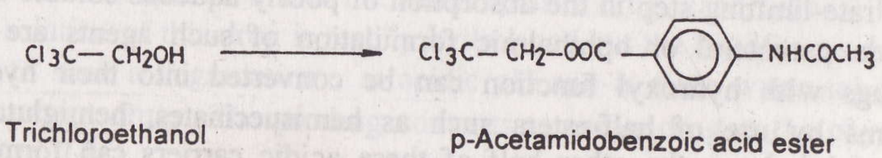


which has higher b.p. and is odorless. The prodrug is administered by rubbing on the skin. After absorption, the ester is metabolized to parent drug by thioesterases.

Change of Physical Form of the Drug

Some drugs which are in liquid form, are unsuitable for formulation as a tablet especially if their dose is high. The method of converting such





liquid drugs into solid prodrugs involves formation of symmetrical molecules having a higher tendency to crystallize; for example, esters of ethyl mercaptan and trichloroethanol.

Reduction of GI Irritation

Several drugs cause irritation and damage to the gastric mucosa through direct contact, increased stimulation of acid secretion or through interference with the protective mucosal layer. The NSAIDs, especially the salicylates, have such a tendency. They lower the gastric pH and induce or aggravate ulceration. Examples of prodrugs designed to overcome such problems of gastric distress are given in Table 6.2.

TABLE 6.2
Prodrugs to Reduce Gastric Irritation

Parent Drug	*Prodrugs That Cause Little/ No Gastric Distress*
Salicylic acid	Salsalate, Aspirin
Diethyl stilbestrol	Fosfestrol
Kanamycin	Kanamycin pamoate
Phenylbutazone	N-methyl piperazine salt
Nicotinic acid	Nicotinic acid hydrazide
Oleandrin	Oleandrin acetate

Reduction of Pain on Injection

Intramuscular injections are particularly painful when the drug precipitates or penetrates into the surrounding cells or when the solution is strongly acidic, alkaline or alcoholic; for example, the low aqueous solubility of clindamycin hydrochloride and the alkaline solution of phenytoin are responsible for pain on injection. This can be overcome by use of more water soluble prodrugs of such agents, for example, the 2'-phosphate ester of clindamycin.

Enhancement of Solubility and Dissolution Rate (Hydrophilicity) of Drug

Hydrophilic or water-soluble drugs are desired where dissolution is the rate-limiting step in the absorption of poorly aqueous soluble agents or when parenteral or ophthalmic formulation of such agents are desired. Drugs with hydroxyl function can be converted into their hydrophilic forms by use of half-esters such as hemisuccinates, hemiglutarates or hemiphthalates; the other half of these acidic carriers can form sodium,

potassium or amine salts and render the moiety water soluble. For phenolic drugs and some alcohols as in the case of steroidal drugs such as cortisol, prednisolone, betamethasone and dexamethasone, the sodium succinate salts have poor chemical stability and hence phosphate esters are preferred. Glycosidic prodrugs of some agents and L-lysine esters of benzodiazepines are also water soluble. Such hydrophilic promoieties when meant for parenteral use are advantageous over their propylene glycol solutions which are toxic or painful. Examples of hydrophilized prodrugs are given in Table 6.3.

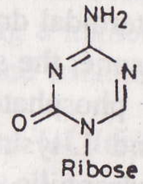
TABLE 6.3
Hydrophilic Prodrugs of Poorly Aqueous Soluble Drugs

Parent Drug	*Prodrugs with Enhanced Hydrophilicity*
Chloramphenicol	Sodium succinate ester
Tocopherols	Sodium succinate ester
Corticosteroids	21-sodium succinates, 21-phosphate esters
Testosterone	Phosphate ester
Menthol	β-Glucoside
Sulfanilamide	Glucosyl sulfanilamide
Tetracycline	Tetralysine
Diazepam	L-lysine ester
Metronidazole	Amino acid esters

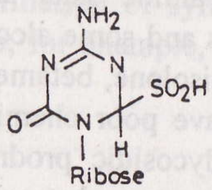
Many of the water-soluble prodrugs for oral use act as substrates for enzymes in the brush border region of microvilli where they are biotransformed into free active drug having lipophilic property facilitating permeation across the biomembrane and rapid absorption.

Enhancement of Chemical Stability

A drug may destabilize either during its shelf-life or in the GIT when used orally. Shelf-life stability is particularly important in case of drugs for intravenous use. The conventional approach is to lyophilize such solutions into a powder which can be reconstituted before use. The prodrug design of such agents is also a good alternative to improve stability. An example of this is the antineoplastic drug, azacytidine. The aqueous solution of this drug is readily hydrolyzed but the bisulfite prodrug is stable to such a degradation at acidic pH and is more water soluble than the parent drug. The prodrug converts to active drug at the physiologic pH of 7.4.



Azacytidine



Stable bisulfite prodrug

The dry powder of nafate ester prodrug of cefamandole has improved shelf-life stability over the parent drug. The prodrug rapidly converts to the active drug upon reconstitution for parenteral administration.

A class of drugs susceptible to hydrolysis and destabilization in gastric acid is penicillins. Carbenicillin, a broad spectrum penicillin, cannot be given orally for the same reason. Its ester prodrugs—carindacillin (α-indanol ester) and carfecillin (α-phenyl ester) are however stable at gastric pH. In the intestine, hydrolysis of these agents release carbenicillin at pH above 7.0. The latter is stable under such a condition and is absorbed intact. Erythromycin also has a similar acid instability problem. Its stearate, ethyl succinate and estolate (lauryl sulfate salt of erythromycin propionate) ester prodrugs are stable to hydrolysis in stomach.

Enhancement of Bioavailability (Lipophilicity)

Most drugs are absorbed by passive diffusion for which lipophilicity is an important prerequisite. Two reasons can be attributed to the enhanced oral bioavailability of lipophilic compounds:

1. The lipophilic form of a drug has enhanced membrane/water partition coefficient as compared to the hydrophilic form thus favoring passive diffusion; for example, the pivampicillin, bacampicillin and talampicillin prodrugs of ampicillin are more lipophilic, better absorbed (above 98%) and rapidly hydrolyzed to the parent drug in blood, and
2. The lipophilic prodrugs, for example, the esters of erythromycin, have poor solubility in gastric fluids and thus greater stability and better absorption.

The dipalmitoyl glycerol ester of the NSAID naproxen produces less gastric irritation and higher plasma concentration. The intraocular penetration of polar drugs such as β-blockers and epinephrine, in the treatment of glaucoma, can be promoted by use of lipophilic carrier prodrugs of such agents; for example, the diacetate ester of nadolol is 20 times more lipophilic and 10 times more readily absorbed ocularly. The dipivalyl ester of epinephrine has good ocular penetrability (8 to 17 times) in comparison to the parent drug.

A big *advantage* of increased bioavailability through increased lipophilicity is reduction in drug dosage; for example, bacampicillin is as effective as ampicillin in just one-third the dose of latter.

The bioavailability of topically administered drugs also depend upon lipid solubility. Skin penetrability of polar drugs can be improved by esterification to form lipid soluble compounds. One of the best approaches in enhancing topical availability of drugs with carboxyl functions is their esterification with one of the hydroxyl groups of propylene glycol or glycerol. The latter are common penetration enhancer components of topical formulations, e.g. glyceryl ester of naproxen.

Prevention of Presystemic Metabolism

Several corticosteroids undergo extensive first-pass hepatic metabolism which can be prevented by use of their ester or ether prodrugs - for example, triamcinolone acetonide. Propranolol is another drug with high first-pass hepatic extraction; its hemisuccinate prodrug is resistant to esterases of liver. The first-pass hepatic metabolism of opiate narcotic agonists or antagonists can be reduced by protecting the enzyme labile phenolic hydroxy group (that undergoes rapid glucuronidation) by forming a prodrug with aspirin. The parent drug is regenerated during first-pass transit of the prodrug through the liver.

Prolongation of Duration of Action

Frequent dosing is required for drugs having short biological half-lives; this can be overcome by use of both controlled release and prodrug approaches. The two rate-controlling steps (Fig. 6.2) in the enhancement of duration of drug action are:

1. **The rate of release of prodrug** from the site of application or administration into the systemic circulation, and
2. **The rate of conversion** of prodrug into active drug in the blood.

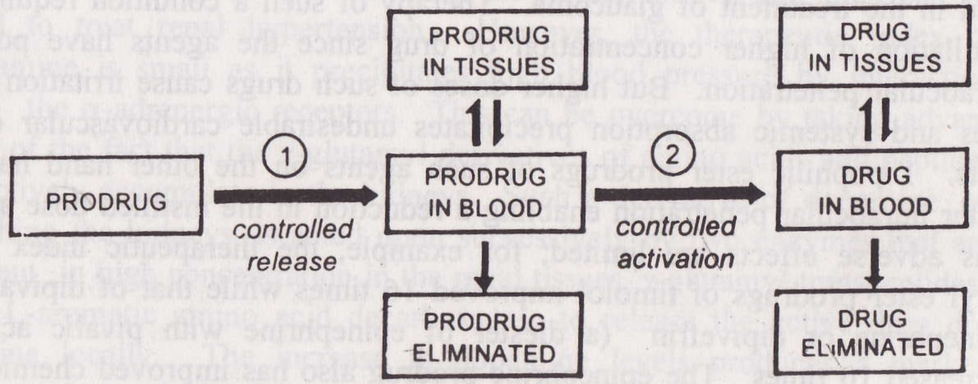


Fig. 6.2 Rate-limiting steps in the release of a drug from prodrugs

The control of either of these two steps would result in prolongation of drug action. The *easier approach*, that of controlling the release rate of prodrug, is useful when *in vivo* conversion of the latter into active drug is rapid. Examples include the i.m. depot injections of lipophilic ester prodrugs of steroids (testosterone cypionate and propionate, estradiol propionate) and antipsychotics (fluphenazine enanthate and decanoate). *Since testosterone and estradiol are natural soft drugs, their lipophilic prodrugs are sometimes called as* **prodrug-soft drugs**.

The *second approach* of controlled conversion of prodrug to active drug though *difficult*, was successfully utilized to deliver pilocarpine to eyes in the treatment of glaucoma. The diesters of the drug when applied as ophthalmic solution showed better intraocular penetration due to improved lipophilicity, and slow conversion of the ester prodrug to active pilocarpine prolonged the therapeutic effect. The rate of conversion however, is greatly dependent upon the ester group. Bitolterol, a di-p-toluate ester prodrug of N-t-butyl noradrenaline has a longer duration of bronchodilator activity than the parent drug. Moreover, the drug preferentially distributes in the pulmonary tissues and, therefore, does not show adverse cardiovascular effects.

Reduction of Toxicity

An important objective of drug design is to develop one with high activity and low toxicity. Examples of drugs for systemic use with local side effects such as gastric distress with NSAIDs, which can be overcome by prodrug design, have already been discussed. Another example of this is the bioprecursor sulindac. As a sulfoxide, it does not cause any gastric irritation and is absorbed better. In blood, it is converted to its active sulfide form.

The utility of some drugs for local use is limited by the incidence of systemic side effects such as those with timolol and epinephrine which are used in the treatment of glaucoma. Therapy of such a condition requires instillation of higher concentration of drug since the agents have poor intraocular penetration. But higher doses of such drugs cause irritation to eyes and systemic absorption precipitates undesirable cardiovascular effects. Lipophilic ester prodrugs of such agents on the other hand have better intraocular penetration enabling a reduction in the instilled dose and thus adverse effects are limited; for example, the therapeutic index of alkyl ester prodrugs of timolol improved 16 times while that of dipivalyl epinephrine or dipivefrin (a diester of epinephrine with pivalic acid) increased 10 times. The epinephrine prodrug also has improved chemical (i.e. resistance to oxidation) and biochemical stability (decreased metabolic rate in ocular tissues). Ibuterol, the diisobutyrate ester of terbutaline, a selective β_2-agonist, is also useful in glaucoma. This prodrug is 100 times more potent, has longer duration of action and is free of both local and systemic toxicity (tachyphylaxis).

Site-Specific Drug Delivery (Drug Targeting)

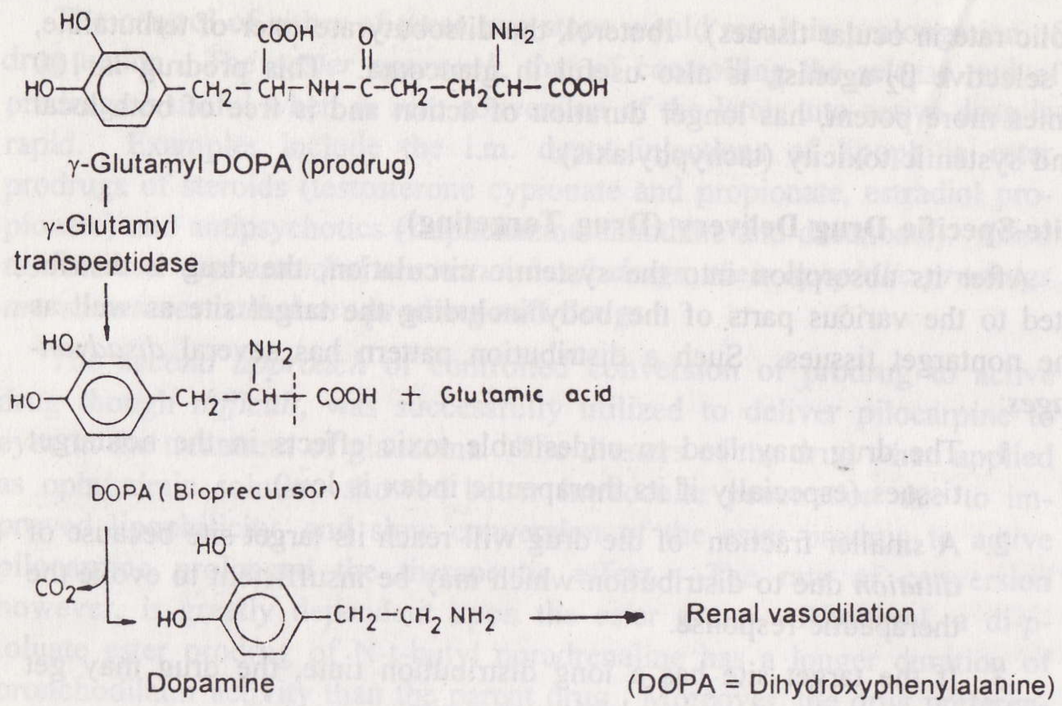
After its absorption into the systemic circulation, the drug is distributed to the various parts of the body including the target site as well as the nontarget tissues. Such a distribution pattern has several *disadvantages*:

1. The drug may lead to undesirable toxic effects in the nontarget tissues (especially if its therapeutic index is low).
2. A smaller fraction of the drug will reach its target site because of *dilution* due to distribution which may be insufficient to evoke the therapeutic response.
3. If the target site has a long distribution time, the drug may get eliminated without reaching such a site.
4. Even if the drug reaches the target cells in sufficient amounts, it may not be able to penetrate into them.

These problems can be overcome by targeting the drug to its site of action by altering its disposition characteristics. There are several approaches to drug targeting and prodrug design is one of them. The prodrug is converted into its active form only in the target organ/tissue by utilizing either specific enzymes or a pH value different from the normal pH for activation. Some of the important examples of site specific drug delivery are discussed below.

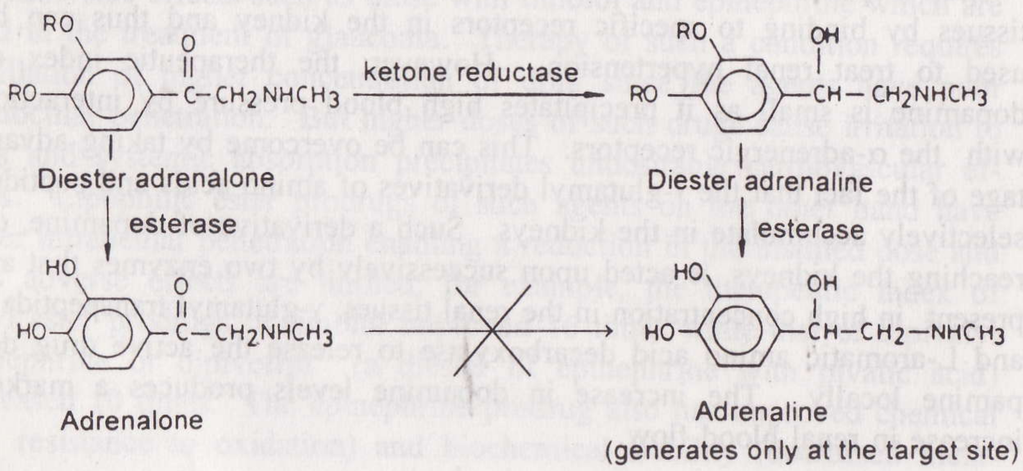
1. Selective Uptake Systems

[A] Dopamine, a neurotransmitter, produces vasodilation of renal tissues by binding to specific receptors in the kidney and thus can be used to treat renal hypertension. However, the therapeutic index of dopamine is small as it precipitates high blood pressure by interaction with the α-adrenergic receptors. This can be overcome by taking advantage of the fact that the γ-glutamyl derivatives of amino acids and peptides selectively accumulate in the kidneys. Such a derivative of dopamine, on reaching the kidneys, is acted upon successively by two enzymes that are present in high concentration in the renal tissues, γ-glutamyl transpeptidase and L-aromatic amino acid decarboxylase to release the active drug dopamine locally. The increase in dopamine levels produces a marked increase in renal blood flow.



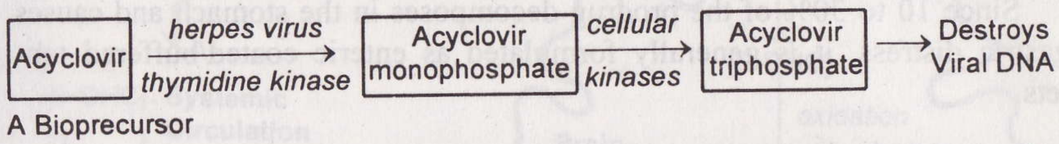
The same principle can be used to deliver sulfonamides selectively to kidneys; the prodrugs used are N-acyl-γ-glutamyl sulfonamides.

[B] Enzymatic activation of the prodrug at the site of action or target is also utilized to deliver epinephrine to eyes in the treatment of glaucoma. The disadvantages of direct ocular administration of adrenaline are known. The drug is also known to cause corneal staining. Use of a simple ester prodrug has the disadvantage of being hydrolyzed rapidly because of easy availability of esterases in several tissues. Specific delivery to the eyes necessitates presence of a specific ocular enzyme different from esterases that acts on a specific prodrug to activate it. The iris-ciliary body of the eyes where the epinephrine acts, contains an enzyme ketone reductase in addition to the usual esterase. Hence, when a diester



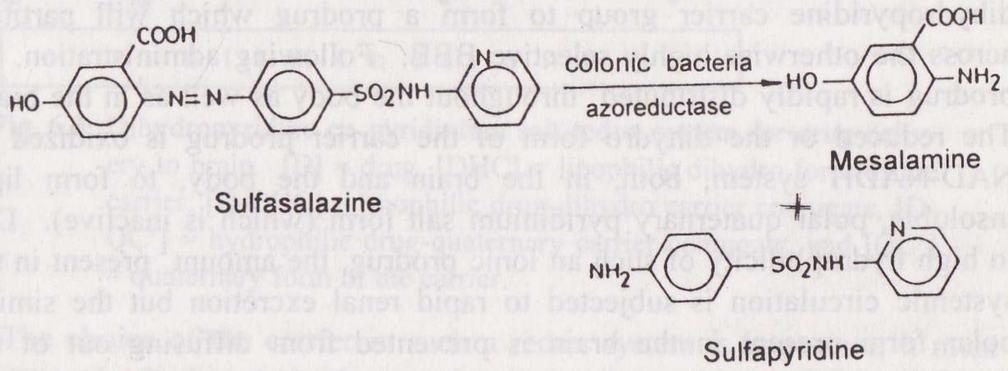
ketone prodrug, diisovaleryl adrenalone is administered, the sequential reduction-hydrolysis by the two enzymes regenerate adrenaline that shows its pharmacologic action at the target site. The drug is generated only when reduction precedes hydrolysis. If hydrolysis occurs first, the adrenalone formed will not be reduced to epinephrine.

[C] Another example of site-specific delivery is that of acyclovir, the antiviral drug useful in herpes infections. After entry into the infected cells, the drug is acted upon by the viral enzyme thymidine kinase to form acyclovir monophosphate that cannot diffuse back out of the cell. The



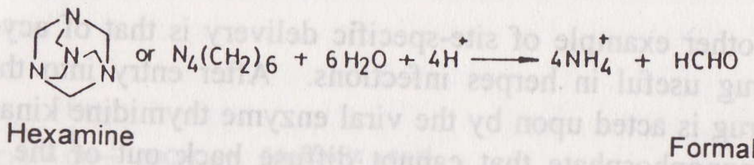
monophosphate is further converted to the active triphosphate form by the cellular enzymes. The triphosphate then destroys the viral DNA. Thus, activation of acyclovir occurs only in the cells infected with the virus.

[D] Mesalamine (mesalazine, 5-amino salicylic acid) is a drug useful in the treatment of inflammatory bowel disease (ulcerative colitis) since it is not absorbed into the systemic circulation. However, following oral administration, the drug is inactivated before reaching the lower intestine, the site of action. Covalent binding of this agent to sulfapyridine yields the prodrug sulfasalazine, an azo compound. This prodrug reaches the colon intact where cleavage by the bacterial enzyme azo reductase releases the active mesalamine for local action.



A disadvantage of the carrier moiety—sulfasalazine, is its systemic toxicity. More inert carriers can be used to deliver mesalamine to colon e.g. 4-amino benzyl alanine (prodrug is basalazide). Another much improved prodrug is a dimer of mesalamine known as olsalazine which is also an azo compound

[E] Hexamine (methenamine) is a prodrug of the urinary tract antiseptic, formaldehyde. After oral absorption, hexamine remains inert and stable in blood at pH 7.4. When excreted in urine, the prodrug decomposes in the acidic pH to generate active formaldehyde which exerts its antibacterial action in the urinary tract.



Since 10 to 30% of the prodrug decomposes in the stomach and causes gastric distress, it is generally formulated as enteric coated/buffered tablets.

2. Redox System for Drug Delivery to Brain

The high selectivity and poor permeability of the blood-brain barrier limits the delivery of hydrophilic drugs to the brain and thus, therapeutic concentration is difficult to achieve. Conversion into their lipophilic forms leads to simultaneous enhancement of transport of drug to other tissues thereby greatly increasing the chances of systemic toxicity. Moreover, to achieve therapeutic levels in the brain, the plasma concentration of the drug has to be very high. Thus, specific delivery of the drug to the brain is very difficult.

A more recent, novel and smart approach for delivery of drugs to brain is use of **dihydropyridine ⇔ pyridinium salt redox system**. The drug to be delivered to the brain is covalently linked to the lipoidal dihydropyridine carrier group to form a prodrug which will partition across the otherwise highly selective BBB. Following administration, the prodrug is rapidly distributed throughout the body as well as in the brain. The reduced or the dihydro form of the carrier prodrug is oxidized by NAD-NADH system, both, in the brain and the body, to form lipid insoluble, polar quaternary pyridinium salt form (which is inactive). Due to high hydrophilicity of such an ionic prodrug, the amount present in the systemic circulation is subjected to rapid renal excretion but the similar polar form present in the brain is prevented from diffusing out of the lipophilic BBB resulting in its **lock-in** in the CNS. The drug is slowly released from such an oxidized prodrug into the CSF by chemical/enzymatic process, allowing the therapeutic concentration to be maintained over a prolonged period of time. Thus, the drug is preferentially targeted to the brain whereas its systemic concentration is negligible (Fig. 6.3.).

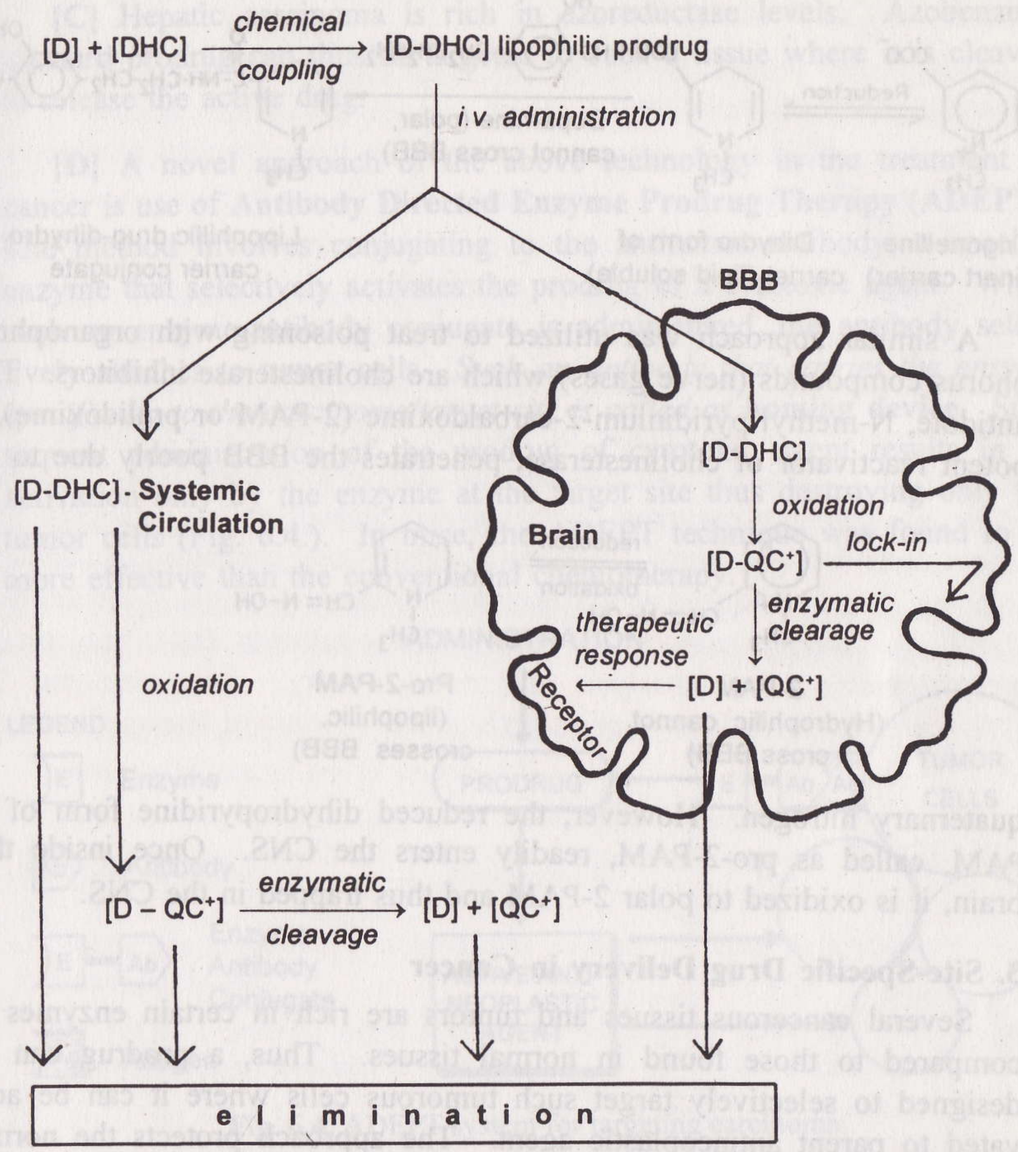
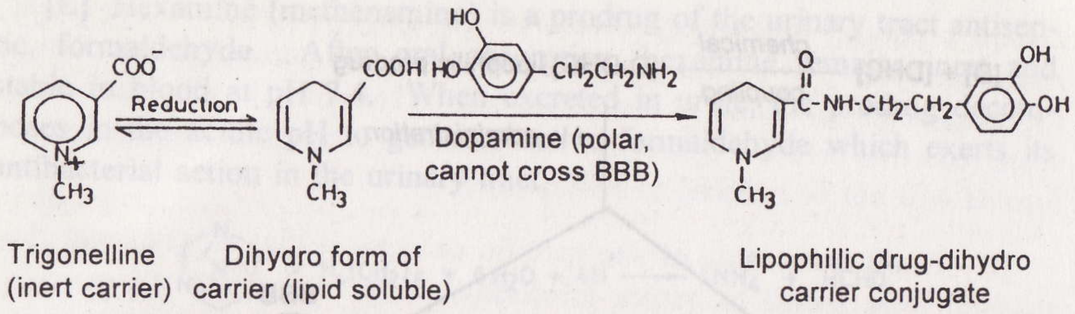
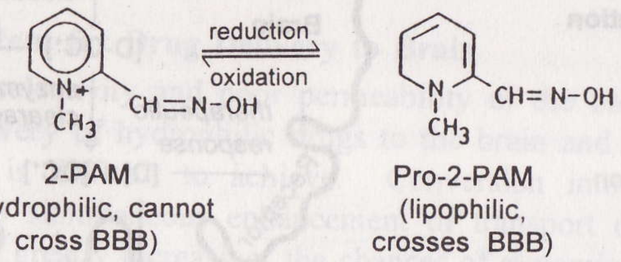


Fig. 6.3 Dihydropyridine ⇔ pyridinium salt redox system for drug delivery to brain. [D] = drug, [DHC] = lipophilic dihydro form of the carrier, [D-DHC] = lipophilic drug-dihydro carrier conjugate, [D-QC⁺] = hydrophilic drug-quaternary carrier conjugate, and [QC⁺] = quaternary form of the carrier.

The choice of the carrier in such a redox system is important; it must be nontoxic, both, when alone and as a conjugate with the drug. The carrier that was used successfully is trigonelline (N-methyl nicotinic acid). The technology holds great potential in treating various conditions of brain caused by neurotransmitter disorders such as Parkinsonism as was proved by successful delivery of dopamine to the brain of rats.



A similar approach was utilized to treat poisoning with organophosphorus compounds (nerve gases) which are cholinesterase inhibitors. The antidote, N-methyl pyridinium-2-carbaldoxime (2-PAM or pralidoxime), a potent reactivator of cholinesterase, penetrates the BBB poorly due to its



quaternary nitrogen. However, the reduced dihydropyridine form of 2-PAM, called as pro-2-PAM, readily enters the CNS. Once inside the brain, it is oxidized to polar 2-PAM and thus trapped in the CNS.

3. Site-Specific Drug Delivery in Cancer

Several cancerous tissues and tumors are rich in certain enzymes as compared to those found in normal tissues. Thus, a prodrug can be designed to selectively target such tumorous cells where it can be activated to parent antineoplastic agent. The approach protects the normal cells from the cytotoxic effects of the drug.

[A] Prostrate carcinomas are particularly rich in enzymes acid phosphatases. This fact was used to design stilbestrol diphosphate to treat such conditions. The prodrug is activated to yield stilbestrol exclusively in the target organ.

[B] Prostrate cancer can also be treated by utilizing the fact that estrogen has strong and specific affinity for such a tissue. Thus, nitrogen mustard linked to estradiol can be used for selective targeting of prostrate where hydrolysis of the prodrug will release the cytotoxic mustard to destroy the cancer cells.

[C] Hepatic carcinoma is rich in azoreductase levels. Azobenzene mustard prodrug can thus be targeted to such a tissue where it is cleaved to release the active drug.

[D] A novel approach of the above technology in the treatment of cancer is use of **Antibody Directed Enzyme Prodrug Therapy (ADEPT)**. The method involves conjugating to the antitumor antibody, a specific enzyme that selectively activates the prodrug of a cytotoxic agent. When such an enzyme-antibody conjugate is administered, the antibody selectively attaches to tumor cells. *Such an antibody that carries the enzyme specifically to the carcinoma/target site is called as* **homing device**. Subsequent administration of the prodrug of cytotoxic agent results in its activation only by the enzyme at the target site thus destroying only the tumor cells (Fig. 6.4.). In mice, the ADEPT technique was found to be more effective than the conventional chemotherapy.

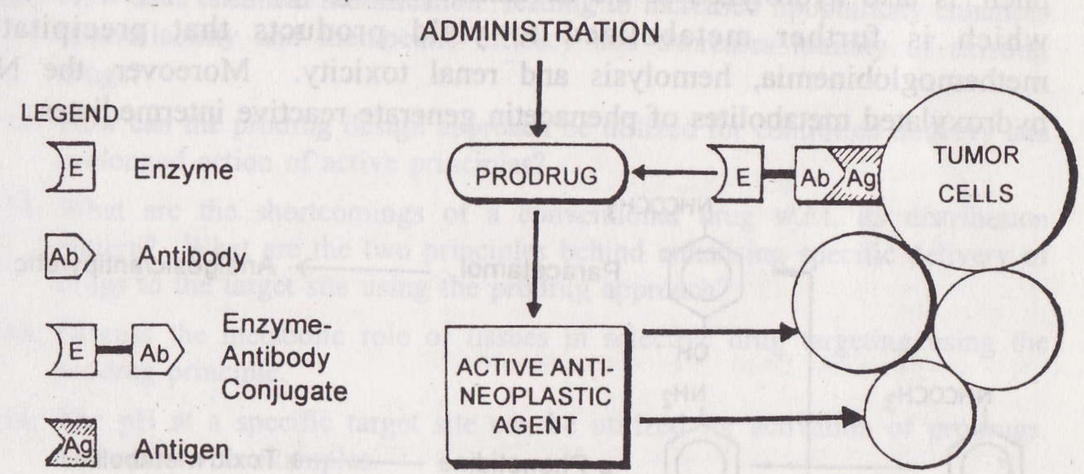


Fig. 6.4 ADEPT system for targeting carcinoma

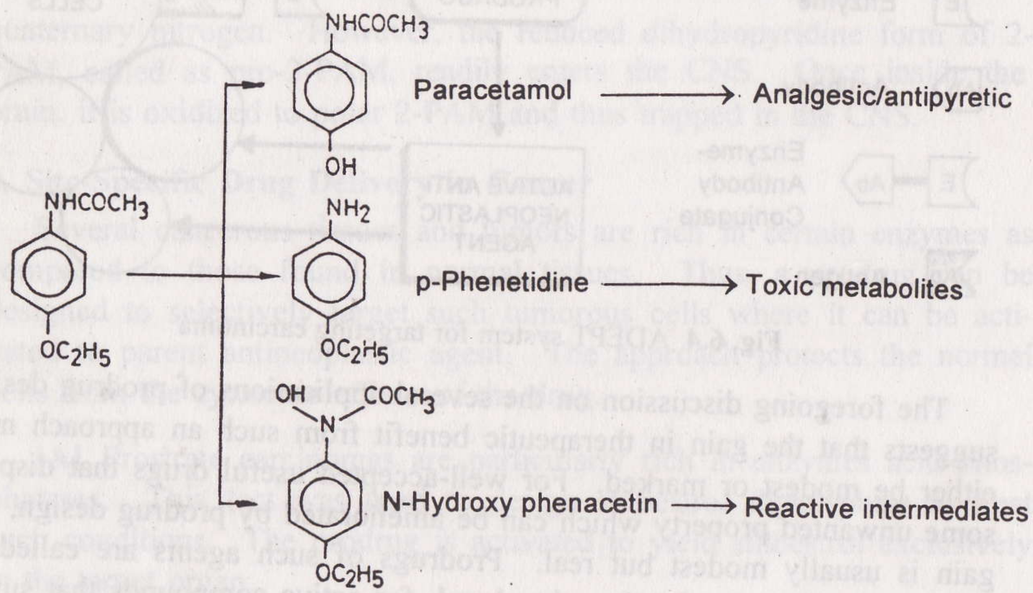
The foregoing discussion on the several applications of prodrug design suggests that the gain in therapeutic benefit from such an approach may either be modest or marked. For well-accepted useful drugs that display some unwanted property which can be ameliorated by prodrug design, the gain is usually modest but real. Prodrugs of such agents are called as **post hoc designed**. On the other hand, for active compounds that suffer from some severe limitation, for example, high hydrophilicity restricting bioavailability, prodrug design leads to a marked therapeutic gain. Prodrugs of such agents are called as **ad hoc designed**.

Limitations of Prodrug Design

One of the biggest problems that can arise in prodrug design is the toxicity which may be due to:

1. Formation of an unexpected metabolite from the total prodrug that may be toxic.
2. The inert carrier generated following cleavage of prodrug may also transform into a toxic metabolite.
3. During its activation stage, the prodrug might consume a vital cell constituent such as glutathione leading to its depletion.

An example of generation of reactive intermediates from prodrug is phenacetin. The activity of this agent is due to formation of paracetamol by de-ethylation. Compared to phenacetin, paracetamol is relatively safe unless used in high doses. Phenacetin, apart from generating acetaminophen, is also hydrolyzed at the acetamido linkage to form p-phenetidine which is further metabolized to yield products that precipitate methemoglobinemia, hemolysis and renal toxicity. Moreover, the N-hydroxylated metabolites of phenacetin generate reactive intermediates.



QUESTIONS

1. What are the various approaches to overcome the undesirable physiochemical, pharmacokinetic and pharmacodynamic properties of a drug?
2. What are hard drugs? What are their advantages over a conventional drug?
3. How do prodrugs differ from soft drugs?

4. The prodrug design approach is called as drug latentiation or chemical formulation. Explain.
5. Name, define and compare the two categories of prodrugs. Why sometimes it becomes necessary to design a double prodrug? What do you understand by mutual prodrugs?
6. Enumerate the properties of an ideal prodrug.
7. What criteria are necessary for a drug to be a candidate for prodrug design?
8. List the various pharmaceutical and pharmacokinetic applications of prodrug design.
9. What principles are utilized in prodrug design for taste masking and odor improvement of drugs?
10. Cite examples of prodrugs used to overcome gastric distress.
11. How are poorly water soluble drugs containing hydroxyl function converted into hydrophilic prodrugs? Cite examples.
12. How does chemical modification leading to increased lipophilicity enhances bioavailability and therapeutic efficacy and decreases toxicity of existing drugs?
13. How can the prodrug design approach be utilized for controlled delivery and prolonged action of active principles?
14. What are the shortcomings of a conventional drug w.r.t. its distribution pattern? What are the two principles behind enhancing specific delivery of drugs to the target site using the prodrug approach?
15. Discuss the metabolic role of tissues in selective drug targeting using the prodrug principle.
16. The pH at a specific target site can be utilized for activation of prodrugs. Elaborate with examples.
17. How is the redox system used successfully for drug targeting to brain?
18. Comment on the ADEPT system for targeting of antineoplastics to cancer cells.
19. What are post hoc and ad hoc designed prodrugs?
20. What could be the most important drawback of prodrug design?

7

Excretion of Drugs

Drugs and/or their metabolites are removed from the body by excretion. **Excretion** *is defined as the process whereby drugs and/or their metabolites are irreversibly transferred from internal to external environment.* Excretion of unchanged or intact drug is important in the termination of its pharmacologic action. The principal organs of excretion are kidneys. *Excretion by organs other than kidneys such as lungs, biliary system, intestine, salivary glands and sweat glands is known as* **nonrenal excretion.**

RENAL EXCRETION OF DRUGS

Almost all drugs and their metabolites are excreted by the kidneys to some extent or the other. Some drugs such as gentamicin are exclusively eliminated by renal route only. Agents that are water-soluble, nonvolatile, small in molecular size (less than 500 daltons) and which are metabolized slowly, are excreted in the urine.

The basic functional unit of the kidney involved in excretion is the **nephron**. Each kidney comprises of one million nephrons. Each nephron is made up of the glomerulus, the proximal tubule, the loop of Henle, the distal tubule and the collecting tubule.

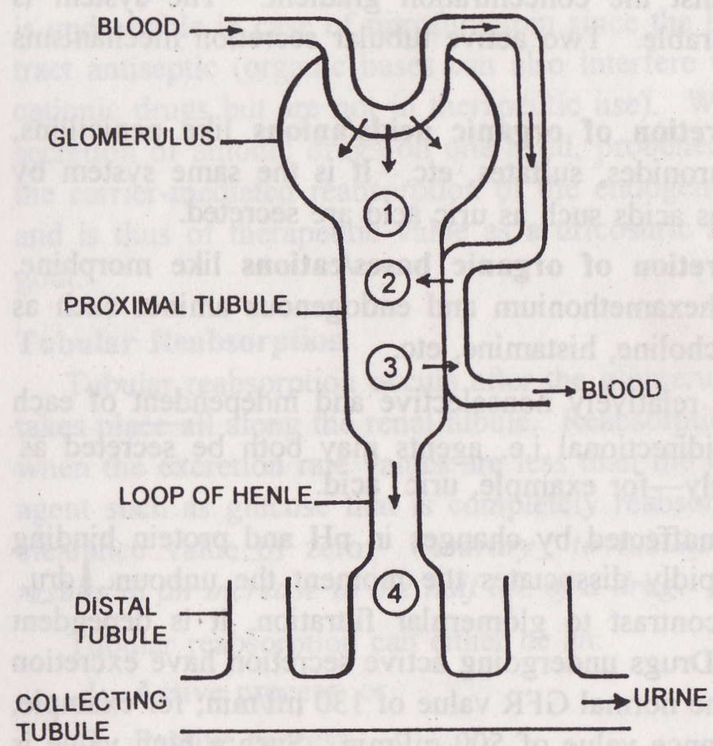
The **principal processes** that determine the urinary excretion of a drug are:

1. Glomerular filtration,
2. Active tubular secretion, and
3. Active or passive tubular reabsorption.

These processes are depicted in Fig.7.1.

Glomerular filtration and active tubular secretion tend to increase the concentration of drugs in lumen and hence facilitate excretion whereas tubular reabsorption decreases it and prevents the movement of drug out of the body. Thus, the rate of excretion can be given by equation:

$$\text{Rate of excretion} = \text{Rate of filtration} + \text{Rate of secretion} - \text{Rate of reabsorption} \quad (7.1)$$



1 Glomerular filtration of water and unbound drugs and metabolites

2 Active tubular secretion of acidic and basic drugs and metabolites

3 Active reabsorption of acidic and basic endogenous compounds and passive reabsorption of lipophilic drugs.

4 Urinary excretion of drugs and metabolites that are filtered and/or actively secreted and not reabsorbed.

Fig. 7.1 A simplified diagram illustrating processes involved in the urinary excretion of drugs

Glomerular Filtration

Glomerular filtration is a nonselective, unidirectional process whereby most compounds, ionized or unionized, are filtered except those that are bound to plasma proteins or blood cells and thus behave as macromolecules. The glomerulus also acts as a negatively charged selective barrier promoting retention of anionic compounds. The driving force for filtration through the glomerulus is the hydrostatic pressure of the blood flowing in the capillaries. Out of the 25% of cardiac output or 1.2 liters of blood/min that goes to the kidneys via renal artery, only 10% or 120 to 130 ml/min is filtered through the glomeruli, the rate being called as the **glomerular filtration rate** (GFR). Though some 180 liters of protein and cell free ultrafiltrate pass through the glomeruli each day, only about 1.5 liters is excreted as urine, the remainder being reabsorbed from the tubules.

The GFR can be determined by an agent that is excreted exclusively by filtration and is neither secreted nor reabsorbed in the tubules. The excretion rate value of such an agent is 120 to 130 ml/min. Creatinine, inulin, mannitol and sodium thiosulfate are used to estimate GFR of which the former two are widely used to estimate renal function.

Active Tubular Secretion

It is a carrier-mediated process which requires energy for transportation of compounds against the concentration gradient. The system is capacity-limited and saturable. Two active tubular secretion mechanisms have been identified:

1. **System for secretion of organic acids/anions** like penicillins, salicylates, glucuronides, sulfates, etc. It is the same system by which endogenous acids such as uric acid are secreted.
2. **System for secretion of organic bases/cations** like morphine, mecamylamine, hexamethonium and endogenous amines such as catecholamines, choline, histamine, etc.

Both the systems are relatively nonselective and independent of each other but both can be bidirectional i.e. agents may both be secreted as well as reabsorbed actively—for example, uric acid.

Active secretion is unaffected by changes in pH and protein binding since the bound drug rapidly dissociates the moment the unbound drug gets excreted. But in contrast to glomerular filtration, it is dependent upon renal blood flow. Drugs undergoing active secretion have excretion rate values greater than the normal GFR value of 130 ml/min; for example, penicillin has renal clearance value of 500 ml/min. Such a high value is indicative of both glomerular filtration as well as tubular secretion.

Agents that are used to measure active tubular secretion are the ones that are filtered as well as secreted to such an extent that they are removed from the blood in a single pass through the kidneys i.e. their clearance reflects the renal plasma flow rate which is 600 to 700 ml/min. Para amino hippuric acid (PAH), a highly polar agent and iodopyracet are used to determine active secretion. Active secretion occurs predominantly in the proximal tubule region of the nephron.

Any two structurally similar drugs having similar ionic charge and employing the same carrier-mediated process for excretion, enter into *competition*. A drug with greater rate of clearance will retard the excretion of the other drug with which it competes. The half-life of both the drugs is increased since the total sites for active secretion are limited. This may result in accumulation of drugs and thus, precipitation of toxicity. However, the principle of competition can be exploited for therapeutic benefits. An interesting example of this is the anionic agent probenecid. *Probenecid inhibits the active tubular secretion of organic acids* such as penicillins, PAS, PAH, 17-keto steroids, etc. thus increasing their concentration in plasma by at least two fold. A 50% reduction in penicillin G dose is suggested, especially when the drug is meant to be consumed in

large doses as in gonococcal infections. The actively secreted and filtered probenecid, if unionized in tubular fluid, is highly lipid soluble and therefore will get reabsorbed passively. Inhibition of drug secretion by probenecid is undesirable in case of nitrofurantoin since the latter is used as a urinary tract antiseptic (organic bases can also interfere with tubular secretion of cationic drugs but are not in therapeutic use). While inhibiting the active secretion of anionic drugs on one hand, probenecid is known to suppress the carrier-mediated reabsorption of the endogenous metabolite, uric acid and is thus of therapeutic value as a uricosuric agent in the treatment of gout.

Tubular Reabsorption

Tubular reabsorption occurs after the glomerular filtration of drugs. It takes place all along the renal tubule. Reabsorption of a drug is indicated when the excretion rate values are less than the GFR of 130 ml/min. An agent such as glucose that is completely reabsorbed after filtration has a clearance value of zero. *Contrary to tubular secretion, reabsorption results in an increase in the half life of a drug.*

Tubular reabsorption can either be an:

1. Active process, or
2. Passive process.

Active tubular reabsorption is commonly seen with high threshold endogenous substances or nutrients that the body needs to conserve such as electrolytes, glucose, vitamins, amino acids, etc. Uric acid is also actively reabsorbed (inhibited by the uricosuric agents). Very few drugs are known to undergo reabsorption actively e.g. oxopurinol.

Passive tubular reabsorption is common for a large number of exogenous substances including drugs. The driving force for such a process i.e. the concentration gradient is established by the back diffusion or reabsorption of water along with sodium and other inorganic ions . Understandably, if a drug is neither secreted nor reabsorbed, its concentration in the urine will be 100 times that of free drug in plasma due to water reabsorption since less than 1% of glomerular filtrate is excreted as urine.

The primary determinant in the passive reabsorption of drugs is their lipophilicity. Lipophilic substances are extensively reabsorbed while polar molecules are not. Since a majority of drugs are weak electrolytes (weak acids or weak bases), diffusion of such agents through the lipoidal tubular membrane depend upon the degree of ionization which in turn depends on two important factors:

1. pH of the urine
2. pK_a of the drug

Urine pH : It is an important factor in the sense that it is not constant like the plasma pH but varies between 4.5 to 7.5, the two extremes. Thus, a large pH gradient may exist between urine and plasma.

The pH of the urine is dependent upon diet, drug intake and pathophysiology of the patient. Food rich in carbohydrates result in higher urinary pH whereas proteins lower it. Drugs such as acetazolamide and antacids such as sodium bicarbonate produce alkaline urine while ascorbic acid makes it acidic. More significant alteration in urine pH is brought about by i.v. infusion of solutions of sodium bicarbonate and ammonium chloride which are used in the treatment of acid-base imbalance. Respiratory and metabolic acidosis and alkalosis result in acidification and alkalinization of the urine respectively.

The relative amount of ionized and unionized drug in the urine at a particular pH and the percent of drug ionized at this pH can be computed from the *Henderson-Hasselbach equations*:

for weak acids,

$$pH = pK_a + \log \frac{[\text{ionized}]}{[\text{unionized}]} \quad (7.2)$$

$$\% \text{ Drug ionized} = \frac{10^{pH-pK_a}}{1 + 10^{pH-pKa}} \times 100 \quad (7.3)$$

for weak bases,

$$pH = pK_a + \log \frac{[\text{unionized}]}{[\text{ionized}]} \quad (7.4)$$

$$\% \text{ Drug ionized} = \frac{10^{pK_a-pH}}{1 + 10^{pK_a-pH}} \times 100 \quad (7.5)$$

The concentration ratio R of the drug in urine to that in plasma (U:P) can be given by equations derived by *Shore et al*:

for weak acids,

$$R_a = \frac{U}{P} = \frac{1 + 10^{pHurine-pK_a}}{1 + 10^{pHplasma-pK_a}} \quad (7.6)$$

for weak bases,

$$R_b = \frac{U}{P} = \frac{1 + 10^{pK_a-pHurine}}{1 + 10^{pK_a-pHplasma}} \quad (7.7)$$

Note : The above equations from 7.2 to 7.7 are identical to equations 2.10 to 2.15 of chapter 2.

The relationship between drug pK_a, urine pH, degree of ionization and renal clearance is illustrated in Table 7.1. Table 7.1 shows percent drug ionized and renal clearance values (in ml/min) of several acidic and basic

drugs at various values of urine pH, assuming that the drug does not bind to plasma proteins, urine flow of 1 ml/min, plasma pH 7.4 and that equilibrium is achieved by diffusion of unionized drug only. The renal clearance values Cl_R are computed by use of equation 7.8.

TABLE 7.1

Percent Drug Ionized and Renal Clearance Values (in ml/min)

Drugs	*pK_a*	*Nature*	*Urine pH Values*					
			4.5		*6.3*		*7.5*	
			% Ionized	*Cl_R*	*% Ionized*	*Cl_R*	*% Ionized*	*Cl_R*
Acids								
A	2.0	Strong	99.7	0.001	99.99	0.8	100.0	1.26
B	6.0	Weak	3.0	0.04	66.6	0.115	97.0	1.25
C	10.0	V.Weak	0.0	0.99	0.02	0.99	0.3	1.0
Bases								
D	12.0	Strong	100.0	794.3	100.0	12.6	99.99	0.79
E	8.0	Weak	99.9	635.2	98.0	10.26	76.0	0.83
F	4.0	V.Weak	24.0	1.32	0.0	1.0	0.0	1.0

$$\underset{Cl_R \text{ (ml/min)}}{\text{Renal Clearance}} = \frac{U}{P} \times \underset{\text{(ml/min)}}{\text{Urine Flow Rate}} \quad (7.8)$$

Drug pK_a : The significance of pH dependent excretion for any particular compound is greatly dependent upon its pK_a and lipid solubility. A characteristic of drugs, pK_a values govern the degree of ionization at a particular pH. A polar and ionized drug will be poorly reabsorbed passively and excreted rapidly (*see* Table 7.1). Reabsorption is also affected by the lipid solubility of drug; an ionized but lipophilic drug will be reabsorbed while an unionized but polar one will be excreted.

The combined effect of urine pH and drug pK_a and lipid solubility on reabsorption of drugs is *summarized* as follows:

1. An acidic drug such as penicillin or a basic drug such as gentamicin which is polar in its unionized form, is not reabsorbed passively, irrespective of the extent of ionization in urine. Excretion of such drugs is independent of pH of urine and its flow rate. Their rate of excretion is the sum of rate of filtration and rate of active secretion.

2. Very weakly acidic, nonpolar drugs ($pK_a > 8.0$) such as phenytoin or very weakly basic, nonpolar drugs ($pK_a < 6.0$) such as propoxyphene

are mostly unionized throughout the entire range of urine pH and are therefore extensively reabsorbed passively at all values of urine pH. The rate of excretion of such drugs is always low and insensitive to urine pH.

3. A strongly acidic drug ($pK_a \leq 2.0$) such as cromoglycic acid or a strongly basic drug ($pK_a \geq 12.0$) such as guanethidine, is completely ionized at all values of urine pH and are, therefore, not reabsorbed. Their rate of excretion is always high and insensitive to pH of urine.

4. Only for an acidic drug in the pK_a range 3.0 to 8.0 (e.g. several NSAIDs) and for a basic drug in the pK_a range 6.0 to 12.0 (e.g. morphine analogs, tricyclic antidepressants, etc.) the extent of reabsorption is greatly dependent upon urine pH and varies from negligible to almost complete; for example, the amount of dexamphetamine excreted in the urine varies from 3 to 55% of the administered dose as the urine pH varies from 8.0 to 5.0. Fig. 7.2 illustrates the influence of urine pH on drug excretion.

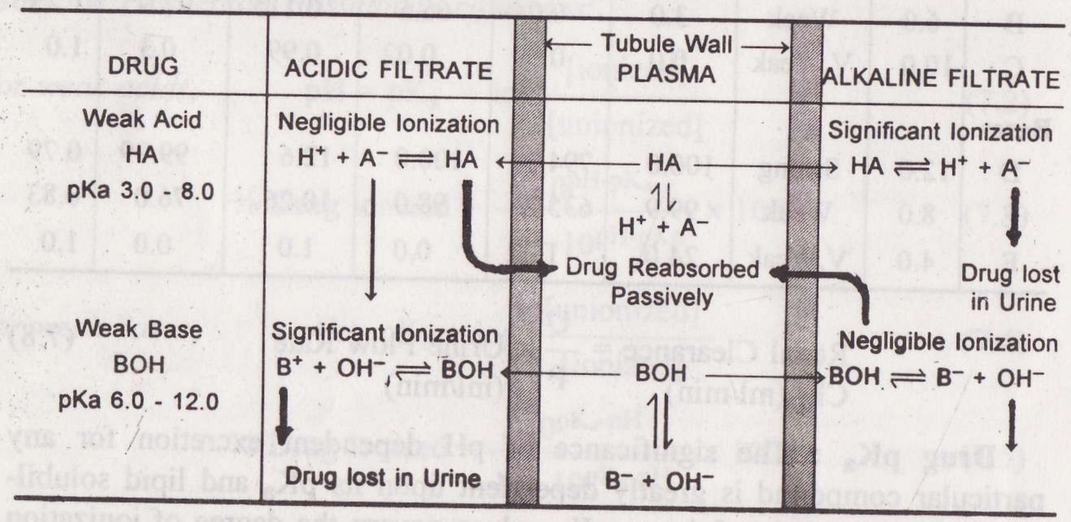


Fig. 7.2 Influence of urinary pH on excretion of weakly acidic and weakly basic drugs. Bold arrows indicate that the process is predominant.

The toxicity due to overdosage of drugs whose excretion is sensitive to pH change can be treated by acidification or alkalinization of the urine with ammonium chloride or sodium bicarbonate respectively. Thus, crystalluria caused by precipitation of sulfonamides in the renal tubules and subsequent kidney damage can be overcome by alkalinizing the urine. Excretion of basic drugs can be promoted by acidification of urine. The therapeutic activity of the urinary antiseptic hexamine also depends on the urine pH. It is not converted to active form i.e. formaldehyde unless the urine is acidic.

Urine Flow Rate : In addition to urine pH and drug pK_a, the rate of urine flow also influences the extent of reabsorption. Polar drugs whose

excretion is independent of urine pH and are not reabsorbed, are unaffected by urine flow rate. An increase in urine flow in case of such drugs will only produce a more dilute urine. Only those drugs whose reabsorption is pH-sensitive, for example, weak acids and weak bases, show dependence on urine flow rate. For such agents, reabsorption is inversely proportional to the urinary flow. These compounds can be divided into two types based on their extent of reabsorption in relation to that of water:

1. Drugs which are reabsorbed to an extent equal to or greater than the reabsorption of water e.g. phenobarbital. In such cases, the relationship between renal clearance and urinary excretion is linear.

2. Drugs which are reabsorbed to an extent lower than the reabsorption of water e.g. theophylline and many more drugs. In these cases, the relationship between renal clearance and urinary excretion is convex curvilinear.

In addition to modification of pH, urinary elimination of an agent can also be enhanced by forced diuresis. **Forced diuresis** *is the increase in urine flow induced by large fluid intake or administration of mannitol or other diuretics.* The principle can be used in an intoxicated person to remove excessive drug by promoting its excretion and decreasing the time for reabsorption.

Both urine pH control and forced diuresis can be used to treat toxicity with drug overdose when:

1. Urinary excretion is the major route for elimination of drug
2. The drug is extensively reabsorbed passively from the renal tubules
3. The reabsorption is sensitive to urine pH (and urine flow rate)

Apart from the foregoing discussion on the passive reabsorption of drugs, the process is also important in the reabsorption of low threshold substances such as urea, certain phosphates and sulfates, etc.

CONCEPT OF CLEARANCE

The clearance concept was first introduced to describe renal excretion of endogenous compounds in order to measure the kidney function. The term is now applied to all organs involved in drug elimination such as liver, lungs, the biliary system, etc. and referred to as hepatic clearance, pulmonary clearance, biliary clearance and so on. *The sum of individual clearances by all eliminating organs is called as* **total body clearance** or **total systemic clearance**. It is sometimes expressed as a sum of renal clearance and nonrenal clearance.

Clearance *is defined as the hypothetical volume of body fluids containing drug from which the drug is removed or cleared completely in a specific period of time.* It is expressed in ml/min and is a constant for any given plasma drug concentration. In comparison to apparent volume of distribution which relates plasma drug concentration to the amount of drug in the body, clearance relates plasma concentration to the rate of drug elimination.

$$\text{Clearance (Cl)} = \frac{\text{Elimination rate}}{\text{Plasma drug concentration}} \quad (7.9)$$

Renal Clearance (Cl_R) : *It can be defined as the volume of blood or plasma which is completely cleared of the unchanged drug by the kidney per unit time.* It is expressed mathematically as:

$$Cl_R = \frac{\text{Rate of urinary excretion}}{\text{Plasma drug concentration}} \quad (7.10)$$

Physiologically speaking, **renal clearance** *is the ratio of "sum of rate of glomerular filtration and active secretion minus rate of reabsorption"* to *"plasma drug concentration C".*

$$Cl_R = \frac{\text{Rate of filtration} + \text{Rate of secretion} - \text{Rate of reabsorption}}{C} \quad (7.11)$$

TABLE 7.2

Relationship between Renal Clearance Values and Mechanism of Clearance

Renal Clearance (ml/min)	*Renal Clearance Ratio*	*Mechanism of Renal Clearance*	*Example(s)*
0 (least value)	0	Drug filtered and reabsorbed completely	Glucose
< 130	Above 0, Below 1	Drug filtered and reabsorbed partially	Lipophilic drugs
130 (GFR)	1	Drug is filtered only	Creatinine, Inulin
> 130	> 1	Drug filtered as well as secreted actively	Polar, ionic drugs
650 (Highest value)	5	Clearance equal to renal plasma flow rate	Iodopyracet, PAH

The contribution of each of the above physiologic processes in clearing a drug cannot be determined by direct measurement. It can however be determined by comparing the clearance values obtained for a drug with that of an agent such as creatinine or inulin which is cleared by glomerular filtration only. The ratio of these two values is called as **renal clearance ratio** or **excretion ratio**.

$$\text{Renal Clearance Ratio} = \frac{Cl_R \text{ of drug}}{Cl_R \text{ of creatinine}} \quad (7.12)$$

Thus, depending upon whether the drug is only filtered, filtered and secreted or filtered and reabsorbed, the clearance ratio will vary (Table 7.2.). The renal clearance values range from zero to 650 ml/min and the clearance ratio from zero to five.

FACTORS AFFECTING RENAL EXCRETION OR RENAL CLEARANCE

Apart from the three physiologic processes that govern the urinary excretion, other factors influencing renal clearance of drugs and metabolites are:

1. Physicochemical properties of the drug
2. Plasma concentration of the drug
3. Distribution and binding characteristics of the drug
4. Urine pH
5. Blood flow to the kidneys
6. Biological factors
7. Drug interactions
8. Disease states

Physicochemical Properties of the Drug

Important physicochemical factors affecting renal excretion of a drug are - molecular size, pK_a and lipid solubility. The molecular weight of a drug is very critical in its urinary elimination. An agent of small molecular size can be easily filtered through the glomerulus. Compounds of weights below 300 daltons, if water soluble, are readily excreted by the kidneys. Drugs in the molecular weight range 300 to 500 daltons can be excreted both in urine and bile. Molecules of size greater than 500 daltons are excreted in urine to a lesser extent.

The influence of drug pK_a on excretion has already been discussed. Urinary excretion of an unchanged drug is inversely related to its lipophilicity. This is because, a lipophilic drug is passively reabsorbed to a large extent.

Plasma Concentration of the Drug

Glomerular filtration and reabsorption are directly affected by plasma drug concentration since both are passive processes. A drug that is not bound to plasma proteins and excreted by filtration only, shows a linear relationship between rate of excretion and plasma drug concentration. In case of drugs which are secreted or reabsorbed actively, the rate process increases with an increase in plasma concentration to a point when saturation of carrier occurs. In case of actively reabsorbed drugs, excretion is negligible at low plasma concentrations. Such agents are excreted in urine only when their concentration in the glomerular filtrate exceeds the active reabsorption capacity, e.g. glucose. With drugs that are actively secreted, the rate of excretion increases with increase in plasma concentration upto a saturation level. These situations are depicted in Fig. 7.3.

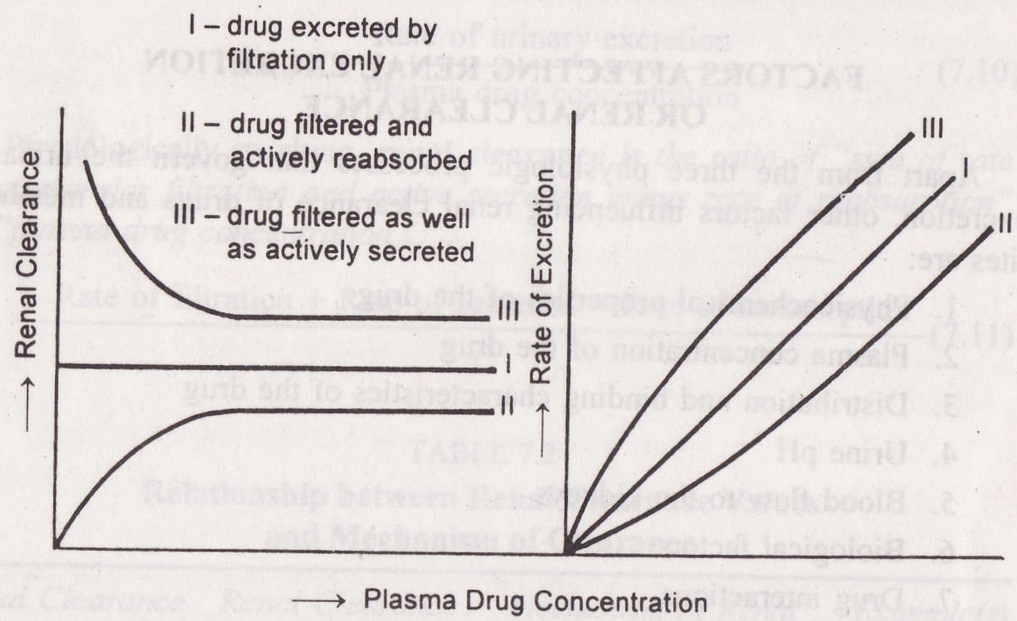


Fig. 7.3 Renal clearance and rate of excretion of a drug in relation to its plasma concentration as affected by the physiologic processes —filtration, active reabsorption and active secretion

Distribution and Binding Characteristics of the Drug

Clearance is inversely related to apparent volume of distribution of drugs. A drug with large V_d is poorly excreted in urine. Drugs restricted to blood compartment have higher excretion rates.

Drugs that are bound to plasma proteins behave as macromolecules and thus cannot be filtered through the glomerulus. Only unbound or free drug appear in the glomerular filtrate. An earlier equation given for renal clearance is:

$$Cl_R = \frac{\text{Urine concentration}}{\text{Plasma concentration}} \times \text{Urine flow rate} \quad (7.8)$$

Since only free drug can be excreted in the urine, the fraction of drug bound to plasma proteins is important and can be computed from equation:

$$f_u = \frac{C_u}{C} \quad (7.13)$$

where, f_u = fraction of unbound drug in plasma,

C_u = concentration of unbound drug in plasma, and

C = total plasma concentration of drug.

Thus, equation 7.8 can be written as:

$$Cl_R = f_u \cdot \text{Urine Flow Rate} \quad (7.14)$$

Drugs extensively bound to proteins have long half-lives because the renal clearance is small and urine flow rate is just 1 to 2 ml/min. The renal clearance of oxytetracycline which is 66% unbound is 99 ml/min while that of doxycycline (7% unbound) is just 16 ml/min.

Actively secreted drugs are much less affected by protein binding, e.g. penicillins. The free fraction of such drugs are filtered as well as secreted actively and dissociation of drug-protein complex occurs rapidly.

The influence of urine pH on renal clearance has already been discussed.

Blood Flow to the Kidneys

The renal blood flow is important in case of drugs excreted by glomerular filtration only and those that are actively secreted. In the latter case, increased perfusion increases the contact of drug with the secretory sites and enhances their elimination. Renal clearance in such instances is said to be **perfusion rate-limited**.

Biological Factors

Age, sex, species and strain differences, differences in the genetic make-up, circadian rhythm, etc. alter drug excretion. Renal excretion is approximately 10% lower in females than in males. The renal function of newborns is 30 to 40% less in comparison to adults and attains maturity between 2.5 to 5 months of age. In old age, the GFR is reduced and tubular function is altered, the excretion of drugs is thus slowed down and half-life is prolonged.

Drug Interactions

Any drug interaction that results in alteration of binding characteristics, renal flood flow, active secretion, urine pH and intrinsic clearance and forced diuresis would alter renal clearance of a drug. The renal clearance of a drug extensively bound to plasma proteins is increased after displacement with another drug. An interesting example of this is gentamicin induced nephrotoxicity by furosemide. Furosemide does not precipitate this effect by its diuretic effect but by displacing gentamicin from binding sites. The increased free antibiotic concentration accelerates its renal clearance. Acidification of urine with ammonium chloride, methionine or ascorbic acid enhances excretion of basic drugs. Alkalinization of urine with citrates, tartarates, bicarbonates and carbonic anhydrase inhibitors promote excretion of acidic drugs. Phenylbutazone competes with hydroxyhexamide, the active metabolite of antidiabetic agent acetohexamide, for active secretion and thus prolongs its action. Urinary excretion of digoxin is reduced by diazepam. All diuretics increase elimination of drugs whose renal clearance gets affected by urine flow rate.

Disease States—Renal Impairment

Renal dysfunction greatly impairs the elimination of drugs especially those that are primarily excreted by the kidneys. Some of the causes of renal failure are hypertension, diabetes mellitus, hypovolemia (decreased blood supply to the kidneys), pyelonephritis (inflammation of kidney due to infections, etc.), nephroallergens (e.g. nephrotoxic serum) and nephrotoxic agents such as aminoglycosides, phenacetin and heavy metals such as lead and mercury.

Uremia, characterized by impaired glomerular filtration and accumulation of fluids and protein metabolites, also impairs renal clearance of drugs. In both these conditions, the half-lives of drugs are increased. As a consequence, drug accumulation and toxicity may result. Determination of renal function is therefore important in such conditions in order to monitor the dosage regimen.

Renal function can be determined by measuring the GFR. Both endogenous and exogenous substances have been used as markers to measure GFR. *In order to be useful as a marker, the agent should entirely get excreted in unchanged form by glomerular filtration only and should be physiologically and pharmacologically inert.* The rate at which these markers are excreted in urine reflects the GFR and changes in GFR reflects renal dysfunction. Inulin (the exogenous fructose polysaccharide) and serum creatinine level have been used successfully for such purposes.

Inulin clearance provides an accurate measure of GFR but has the disadvantage of being a tedious method. Clinically, creatinine clearance is widely used to assess renal function.

Creatinine is an endogenous amine produced as a result of muscle catabolism. It is excreted unchanged in the urine by glomerular filtration only. An advantage of this test is that it can be correlated to the steady-state concentration of creatinine in plasma and needs no collection of urine. The method involves determination of serum creatinine levels. Since creatinine production varies with age, weight and gender, different formulae are used to calculate creatinine clearance from the serum creatinine values.

For Children (between 1 to 20 years),

$$Cl_{cr} = \frac{0.48\ H}{S_{cr}} \times \left[\frac{W}{70}\right]^{0.7} \quad (7.15)$$

For Adults (above 20 years):

Males, $$Cl_{cr} = \frac{(140 - Age)\ W}{72 \times S_{cr}} \quad (7.16)$$

Females, $$Cl_{cr} = \frac{(140 - Age)\ W}{85 \times S_{cr}} \quad (7.17)$$

$$= 0.9\ .\ Cl_{cr} \text{ of Male}$$

where, Cl_{cr} = creatinine clearance in ml/min.

S_{cr} = serum creatinine in mg%,

H = height in cms, and

W = weight in Kg.

Age is measured in years.

A direct method for determining creatinine clearance is determination of the amount of creatinine excreted in urine in 24 hours (to calculate the rate of creatinine excretion) and the mean of serum creatinine from blood samples taken just before and immediately after the urine collection period. Following formula is used:

$$Cl_{cr} = \frac{\text{Rate of creatinine excretion}}{\text{Serum creatinine in mg \%}} \quad (7.18)$$

The normal creatinine clearance value is 120 to 130 ml/min. A value of 20 to 50 ml/min denotes moderate renal failure and values below 10 ml/min indicate severe renal impairment.

The renal function, RF is calculated by equation 7.19.

$$RF = \frac{Cl_{cr} \text{ of patient}}{Cl_{cr} \text{ of a normal person}} \quad (7.19)$$

Dose Adjustment in Renal Failure

Generally speaking, drugs in patients with renal impairment have altered pharmacokinetic profile. Their renal clearance and elimination rate are reduced, the elimination half-life is increased and the apparent volume of distribution is altered. Thus, dose must be altered depending upon the renal function in such patients. However, except for drugs having low therapeutic indices, the therapeutic range of others is sufficiently large and dosage adjustment is not essential. Dosage regimen need not be changed when the fraction of drug excreted unchanged, f_u is ≤ 0.3 and the renal function RF is ≥ 0.7 of normal. This generalization is based on the assumption that the metabolites are inactive and binding characteristics and drug availability are unaltered and so is the renal function in kidney failure conditions. When the f_u value approaches unity and RF approaches zero, elimination is extremely slowed down and dosing should be reduced drastically. The significance of nonrenal clearance increases in such conditions.

The required dose in patients with renal impairment can be calculated by the simple formula:

$$\text{Normal dose} \times RF \quad (7.20)$$

The dosing interval in hours can be computed from the following equation:

$$\frac{\text{Normal interval (in hours)}}{RF} \quad (7.21)$$

When the drug is eliminated both by renal and nonrenal mechanisms, the dose to be administered in patients with renal failure is obtained from equation 7.22.

$$\text{Normal dose} \left[RF \times \text{Fraction excreted in urine} + \text{Fraction eliminated nonrenally} \right] \quad (7.22)$$

Dialysis and Hemoperfusion

In severe renal failure, the patients are put on dialysis to remove toxic waste products and drugs and their metabolites which accumulate in the body.

Dialysis *is a process in which easily diffusible substances are separated from poorly diffusible ones by the use of semipermeable membrane.*

There are two procedures for dialysis:

1. Peritoneal dialysis, and
2. Hemodialysis.

In the former, the semipermeable membrane is the natural membrane of the peritoneal cavity. The method involves introduction of the dialysate fluid into the abdomen by inserting the catheter and draining and discarding the same after a certain period of time. In hemodialysis, the semipermeable membrane is an artificial membrane. Since the system is outside the body, it is also called as **extracorporeal dialysis**. The equipment is referred to as **artificial kidney** or **hemodialyzer**. Apart from the removal of toxic waste from the body, hemodialysis is also useful in the treatment of overdose or poisoning situations where rapid removal of drug becomes necessary to save the life of the patient. Patients of kidney failure require dialysis of blood every 2 days. Each treatment period lasts for 3 to 4 hours.

Factors that govern the removal of substances by hemodialysis are:

Water Solubility : Only water soluble substances are dialyzed; lipid soluble drugs such as glutethimide cannot be removed by dialysis.

Molecular Weight : Molecules with size less than 500 daltons are dialyzed easily, e.g. many unbound drugs; drugs having large molecular weight such as vancomycin cannot be dialyzed.

Protein Binding : Drugs bound to plasma proteins or blood cells cannot be dialyzed since dialysis is a passive diffusion process.

Volume of Distribution : Drugs with large volume of distribution are extensively distributed throughout the body and therefore less easily removed by dialysis, e.g. digoxin.

The Fig. 7.4 shows schematic representation of hemodialysis.

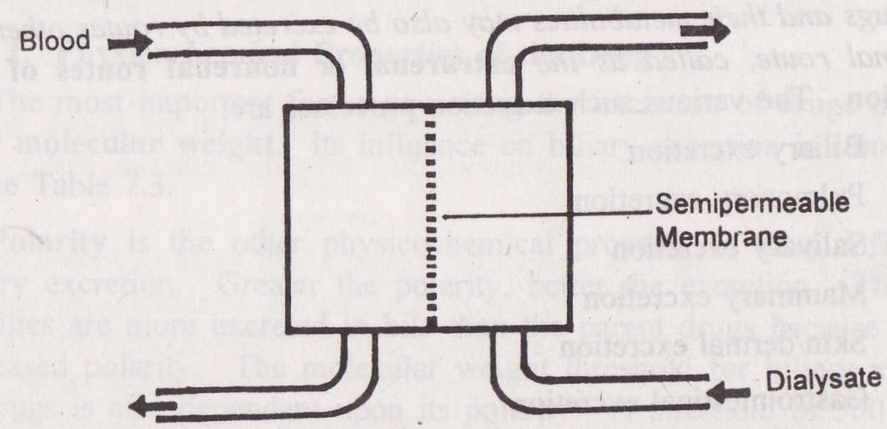


Fig. 7.4 Diagrammatic representation of a hemodialyzer. The blood and the dialysate flow counter-currently.

The dialyzing fluid contains sodium, potassium, calcium, chloride and acetate ions, and dextrose and other constituents in the same concentration as that in plasma. The unwanted metabolites in the patient's blood such as urea, uric acid, creatinine, etc. diffuse into the dialysate until equilibrium. Since the volume of dialysate is much greater than that of blood and since it is replenished with fresh fluid from time to time, almost complete removal of unwanted substances from the blood is possible. Drugs which can be removed by hemodialysis are barbiturates, aminoglycosides, chloral hydrate, lithium, etc.

The rate at which a drug is removed by the dialyzer depends upon the flow rate of blood to the machine and its performance. The term **dialysance**, also called as **dialysis clearance**, is used to express the ability of machine to clear the drug from blood. It is defined in a manner similar to clearance by equation:

$$Cl_d = \frac{Q\,(C_{in} - C_{out})}{C_{in}} \qquad (7.23)$$

where, Cl_d = dialysance or dialysis clearance

Q = blood flow rate to dialyzer

C_{in} = concentration of drug in blood entering the dialyzer

C_{out} = concentration of drug in blood leaving the dialyzer

In **hemoperfusion**, the blood is passed through a bed of adsorbent such as charcoal or resin; as a result, drugs and other unwanted molecules are adsorbed while plasma proteins are not. It is also useful in treating severe drug intoxication. The limitation of hemoperfusion is that it also removes the blood platelets, white cells and endogenous steroids.

NON-RENAL ROUTES OF DRUG EXCRETION

Drugs and their metabolites may also be excreted by routes other than the renal route, called as the **extrarenal** or **nonrenal routes of drug excretion**. The various such excretion processes are:

1. Biliary excretion
2. Pulmonary excretion
3. Salivary excretion
4. Mammary excretion
5. Skin/dermal excretion
6. Gastrointestinal excretion
7. Genital excretion

Biliary Excretion of Drugs—Enterohepatic Cycling

The hepatic cells lining the bile canaliculi produce bile. The production and secretion of bile are active processes. The bile secreted from liver, after storage in the gall bladder, is secreted in the duodenum. In humans, the bile flow rate is a steady 0.5 to 1 ml/min. Bile is important in the digestion and absorption of fats. Almost 90% of the secreted bile acids are reabsorbed from the intestine and transported back to the liver for resecretion. The rest is excreted in feces.

Being an active process, bile secretion is capacity-limited and subject to saturation. The process is exactly analogous to active renal secretion. Different transport mechanisms exist for the secretion of organic anions, cations and neutral polar compounds. A drug whose biliary concentration is less than that in plasma, has a small biliary clearance and *vice versa*. In some instances, the bile to plasma concentration ratio of drug can approach 1000 in which cases, the biliary clearance can be as high as 500 ml/min or more.

Compounds that are excreted in bile have been classified into 3 categories on the basis of their bile/plasma concentration ratios:

Group A compounds whose ratio is approximately 1, e.g. sodium, potassium and chloride ions and glucose.

Group B compounds whose ratio is >1, usually from 10 to 1000, e.g. bile salts, bilirubin glucuronide, creatinine, sulfobromophthalein conjugates, etc.

Group C compounds with ratio < 1, e.g. sucrose, inulin, phosphates, phospholipids and mucoproteins.

Drugs can fall in any of the above three categories.

Several *factors* influence secretion of drugs in bile:

1. Physicochemical Properties of the Drug

The most important factor governing the excretion of drugs in bile is their **molecular weight**. Its influence on biliary excretion is summarized in the Table 7.3.

Polarity is the other physicochemical property of drug influencing biliary excretion. Greater the polarity, better the excretion. Thus, metabolites are more excreted in bile than the parent drugs because of their increased polarity. The molecular weight threshold for biliary excretion of drugs is also dependent upon its polarity. A threshold of 300 daltons and greater than 300 daltons is necessary for organic cations (e.g. quaternaries) and organic anions respectively. Nonionic compounds should also be highly polar for biliary excretion, e.g. cardiac glycosides.

TABLE 7.3
Influence of Molecular Weight on Excretion Behavior of Drugs

Molecular Weight of Drug/Metabolite	*Excretion Pattern*
Below 300 daltons	Excreted mainly in urine; less than 5% is excreted in bile
Above 500 daltons	Excreted mainly in bile; less than 5% is excreted in urine
Between 300 to 500 daltons	Excreted both in urine and in bile; a decrease in one excretory route is compensated by excretion through the other route

2. Nature of Biotransformation Process

A metabolic reaction that greatly increases the polarity as well as the molecular weight of drug favors biliary excretion of the metabolite. Thus, phase II reactions, mainly glucuronidation and conjugation with glutathione, result in metabolites with increased tendency for biliary excretion (increase the molecular weight by 176 and 300 daltons respectively). Examples of drugs excreted in the bile as glucuronides are morphine chloramphenicol and indomethacin. Stilbestrol glucuronide is almost entirely excreted in bile. Glutathione conjugates are exclusively excreted via bile and are not observable in the urine because of their large molecular size. Conjugation with amino acids and acetylation and methylation reactions do not result in metabolites with greatly increased molecular weight and therefore have little influence on biliary excretion of xenobiotics. For a drug to be excreted unchanged in the bile, it must have a highly polar functional group such as -COOH (cromoglycic acid), $-SO_3H$ (amaranth), $-NH_4^+$ (oxyphenonium), etc. Clomiphene citrate, an ovulation inducer, is almost completely removed from the body via biliary excretion.

3. Other Factors

Miscellaneous factors influencing biliary excretion of drugs include sex and species differences, protein-drug binding, disease states, drug interactions, etc.

Substances having high molecular weight show good excretion in bile in case of rats, dogs, and hen and poor excretion in rabbits, guinea pigs and monkeys. The route is more important for the excretion of drugs in laboratory animals than in man. Protein bound drugs can also be excreted in the bile since the secretion is an active process. In cholestasis, the bile flow rate is reduced thereby decreasing biliary excretion of drugs. Agents

such as phenobarbital stimulate biliary excretion of drugs, firstly, by enhancing the rate of glucuronidation, and secondly, by promoting bile flow. The route of drug administration also influences biliary drug excretion. Orally administered drugs which during absorption process go to the liver, are excreted more in bile in comparison to parenterally administered drugs. Food also has a direct influence on biliary excretion of drugs. Protein and fat rich food increase bile flow.

The efficacy of drug excretion by the biliary system and hepatic function can be tested by an agent that is exclusively and completely eliminated unchanged in the bile, e.g. sulfobromophthalein. This marker is excreted within half an hour in the intestine when the hepatic function is normal. A delay in its excretion is indicative of hepatic and biliary malfunction. The marker is also useful in determining hepatic blood flow rate.

The ability of liver to excrete the drug in the bile is expressed by **biliary clearance** (equation 7.24).

$$\text{Biliary clearance} = \frac{\text{Biliary excretion rate}}{\text{Plasma drug concentration}} \quad (7.24a)$$

$$= \frac{\text{Bile flow} \times \text{Biliary drug concentration}}{\text{Plasma drug concentration}} \quad (7.24b)$$

Just as the major portion of bile salts excreted in intestine is reabsorbed, several drugs which are excreted unchanged in bile are also absorbed back into the circulation. Some drugs which are excreted as glucuronides or as glutathione conjugates are hydrolyzed by the intestinal or bacterial enzymes to the parent drugs which are then reabsorbed. The reabsorbed drugs are again carried to the liver for resecretion via bile

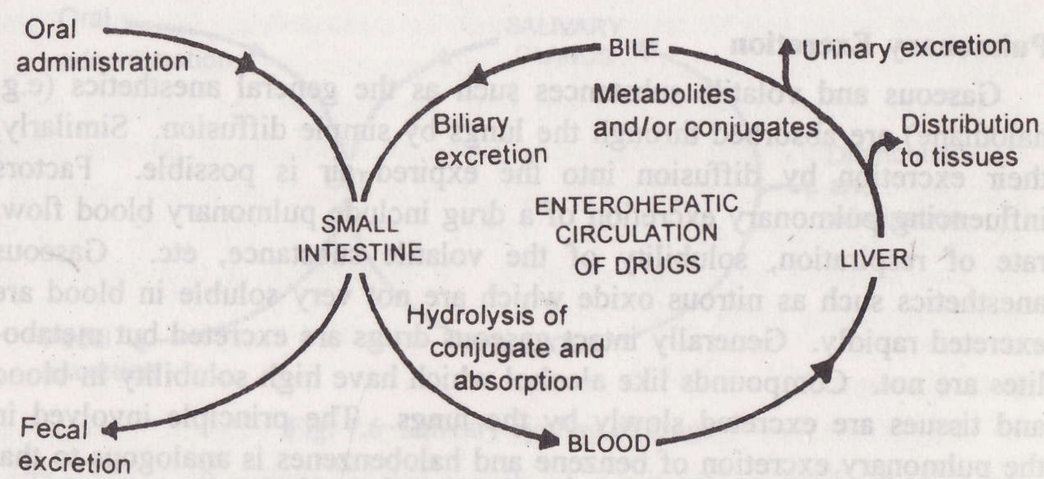


Fig. 7.5 Enterohepatic cycling of drugs

into the intestine. *This phenomenon of drug cycling between the intestine and the liver is called as* **enterohepatic cycling** or **enterohepatic circulation of drugs** (Fig. 7.5.).

Such a recycling process continues until the drug is biotransformed in the liver or is excreted in the urine or both. The drugs which are secreted via bile in the intestine but not reabsorbed, are finally excreted in the feces.

Enterohepatic circulation is important in the conservation of important endogenous substances such as vitamin B_{12}, vitamin D_3, folic acid, several steroid hormones and bile salts. The process results in prolongation of half-lives of several drugs (e.g. carbenoxolone) which are extensively excreted in bile. The half-life of agents such as DDT, which are resistant to biotransformation and are highly lipophilic, may increase to several days due to such a recycling phenomenon. The prolonged therapeutic activity of oral contraceptives (upto 12 hours) is also due to such a recirculation. Other examples of drugs undergoing enterohepatic circulation are cardiac glycosides, rifampicin, chlorpromazine and indomethacin. Drug interactions affecting enterohepatic cycling occur when agents such as antibiotics kill the intestinal microflora and thus retard hydrolysis of drug conjugates and their subsequent reabsorption, or the unabsorbable ion exchange resins such as cholestyramine which bind strongly to the acidic and neutral drugs (e.g. digitoxin) and thus prevent their reabsorption. The principle of adsorption onto the resins in the GIT can however be used to treat pesticide poisoning by promoting their fecal excretion.

Biliary excretion of drugs can be assessed by giving the drugs parenterally and detecting their presence in feces. This also rules out the doubt about the incomplete absorption of such drugs when given orally and observed in feces.

Pulmonary Excretion

Gaseous and volatile substances such as the general anesthetics (e.g. halothane) are absorbed through the lungs by simple diffusion. Similarly, their excretion by diffusion into the expired air is possible. Factors influencing pulmonary excretion of a drug include pulmonary blood flow, rate of respiration, solubility of the volatile substance, etc. Gaseous anesthetics such as nitrous oxide which are not very soluble in blood are excreted rapidly. Generally intact gaseous drugs are excreted but metabolites are not. Compounds like alcohol which have high solubility in blood and tissues are excreted slowly by the lungs. The principle involved in the pulmonary excretion of benzene and halobenzenes is analogous to that of steam distillation.

Salivary Excretion

Excretion of drugs in saliva is also a passive diffusion process and therefore predictable on the basis of pH-partition hypothesis. The pH of saliva varies from 5.8 to 8.4. The mean salivary pH in man is 6.4. Unionized, lipid soluble drugs at this pH are excreted passively in the saliva. Equations analogous to 7.3, 7.5, 7.6 and 7.7 can be written for drugs with known pK_a at the salivary pH and percent ionization and saliva/plasma drug concentration ratio (S/P) can be computed.

for weak acids,

$$R_a = \frac{S}{P} = \frac{1 + 10^{(pHsaliva-pK_a)}}{1 + 10^{(pHplasma-pK_a)}} \times \frac{f_{plasma}}{f_{saliva}} \quad (7.25)$$

for weak bases,

$$R_b = \frac{S}{P} = \frac{1 + 10^{(pK_a-pHsaliva)}}{1 + 10^{(pK_a-pHplasma)}} \times \frac{f_{plasma}}{f_{saliva}} \quad (7.26)$$

where, f_{plasma} and f_{saliva} are free drug fractions in plasma and in saliva respectively.

The S/P ratios have been found to be less than 1 for weak acids and greater than 1 for weak bases i.e. basic drugs are excreted more in saliva as compared to acidic drugs. The salivary concentration of some drugs reaches as high as 0.1%. Since the S/P ratio is fairly constant for several drugs, their blood concentration can be determined by detecting the amount of drug excreted in saliva, e.g. caffeine, theophylline, phenytoin, carbamazepine, etc. Some drugs are actively secreted in saliva, e.g. lithium, the concentration of which is sometimes 2 to 3 times that in plasma. Penicillin and phenytoin are also actively secreted in saliva.

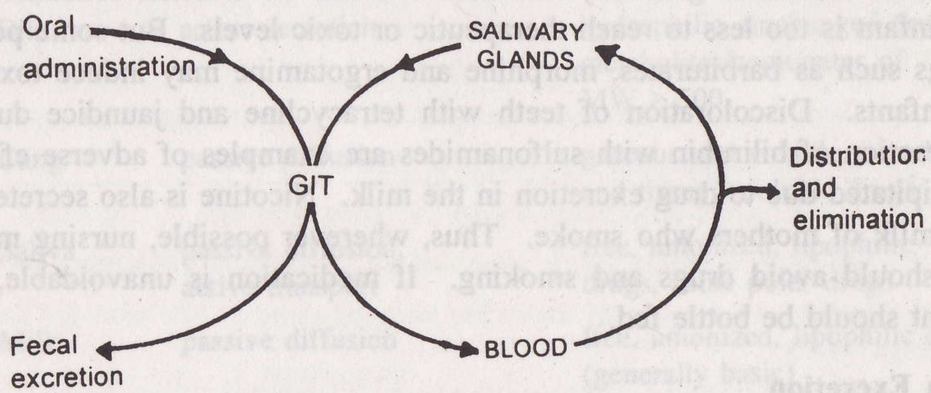


Fig. 7.6 Salivary cycling of drugs

The bitter after taste in the mouth of a patient on medication is an indication of drug excretion in saliva. In few instances, the process is

responsible for side effects such as black hairy tongue in patients receiving antibiotics, gingival hyperplasia due to phenytoin, etc. Some basic drugs inhibit saliva secretion and are responsible for dryness of mouth.

Drugs excreted in saliva can undergo cycling in a fashion similar to enterohepatic cycling, e.g. sulfonamides, antibiotics, clonidine, etc. (Fig. 7.6.).

Mammary Excretion

Excretion of a drug in milk is important since it can gain entry into the breast feeding infant.

Milk consists of lactic secretions originating from the extracellular fluid and is rich in fats and proteins. About 0.5 to 1 liter/day of milk is secreted in lactating mothers

Excretion of drugs in milk is a passive process and is dependent upon pH-partition behavior, molecular weight, lipid solubility and degree of ionization. The pH of milk varies from 6.4 to 7.6 with a mean pH of 7.0. Free, unionized, lipid soluble drugs diffuse into the mammary alveolar cells passively. The extent of drug excretion in milk can be determined from milk/plasma drug concentration ratio (M/P). Since milk is acidic in comparison to plasma, as in the case of saliva, weakly basic drugs concentrate more in milk and have M/P ratio greater than 1. The opposite is true for weakly acidic drugs. It has been shown that for acidic drugs, excretion in milk is inversely related to the molecular weight and partition coefficient and that for basic drugs, is inversely related to the degree of ionization and partition coefficient. Drugs extensively bound to plasma proteins, e.g. diazepam, are less secreted in milk. Since milk contains proteins, drugs excreted in milk can bind to it. The amount of drug excreted in milk is generally less than 1% and the fraction consumed by the infant is too less to reach therapeutic or toxic levels. But some potent drugs such as barbiturates, morphine and ergotamine may induce toxicity in infants. Discoloration of teeth with tetracycline and jaundice due to interaction of bilirubin with sulfonamides are examples of adverse effects precipitated due to drug excretion in the milk. Nicotine is also secreted in the milk of mothers who smoke. Thus, wherever possible, nursing mothers should avoid drugs and smoking. If medication is unavoidable, the infant should be bottle fed.

Skin Excretion

Drugs excreted through the skin via sweat also follow pH-partition hypothesis. Passive excretion of drugs and their metabolites through skin is responsible to some extent for the urticaria and dermatitis and other

hypersensitivity reactions. Compounds such as benzoic acid, salicylic acid, alcohol and antipyrine and heavy metals like lead, mercury and arsenic are excreted in sweat.

Gastrointestinal Excretion

Excretion of drugs into the GIT usually occurs after parenteral administration when the concentration gradient for passive diffusion is favorable. The process is reverse of GI absorption of drugs. Water soluble and ionized form of weakly acidic and basic drugs are excreted in the GIT, e.g. nicotine and quinine are excreted in stomach. Orally administered drugs can also be absorbed and excreted in the GIT. Drugs excreted in the GIT are reabsorbed into the systemic circulation and undergo recycling.

Genital Excretion

Reproductive tract and genital secretions may contain the excreted drugs. Some drugs have been detected in semen.

Drugs can also get excreted via the lacrymal fluid.

A summary of drugs excreted by various routes is given in Table 7.4.

TABLE 7.4

Excretion Pathways, Transport Mechanisms and Drugs Excreted

Excretory Route	*Mechanism*	*Drugs Excreted*
Urine	glomerular filtration, active secretion, active/passive reabsorption	free, hydrophilic, unchanged drugs/metabolites/conjugates of MW < 500
Bile	active secretion	hydrophilic, unchanged drugs/ metabolites/conjugates of MW ≥ 500
Lung	passive diffusion	gaseous and volatile, blood and tissue insoluble drugs
Saliva	passive diffusion, active transport	free, unionized, lipophilic drugs, some polar drugs
Milk	passive diffusion	free, unionized, lipophilic drugs (generally basic)
Sweat	passive diffusion	free, unionized, lipophilic drugs
Intestine	passive diffusion	water soluble, ionized drugs

QUESTIONS

1. What physicochemical properties of a drug/metabolite govern its excretion in urine?
2. Name the principal processes involved in urinary excretion of drugs. How do the excretion rate values correlate with the contribution of each of these processes in drug excretion? Quote with examples the markers used to estimate these parameters.
3. Secretion of an exogenous compound in tubules is an active process but reabsorption is generally a passive phenomenon. Explain?
4. Unlike glomerular filtration, active secretion of a drug is unaffected by protein binding. Explain.
5. How can the principle of competitive inhibition of tubular secretion be put to therapeutic use?
6. How is the driving force for passive reabsorption of drugs from tubules established?
7. Discuss the factors influencing passive reabsorption of drugs from tubules.
8. Based on the extent of ionization or pK_a of a drug, reabsorption of which drugs is affected and which remain unaffected by a change in urine pH?
9. How can the principle of urine pH control and forced diuresis be utilized to treat drug intoxication? To which drugs is such an approach applicable?
10. Define clearance and renal clearance ratio of a drug. Based on the renal clearance ratio values, how can one estimate the mechanism of renal clearance of a drug?
11. List the factors influencing renal excretion of drugs.
12. What criteria are necessary for an agent to be useful as a marker to measure kidney function? Why is creatinine clearance preferred over inulin clearance in estimating renal function?
13. Based on fraction of drug excreted unchanged and renal excretion values, in what situations does dose adjustment becomes necessary in renal failure? What assumptions are made when adjusting a dose in renal failure patient?
14. What factors govern removal of a substance by hemodialysis?
15. What are the various nonrenal routes of drug excretion?
16. Discuss the factors influencing biliary excretion of drugs.
17. How are drugs excreted in bile classified on the basis of bile/plasma ratio?
18. Metabolites and conjugated forms are excreted more in bile than the parent drugs. Why?
19. How can the efficiency of biliary system be tested?
20. Explain enterohepatic circulation of drugs. What is the significance of such a cycling? How can interaction at the cycling level be used therapeutically to treat pesticide poisoning?

21. What factors determine the pulmonary excretion of drugs?
22. Why should a nursing mother refrain from smoking and taking medication?
23. What is the reason for bitter after taste of medicaments in a patient on drug therapy?
24. What is the cause of hypersensitivity skin reactions with some drugs?
25. The normal dose of a drug is 200 mg. If the fraction excreted unchanged in urine is 0.75, what would be the dose for a patient whose creatinine clearance is 13 ml/min? Calculate the new dosing interval if the normal dosing frequency is every two hours.

    Answer : Dose = 65 mg; Dosing interval = 20 hours.
26. Estimate the creatinine clearance of a 30 years old, 70 Kg man with serum creatinine value 2.0 mg%. What is the renal function value of such a patient?

    Answer : Cl_{cr} = 53.5 ml/min; RF = 0.4.
27. The pK_a of an acidic drug is 6.0. Determine the % drug ionized in plasma pH 7.4 and urine pH 6.0. Calculate the U/P ratio and compare it with the value obtained when urine pH is 7.4. At what pH more of such a drug is expected to be excreted in urine?

    Answer : % ionized at pH 7.4 = 96.2; % ionized at pH 6.0 = 50; U/P when urine pH 6.0 = 0.076; U/P ratio when urine pH 7.4 = 1.0; the drug will be excreted more when urine pH is 7.4.
28. Calculate the dialysis clearance if the blood flow rate to the dialyzer is 50 ml/min and concentration of drug entering and leaving the dialyzer is 100 and 20 mcg/ml respectively.

    Answer : Dialysis clearance = 40 ml/min.
29. Given in the table below are four fluoroquinolone antibiotics alongwith their fractions excreted unchanged in urine and the renal function of the patient to whom they are to be administered. Rank the situations from most important to least important for considering a change in dosage regimen and name the situation for which you recommend a change in usual dosage regimen. Assume that all drugs are administered in same doses of 1000 mg/day, have similar therapeutic indices and inactive metabolites. (*Hint*: Use equation 7.22).

Situation	*Drug*	f_u	*RF*
A	Pefloxacin	0.15	0.1
B	Ciprofloxacin	0.40	0.3
C	Norfloxacin	0.70	0.5
D	Ofloxacin	0.80	0.7

Answer : B > C > D > A.

8

Pharmacokinetic Drug Interactions

Drug interactions are said to occur when the pharmacologic activity of a drug is altered by the concomitant use of another drug or by the presence of food, drink or environmental chemicals. *The drug whose activity is affected by such an interaction is called as the* **object drug** *and the agent which precipitates such an interaction is referred to as the* **precipitant**. Drug interactions are generally quantitative whereby the intensity of effect is changed (for example, an increase or a decrease) and seldom qualitative. Most interactions are specific types of *adverse reactions* with altered efficacy of the drug, for example an enhanced pharmacologic activity (e.g. hemorrhagic tendency of warfarin when phenylbutazone is given subsequently) or a decrease in the therapeutic activity resulting in loss of efficacy like that of tetracycline when concomitantly administered with food, antacids or mineral supplements containing heavy metal ions. In some instances, drug interactions may be beneficial—for example, enhancement of activity of penicillins when administered with probenecid.

Mechanisms of Drug Interactions

The two major mechanisms by which an interaction can develop are —pharmacokinetic and pharmacodynamic.

Pharmacokinetic interactions *are those in which the absorption, distribution, metabolism and/or excretion (i.e. ADME) of the object drug are altered by the precipitant.* The resultant effect is altered plasma concentration of the object drug.

Pharmacodynamic interactions *are those in which the activity of the object drug at its site of action is altered by the precipitant.* Such interactions may be direct or indirect. *A* **direct pharmacodynamic interaction** *is the one in which drugs having similar or opposing pharmacologic effects are used concurrently.* The three consequences of direct interactions are:

1. **Antagonism** : The interacting drugs have opposing actions, e.g. acetyl choline and noradrenaline have opposing effects on heart rate.
2. **Addition** or **Summation** : The interacting drugs have similar actions and the resultant effect is the sum of individual drug responses, e.g. CNS depressants like sedatives, hypnotics, etc.
3. **Synergism** or **Potentiation** : It is enhancement of action of one drug by another, e.g. alcohol enhances the analgesic activity of aspirin.

Indirect pharmacodynamic interactions *are situations in which both the object and the precipitant drugs have unrelated effects but the latter in some way alters the effects of the former* -for example, salicylates decrease the ability of the platelets to aggregate thus impairing the hemostasis if warfarin induced bleeding occurs.

The resultant effect of all pharmacodynamic interactions is thus altered drug action without a change in plasma concentration.

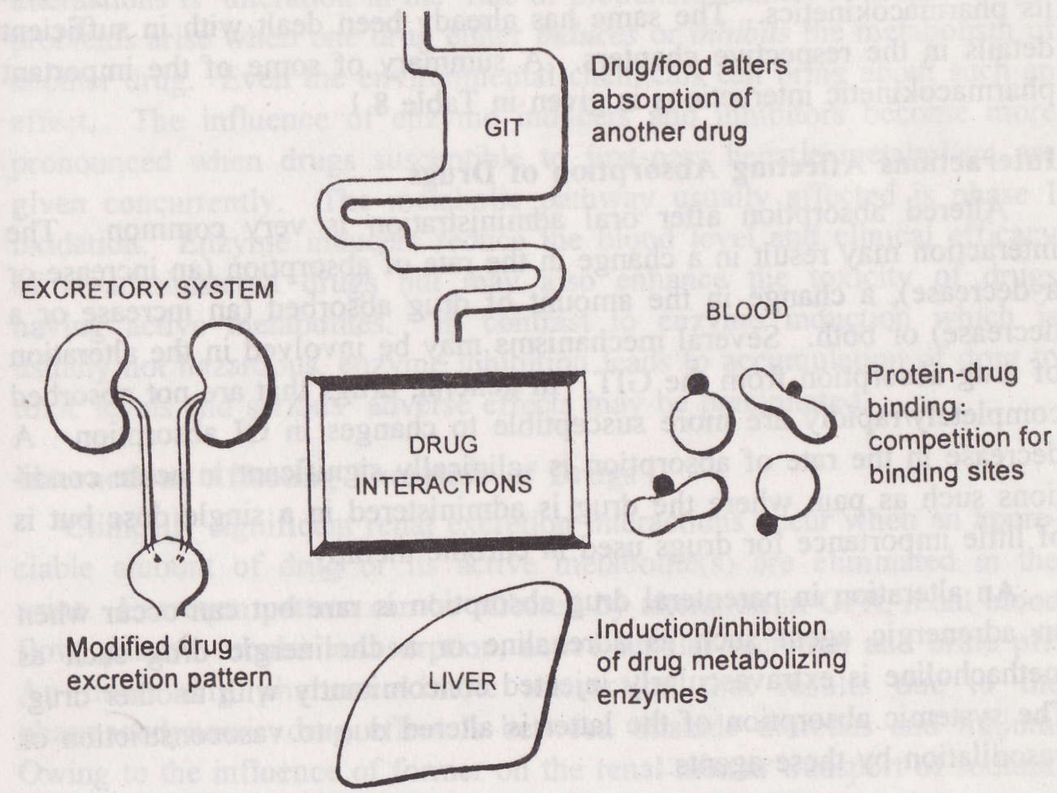


Fig. 8.1 Major sites of pharmacokinetic interactions

Apart from the pharmacokinetic and pharmacodynamic interactions, the third type of interaction is **pharmaceutical interaction**. *It is a*

physicochemical interaction that occurs when drugs are mixed in intravenous infusions causing precipitation or inactivation of the active principles. Such interactions are better expressed by the term **incompatibility** e.g. ampicillin, chlorpromazine and barbiturates interact with dextran in solutions and are broken down or form chemical complexes.

Of the various types of interactions, *the pharmacokinetic interactions are most common* and often result in differences in pharmacologic effects. Several examples of such interactions are known but few are clinically significant. Clinically important effects are precipitated by drugs having low therapeutic indices, e.g. digoxin or those having poorly defined therapeutic end-points, e.g. antipsychotics.

All pharmacokinetic interactions result due to alteration in the rate of absorption, distribution, metabolism or excretion of drugs (and therefore also called as **ADME interactions**) (*see* Fig.8.1). Such a change is reflected in the altered duration and intensity of pharmacologic action of the drug due to variation in the plasma concentration precipitated by altered ADME. All factors which influence the ADME of a drug affect its pharmacokinetics. The same has already been dealt with in sufficient details in the respective chapters. A summary of some of the important pharmacokinetic interactions is given in Table 8.1.

Interactions Affecting Absorption of Drugs

Altered absorption after oral administration is very common. The interaction may result in a change in the rate of absorption (an increase or a decrease), a change in the amount of drug absorbed (an increase or a decrease) or both. Several mechanisms may be involved in the alteration of drug absorption from the GIT. In general, drugs that are not absorbed completely/rapidly are more susceptible to changes in GI absorption. A decrease in the rate of absorption is clinically significant in acute conditions such as pain where the drug is administered in a single dose but is of little importance for drugs used in chronic therapy.

An alteration in parenteral drug absorption is rare but can occur when an adrenergic agent such as adrenaline or a cholinergic drug such as methacholine is extravascularly injected concomitantly with another drug. The systemic absorption of the latter is altered due to vasoconstriction or vasodilation by these agents.

Interactions Affecting Distribution of Drugs

Though several factors govern the distribution of drugs to various tissues, clinically significant interactions result due to competition between drugs for binding to proteins/tissues and displacement of one drug by the

other. Competitive displacement which results when two drugs are capable of binding to the same site on the protein, causes the most significant interactions. Greater risk of interactions exists when the displaced drug is highly protein bound (more than 95%), has a small volume of distribution and has a narrow therapeutic index (e.g. tolbutamide, warfarin and phenytoin), and when the displacer drug has a higher degree of affinity than the drug to be displaced. In such situations, displacement of even a small percent of drug results in a tremendous increase in the free form of the drug which precipitates increased therapeutic or toxic effects.

Drugs may also be displaced from binding sites in tissues. An interesting example of this are oral hypoglycemics such as the sulfonyl ureas (tolbutamide, glibenclamide, etc.). These agents exert their therapeutic effects by displacing insulin from protein binding sites in pancreas, plasma and other regions resulting in its elevated levels.

Interactions Affecting Metabolism of Drugs

The most important and the most common cause of pharmacokinetic interactions is alteration in the rate of biotransformation of drugs. Major problems arise when one drug either *induces* or *inhibits* the metabolism of another drug. Even the environmental chemicals can bring about such an effect. The influence of enzyme inducers and inhibitors become more pronounced when drugs susceptible to first-pass hepatic metabolism are given concurrently. The metabolic pathway usually affected is phase I oxidation. Enzyme inducers reduce the blood level and clinical efficacy of co-administered drugs but may also enhance the toxicity of drugs having active metabolites. In contrast to enzyme induction which is usually not hazardous, enzyme inhibition leads to accumulation of drug to toxic levels and serious adverse effects may be precipitated.

Interactions Affecting Excretion of Drugs

Clinically significant renal excretion interactions occur when an appreciable amount of drug or its active metabolite(s) are eliminated in the urine. Excretion pattern can be affected by alteration in GFR, renal blood flow, passive tubular reabsorption, active tubular secretion and urine pH. An interesting pharmacokinetic interaction that results due to the pharmacodynamic drug effect is between thiazide diuretics and lithium. Owing to the influence of former on the renal tubular transport of sodium, the lithium ions are retained in the body resulting in its toxicity.

Biliary excretion, the other major mechanism of drug excretion, is altered by agents that inhibit biliary transport or modify bile flow rate.

TABLE 8.1

List of Some of the Important Pharmacokinetic (ADME) Interactions

Object Drug(s)	*Precipitant Drug(s)*	*Influence on Object Drug(s)*
ABSORPTION INTERACTIONS		
1. Complexation and Adsorption		
tetracycline, penicillamine	antacids, food and mineral supplements containing Al, Mg, Fe, Zn, Bi and Ca ions	formation of poorly soluble and unabsorbable complex with such heavy metal ions
ciprofloxacin, norfloxacin	antacids containing Al, Mg and Ca and sucralfate	reduced absorption due to complexation with metal ions
cephalexin, sulfamethoxazole, trimethoprim, warfarin and thyroxine	cholestyramine	reduced absorption due to adsorption and binding
2. Alteration of GI pH		
sulfonamides, aspirin	antacids	enhanced dissolution and absorption rate
ferrous sulfate	sodium bicarbonate, calcium carbonate	decreased dissolution and hence absorption
ketoconazole, tetracycline, atenolol	antacids	decreased dissolution and bioavailability
3. Alteration of Gut Motility		
aspirin, diazepam, levodopa, lithium carbonate, paracetamol, mexiletine	metoclopramide	rapid gastric emptying; increased rate of absorption
levodopa, lithium carbonate, mexiletine	anticholinergics (atropine, homatropine)	delayed gastric emptying; decreased rate of absorption
4. Inhibition of GI Enzymes (*see* metabolism interactions)		
5. Alteration of GI Microflora		
digoxin	antibiotics (erythromycin, tetracycline)	increased bioavailability due to destruction of bacterial flora that inactivates digoxin in lower intestine

...*continued*

Object Drug(s)	*Precipitant Drug(s)*	*Influence on Object Drug(s)*
oral contraceptives	antibiotics (ampicillin)	decreased reabsorption of drugs secreted as conjugates via bile in the intestine
6. Malabsorption Syndrome		
vitamin A, B_{12}, digoxin	neomycin (and colchicine)	inhibition of absorption due to malabsorption/steatorrhea caused by neomycin
DISTRIBUTION INTERACTIONS (resulting from altered P-D binding)		
1. Competitive Displacement Interactions		
Displaced drug(s)	**Displacer(s)**	
anticoagulants (warfarin)	phenylbutazone, chloral hydrate, salicylates	increased clotting time; increased risk of hemorrhage
tolbutamide (long acting)	sulfonamides	increased hypoglycemic effect
methotrexate	sulfonamides, salicylic acid	increased methotrexate toxicity
phenytoin	valproic acid	phenytoin toxicity
METABOLISM INTERACTIONS		
1. Enzyme Induction		
corticosteroids, oral contraceptives, coumarins, phenytoin, tolbutamide, tricyclics	barbiturates	decreased plasma levels; decreased efficacy of object drugs
corticosteroids, oral contraceptives theophylline, cyclosporin	phenytoin	-do-
oral contraceptives, oral hypoglycemics, coumarins	rifampicin	-do-

...*continued*

Object Drug(s)	*Precipitant Drug(s)*	*Influence on Object Drug(s)*
2. Enzyme Inhibition		
tyramine rich food (cheese, liver, yeast products)	MAO inhibitors (phenelzine, pargyline, etc.)	enhanced absorption of unmetabolized tyramine; increased pressor activity, potentially fatal risk of hypertensive crisis
folic acid	phenytoin	decreased absorption of folic acid due to inhibition of an enzyme responsible for its efficient absorption
tricyclic antidepressants	chlorpromazine, haloperidol	increased plasma half-life of tricyclics; increased risk of sudden death from cardiac disease in such patients
coumarins	metronidazole, phenylbutazone	increased anticoagulant activity; risk of hemorrhage
oral hypoglycemics	phenylbutazone, sulfaphenazole, chloramphenicol	hypoglycemia may be precipitated
alcohol	disulfiram, metronidazole, tinidazole	disulfiram like reactions due to increase in plasma acetaldehyde levels
azathioprine, mercaptopurine	xanthine oxidase inhibitors (allopurinol)	increased toxicity of antineoplastics
alcohol, benzodiazepines, warfarin, phenytoin, theophylline, phenobarbital	cimetidine	increased blood levels of object drugs
EXCRETION INTERACTIONS		
1. Changes in Active Tubular Secretion		
penicillin, PAS, cephalosporin, nalidixic acid, methotrexate, dapsone	probenecid (acid)	elevated plasma levels of acidic drugs; risk of toxic reactions

...*continued*

Object Drug(s)	*Precipitant Drug(s)*	*Influence on Object Drug(s)*
procainamide, ranitidine	cimetidine (base)	increased plasma levels of basic object drugs; risk of toxicity
acetohexamide	phenylbutazone	increased hypoglycemic effect
2. Changes in urine pH		
amphetamine, tetracycline, quinidine	antacids, thiazides, acetazolamide	increased passive reabsorption of basic drugs; increased toxicity
3. Changes in Renal Blood Flow		
lithium	NSAIDs (inhibitors of prostaglandin synthesis; the latter control renal blood flow partially by vasoconstriction)	decreased renal clearance of lithium; risk of toxicity

QUESTIONS

1. Define drug interactions. Explain how drug interactions are of great concern in drug therapy.
2. Quote examples of beneficial drug interactions.
3. What are the two major mechanisms by which drug-drug interactions can develop? Which one is the most common of the two?
4. Define pharmacodynamic interactions. What are the three consequences of direct pharmacodynamic interactions?
5. What is the basic difference between pharmacodynamic and pharmacokinetic drug interactions?
6. What type of interaction is called pharmaceutical incompatibility?
7. What are ADME interactions? Enumerate some of the major causes of each of these interactions.

9

Pharmacokinetics : Basic Considerations

The duration of drug therapy ranges from a single dose of a drug taken for relieving an acute condition such as headache to drugs taken lifelong for chronic conditions such as hypertension, diabetes, asthma or epilepsy. *The frequency of administration of a drug in a particular dose is called as* **dosage regimen**. Depending upon the therapeutic objective to be attained, the duration of drug therapy and the dosage regimen are decided.

Rational and optimal therapy with a drug depends upon:

1. Choice of a suitable drug, and
2. A balance between the therapeutic and the toxic effects.

Both, the therapeutic and the toxic effects, depend upon the concentration of drug at the site of action which is difficult to measure. However, it corresponds to a specific concentration of drug in plasma which can be measured with accuracy. The drug fails to elicit a therapeutic response when the concentration is below the effective level and precipitates adverse reactions when above the toxic level. The plasma drug concentration between these two limits is called as the **therapeutic concentration range** or **therapeutic window** (*the ratio of maximum safe concentration to minimum effective concentration of the drug is called as the* **therapeutic index**). Thus, in order to achieve therapeutic success, plasma concentration of the drug should be maintained within the therapeutic window. For this, knowledge is needed not only of the mechanisms of drug absorption, distribution, metabolism and excretion, but also of the kinetics of these processes i.e. pharmacokinetics. **Pharmacokinetics** *is defined as the kinetics of drug absorption, distribution, metabolism and excretion (KADME) and their relationship with the pharmacologic, therapeutic or toxicologic response in man and animals. The applications of pharmacokinetic principles in the safe and effective management of individual patient is called as* **clinical pharmacokinetics**.

Plasma Drug Concentration-Time Profile

A direct relationship exists between the concentration of drug at the biophase (site of action) and the concentration of drug in plasma. A typical plasma drug concentration-time curve obtained after a single oral dose of a drug and showing various pharmacokinetic and pharmacodynamic parameters is depicted in Fig. 9.1. Such a profile can be obtained by measuring the concentration of drug in plasma samples taken at various intervals of time after administration of a dosage form and plotting the concentration of drug in plasma (*Y*-axis) versus the corresponding time at which the plasma sample was collected (*X*-axis).

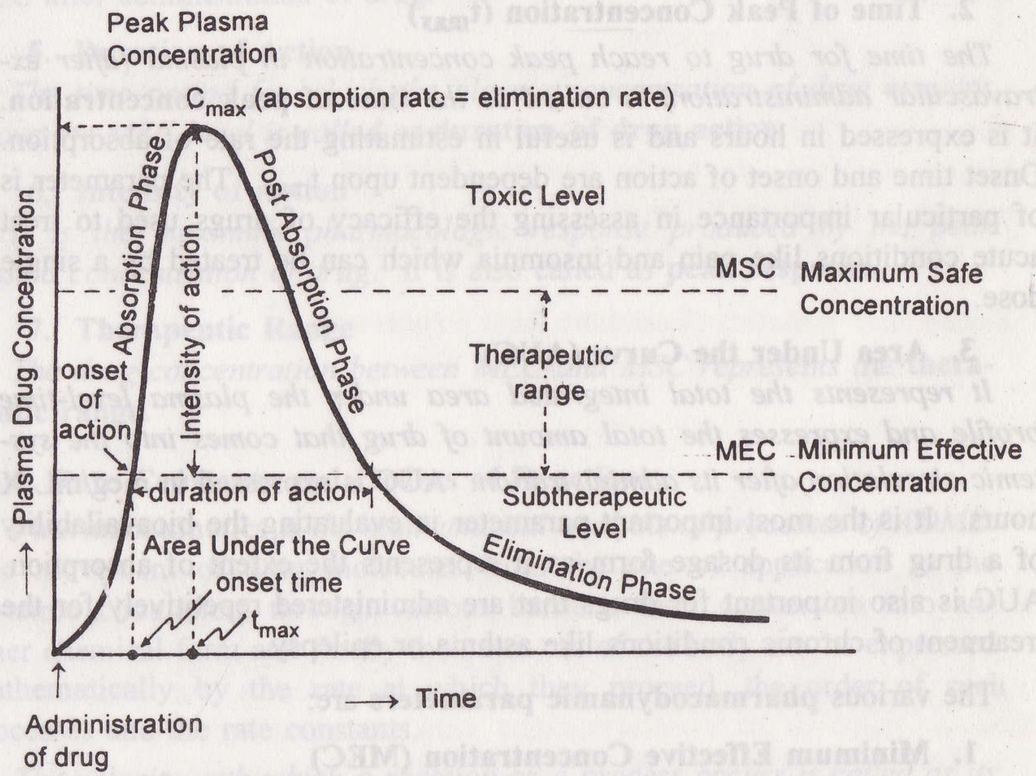


Fig. 9.1 A typical plasma concentration-time profile showing pharmacokinetic and pharmacodynamic parameters, obtained after oral administration of single dose of a drug.

The three important **pharmacokinetic parameters** *that describe the plasma level-time curve and useful in assessing the bioavailability of a drug from its formulation are*:

1. Peak Plasma Concentration (C_{max})

The point of maximum concentration of drug in plasma is called as the **peak** *and the concentration of drug at peak is known as* **peak plasma concentration**. It is also called as **peak height concentration** and **maximum drug concentration**. C_{max} is expressed in mcg/mL. The peak

level depends upon the administered dose and rate of absorption and elimination. The peak represents the point of time when absorption rate equals elimination rate of drug. The portion of curve to the left of peak represents **absorption phase** i.e. when the rate of *absorption is greater than the rate of elimination.* The section of curve to the right of peak generally represents **elimination phase** i.e. *when the rate of elimination exceeds rate of absorption.* Peak concentration is often related to the intensity of pharmacologic response and should ideally be above minimum effective concentration (MEC) but less than the maximum safe concentration (MSC).

2. Time of Peak Concentration (t_{max})

The time for drug to reach peak concentration in plasma (after extravascular administration) is called as the **time of peak concentration**. It is expressed in hours and is useful in estimating the rate of absorption. Onset time and onset of action are dependent upon t_{max}. The parameter is of particular importance in assessing the efficacy of drugs used to treat acute conditions like pain and insomnia which can be treated by a single dose.

3. Area Under the Curve (AUC)

It represents the total integrated area under the plasma level-time profile and expresses the total amount of drug that comes into the systemic circulation after its administration. AUC is expressed in mcg/mL X hours. It is the most important parameter in evaluating the bioavailability of a drug from its dosage form as it represents the extent of absorption. AUC is also important for drugs that are administered repetitively for the treatment of chronic conditions like asthma or epilepsy.

The various **pharmacodynamic parameters** are:

1. Minimum Effective Concentration (MEC)

It is defined as the minimum concentration of drug in plasma required to produce the therapeutic effect. It reflects the minimum concentration of drug at the receptor site to elicit the desired pharmacologic response. *The concentration of drug below MEC is said to be in the* **subtherapeutic level**.

In case of antibiotics, the term **minimum inhibitory concentration** (MIC) is used. *It describes the minimum concentration of antibiotic in plasma required to kill or inhibit the growth of microorganisms.*

2. Maximum Safe Concentration (MSC)

Also called as **minimum toxic concentration (MTC)**, *it is the concentration of drug in plasma above which adverse or unwanted effects are precipitated. Concentration of drug above MSC is said to be in the* **toxic level**.

3. Onset of Action

The beginning of pharmacologic response is called as **onset of action**. It occurs when the plasma drug concentration just exceeds the required MEC.

4. Onset Time

It is the time required for the drug to start producing pharmacologic response. It corresponds to the time for the plasma concentration to reach MEC after administration of drug.

5. Duration of Action

The time period for which the plasma concentration of drug remains above the MEC level is called as **duration of drug action**.

6. Intensity of Action

It is the maximum pharmacologic response produced by the peak plasma concentration of drug. It is also called as **peak response**.

7. Therapeutic Range

The drug concentration between MEC and MSC represents the **therapeutic range**.

Rate, Rate Constants and Orders of Reactions

Pharmacokinetics *is the mathematical analysis of processes of ADME.* The movement of drug molecules from the site of application to the systemic circulation, through various barriers, their conversion into another chemical form and finally their exit out of the body can be expressed mathematically by the rate at which they proceed, the order of such processes and the rate constants.

The velocity with which a reaction or a process occurs is called as its **rate**. Consider the following chemical reaction:

$$\text{drug A} \longrightarrow \text{drug B} \tag{9.1}$$

The rate of forward reaction is expressed as:

$$\frac{-dA}{dt} \tag{9.2}$$

Negative sign indicates that the concentration of drug A decreases with time t. As the reaction proceeds, the concentration of drug B increases and the rate of reaction can also be expressed as:

$$\frac{dB}{dt} \tag{9.3}$$

Experimentally, the rate of reaction is determined by measuring the decrease in concentration of drug A with time t.

The manner in which the concentration of drug (or reactants) influence the rate of reaction or process is called as the **order of reaction** or **order of process.** If C is the concentration of drug A, the rate of decrease in C of drug A as it is changed to B can be described by a general expression as a function of time t.

$$\frac{dC}{dt} = -K\,C^n \qquad (9.4)$$

where, K = rate constant

n = order of reaction

If n = 0, its a zero-order process, if n = 1, it is a first-order process and so on. The three commonly encountered rate processes in a physiologic system are—zero-order process, first-order process and mixed-order process. The pharmacokinetics of most drugs can be adequately described by zero- and first-order processes of which the latter are more important.

Zero-order Kinetics (Constant Rate Processes)

If n = 0, equation 9.4 becomes:

$$\frac{dC}{dt} = -K_o\,C^o = -K_o \qquad (9.5)$$

where K_o = zero-order rate constant (in mg/min)

From equation 9.5, the **zero-order process** *can be defined as the one whose rate is independent of the concentration of drug undergoing reaction i.e. the rate of reaction cannot be increased further by increasing the concentration of reactants.*

Rearrangement of equation 9.5 yields:

$$dC = -K_o\,dt \qquad (9.6)$$

Integration of equation 9.6 gives:

$$C - C_o = -K_o\,t$$

$$\text{or simply,} \quad C = C_o - K_o\,t \qquad (9.7)$$

where C_o = concentration of drug at t = 0, and

C = concentration of drug yet to undergo reaction at time t.

Equation 9.7 is that of a straight line and states that the concentration of reactant decreases linearly with time. A plot of C versus t yields such a straight line having slope $-K_o$ and y-intercept C_o (Fig.9.2.).

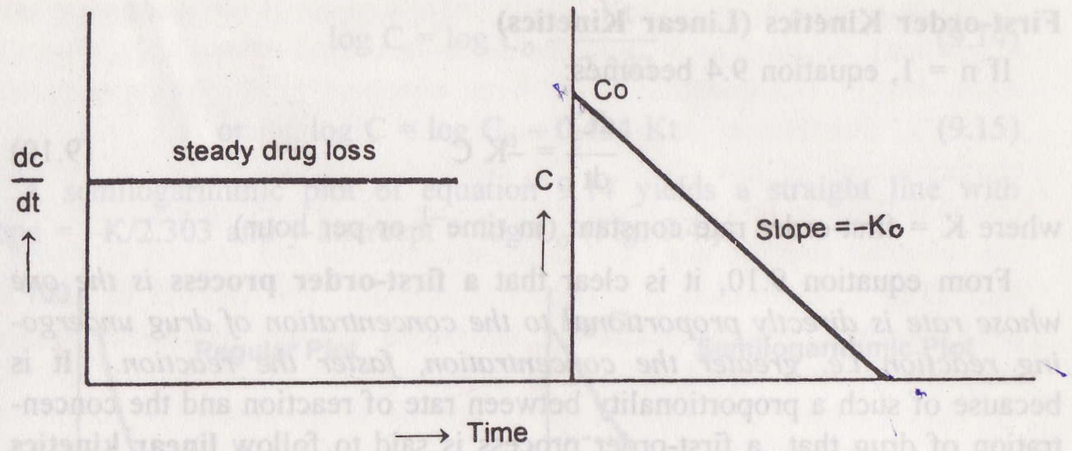


Fig. 9.2 Graphs of zero-order kinetics (equations 9.5 and 9.7)

Zero-Order Half-Life

Half-life ($t_{½}$) or **half-time** *is defined as the time period required for the concentration of drug to decrease by one-half.* When $t = t_{½}$, $C = C_o/2$ and the equation 9.7 becomes:

$$\frac{C_o}{2} = C_o - K_o t_{½} \qquad (9.8)$$

Solving 9.8, we get:

$$t_{½} = \frac{C_o}{2\,K_o} = \frac{0.5\,C_o}{K_o} \qquad (9.9)$$

Equation 9.9 shows that the $t_{½}$ of a zero-order process is not constant but proportional to the initial concentration of drug C_o and inversely proportional to the zero-order rate constant K_o. Since the zero-order $t_{½}$ changes with the decline in drug concentration, it is of little practical importance. Zero-order equations do not require logarithmic transformations.

Examples of zero-order processes are:

1. Metabolism/protein-drug binding/enzyme or carrier-mediated transport under saturated conditions. The rate of metabolism, binding or transport of drug remains constant as long as its concentration is in excess of saturating concentration.
2. Administration of a drug as a constant rate i.v. infusion.
3. Controlled drug delivery such as that from i.m. implants or osmotic pumps.

First-order Kinetics (Linear Kinetics)

If n = 1, equation 9.4 becomes:

$$\frac{dC}{dt} = -K\,C \qquad (9.10)$$

where K = first-order rate constant (in time^{-1} or per hour)

From equation 9.10, it is clear that **a first-order process** *is the one whose rate is directly proportional to the concentration of drug undergoing reaction i.e. greater the concentration, faster the reaction.* It is because of such a proportionality between rate of reaction and the concentration of drug that a first-order process is said to follow **linear kinetics** (Fig. 9.3.).

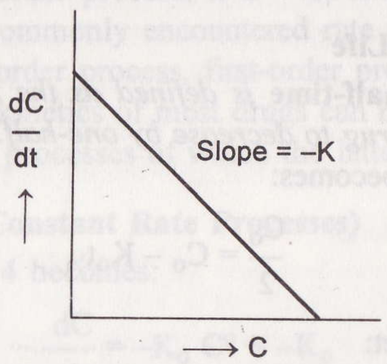


Fig. 9.3 Graph of first-order kinetics showing linear relationship between rate of reaction and concentration of drug (equation 9.10).

Rearrangement of equation 9.10 yields:

$$\frac{dC}{C} = -K\,dt \qquad (9.11)$$

Integration of equation 9.11 gives:

$$\ln C = \ln C_o - Kt \qquad (9.12)$$

Equation 9.12 can also be written in exponential form as:

$$C = C_o\,e^{-Kt} \qquad (9.13)$$

where e = natural (Naperian) log base.

Since equation 9.13 has only one exponent, the first-order process is also called as **monoexponential rate process**. Thus, a first-order process is characterized by **logarithmic** or **exponential kinetics** i.e. *a constant fraction of drug undergoes reaction per unit time.*

Since ln = 2.303 log, equation 9.12 can be written as:

$$\log C = \log C_o - \frac{Kt}{2.303} \qquad (9.14)$$

or

$$\log C = \log C_o - 0.434\,Kt \qquad (9.15)$$

A semilogarithmic plot of equation 9.14 yields a straight line with slope = –K/2.303 and y-intercept = log C_o (Fig. 9.4.).

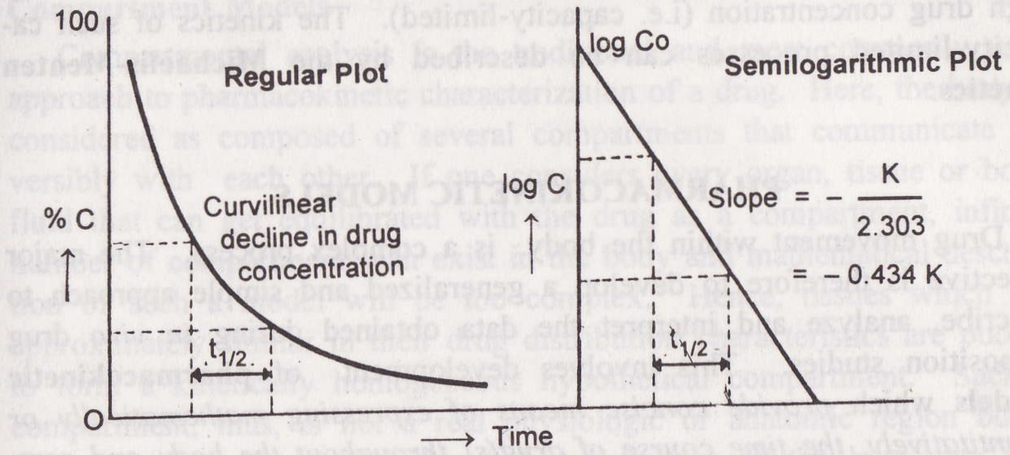


Fig. 9.4 Regular and semilog graphs of first-order kinetics

First-Order Half-Life

Substituting the value of C = $C_o/2$ at $t_{½}$ in equation 9.14 and solving it yields:

$$t_{½} = \frac{0.693}{K} \qquad (9.16)$$

Equation 9.16 shows that, in contrast to zero-order process, the half-life of a first-order process is a constant and independent of initial drug concentration i.e. irrespective of what the initial drug concentration is, the time required for the concentration to decrease by one-half remains the same (see Fig. 9.4.). The $t_{½}$ of a first-order process is an important pharmacokinetic parameter.

Most pharmacokinetic processes viz. absorption, distribution and elimination follow first-order kinetics.

Mixed-Order Kinetics (Nonlinear Kinetics)

In some instances, the kinetics of a pharmacokinetic process change from predominantly first-order to predominantly zero-order with increasing dose or chronic medication. A *mixture* of both first-order and zero-order kinetics is observed in such cases and therefore the process is said to follow **mixed-order kinetics**. Since deviations from an originally linear pharmacokinetic profile is observed, the rate process of such a drug is

called as **nonlinear kinetics**. Mixed order kinetics is also termed as **dose dependent kinetics** as it is observed at increased or multiple doses of some drugs. Nonlinearities have been observed in absorption (e.g. vitamin C), distribution (e.g. naproxen) and elimination (e.g. riboflavin) characteristics of few drugs. The phenomena is seen when a particular pharmacokinetic process involves presence of carriers or enzymes which are substrate specific and have definite capacities and can get saturated at high drug concentration (i.e. capacity-limited). The kinetics of such capacity-limited processes can be described by the **Michaelis-Menten kinetics**.

PHARMACOKINETIC MODELS

Drug movement within the body is a complex process. The major objective is therefore to develop a generalized and simple approach to describe, analyze and interpret the data obtained during *in vivo* drug disposition studies. This involves development of **pharmacokinetic models** which *provide concise means of expressing mathematically or quantitatively, the time course of drug(s) throughout the body and compute meaningful pharmacokinetic parameters.*

Pharmacokinetic models are useful in—

1. Characterizing the behavior of drugs in patients
2. Predicting the concentration of drug in various body fluids with any dosage regimen
3. Predicting the multiple-dose concentration curves from single dose experiments
4. Calculating the optimum dosage regimen for individual patients
5. Evaluating the risk of toxicity with certain dosage regimens
6. Correlating plasma drug concentration with pharmacologic response
7. Evaluating the bioequivalence/bioinequivalence between different formulations of the same drug
8. Estimating the possible drug and/or metabolite(s) accumulation in the body
9. Determining the influence of altered physiology/disease state on drug ADME
10. Explaining drug interactions

Caution must however be exercised in ensuring that the model fits the experimental data; otherwise, a new, more complex and suitable model may be proposed and tested.

The three different approaches to pharmacokinetic analysis of experimental data are:

1. Compartment modeling
2. Noncompartmental analysis
3. Physiologic modeling

Compartment Models

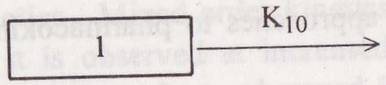
Compartmental analysis is the traditional and most commonly used approach to pharmacokinetic characterization of a drug. Here, the body is considered as composed of several compartments that communicate reversibly with each other. If one considers every organ, tissue or body fluid that can get equilibrated with the drug as a compartment, infinite number of compartments can exist in the body and mathematical description of such a model will be too complex. Hence, tissues which are approximately similar in their drug distribution characteristics are pooled to form a kinetically homogeneous hypothetical compartment. Such a compartment, thus, is not a real physiologic or anatomic region but a fictive or virtual one, considered as a reflection of the biosystem and assumed to comprise of tissues having similar blood flow and drug affinity. The kinetics of most drugs can be described by a hypothetical model consisting of one, two or at the most, three functional compartments arranged either in series or parallel to each other. It is also assumed that the rate of drug movement between compartments (i.e. entry and exit) follow first-order kinetics.

Depending upon whether the compartments are arranged parallel or in a series, compartment models are divided into two categories—mammillary model and caternary model.

Mammillary Model

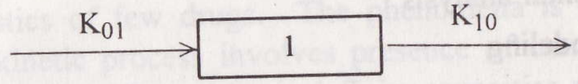
This model is the most common compartment model used in pharmacokinetics. It consists of one or more peripheral compartments connected to the central compartment in a manner similar to connection of satellites to a planet (i.e. they are joined parallel to the central compartment). The **central compartment** (or **compartment 1**) *comprises of plasma and highly perfused tissues* such as lungs, liver, kidneys, etc. which rapidly equilibrate with the drug. The drug is directly absorbed into this compartment (i.e. blood). Elimination too occurs from this compartment since the chief organs involved in drug elimination are liver and kidneys, the highly perfused tissues and therefore presumed to be rapidly accessible to drug in the systemic circulation. The **peripheral compartments** or **tissue compartments** (denoted by numbers 2, 3, etc.) *are those with low vascularity*

Model 1



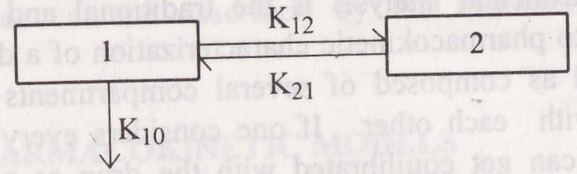
One-compartment open model, intravenous administration

Model 2



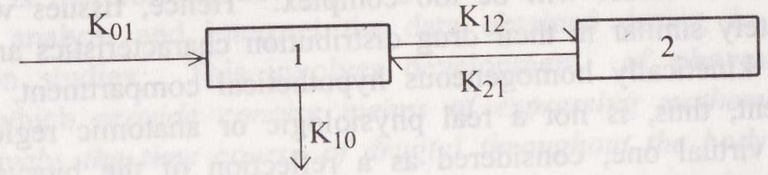
One-compartment open model, extravascular administration (oral, rectal, etc.)

Model 3



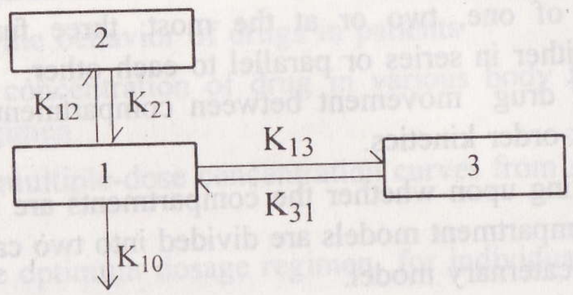
Two-compartment open model, intravenous administration

Model 4



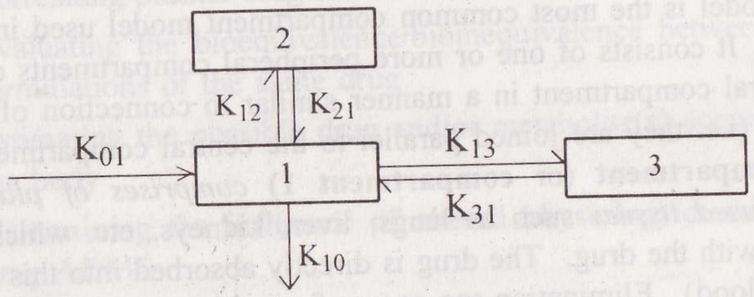
Two-compartment open model, extravascular administration

Model 5



Three-compartment open model, intravenous administration

Model 6



Three-compartment open model, extravascular administration

Fig. 9.5 Various mammillary compartment models. The rate constant K_{01} is basically K_a, the first-order absorption rate constant and K_{10} is K_E, the first-order elimination rate constant.

and poor perfusion. Distribution of drugs to these compartments is through blood. Movement of drug between compartments is defined by characteristic first-order rate constants denoted by letter K (*see* Fig. 9.5). The subscript indicates the direction of drug movement; thus, K_{12} (K-one-two) refers to drug movement from compartment 1 to compartment 2 and reverse for K_{21}.

The number of rate constants which will appear in a particular compartment model is given by R.

For intravenous administration, $R = 2n - 1$ (9.17)

For extravascular administration, $R = 2n$ (9.18)

where n = number of compartments.

Caternary Model

In this model, the compartments are joined to one another in a series like compartments of a train (Fig. 9.6). This is however not observable physiologically/anatomically as the various organs are directly linked to the blood compartment. Hence this model is rarely used.

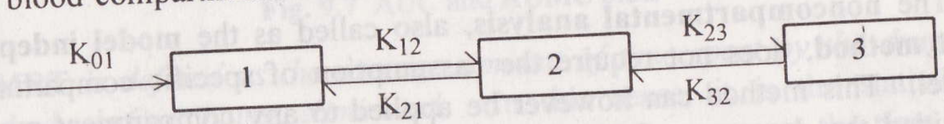


Fig. 9.6 A caternary model

The compartment modeling approach has several **advantages**—

1. It gives a visual representation of various rate processes involved in drug disposition.
2. It shows how many rate constants are necessary to describe these processes.
3. It enables the pharmacokineticist to write differential equations for each of the rate processes in order to describe drug-concentration changes in each compartment.
4. It is useful in predicting drug concentration-time profile in both normal physiologic and in pathologic conditions.
5. It is important in the development of dosage regimens.

Disadvantages of compartment modeling include—

1. The compartments and parameters bear no relationship with the physiologic functions or the anatomic structure of the species; several assumptions have to be made to facilitate data interpretation.

2. Extensive efforts are required in the development of an exact model that predicts and describes correctly the ADME of a certain drug.
3. The model is based on curve fitting of plasma concentration with complex multiexponential mathematical equations.
4. The model may vary within a study population.
5. The approach can be applied only to a specific drug under study.
6. The drug behavior within the body may fit different compartmental models depending upon the route of administration.
7. Difficulties generally arise when using models to interpret the differences between results from human and animal experiments.

Noncompartmental Analysis

Because of the several drawbacks of and difficulties with the classical compartment modeling, newer approaches have been devised to study the time course of drugs in the body. They are—physiologic models and noncompartmental methods.

The **noncompartmental analysis**, also called as the **model independent method**, does not require the assumption of specific compartment model. This method can however be applied to any compartment model provided the drugs or metabolites follow linear kinetics. *The approach, based on the* **statistical moments theory**, involves collection of experimental data following a single dose of drug. If one considers the time course of drug concentration in plasma as a statistical distribution curve, then:

$$MRT = \frac{AUMC}{AUC} \qquad (9.19)$$

where MRT = mean residence time

AUMC = area under the *first-moment curve*

AUC = area under the *zero-moment curve*

AUMC is obtained from a plot of product of plasma drug concentration and time (i.e. C.t) versus time t from zero to infinity (Fig. 9.7). Mathematically, it is expressed by equation:

$$AUMC = \int_0^\infty C\,t\,dt \qquad (9.20)$$

AUC is obtained from a plot of plasma drug concentration versus time from zero to infinity. Mathematically, it is expressed by equation:

$$AUC = \int_0^\infty C\,dt \qquad (9.21)$$

Practically, the AUMC and AUC can be calculated from the respective graphs by the **trapezoidal rule** (the method involves dividing the curve by a series of vertical lines into a number of trapezoids, calculating separately the area of each trapezoid and adding them together).

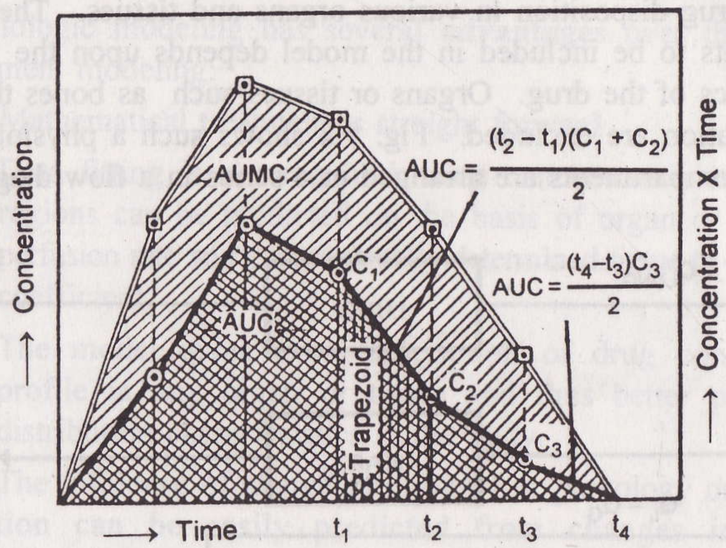


Fig. 9.7 AUC and AUMC plots

MRT *is defined as the average amount of time spent by the drug in the body before being eliminated.* In this sense, it is the statistical moment analogy of half-life, $t_{1/2}$. In effect, MRT represents the time for 63.2% of the intravenous bolus dose to be eliminated. The values will always be greater when the drug is administered in a fashion other than i.v. bolus.

Noncompartmental technique is widely used to estimate the important pharmacokinetic parameters like bioavailability, clearance and apparent volume of distribution. The method is also useful in determining half-life, rate of absorption and first-order absorption rate constant of the drug.

Advantages of noncompartmental method include—

1. Ease of derivation of pharmacokinetic parameters by simple algebraic equations.
2. The same mathematical treatment can be applied to almost any drug or metabolite provided they follow first-order kinetics.
3. A detailed description of drug disposition characteristics is not required.

The **disadvantage** of this method is that it provides limited information regarding the plasma drug concentration-time profile; more often, it deals with averages.

Physiologic Models

These models are also termed as **blood flow rate-limited models** and **perfusion rate-limited models**. The model is drawn on the basis of known anatomic and physiologic data and thus presents a more realistic picture of drug disposition in various organs and tissues. The number of compartments to be included in the model depends upon the disposition characteristics of the drug. Organs or tissues such as bones that have no drug penetration are excluded. Fig. 9.8 shows such a physiologic model where the compartments are arranged in a series in a flow diagram.

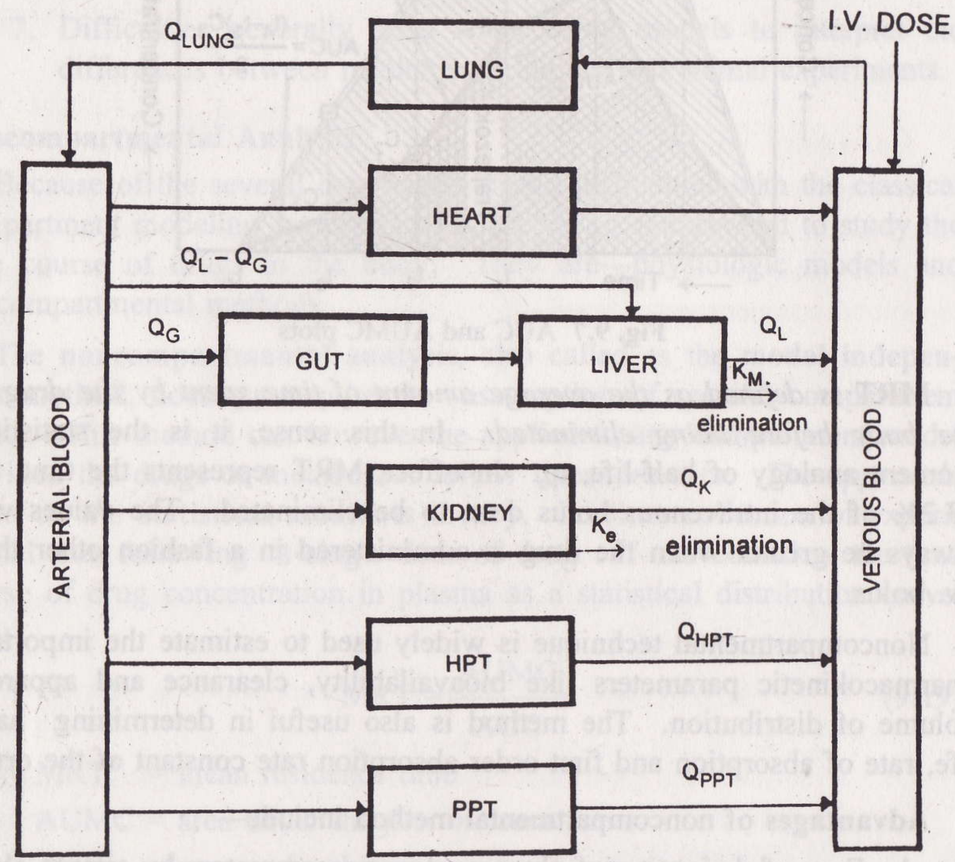


Fig. 9.8 Schematic representation of a physiologic pharmacokinetic model. The term Q indicates blood flow rate to a body region. HPT stands for other highly perfused tissues and PPT for poorly perfused tissues. K_m is rate constant for hepatic elimination and K_e is first-order rate constant for urinary excretion.

Most physiologic models are based on the assumption that the drug movement within a body region is much more rapid than its rate of delivery to that region by the perfusing blood and therefore, the model is said to be perfusion rate-limited. The assumption is however applicable

only to the highly membrane permeable drugs i.e. low molecular weight, poorly ionized and highly lipophilic drugs. *For highly polar, ionized and charged drugs, the model is referred to as* **membrane permeation rate-limited**.

Physiologic modeling has several **advantages** over the conventional compartment modeling:

1. Mathematical treatment is straight forward.
2. Data fitting is not required; drug concentration in various body regions can be predicted on the basis of organ or tissue volume, perfusion rate and experimentally determined tissue-to-plasma partition coefficient.
3. The model gives exact description of drug concentration-time profile in any organ or tissue and thus better picture of drug distribution characteristics in the body.
4. The influence of altered physiology or pathology on drug disposition can be easily predicted from changes in the various pharmacokinetic parameters since the parameters correspond to actual physiologic and anatomic measures.
5. Correlation of data in several animal species is possible and with some drugs, can be extrapolated to humans.

The only **disadvantage** of these comprehensive models is obtaining experimental data which is very exhaustive.

QUESTIONS

1. In addition to mechanisms of drug ADME, explain how the knowledge about the kinetics of these processes is also important.
2. Define pharmacokinetics. Name and define the three pharmacokinetic parameters that describe a typical plasma level-time curve.
3. On what parameters/variables are C_{max}, t_{max} and AUC dependent? What is the importance of these parameters in expressing pharmacodynamic behavior of a drug?
4. What are the various pharmacodynamic parameters?
5. Quote examples of zero-order rate processes.
6. In contrast to zero-order process, the half-life of a first-order process is considered to be an important pharmacokinetic parameter. Why?
7. Why are first-order processes said to follow linear kinetics?
8. What are mixed-order processes? By what other names such processes are identified? Quote examples of such processes.

9. What are pharmacokinetic models? What is the importance and utility of developing such models?
10. Name the different approaches to pharmacokinetic analysis of experimental data.
11. What assumptions are made in the development of compartment models? Discuss the advantages and disadvantages of such an approach.
12. In compartment modeling, elimination is presumed to occur from central compartment only. Why?
13. In comparison to a mammillary model, the caternary model is less useful. Explain.
14. On what theory is the noncompartmental analysis of pharmacokinetic data based? Discuss the merits and demerits of such an approach.
15. What are the advantages of physiologic models over compartment models? On what assumptions are such models based?
16. The half-life for first-order photolysis of cefotaxime solution containing 150 mg drug is 50 minutes.
    a. How long will it take for the drug to decompose to 20% of its original amount?

       Answer : 116 minutes.

    b. If one mL aliquot taken after 90 min of exposure to light was found to contain 0.43 mg of cefotaxime, what was the original volume of the solution ?

       Answer : 100 ml.
17. The following data for decomposition of two drugs, A and B are given in the table below :

Time (hours)	*Drug A (mg)*	*Drug B (mg)*
0.5	379	181.2
1.0	358	164.0
1.5	337	148.6
2.0	316	134.6
3.0	274	110.4
4.0	232	90.6
6.0	148	61.0
8.0	64	41.0

    a. Determine (by plotting or otherwise) the rate of decomposition of both drugs.

       Answer : Drug A = zero-order, and Drug B = first-order.

    b. What is the rate constant for decomposition?

       Answer : Drug A = 42 mg/hour, and Drug B = 0.198/hour.

    c. What is their half-life?

       Answer : Drug A = 4.76 hours, and Drug B = 3.5 hours.

    d. What were the original amounts of drug before decomposition?

       Answer : Drug A = 400 mg, and Drug B = 200 mg.

    e. If the original quantities of drug taken were 800 mg for A and 400 mg for B then what will be their new half-lives?

       Answer : Drug A = 9.52 hours, and Drug B = $t_{1/2}$ will remain unchanged i.e. 3.5 hours.

    f. Write equations for the line that best fits the experimental data for both drugs.

       Answer : Drug A : C = 400 – 42t, and

       Drug B : log C = log 200 – 0.198t/2.303.

10

Compartment Modeling

The time course of drug concentration determined after its administration can be satisfactorily explained by assuming the body as a single, well mixed compartment with first-order disposition processes. In case of other drugs, two or more body compartments may be postulated to describe mathematically the data collected.

ONE-COMPARTMENT OPEN MODEL
(Instantaneous Distribution Model)

The one-compartment open model is the simplest model which depicts the body as a single, kinetically homogeneous unit that has no barriers to the movement of drug and final distribution equilibrium between the drug in plasma and other body fluids is attained instantaneously and maintained at all times. This model thus applies only to those drugs that distribute rapidly throughout the body. The anatomical **reference compartment** is the plasma and concentration of drug in plasma is representative of drug concentration in all body tissues i.e. any change in plasma drug concentration reflects a proportional change in drug concentration throughout the body. However, the model does not assume that the drug concentration in plasma is equal to that in other body tissues. *The term* **open** *indicates that the input (availability) and output (elimination) are unidirectional and that the drug can be eliminated from the body.* Fig. 10.1 shows such a one-compartment model.

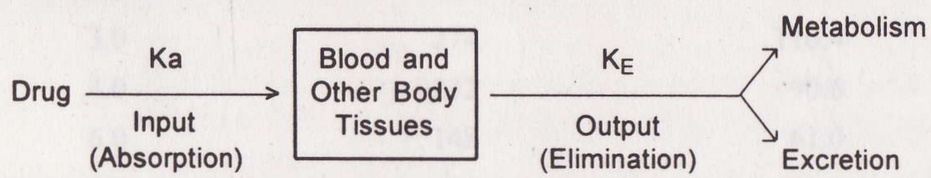


Fig. 10.1 Representation of one-compartment open model showing input and output processes.

One-compartment open model is generally used to describe plasma levels following administration of a single dose of a drug. Depending

upon the rate of input, several one-compartment open models can be defined:

One-compartment open model, intravenous bolus administration

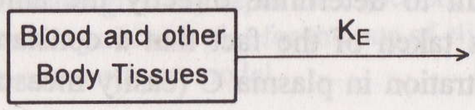
One-compartment open model, continuous intravenous infusion

One-compartment open model, extravascular administration, zero-order absorption, and

One-compartment open model, extravascular administration, first-order absorption.

One-Compartment Open Model—Intravenous Bolus Administration

When a drug that distributes rapidly in the body is given in the form of a rapid intravenous injection (i.e. i.v. bolus or slug), it takes about one to three minutes for complete circulation and therefore the rate of absorption is neglected in calculations. The model can be depicted as follows:



The general expression for **rate of drug presentation** to the body is:

$$\frac{dX}{dt} = \text{Rate in (availability)} - \text{Rate out (elimination)} \qquad (10.1)$$

Since **rate in** or absorption is absent, the equation becomes:

$$\frac{dX}{dt} = -\text{ Rate out} \qquad (10.2)$$

If the **rate out** or elimination follows first-order kinetics, then:

$$\frac{dX}{dt} = -K_E X \qquad (10.3)$$

where, K_E = first-order elimination rate constant, and
X = amount of drug in the body at any time t remaining to be eliminated.

Negative sign indicates that the drug is being lost from the body. The various related pharmacokinetic parameters can now be estimated.

Elimination Rate Constant : For a drug that follows one-compartment kinetics and administered as rapid i.v. injection, the decline in plasma drug concentration is only due to elimination of drug from the body (and not due to distribution), the phase being called as elimination phase. **Elimination phase** can be characterized by 3 parameters—elimination rate constant, elimination half-life and clearance.

Integration of equation 10.3 yields:

$$\ln X = \ln X_o - K_E\, t \qquad (10.4)$$

where, X_o = amount of drug at time t = zero i.e. the initial amount of drug injected.

Equation 10.4 can also be written in the exponential form as:

$$X = X_o\, e^{-K_E t} \qquad (10.5)$$

The above equation shows that *disposition of a drug that follows one-compartment kinetics is* **monoexponential.**

Transforming equation 10.4 into common logarithms (log base 10), we get:

$$\log X = \log X_o - \frac{K_E\, t}{2.303} \qquad (10.6)$$

Since it is difficult to determine directly the amount of drug in the body X, advantage is taken of the fact that a constant relationship exists between drug concentration in plasma C (easily measurable) and X; thus:

$$X = V_d\, C \qquad (10.7)$$

where, V_d = proportionality constant popularly known as the *apparent volume of distribution.* It is a pharmacokinetic parameter that permits the use of plasma drug concentration in place of amount of drug in the body. The equation 10.6 therefore becomes:

$$\log C = \log C_o - \frac{K_E\, t}{2.303} \qquad (10.8)$$

where, C_o = plasma drug concentration immediately after i.v. injection.

Equation 10.8 is that of a straight line and indicates that a semilogarithmic plot of log C versus t will be linear with *Y*-intercept log C_o. The elimination rate constant is directly obtained from the slope of the line (Fig. 10.2*b*). It has units of min^{-1}. Thus, a linear plot is easier to handle mathematically than a curve which in this case will be obtained from a plot of C versus t on regular (Cartesian) graph paper (Fig. 10.2*a*).

Thus, C_o, K_E (and $t_{½}$) can be readily obtained from log C versus t graph. The elimination or removal of the drug from the body is the sum of urinary excretion, metabolism, biliary excretion, pulmonary excretion, and other mechanisms involved therein. Thus, K_E is an additive property of rate constants for each of these processes and better called as **overall elimination rate constant.**

$$K_E = K_e + K_m + K_b + K_l + \ldots\ldots \qquad (10.9)$$

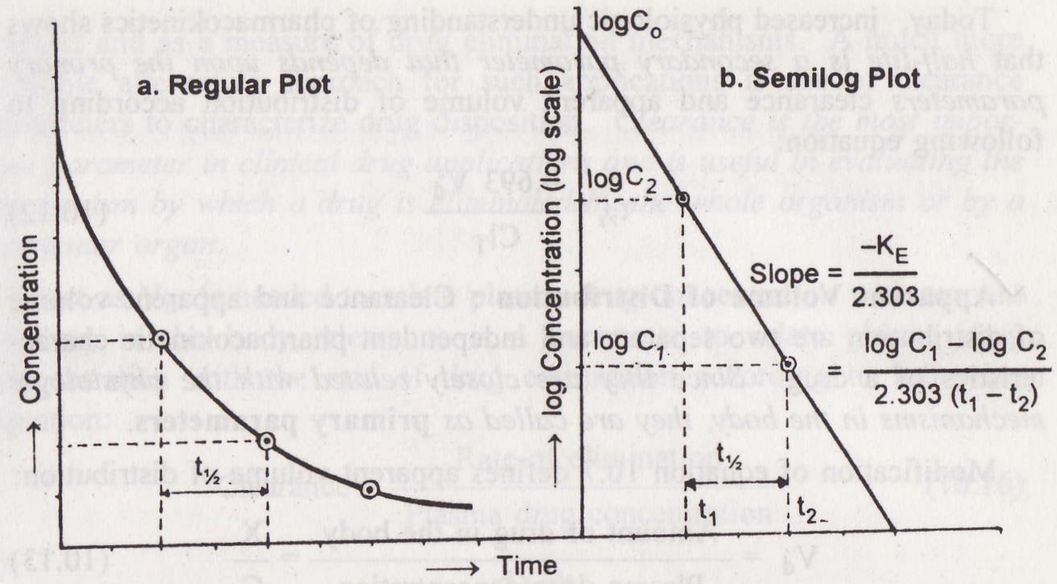


Fig. 10.2 (*a*) Cartesian plot of a drug that follows one-compartment kinetics and given by rapid i.v. injection, and (*b*) Semilogarithmic plot for the rate of elimination in a one-compartment model.

The fraction of drug eliminated by a particular route can be evaluated if the number of rate constants involved and their values are known. For example, if a drug is eliminated by urinary excretion and metabolism only, then, the fraction of drug excreted unchanged in urine F_e and fraction of drug metabolized F_m can be given as:

$$F_e = \frac{K_e}{K_E} \qquad (10.10a)$$

$$F_m = \frac{K_m}{K_E} \qquad (10.10b)$$

Elimination Half-Life : Also called as **biological half-life**, it is the oldest and the best known of all pharmacokinetic paramters and was once considered as the most important characteristic of a drug. *It is defined as the time taken for the amount of drug in the body as well as plasma concentration to decline by one-half or 50% its initial value.* It is expressed in hours or minutes. Half-life is related to elimination rate constant by the following equation:

$$t_{½} = \frac{0.693}{K_E} \qquad (10.11)$$

Elimination half-life can be readily obtained from the graph of log C versus t as shown in Fig 10.2.

Today, increased physiologic understanding of pharmacokinetics shows that *half-life is a secondary parameter that depends upon the primary parameters* clearance and apparent volume of distribution according to following equation:

$$t_{1/2} = \frac{0.693\ V_d}{Cl_T} \qquad (10.12)$$

Apparent Volume of Distribution : Clearance and apparent volume of distribution are two separate and independent pharmacokinetic characteristics of a drug. *Since they are closely related with the physiologic mechanisms in the body, they are called as* **primary parameters**.

Modification of equation 10.7 defines apparent volume of distribution:

$$V_d = \frac{\text{Amount of drug in the body}}{\text{Plasma drug concentration}} = \frac{X}{C} \qquad (10.13)$$

V_d is a measure of the extent of distribution of drug and is expressed in liters. The best and the simplest way of estimating V_d of a drug is administering it by rapid i.v. injection and using the following equation:

$$V_d = \frac{X_o}{C_o} = \frac{\text{i.v. bolus dose}}{C_o} \qquad (10.14)$$

Equation 10.14 can only be used for drugs that obey one-compartment kinetics. This is because the V_d can only be estimated when distribution equilibrium is achieved between drug in plasma and that in tissues and such an equilibrium is established instantaneously for a drug that follows one-compartment kinetics. A more general, more useful noncompartmental method that can be applied to many compartment models for estimating the V_d is:

For drugs given as i.v. bolus,

$$V_{d(area)} = \frac{X_o}{K_E.AUC} \qquad (10.15a)$$

For drugs administered extravascularly (e.v.),

$$V_{d(area)} = \frac{F\ X_o}{K_E\ AUC} \qquad (10.15b)$$

where, X_o = dose administered, and F = fraction of drug absorbed into the systemic circulation. F is equal to *one* i.e. complete availability when the drug is administered intravenously.

Clearance : Difficulties arise when one applies elimination rate constant and half-life as pharmacokinetic parameters in an anatomical/physiological

context and as a measure of drug elimination mechanisms. A much more valuable alternative approach for such applications is use of clearance parameters to characterize drug disposition. *Clearance is the most important parameter in clinical drug applications and is useful in evaluating the mechanism by which a drug is eliminated by the whole organism or by a particular organ.*

Just as V_d is needed to relate plasma drug concentration with amount of drug in the body, clearance is a parameter to relate plasma drug concentration with the rate of drug elimination according to following equation:

$$\text{Clearance} = \frac{\text{Rate of elimination}}{\text{Plasma drug concentration}} \qquad (10.16)$$

or

$$Cl = \frac{dX/dt}{C} \qquad (10.17)$$

Clearance *is defined as the theoretical volume of body fluid containing drug* (i.e. that fraction of apparent volume of distribution) *from which the drug is completely removed in a given period of time.* It is expressed in ml/min or liters/hour. Clearance is usually further defined as **blood clearance** (Cl_b), **plasma clearance** (Cl_p) or clearance based on unbound or free drug concentration (Cl_u) depending upon the concentration C measured for the right side of the equation 10.17.

Total Body Clearance : Elimination of a drug from the body involves processes occurring in kidney, liver, lungs and other eliminating organs. *Clearance at an individual organ level is called as* **organ clearance**. It can be estimated by dividing the rate of elimination by each organ with the concentration of drug presented to it. Thus,

Renal Clearance

$$Cl_R = \frac{\text{Rate of elimination by kidney}}{C} \qquad (10.18a)$$

Hepatic Clearance

$$Cl_H = \frac{\text{Rate of elimination by liver}}{C} \qquad (10.18b)$$

Other Organ Clearance

$$Cl_{others} = \frac{\text{Rate of elimination by other organs}}{C} \qquad (10.18c)$$

The **total body clearance**, Cl_T, *also called as* **total systemic clearance**, *is an additive property of individual organ clearances.* Hence,

Total Systemic Clearance, $Cl_T = Cl_R + Cl_H + Cl_{others}$ (10.18*d*.)

Because of the additivity of clearance, the relative contribution by any organ in eliminating a drug can be easily calculated. *Clearance by all organs other than kidney is sometimes known as* **nonrenal clearance** Cl_{NR}. It is the difference between total clearance and renal clearance.

According to an earlier definition (equation 10.17),

$$Cl_T = \frac{dX/dt}{C} \qquad (10.17)$$

Substituting $dX/dt = K_E X$ from equation 10.3 in above equation, we get:

$$Cl_T = \frac{K_E X}{C} \qquad (10.19)$$

Since $X/C = V_d$ (from equation 10.13), the equation 10.19 can be written as:

$$Cl_T = K_E V_d \qquad (10.20a)$$

Parallel equations can be written for renal and hepatic clearances as:

$$Cl_R = K_e V_d \qquad (10.20b)$$

$$Cl_H = K_m V_d \qquad (10.20c)$$

Since $K_E = 0.693/t_{½}$ (from equation 10.11), clearance can be related to half-life by the following equation:

$$Cl_T = \frac{0.693\ V_d}{t_{½}} \qquad (10.21)$$

Identical equations can be written for Cl_R and Cl_H in which cases the $t_{½}$ will be urinary excretion half-life for unchanged drug and metabolism half-life respectively. Equation 10.21. shows that as Cl_T decreases, as in renal insufficiency, $t_{½}$ of the drug increases. As the Cl_T takes into account V_d, changes in V_d as in obesity or edematous condition will reflect changes in Cl_T.

The noncompartmental method of computing total clearance for a drug that follows one-compartment kinetics is:

For drugs given as i.v. bolus,

$$Cl_T = \frac{X_o}{AUC} \qquad (10.22a)$$

For drugs administered e.v.,

$$Cl_T = \frac{F\ X_o}{AUC} \qquad (10.22b)$$

For a drug given by i.v. bolus, the renal clearance Cl_R may be estimated by determining the total amount of unchanged drug excreted in urine, X_u^∞ and AUC.

$$Cl_R = \frac{X_u^\infty}{AUC} \qquad (10.23)$$

Organ Clearance : The best way of understanding clearance is at individual organ level. Such a physiologic approach is advantageous in predicting and evaluating the influence of pathology, blood flow, P-D binding, enzyme activity, etc. on drug elimination. At an organ level, the rate of elimination can be written as:

Rate of elimination by an organ = Rate of presentation to the organ – Rate of exit from the organ (10.24)

Rate of presentation (input) = Organ blood flow x Entering concentration = $Q.C_{in}$ (10.25)

Rate of exit (output) = Organ blood flow x Exiting concentration = $Q.C_{out}$ (10.26)

Substitution of equations 10.25 and 10.26 in equation 10.24 yields:

Rate of elimination (also called as **rate of extraction**) = $Q.C_{in} - Q.C_{out}$ = $Q\ (C_{in} - C_{out})$ (10.27)

Division of above equation by concentration of drug that enters the organ of elimination C_{in} yields an expression for clearance of drug by the organ under consideration. Thus:

$$\frac{\text{Rate of extraction}}{C_{in}} = Cl_{organ} = \frac{Q\ (C_{in} - C_{out})}{C_{in}} = Q.ER \qquad (10.28)$$

where, $ER = (C_{in} - C_{out})/C_{in}$ is called as **extraction ratio**. It has no units and its value ranges from zero (no elimination) to one (complete elimination). Based on ER values, drugs can be classified into 3 groups:

—drugs with **high ER** (above 0.7),
—drugs with **intermediate ER** (between 0.7 to 0.3), and
—drugs with **low ER** (below 0.3).

ER *is an index of how efficiently the eliminating organ clears the blood flowing through it of drug.* For example, an ER of 0.6 tells that 60% of the blood flowing through the organ will be completely cleared of drug. The fraction of drug that *escapes removal* by the organ is expressed as:

$$F = 1 - ER \qquad (10.29)$$

where, **F = systemic availability** when the eliminating organ is liver.

Hepatic Clearance : For certain drugs, the nonrenal clearance can be assumed as equal to hepatic clearance Cl_H. It is given as:

$$Cl_H = Cl_T - Cl_R \qquad (10.30)$$

An equation parallel to equation 10.28 can also be written for hepatic clearance:

$$Cl_H = Q_H.ER_H \qquad (10.31)$$

where, Q_H = hepatic blood flow (about 1.5 liters/min), and
ER_H = hepatic extraction ratio.

The hepatic clearance of drugs can be divided into two groups:
—drugs with hepatic blood flow rate-limited clearance, and
—drugs with intrinsic capacity-limited clearance.

1. Hepatic Blood Flow : When ER_H is one, Cl_H approaches its maximum value i.e. hepatic blood flow. In such a situation, hepatic clearance is said to be **perfusion rate-limited** or **flow-dependent**. Alteration in hepatic blood flow significantly affects the elimination of drugs with high ER_H e.g. propranolol, lidocaine, etc. Such drugs are removed from the blood as rapidly as they are presented to the liver (high first-pass hepatic metabolism). Indocyanine green is so rapidly eliminated by the human liver that its clearance is often used as an indicator of hepatic blood flow rate. First-pass hepatic extraction is suspected when there is lack of unchanged drug in systemic circulation after oral administration. **Maximum oral availability F** for such drugs can be computed from equation 10.29. An extension of the same equation is the noncompartmental method of estimating F:

$$F = 1 - ER_H = \frac{AUC_{oral}}{AUC_{i.v.}} \qquad (10.32)$$

TABLE 10.1

Influence of Blood Flow Rate and Protein Binding on Total Clearance of Drugs with High and with Low ER Values.

Drugs with	*Changes in Total Clearance due to*			
	↑ Blood Flow	*↓ Blood Flow*	*↑ Binding*	*↓ Binding*
High ER (above 0.7)	↑	↓	No Change	No Change
Low ER (below 0.3)	No Change	No Change	↓	↑

where, ↑ = increase, and ↓ = decrease.

On the contrary, hepatic blood flow has very little or no effect on drugs with low ER_H e.g. theophylline. For such drugs, whatever concentration of drug present in the blood perfuses liver, is more than what the liver can eliminate (low first-pass hepatic metabolism). Similar discussion can be extended to the influence of blood flow on renal clearance of drugs. This is illustrated in Table 10.1. Hepatic clearance of a drug with high ER is independent of protein binding.

2. Intrinsic Capacity Clearance : *Denoted as* Cl_{int}, *it is defined as the inherent ability of an organ to irreversibly remove a drug in the absence of any flow limitation.* It depends, in this case, upon the hepatic enzyme activity. Drugs with low ER_H and with elimination primarily by metabolism are greatly affected by changes in enzyme activity. Hepatic clearance of such drugs is said to be **capacity-limited**, e.g. theophylline. The $t_{1/2}$ of such drugs show great intersubject variability. Hepatic clearance of drugs with low ER is independent of blood flow rate but sensitive to changes in protein binding.

The hepatic and renal extraction ratios of some drugs and metabolites are given in Table 10.2.

TABLE 10.2

Hepatic and Renal Extraction Ratio of Some Drugs and Metabolites

	Extraction Ratio		
	High	*Intermediate*	*Low*
Hepatic Extraction	Propranolol Lidocaine Nitroglycerine Morphine Isoprenaline	Aspirin Codeine Nortriptyline Quinidine	Diazepam Phenobarbital Phenytoin Procainamide Theophylline
Renal Extraction	Some Penicillins Hippuric acid Several Sulfates Several Glucuronides	Some Penicillins Procainamide Cimetidine	Digoxin Furosemide Atenolol Tetracycline

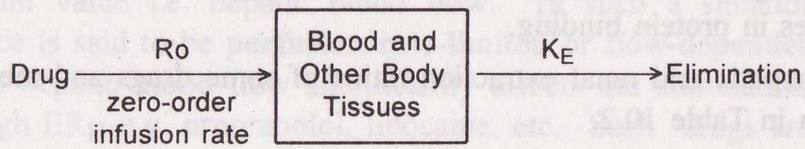
One-Compartment Open Model —Intravenous Infusion

Rapid i.v. injection is unsuitable when the drug has potential to precipitate toxicity or when maintenance of a stable concentration or amount

of drug in the body is desired. In such a situation, the drug (for example, several antibiotics, theophylline, procainamide, etc.) is administered at a constant rate (zero-order) by i.v. infusion. In contrast to the short duration of infusion of an i.v. bolus (few seconds), the duration of constant rate infusion is usually much longer than the half-life of the drug. *Advantages* of such a zero-order infusion of drugs include—

1. Ease of control of rate of infusion to fit individual patient needs.
2. Prevents fluctuating maxima and minima (peak and valley) plasma level, desired especially when the drug has a narrow therapeutic index.
3. Other drugs, electrolytes and nutrients can be conveniently administered simultaneously by the same infusion line in critically ill patients.

The model can be represented as follows:



At any time during infusion, the rate of change in the amount of drug in the body, dX/dt is the difference between the zero-order rate of drug infusion R_o and first-order rate of elimination, $-K_EX$:

$$\frac{dX}{dt} = R_o - K_EX \qquad (10.33)$$

Integration and rearrangement of above equation yields:

$$X = \frac{R_o}{K_E}(1 - e^{-K_Et}) \qquad (10.34)$$

Since $X = V_d\,C$, the equation 10.34 can be transformed into concentration terms as follows:

$$C = \frac{R_o}{K_EV_d}(1 - e^{-K_Et}) = \frac{R_o}{Cl_T}(1 - e^{-K_Et}) \qquad (10.35)$$

At the start of constant rate infusion, the amount of drug in the body is zero and hence, there is no elimination. As time passes, the amount of drug in the body rises gradually (elimination rate less than the rate of infusion) until a point after which the rate of elimination equals the rate of infusion i.e. the concentration of drug in plasma approaches a constant value called as **steady-state**, **plateau** or **infusion equilibrium** (Fig. 10.3.).

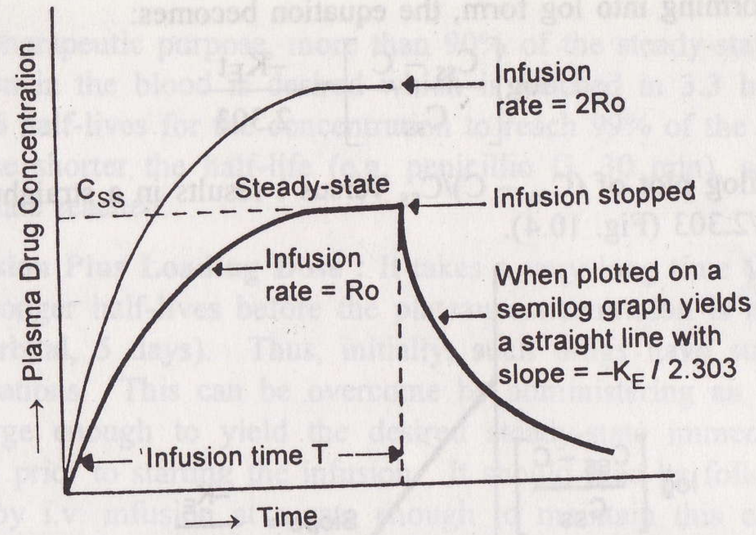


Fig. 10.3 Plasma concentration-time profile for a drug given by constant rate i.v. infusion (the two curves indicate different infusion rates R_o and $2R_o$ for the same drug)

At steady-state, the rate of change of amount of drug in the body is zero, hence, the equation 10.33 becomes:

$$\text{Zero} = R_o - K_E\,X_{ss}$$

or

$$K_E\,X_{ss} = R_o \qquad (10.36)$$

Transforming to concentration terms and rearranging the equation:

$$C_{ss} = \frac{R_o}{K_EV_d} = \frac{R_o}{Cl_T} \text{ i.e. } \frac{\text{Infusion rate}}{\text{Clearance}} \qquad (10.37)$$

where, X_{ss} and C_{ss} are amount of drug in the body and concentration of drug in plasma at steady-state respectively. The value of K_E (and hence $t_{½}$) can be obtained from the slope of straight line obtained after a semilogarithmic plot (log C versus t) of the plasma concentration-time data gathered from the time when infusion is stopped (Fig. 10.3). Alternatively, K_E can be calculated from the data collected during infusion to steady-state as follows:

Substituting $R_o/Cl_T = C_{ss}$ from equation 10.37 in equation 10.35 we get:

$$C = C_{ss}(1 - e^{-K_Et}) \qquad (10.38)$$

Rearrangement yields:

$$\left[\frac{C_{ss} - C}{C_{ss}}\right] = e^{-K_Et} \qquad (10.39)$$

Transforming into log form, the equation becomes:

$$\log\left[\frac{C_{ss} - C}{C_{ss}}\right] = \frac{-K_E t}{2.303} \qquad (10.40)$$

A semilog plot of $(C_{ss} - C)/C_{ss}$ versus t results in a straight line with slope $-K_E/2.303$ (Fig. 10.4).

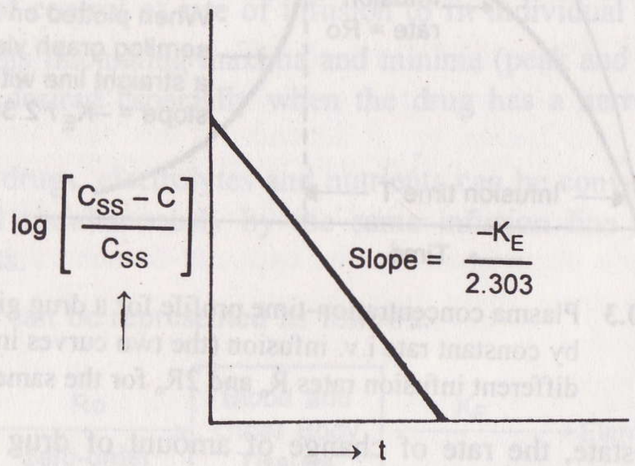


Fig. 10.4 Semilog plot to compute K_E from infusion data upto steady-state

The time to reach steady-state concentration is dependent upon the elimination half-life and not infusion rate. An increase in infusion rate will merely increase the plasma concentration attained at steady-state (Fig. 10.3). If n is the number of half-lives passed since the start of infusion ($t/t_{½}$), equation 10.38 can be written as:

$$C = C_{ss}\,[1 - (½)^n] \qquad (10.41)$$

The percent of C_{ss} achieved at the end of each $t_{½}$ is the sum of C_{ss} at previous $t_{½}$ and the concentration of drug remaining after a given $t_{½}$ (Table 10.3).

TABLE 10.3

Percent of C_{ss} attained at the end of a given $t_{½}$

Half-life	*% Remaining*	*% C_{ss} Achieved*
1	50	50
2	25	50 + 25 = 75
3	12.5	75 + 12.5 = 87.5
4	6.25	87.5 + 6.25 = 93.75
5	3.125	93.75 + 3.125 = 96.875
6	1.562	96.875 + 1.562 = 98.437
7	0.781	98.437 + 0.781 = 99.218

For therapeutic purpose, more than 90% of the steady-state drug concentration in the blood is desired which is reached in 3.3 half-lives. It takes 6.6 half-lives for the concentration to reach 99% of the steady-state. Thus, the shorter the half-life (e.g. penicillin G, 30 min), sooner is the steady-state reached.

Infusion Plus Loading Dose : It takes a very long time for the drugs having longer half-lives before the plateau concentration is reached (e.g. phenobarbital, 5 days). Thus, initially, such drugs have subtherapeutic concentrations. This can be overcome by administering an i.v. **loading dose** large enough to yield the desired steady-state immediately upon injection prior to starting the infusion. It should then be followed immediately by i.v. infusion at a rate enough to maintain this concentration (Fig. 10.5).

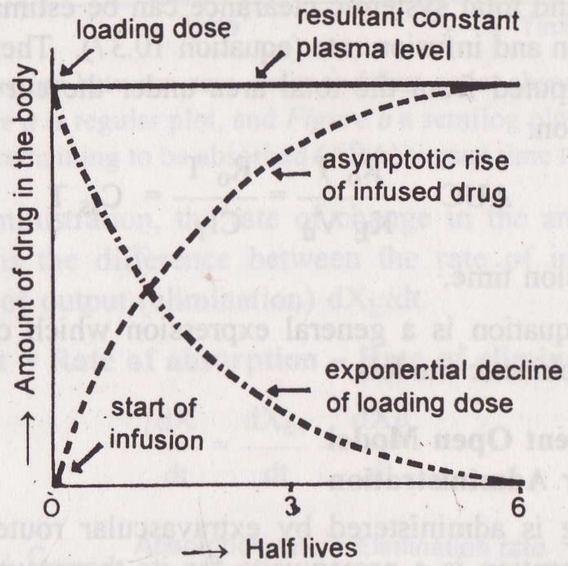


Fig. 10.5 Intravenous infusion with loading dose. As the amount of bolus dose remaining in the body falls, there is a complementary rise resulting from the infusion

Recalling once again the relationship $X = V_d C$, the equation for computing the loading dose $X_{o,L}$ can be given:

$$X_{o,L} = C_{ss} V_d \qquad (10.42)$$

Substitution of $C_{ss} = R_o/K_E V_d$ from equation 10.37 in above equation yields another expression for loading dose in terms of infusion rate:

$$X_{o,L} = \frac{R_o}{K_E} \qquad (10.43)$$

The equation describing the plasma concentration-time profile following simultaneous i.v. loading dose (i.v. bolus) and constant rate i.v. infusion

is the sum of two equations describing each process (i.e. modified equation 10.5 and 10.35):

$$C = \frac{X_{o,L}}{V_d} e^{-K_E t} + \frac{R_o}{K_E V_d}(1 - e^{-K_E t}) \qquad (10.44)$$

If we substitute $C_{ss}V_d$ for $X_{o,L}$ (from equation 10.42) and $C_{ss}K_E V_d$ for R_o (from equation 10.37) in above equation and simplify it, it reduces to $C = C_{ss}$ indicating that the concentration of drug in plasma remains constant (steady) throughout the infusion time.

Assessment of Pharmacokinetic Parameters

The first-order elimination rate constant and elimination half-life can be computed from a semilog plot of post-infusion concentration-time data. Equation 10.40 can also be used for the same purpose. Apparent volume of distribution and total systemic clearance can be estimated from steady-state concentration and infusion rate (equation 10.37). These two parameters can also be computed from the total area under the curve (Fig. 10.3) till the end of infusion:

$$AUC = \frac{R_o T}{K_E V_d} = \frac{R_o T}{Cl_T} = C_{ss} T \qquad (10.45)$$

where, T = infusion time.

The above equation is a general expression which can be applied to several pharmacokinetic models.

One-Compartment Open Model —Extravascular Administration

When a drug is administered by extravascular route (e.g. oral, i.m., rectal, etc.), absorption is a prerequisite for its therapeutic activity. Factors that influence drug absorption have already been discussed in *chapter 2*. The rate of absorption may be described mathematically as a zero-order or first-order process. A large number of plasma concentration-time profiles can be described by a one-compartment model with first-order absorption and elimination. However, under certain conditions, the absorption of some drugs may be better described by assuming zero-order (constant rate) kinetics. Differences between zero-order and first-order kinetics are illustrated in Fig. 10.6.

Zero-order absorption is characterized by a constant rate of absorption. It is independent of amount remaining to be absorbed (ARA), and its regular ARA versus t plot is linear with slope equal to rate of absorption while the semilog plot is described by an ever-increasing gradient with time. In contrast, the first-order absorption process is distinguished

by a decline in the rate with ARA i.e. absorption rate is dependent upon ARA; its regular plot is curvilinear and semilog plot a straight line with absorption rate constant as its slope.

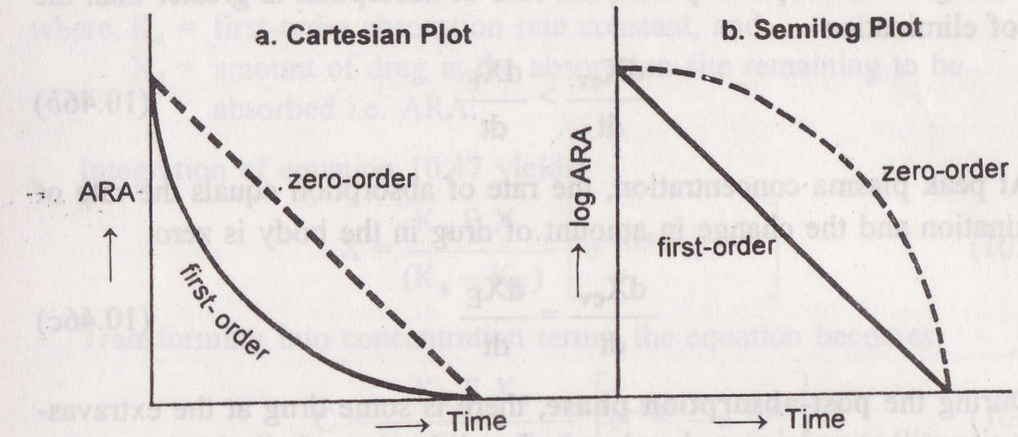


Fig. 10.6 Distinction between zero-order and first-order absorption processes. *Figure a* is regular plot, and *Figure b* a semilog plot of amount of drug remaining to be absorbed (ARA) versus time t.

After e.v. administration, the rate of change in the amount of drug in the body dX/dt is the difference between the rate of input (absorption) dX_{ev}/dt and rate of output (elimination) dX_E/dt.

dX/dt = Rate of absorption – Rate of elimination

$$\frac{dX}{dt} = \frac{dX_{ev}}{dt} - \frac{dX_E}{dt} \qquad (10.46a)$$

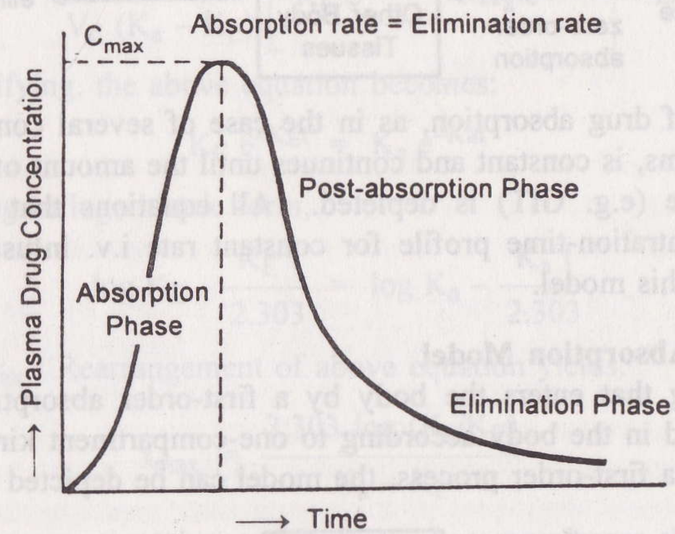


Fig. 10.7 The absorption and elimination phases of the plasma concentration-time profile obtained after extravascular administration of a single dose of a drug.

For a drug that follows one-compartment kinetics, the plasma concentration-time profile is characterized by absorption phase, post-absorption phase and elimination phase (Fig. 10.7.).

During the **absorption phase**, the rate of absorption is greater than the rate of elimination

$$\frac{dX_{ev}}{dt} > \frac{dX_E}{dt} \qquad (10.46b)$$

At peak plasma concentration, the rate of absorption equals the rate of elimination and the change in amount of drug in the body is zero.

$$\frac{dX_{ev}}{dt} = \frac{dX_E}{dt} \qquad (10.46c)$$

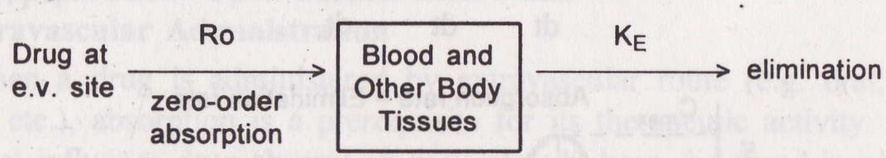
During the **post-absorption phase**, there is some drug at the extravascular site still remaining to be absorbed and the rate of elimination at this stage is greater than the absorption rate.

$$\frac{dX_{ev}}{dt} < \frac{dX_E}{dt} \qquad (10.46d)$$

After completion of drug absorption, its rate becomes zero and the plasma level time curve is characterized only by the **elimination phase**.

Zero-Order Absorption Model

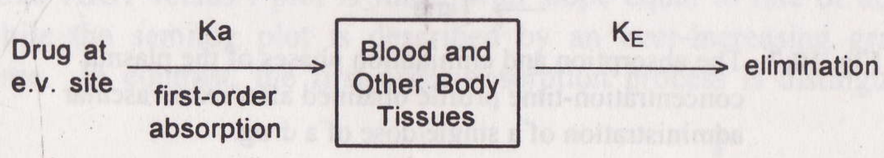
This model is similar to that for constant rate infusion.



The rate of drug absorption, as in the case of several controlled drug delivery systems, is constant and continues until the amount of drug at the absorption site (e.g. GIT) is depleted. All equations that explain the plasma concentration-time profile for constant rate i.v. infusion are also applicable to this model.

First-Order Absorption Model

For a drug that enters the body by a first-order absorption process, gets distributed in the body according to one-compartment kinetics and is eliminated by a first-order process, the model can be depicted as follows:



The differential form of the equation 10.46*a* is:

$$\frac{dX}{dt} = K_a X_a - K_E X \qquad (10.47)$$

where, K_a = first-order absorption rate constant, and
X_a = amount of drug at the absorption site remaining to be absorbed i.e. ARA.

Integration of equation 10.47 yields:

$$X = \frac{K_a F X_o}{(K_a - K_E)}\left[e^{-K_E t} - e^{-K_a t}\right] \qquad (10.48)$$

Transforming into concentration terms, the equation becomes:

$$C = \frac{K_a F X_o}{V_d (K_a - K_E)}\left[e^{-K_E t} - e^{-K_a t}\right] \qquad (10.49)$$

where F = fraction of drug absorbed systemically after e.v. administration. A typical plasma concentration-time profile of a drug administered e.v. is shown in Fig. 10.7.

Assessment of Pharmacokinetic Parameters

C_{max} and t_{max} : At peak plasma concentration, the rate of absorption equals rate of elimination i.e. $K_a X_a = K_E X$ and the rate of change in plasma drug concentration dC/dt = zero. This rate can be obtained by differentiating equation 10.49.

$$\frac{dC}{dt} = \frac{K_a F X_o}{V_d (K_a - K_E)}\left[-K_E e^{-K_E t} + K_a e^{-K_a t}\right] = \text{Zero} \qquad (10.50)$$

On simplifying, the above equation becomes:

$$K_E e^{-K_E t} = K_a e^{-K_a t} \qquad (10.51)$$

Converting to logarithmic form,

$$\log K_E - \frac{K_E t}{2.303} = \log K_a - \frac{K_a t}{2.303} \qquad (10.52)$$

where t is t_{max}. Rearrangement of above equation yields:

$$t_{max} = \frac{2.303 \log (K_a/K_E)}{K_a - K_E} \qquad (10.53)$$

The above equation shows that as K_a becomes larger than K_E, t_{max} becomes smaller since $(K_a - K_E)$ increases much faster than log K_a/K_E.

C_{max} can be obtained by substituting equation 10.53 in equation 10.49. However, a simpler expression for the same is:

$$C_{max} = \frac{F X_o}{V_d} e^{-K_E t_{max}} \quad (10.54)$$

It has been shown that at C_{max}, when $K_a = K_E$, $t_{max} = 1/K_E$. Hence, the above equation further reduces to:

$$C_{max} = \frac{F X_o}{V_d} e^{-1} = \frac{0.37\ F X_o}{V_d} \quad (10.55)$$

Since FX_o/V_d represents C_o following i.v. bolus, the maximum plasma concentration that can be attained after e.v. administration is just 37% of the maximum level attainable with i.v. bolus in the same dose. If bioavailability is less than 100%, still lower concentration will be attained.

Elimination Rate Constant : This parameter can be computed from the elimination phase of the plasma level time profile. For most drugs administered e.v., absorption rate is significantly greater than the elimination rate i.e. $K_a t >> K_E t$. Hence, one can say that $e^{-K_a t}$ approaches zero much faster than does $e^{-K_E t}$. At such a stage, when absorption is complete, the change in plasma concentration is dependent only on elimination rate and equation 10.49 reduces to:

$$C = \frac{K_a F X_o}{V_d (K_a - K_E)} e^{-K_E t} \quad (10.56)$$

Transforming into log form, the equation becomes:

$$\log C = \log \frac{K_a F X_o}{V_d (K_a - K_E)} - \frac{K_E t}{2.303} \quad (10.57)$$

A plot of log C versus t yields a straight line with slope $-K_E/2.303$ (half-life can then be computed from K_E). K_E can also be estimated from urinary excretion data (*see the section on urinary excretion data*).

Absorption Rate Constant : It can be calculated by the **method of residuals**. The technique is also known as **feathering, peeling** and **stripping**. It is commonly used in pharmacokinetics to resolve a multiexponential curve into its individual components. For a drug that follows one-compartment kinetics and administered e.v., the concentration of drug in plasma is expressed by a biexponential equation 10.49.

$$C = \frac{K_a F X_o}{V_d (K_a - K_E)} \left[e^{-K_E t} - e^{-K_a t} \right] \quad (10.49)$$

If $K_a F X_o / V_d (K_a - K_E) = A$, a hybrid constant, then:

$$C = A\ e^{-K_E t} - A\ e^{-K_a t} \quad (10.58)$$

During the elimination phase, when absorption is almost over, $K_a >> K_E$ and the value of second exponential $e^{-K_a t}$ approaches zero whereas the first exponential $e^{-K_E t}$ retains some finite value. At this time, the equation 10.58 reduces to:

$$\overleftarrow{C} = A\ e^{-K_E t} \quad (10.59)$$

In log form, the above equation is:

$$\log \overleftarrow{C} = \log A - \frac{K_E t}{2.303} \quad (10.60)$$

where $\overleftarrow{C}$ represents the back extrapolated plasma concentration values. A plot of log C versus t yields a biexponential curve with a terminal linear phase having slope $-K_E/2.303$ (Fig. 10.8). Back extrapolation of this straight line to time zero yields y-intercept equal to log A.

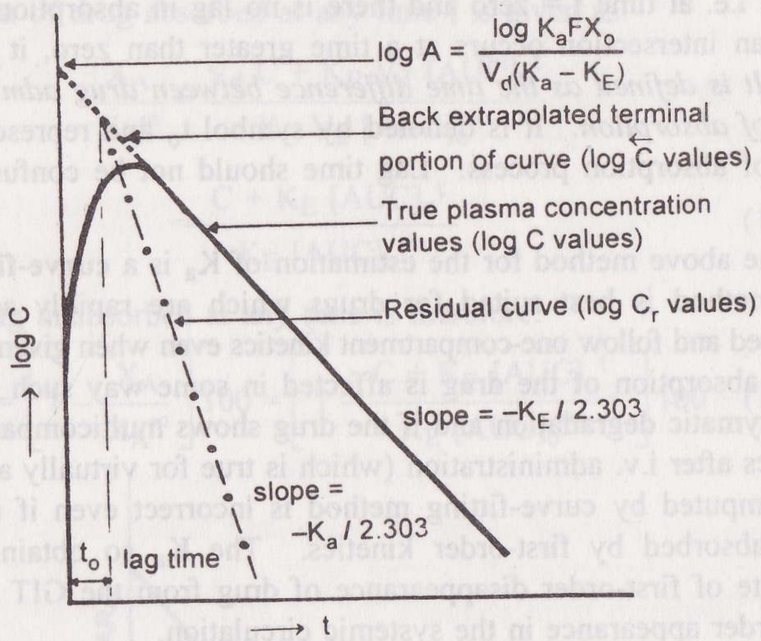


Fig. 10.8 Plasma concentration-time profile after oral administration of a single dose of a drug. The biexponential curve has been resolved into its two components—absorption and elimination.

Subtraction of true plasma concentration values i.e. equation 10.58 from the extrapolated plasma concentration values i.e. equation 10.59 yields a series of residual concentration values C_r :

$$(\overleftarrow{C} - C) = C_r = A\ e^{-K_a t} \quad (10.61)$$

In log form, the equation is:

$$\log C_r = \log A - \frac{K_a t}{2.303} \quad (10.62)$$

A plot of log C_r versus t yields a straight line with slope $-K_a/2.303$ and *Y*-intercept log A (Fig. 10.8). Absorption half-life can then be computed from K_a using the relation $0.693/K_a$. Thus, the method of residuals enables resolution of the biexponential plasma level-time curve into its two exponential components. The technique works best when the difference between K_a and K_E is large ($K_a/K_E \geq 3$). In some instances, the K_E obtained after i.v. bolus of the same drug is very large, much larger than the K_a obtained by the method of residuals (e.g. isoprenaline) and if $K_E/K_a \geq 3$, the terminal slope estimates K_a and not K_E whereas the slope of residual line gives K_E and not K_a. This is called as **flip-flop** *phenomenon since the slopes of the two lines have exchanged their meanings.*

Ideally, the extrapolated and the residual lines intersect each other on y-axis i.e. at time t = zero and there is no lag in absorption. However, if such an intersection occurs at a time greater than zero, it indicates **time lag**. *It is defined as the time difference between drug administration and start of absorption.* It is denoted by symbol t_o and represents the beginning of absorption process. Lag time should not be confused with onset time.

The above method for the estimation of K_a is a **curve-fitting method**. The method is best suited for drugs which are rapidly and completely absorbed and follow one-compartment kinetics even when given i.v. However, if the absorption of the drug is affected in some way such as GI motility or enzymatic degradation and if the drug shows multicompartment characteristics after i.v. administration (which is true for virtually all drugs), then K_a computed by curve-fitting method is incorrect even if the drug were truly absorbed by first-order kinetics. The K_a so obtained is at best, estimate of first-order disappearance of drug from the GIT rather than of first-order appearance in the systemic circulation.

Wagner-Nelson Method for Estimation of K_a

One of the better alternatives to curve-fitting method in the estimation of K_a is Wagner-Nelson method. The method involves determination of K_a from percent unabsorbed-time plots and does not require the assumption of zero- or first-order absorption.

After oral administration of a single dose of a drug, at any given time, the amount of drug absorbed into the systemic circulation X_A, is the sum

of amount of drug in the body X and the amount of drug eliminated from the body X_E. Thus:

$$X_A = X + X_E \quad (10.63)$$

The amount of drug in the body is $X = V_d C$. The amount of drug eliminated at any time t can be calculated as follows:

$$X_E = K_E V_d [AUC]_o^t \quad (10.64)$$

Substitution of values of X and X_E in equation 10.63 yields:

$$X_A = V_d C + K_E V_d [AUC]_o^t \quad (10.65)$$

The total amount of drug absorbed into the systemic circulation from time zero to infinity X_A^∞ can be given as:

$$X_A^\infty = V_d C^\infty + K_E V_d [AUC]_o^\infty \quad (10.66)$$

Since at $t = \infty$, $C^\infty = 0$, the above equation reduces to:

$$X_A^\infty = K_E V_d [AUC]_o^\infty \quad (10.67)$$

The fraction of drug absorbed at any time t is given as:

$$\frac{X_A}{X_A^\infty} = \frac{V_d C + K_E V_d [AUC]_o^t}{K_E V_d [AUC]_o^\infty}$$

$$= \frac{C + K_E [AUC]_o^t}{K_E [AUC]_o^\infty} \quad (10.68)$$

Percent drug unabsorbed at any time is therefore:

$$\% \text{ ARA} = \left[1 - \frac{X_A}{X_A^\infty}\right] 100 = \left[1 - \frac{C + K_E [AUC]_o^t}{K_E [AUC]_o^\infty}\right] 100 \quad (10.69)$$

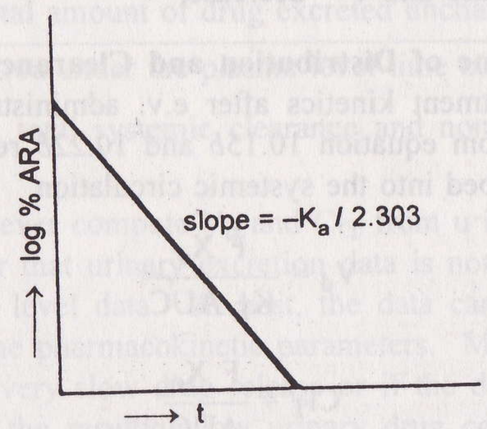


Fig. 10.9 Semilog plot of percent ARA versus t according to Wagner-Nelson method

The method requires collection of blood samples after a single oral dose at regular intervals of time till the entire amount of drug is eliminated from the body. K_E is obtained from log C versus t plot and $[AUC]_0^t$ and $[AUC]_0^\infty$ are obtained from plots of C versus t. A semilog plot of percent unabsorbed (i.e. percent ARA) versus t yields a straight line whose slope is $-K_a/2.303$ (Fig.10.9). If a regular plot of the same is a straight line, then absorption is zero-order.

K_a can similarly be estimated from urinary excretion data (*see the relevant section*). The biggest *disadvantage* of Wagner-Nelson method is that it applies only to drugs with one-compartment characteristics. Problem arises when a drug that obeys one-compartment model after e.v. administration shows multicompartment characteristics on i.v. injection.

Effect of K_a and K_E on C_{max}, t_{max} and AUC

A summary of the influence of changes in K_a at constant K_E and of K_E at constant K_a on C_{max}, t_{max} and AUC of a drug administered e.v. is shown in Table 10.4.

TABLE 10.4

Influence of K_a and K_E on C_{max}, t_{max} and AUC

Parameters Affected	*Influence when K_E is Constant*		*Influence when K_a is Constant*	
	Smaller K_a	*Larger K_a*	*Smaller K_E*	*Larger K_E*
C_{max}	↓	↑	↑	↓
t_{max}	Long	Short	Long	Short
AUC	No Change	No Change	↑	↓

where, ↑ = increase and ↓ = decrease.

Apparent Volume of Distribution and Clearance : For a drug that follows one-compartment kinetics after e.v. administration, V_d and Cl_T can be computed from equation 10.15*b* and 10.22*b* respectively where F is the fraction absorbed into the systemic circulation.

$$V_d = \frac{F\,X_o}{K_E\,AUC} \qquad (10.15b)$$

$$Cl_T = \frac{F\,X_o}{AUC} \qquad (10.22b)$$

URINARY EXCRETION DATA
(Disposition Viewed from Urine only)

In the absence of plasma level-time data, useful information can still be obtained from urine data regarding elimination kinetics of a drug. The method has several *advantages* in the analysis of a pharmacokinetic system:

1. The method is useful when there is lack of sufficiently sensitive analytic techniques to measure concentration of drugs in plasma with accuracy.
2. The method is noninvasive and therefore better subject compliance is assured.
3. Convenience of collecting urine samples in comparison to drawing of blood periodically.
4. Often, a less sensitive analytic method is required for determining urine drug concentration as compared to plasma concentrations; if the urine drug concentrations are low, assaying of larger sample volumes is relatively easy.
5. First-order elimination, excretion and absorption rate constants and fraction excreted unchanged can be computed from such data; first-order metabolism or extrarenal excretion rate constant can also be calculated subsequently from the difference $(K_E - K_e) = K_m$.
6. Direct measurement of bioavailability, both absolute and relative, is possible without the necessity of fitting the data to a mathematical model.
7. When coupled with plasma level-time data, it can also be used to estimate renal clearance of unchanged drug according to following equation:

$$Cl_R = \frac{\text{Total amount of drug excreted unchanged}}{\text{Area under the plasma level-time curve}} \qquad (10.70)$$

If V_d is known, total systemic clearance and nonrenal clearance can also be calculated.

One cannot however compute V_d and Cl_T from urine data alone. One must also remember that urinary excretion data is not an accurate substitute for the plasma level data. At best, the data can be employed as a rough estimate of the pharmacokinetic parameters. Moreover, if the drug product provides a very slow drug release or if the drug has a very long biological half-life, the resulting low urinary drug concentration may be too dilute to be assessed with accuracy. In the latter case, i.e. for drugs

with long $t_{1/2}$, urine may have to be collected for several days to account for total drug excreted.

Criteria for Obtaining Valid Urinary Excretion Data

1. A significant amount of drug must be excreted unchanged in the urine (at least 10%).
2. The analytical method must be specific for the unchanged drug; metabolites should not interfere.
3. *Water-loading* should be done by taking 400 ml of water after fasting overnight, to promote diuresis and enable collection of sufficient urine samples.
4. Before administration of drug, the bladder must be emptied completely after 1 hour from water-loading and the urine sample taken as blank; the drug should then be administered with 200 ml of water and should be followed by 200 ml given at hourly intervals for the next 4 hours.
5. Volunteers must be instructed to completely empty their bladder while collecting urine samples.
6. Frequent sampling should be done in order to obtain a good curve.

TABLE 10.5

Urinary Excretion Data following i.v. Bolus of 100 mg of a Drug

Observation				Treatment of Data					
Sample	Time of urine collection t (hours)	Volume of urine collected (ml)	Concentration of unchanged drug in urine (mcg/ml)	Urine collection interval dt (or Δt)	Mid-point of urine collection t*	Amount excreted in time interval (mg) dXu (or ΔXu)	Excretion rate (mg/H) dXu/dt	Cumulative amount excreted (mg) Xu^t	Amount remaining to be excreted $Xu^\infty - Xu^t$
0	0	-	-	-	-	-	-	0	66.7
1	0 - 2	140	250	2	1	35.0	17.5	35.0	31.7
2	2 - 4	150	100	2	3	15.0	7.5	50.0	16.7
3	4 - 6	90	80	2	5	7.2	3.6	57.2	9.5
4	6 - 8	200	20	2	7	4.0	2.0	61.2	5.5
5	8 -12	310	10	4	10	3.1	0.8	64.3	2.4
6	12 - 24	600	04	12	18	2.4	0.2	66.7 → Xu^∞	-

7. During sampling, the exact time and volume of urine excreted should be noted.
8. An individual collection period should not exceed one biologic half-life of the drug and ideally should be considerably less.
9. Urine samples must be collected for at least 7 biological half-lives in order to ensure collection of more than 99% of excreted drug.
10. Changes in urine pH and urine volume may alter the urinary excretion rate.

The urine data can be set as shown in the Table 10.5. Observations include times of urine collection, volumes collected and concentration of unchanged drug in each sample. These data are treated to derive further information.

Determination of K_E from Urinary Excretion Data

The first-order elimination (and excretion) rate constants can be computed from urine data by two methods:

1. Rate of excretion method, and
2. Sigma-minus method.

Rate of Excretion Method : The rate of urinary drug excretion dX_u/dt is proportional to the amount of drug in the body X and written as:

$$\frac{dX_u}{dt} = K_e X \qquad (10.71)$$

where K_e = first-order urinary excretion rate constant. According to first-order disposition kinetics, $X = X_o e^{-K_E t}$ (equation 10.5). Substituting it in above equation yields:

$$\frac{dX_u}{dt} = K_e X_o e^{-K_E t} \qquad (10.72)$$

where X_o = dose administered (i.v. bolus). Transforming to log form the equation becomes:

$$\log \frac{dX_u}{dt} = \log K_e X_o - \frac{K_E t}{2.303} \qquad (10.73)$$

The above equation states that a semilog plot of rate of excretion versus time yields a straight line with slope $-K_E/2.303$ (Fig. 10.10). It must therefore be remembered that the slope of such an excretion rate versus time plot is related to elimination rate constant K_E and not to excretion rate constant K_e. The excretion rate constant can be obtained from the *Y*-intercept (log $K_e X_o$). Elimination half-life and nonrenal elimination rate constant can then be computed from K_E and K_e.

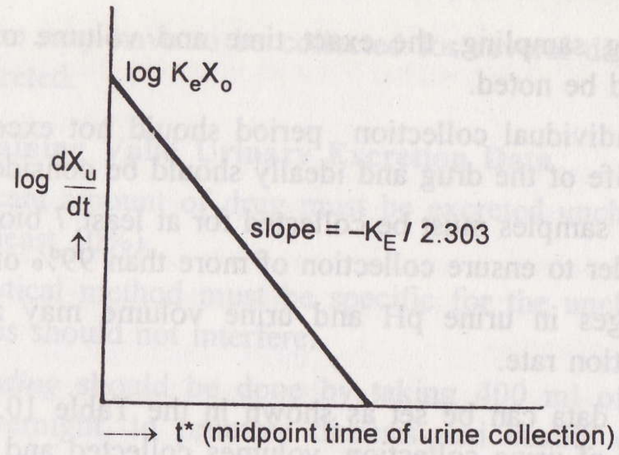


Fig. 10.10 Semilog plot of excretion rate versus mid-point time of urine collection period for computing elimination rate constant after i.v. bolus administration.

An *advantage* of excretion rate method is that for drugs having long half-lives, urine may be collected for only 3 to 4 half-lives. Moreover, there is no need to collect all urine samples since collection of any two consecutive urine samples yield points on the rate plot from which a straight line can be constructed.

Sigma-Minus Method : A *disadvantage of rate of excretion method* in estimating K_E is that fluctuations in the rate of drug elimination are observed to a high degree and in most instances, the data are so scattered that an estimate of half-life is difficult. These problems can be minimized by using the alternative approach called as sigma-minus method.

From an earlier equation:

$$\frac{dX_u}{dt} = K_e X_o e^{-K_E t} \qquad (10.72)$$

Integration of equation 10.72 yields:

$$X_u = \frac{K_e X_o}{K_E}(1 - e^{-K_E t}) \qquad (10.74)$$

where X_u = cumulative amount of drug excreted unchanged in urine at any time t. As time approaches infinity i.e. after 6 to 7 half-lives, the value $e^{-K_E \infty}$ becomes zero and therefore the cumulative amount excreted at infinite time X_u^∞ can be given by equation:

$$X_u^\infty = \frac{K_e X_o}{K_E} \qquad (10.75)$$

Substitution of equation 10.75 in equation 10.74 and rearrangement yields:

$$X_u^\infty - X_u = X_u^\infty e^{-K_E t} \qquad (10.76)$$

Converting to logarithms, we get:

$$\log (X_u^\infty - X_u) = \log X_u^\infty - \frac{K_E t}{2.303} \qquad (10.77)$$

where $(X_u^\infty - X_u)$ = **amount remaining to be excreted** i.e. **ARE** at any given time. A semilog plot of ARE versus t yields a straight line with slope $-K_E/2.303$. The method is, therefore, also called as **ARE plot method**. A *disadvantage* of this method is that total urine collection has to be carried out until no unchanged drug can be detected in the urine i.e upto 7 half-lives, which may be tedious for drugs having long $t_{½}$.

The equations until now for computing K_E from the urinary excretion data apply to a drug that fits one-compartment model and given as i.v. bolus. Similarly, data obtained during constant rate i.v. infusion can be used to evaluate the elimination rate constant. The equation that describes the urinary excretion rate of unchanged drug when administered as i.v. bolus also applies when it is administered as i.v. infusion. Thus:

$$\frac{dX_u}{dt} = K_e X \qquad (10.71)$$

For a drug given as i.v. infusion, the amount of drug in the body X is given by equation (described earlier):

$$X = \frac{R_o}{K_E}(1 - e^{-K_E t}) \qquad (10.34)$$

Substitution of equation 10.34 in equation 10.71 and integration of the same yields:

$$X_u = \frac{K_e R_o t}{K_E} - \frac{K_e R_o}{K_E^2}(1 - e^{-K_E t}) \qquad (10.78)$$

When the drug has been infused for a period long enough to attain steady-state in the plasma, the term $e^{-K_E t}$ approaches zero and the above equation reduces to:

$$X_u = \frac{K_e R_o t}{K_E} - \frac{K_e R_o}{K_E^2} \qquad (10.79)$$

A regular plot of cumulative amount of drug excreted X_u versus t yields a curvilinear plot the linear portion of which has a slope K_eR_o/K_E.

Extrapolation of linear segment to time axis yields x-intercept equal to $1/K_E$ since when $X_u = 0$, $t = 1/K_E$ (Fig. 10.11).

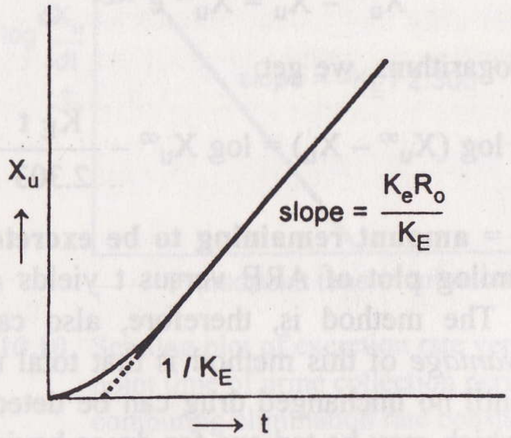


Fig. 10.11 Regular plot of X_u versus t during constant rate i.v. infusion

Relationships for rate of excretion when the drug is administered e.v. can also be given similarly. Thus:

$$\frac{dX_u}{dt} = K_e X \qquad (10.71)$$

For a drug given e.v. and absorbed by a first-order process, X is given as:

$$X = \frac{K_a F X_o}{(K_a - K_E)} \left[e^{-K_E t} - e^{-K_a t} \right] \qquad (10.48)$$

Substitution of equation 10.48 in equation 10.71 and integration of the same yields:

$$X_u = \frac{K_e K_a F X_o}{K_E} \left[\frac{1}{K_a} + \frac{e^{-K_E t}}{(K_E - K_a)} - \frac{K_E . e^{-K_a t}}{K_a (K_E - K_a)} \right] \qquad (10.80)$$

At time infinity, the equation 10.80 reduces to:

$$X_u^{\infty} = \frac{K_e F X_o}{K_E} \qquad (10.81)$$

Substitution of equation 10.81 in equation 10.80 and subsequent rearrangement yields:

$$ARE = (X_u^{\infty} - X_u) = \frac{X_u^{\infty}}{(K_a - K_E)} (K_a e^{-K_E t} - K_E e^{-K_a t}) \qquad (10.82)$$

A semilog plot of $(X_u^{\infty} - X_u)$ versus t results in a biexponential curve and if $K_a > K_E$, the slope of the terminal linear portion of the curve will

define K_E of the drug. The absorption rate constant K_a can be estimated by the method of residuals using the same data i.e. equation 10.82.

Urinary excretion data after oral administration can also be treated according to Wagner-Nelson method to calculate K_a by construction of % ARA plots. The method requires urine collection for sufficiently long time to ensure accurate estimation of K_E but need not be collected to time infinity. The equation derived to relate % ARA with urinary excretion rate is:

$$\%ARA = \left[1 - \frac{X_A}{X_A^{\infty}} \right] 100 = \left[1 - \frac{dX_u/dt + K_E X_u}{K_E X_u^{\infty}} \right] 100 \qquad (10.83)$$

A semilog plot of % ARA versus t yields a straight line with slope $-K_a/2.303$.

Accurate determination of K_a from urinary excretion data is possible only for drugs with slow rate of absorption since for drugs with rapid absorption, collection of urine samples at very short intervals of time is difficult.

MULTICOMPARTMENT MODELS
(Delayed Distribution Models)

The one-compartment model adequately describes pharmacokinetics of many drugs. Instantaneous distribution equilibrium is assumed in such cases and decline in the amount of drug in the body with time is expressed by an equation with a monoexponential term (i.e. elimination). However, instantaneous distribution is not truly possible for an even larger number of drugs and drug disposition is not monoexponential but bi- or multi-exponential. This is because the body is composed of a heterogeneous group of tissues each with different degree of blood flow and affinity for drug and therefore different rates of equilibration. *Ideally, a true pharmacokinetic model should be the one with a rate constant for each tissue undergoing equilibrium*, which is difficult mathematically. The best approach is therefore to **pool** together tissues on the basis of similarity in their distribution characteristics. As for one-compartment models, drug disposition in multicompartment systems is also assumed to occur by first-order. Multicompartment characteristics of a drug are best understood by giving it as i.v. bolus and observing the manner in which the plasma concentration declines with time. The number of exponentials required to describe such a plasma level-time profile determines the number of kinetically homogeneous compartments into which a drug will distribute.

TWO-COMPARTMENT OPEN MODEL

The commonest of multicompartment models is two compartment model. In such a model, the body tissues are broadly classified into 2 categories:

1. **Central Compartment** or **Compartment 1** comprising of blood and highly perfused tissues like liver, lungs, kidneys, etc. that equilibrate with the drug rapidly. Elimination usually occurs from this compartment.
2. **Peripheral** or **Tissue Compartment** or **Compartment 2** comprising of poorly perfused and slow equilibrating tissues such as muscles, skin, adipose, etc. and considered as a hybrid of several functional physiologic units.

Classification of a particular tissue, for example brain, into central or peripheral compartment depends upon the physicochemical properties of the drug. A highly lipophilic drug can cross the BBB and brain would then be included in the central compartment. In contrast, a polar drug cannot penetrate the BBB and brain in this case will be a part of peripheral compartment despite the fact that it is a highly perfused organ.

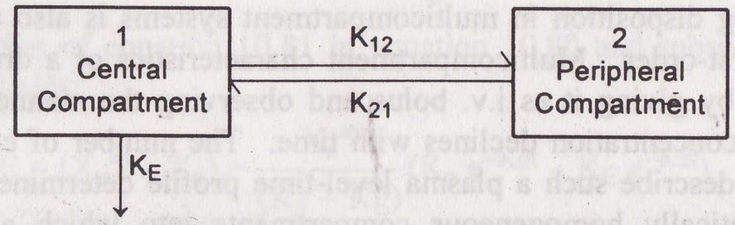
Depending upon the compartment from which the drug is eliminated, the two-compartment model can be categorized into 3 types:

1. Two-compartment model with elimination from central compartment.
2. Two-compartment model with elimination from peripheral compartment.
3. Two-compartment model with elimination from both the compartments.

In the absence of information, elimination is assumed to occur exclusively from central compartment.

Two-Compartment Open Model—Intravenous Bolus Administration

The model can be depicted as shown below with elimination from the central compartment.



After the i.v. bolus of a drug that follows two-compartment kinetics, the decline in plasma concentration is biexponential indicating the presence of *two disposition processes viz. distribution and elimination.* These two processes are not evident to the eyes in a regular arithmetic plot but when a semilog plot of C versus t is made, they can be identified (Fig. 10.12). Initially, the concentration of drug in the central compartment *declines rapidly*; this is due to the distribution of drug from the central compartment to the peripheral compartment. The phase during which this occurs is therefore called as the **distributive phase**. After sometime, a *pseudo-distribution equilibrium* is achieved between the two compartments following which the subsequent loss of drug from the central compartment is slow and mainly due to elimination. This *second, slower rate process, is called as the* **post-distributive** or **elimination phase**. In contrast to the central compartment, the drug concentration in the peripheral compartment first increases and reaches a maximum. This corresponds with the distribution phase. Following peak, the drug concentration declines which corresponds to the post-distributive phase (Fig.10.12).

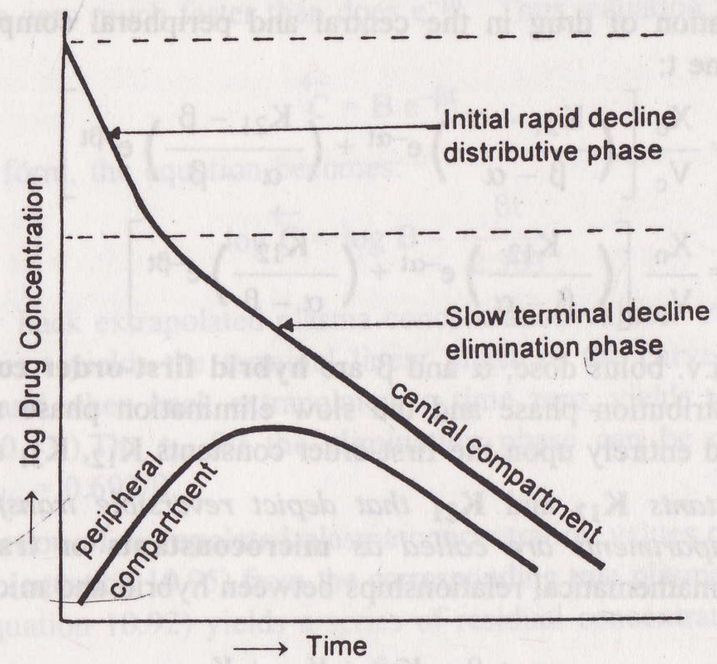


Fig. 10.12 Changes in drug concentration in the central (plasma) and the peripheral compartment after i.v. bolus of a drug that fits two-compartment model.

Let K_{12} and K_{21} be the first-order distribution rate constants depicting drug transfer between the central and the peripheral compartments and let subscript c and p define central and peripheral compartment respectively. The rate of change in drug concentration in the central compartment is given by:

$$\frac{dC_c}{dt} = K_{21}\, C_p - K_{12}\, C_c - K_E\, C_c \qquad (10.84)$$

Extending the relationship $X = V_d C$ to the above equation, we have

$$\frac{dC_c}{dt} = \frac{K_{21} X_p}{V_p} - \frac{K_{12} X_c}{V_c} - \frac{K_E X_c}{V_c} \quad (10.85)$$

where X_c and X_p are the amounts of drug in the central and peripheral compartments respectively and V_c and V_p are the apparent volumes of the central and the peripheral compartment respectively. The rate of change in drug concentration in the peripheral compartment is given by:

$$\frac{dC_p}{dt} = K_{12} C_c - K_{21} C_p \quad (10.86)$$

$$= \frac{K_{12} X_c}{V_c} - \frac{K_{21} X_p}{V_p} \quad (10.87)$$

Integration of equations 10.85 and 10.87 yields equations that describe the concentration of drug in the central and peripheral compartments at any given time t:

$$C_c = \frac{X_o}{V_c}\left[\left(\frac{K_{21} - \alpha}{\beta - \alpha}\right) e^{-\alpha t} + \left(\frac{K_{21} - \beta}{\alpha - \beta}\right) e^{-\beta t}\right] \quad (10.88)$$

$$C_p = \frac{X_o}{V_p}\left[\left(\frac{K_{12}}{\beta - \alpha}\right) e^{-\alpha t} + \left(\frac{K_{12}}{\alpha - \beta}\right) e^{-\beta t}\right] \quad (10.89)$$

where X_o = i.v. bolus dose, α and β are **hybrid first-order constants** for the rapid distribution phase and the slow elimination phase respectively which depend entirely upon the first-order constants K_{12}, K_{21} and K_E.

The constants K_{12} and K_{21} *that depict reversible transfer of drug between compartments are called as* **microconstants** or **transfer constants**. The mathematical relationships between hybrid and microconstants are given as:

$$\alpha + \beta = K_{12} + K_{21} + K_E \quad (10.90)$$

$$\alpha \beta = K_{21} K_E \quad (10.91)$$

Equation 10.88 can be written in simplified form as:

$$C_c = A e^{-\alpha t} + B e^{-\beta t} \quad (10.92)$$

$$C_c = \underset{\text{exponent}}{\textbf{Distribution}} + \underset{\text{exponent}}{\textbf{Elimination}}$$

where A and B are also hybrid constants for the two exponents and can be resolved graphically by the method of residuals.

$$A = \frac{X_o}{V_c}\left[\frac{K_{21} - \alpha}{\beta - \alpha}\right] = C_o\left[\frac{K_{21} - \alpha}{\beta - \alpha}\right] \quad (10.93)$$

$$B = \frac{X_o}{V_c}\left[\frac{K_{21} - \beta}{\alpha - \beta}\right] = C_o\left[\frac{K_{21} - \beta}{\alpha - \beta}\right] \quad (10.94)$$

where C_o = plasma drug concentration immediately after i.v. injection.

Method of Residuals : The biexponential disposition curve obtained after i.v. bolus of a drug that fits two compartment model can be resolved into its individual exponents by the method of residuals. Rewriting the equation 10.92:

$$C = A e^{-\alpha t} + B e^{-\beta t} \quad (10.92)$$

As apparent from the biexponential curve given in Fig. 10.12., the initial decline due to distribution is more rapid than the terminal decline due to elimination i.e. the rate constant $\alpha >> \beta$ and hence the term $e^{-\alpha t}$ approaches zero much faster than does $e^{-\beta t}$. Thus, equation 10.92 reduces to:

$$\overleftarrow{C} = B e^{-\beta t} \quad (10.95)$$

In log form, the equation becomes:

$$\log \overleftarrow{C} = \log B - \frac{\beta t}{2.303} \quad (10.96)$$

where $\overleftarrow{C}$ = back extrapolated plasma concentration values. A semilog plot of C versus t yields the terminal linear phase of the curve having slope $-\beta/2.303$ and when back extrapolated to time zero, yields y-intercept log B (Fig. 10.13.).The $t_{1/2}$ for the elimination phase can be obtained from equation $t_{1/2} = 0.693/\beta$.

Subtraction of extrapolated plasma concentration values of the elimination phase (equation 10.95) from the corresponding true plasma concentration values (equation 10.92) yields a series of residual concentration values C_r.

$$C_r = C - \overleftarrow{C} = A e^{-\alpha t} \quad (10.97)$$

In log form, the equation becomes:

$$\log C_r = \log A - \frac{\alpha t}{2.303} \quad (10.98)$$

A semilog plot of C_r versus t yields a straight line with slope $-\alpha/2.303$ and *Y*-intercept log A (Fig. 10.13).

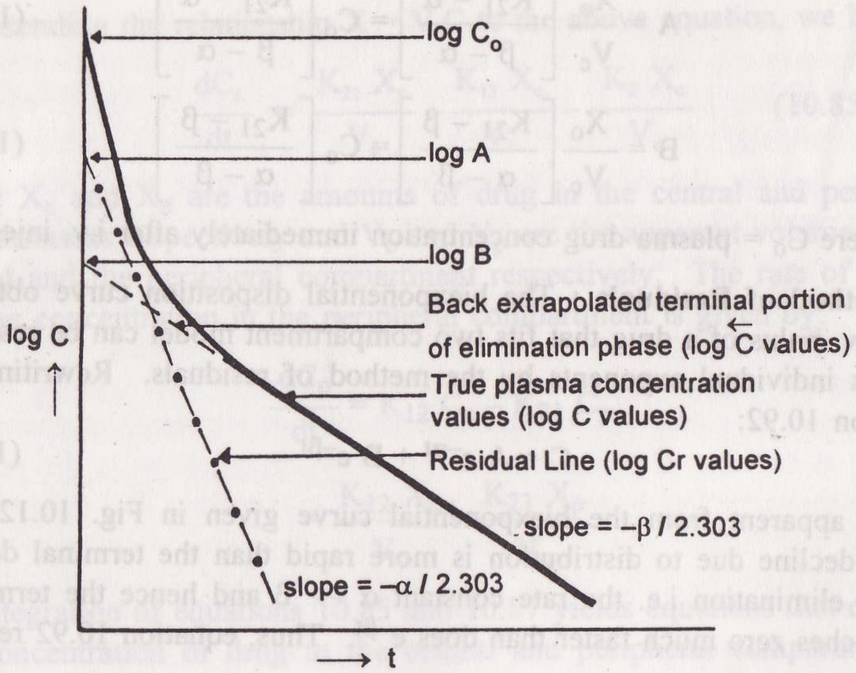


Fig. 10.13 Resolution of biexponential plasma concentration-time curve by the method of residuals for a drug that follows two-compartment kinetics on i.v. bolus administration.

Assessment of Pharmacokinetic Parameters : All the parameters of equation 10.92 can be resolved by the method of residuals as described above. Other parameters of the model viz. K_{12}, K_{21}, K_E, etc. can now be derived by proper substitution of these values.

$$C_o = A + B \tag{10.99}$$

$$K_E = \frac{\alpha \beta C_o}{A \beta + B \alpha} \tag{10.100}$$

$$K_{12} = \frac{A B (\beta - \alpha)^2}{C_o (A \beta + B \alpha)} \tag{10.101}$$

$$K_{21} = \frac{A \beta + B \alpha}{C_o} \tag{10.102}$$

It must be noted that for two-compartment model, K_E is the rate constant for elimination of drug from the central compartment and β *is the rate constant for elimination from the entire body.* Overall elimination $t_{½}$ should therefore be calculated from β.

Area under the plasma concentration-time curve can be obtained by the following equation:

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} \tag{10.103}$$

The apparent volume of central compartment V_c is given as:

$$V_c = \frac{X_o}{C_o} = \frac{X_o}{K_E\ AUC} \tag{10.104}$$

Apparent volume of peripheral compartment can be obtained from equation:

$$V_p = \frac{V_c\ K_{12}}{K_{21}} \tag{10.105}$$

The apparent volume of distribution at steady-state or equilibrium can now be defined as:

$$V_{d,ss} = V_c + V_p \tag{10.106}$$

It is also given as:

$$V_{d,area} = \frac{X_o}{\beta\ AUC} \tag{10.107}$$

Total systemic clearance is given as:

$$Cl_T = \beta\ V_d \tag{10.108}$$

The pharmacokinetic parameters can also be calculated by using urinary excretion data:

$$\frac{dX_u}{dt} = K_e\ V_c \tag{10.109}$$

An equation identical to equation 10.92 can be derived for rate of excretion of unchanged drug in urine:

$$\frac{dX_u}{dt} = K_e\ A\ e^{-\alpha t} + K_e\ B\ e^{-\beta t} \tag{10.110}$$

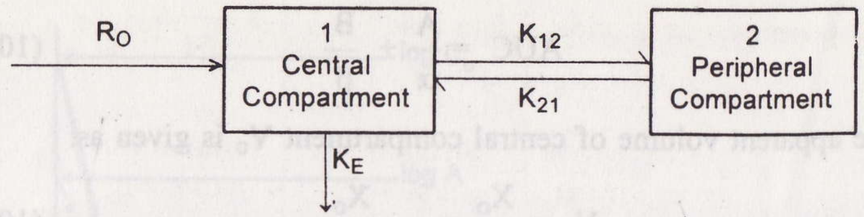
The above equation can be resolved into individual exponents by the method of residuals as described for plasma concentration-time data.

Renal clearance is given as:

$$Cl_R = K_e\ V_c \tag{10.111}$$

Two-Compartment Open Model —Intravenous Infusion

The model can be depicted as shown below with elimination from the central compartment.



The plasma or central compartment concentration of a drug that fits two-compartment model when administered as constant rate (zero-order) i.v. infusion, is given by equation:

$$C = \frac{R_o}{V_c K_E}\left[1 + \left(\frac{K_E - \beta}{\beta - \alpha}\right)e^{-\alpha t} + \left(\frac{K_E - \alpha}{\alpha - \beta}\right)e^{-\beta t}\right] \quad (10.112)$$

At steady-state (i.e. at time infinity), the second and the third term in the bracket becomes zero and the equation reduces to:

$$C_{ss} = \frac{R_o}{V_c K_E} \quad (10.113)$$

Now $V_c K_E = V_d \beta$. Substituting this in equation 10.113, we get:

$$C_{ss} = \frac{R_o}{V_d \beta} = \frac{R_o}{Cl_T} \quad (10.114)$$

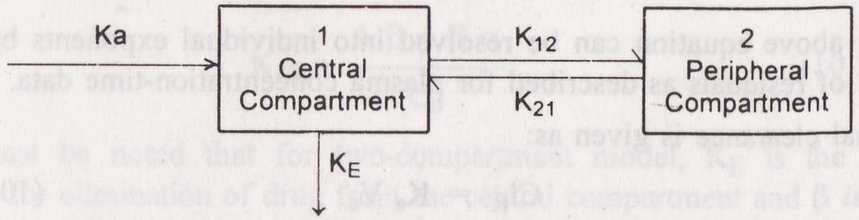
The loading dose $X_{o,L}$ to obtain C_{ss} immediately at the start of infusion can be calculated from equation:

$$X_{o,L} = C_{ss} V_c = \frac{R_o}{K_E} \quad (10.115)$$

Two-Compartment Open Model —Extravascular Administration

First-Order Absorption

The model can be depicted as follows:



For a drug that enters the body by a first-order absorption process and distributed according to two compartment model, the rate of change in drug concentration in the central compartment is described by *3 exponents* —an absorption exponent, and the two usual exponents that describe drug disposition.

The plasma concentration at any time t is given by equation:

$$C = N e^{-Kat} + L e^{-\alpha t} + M e^{-\beta t} \quad (10.116)$$

$$C = \text{Absorption exponent} + \text{Distribution exponent} + \text{Elimination exponent}$$

where K_a, α and β have usual meanings. L, M and N are coefficients.

The 3 exponents can be resolved by stepwise application of method of residuals assuming $K_a > \alpha > \beta$ as shown in Fig. 10.14. The various pharmacokinetic parameters can then be estimated.

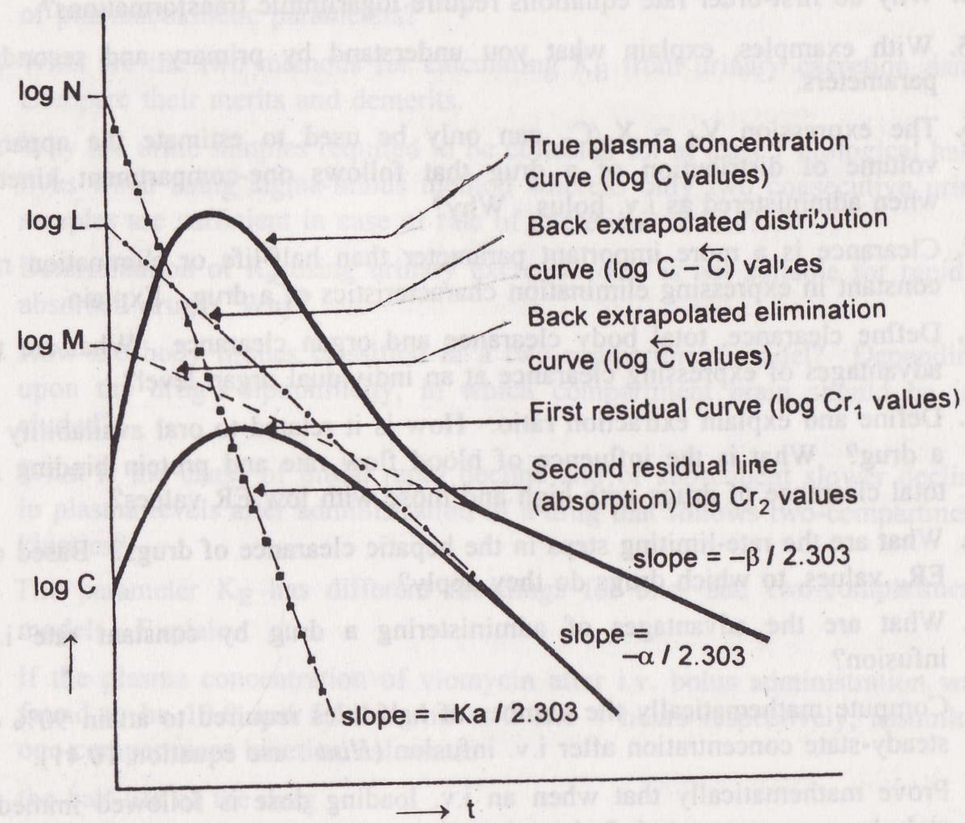


Fig. 10.14 Semilog plot of C versus t of a drug with two-compartment characteristics when administered extravascularly. The various exponents have been resolved by the method of residuals.

Besides the method of residuals, K_a can also be estimated by **Loo-Riegelman method** for a drug that follows two-compartment characteristics. This method is in contrast to the Wagner-Nelson method for determination of K_a of a drug with one-compartment characteristics. The Loo-Riegelman method requires plasma drug concentration-time data both after oral and i.v. administration of the drug to the same subject at different times in order to obtain all the necessary kinetic constants. Despite its complexity,

the method can be applied to drugs that distribute in any number of compartments.

QUESTIONS

1. In one-compartment open model, what do you infer from plasma being called as reference compartment?
2. In compartment modeling, what does the term **open** mean?
3. Disposition of a drug that follows one-compartment kinetics is a monoexponential process. Explain.
4. Why do first-order rate equations require logarithmic transformations?
5. With examples, explain what you understand by primary and secondary parameters.
6. The expression $V_d = X_o/C_o$ can only be used to estimate the apparent volume of distribution of a drug that follows one-compartment kinetics when administered as i.v. bolus. Why?
7. Clearance is a more important parameter than half-life or elimination rate constant in expressing elimination characteristics of a drug. Explain.
8. Define clearance, total body clearance and organ clearance. What are the advantages of expressing clearance at an individual organ level?
9. Define and explain extraction ratio. How is it related to oral availability of a drug? What is the influence of blood flow rate and protein binding on total clearance of drugs with high and those with low ER values?
10. What are the rate-limiting steps in the hepatic clearance of drugs? Based on ER_H values, to which drugs do they apply?
11. What are the advantages of administering a drug by constant rate i.v. infusion?
12. Compute mathematically the number of half-lives required to attain 90% of steady-state concentration after i.v. infusion (*Hint* : use equation 10.41).
13. Prove mathematically that when an i.v. loading dose is followed immediately by a constant rate infusion, the plasma concentration remains steady as long as the infusion is continued.
14. On what parameters does the time to reach steady-state depend?
15. How can the steady-state be attained rapidly? When is it advisable? Give expressions for calculating such doses for a drug that fits one-compartment model.
16. Compare the absorption characteristics of drugs absorbed by zero-order with those absorbed by a first-order process after e.v. administration.
17. Show that after e.v. administration, the C_{max} that can be attained is no more than 37% of maximum level attainable with the same dose given as i.v. bolus. Why is this so?

18. What are the applications and limitations of method of residuals?
19. What is flip-flop phenomenon and when is it observed?
20. Under what circumstances is the value of K_a computed from method of residuals incorrect?
21. What are the merits and demerits of Wagner-Nelson method in computing K_a?
22. What is the influence of K_a and K_E on C_{max}, t_{max} and AUC?
23. What criteria are necessary for obtaining a valid urinary excretion data? What are the advantages and disadvantages of such a method in assessment of pharmacokinetic parameters?
24. What are the two methods for calculating K_E from urinary excretion data? Compare their merits and demerits.
25. Why are urine samples required to be collected for at least 7 biological half-lives when using sigma-minus method whereas only two consecutive urine samples are sufficient in case of rate of excretion method?
26. Determination of K_a using urinary excretion data is not suitable for rapidly absorbed drugs. Why?
27. How are body tissues classified in a two-compartment model? Depending upon the drug's lipophilicity, in which compartment brain should be included?
28. What is the cause of initial rapid decline and of subsequent slower decline in plasma levels after administration of a drug that follows two-compartment kinetics?
29. The parameter K_E has different meanings for one- and two-compartment models. Explain.
30. If the plasma concentration of viomycin after i.v. bolus administration was found to be 10.0 and 5.5 mcg/ml at 2 and 4 hours respectively, assuming one-compartment kinetics, calculate:

    a. the half-life of the drug

    Answer : 2.3 hours.

    b. the concentration of drug in plasma at time zero

    Answer : 18.2 mcg/ml.

    c. the V_d if dose administered was 300 mg

    Answer : 16.5 liters.

    d. the total systemic clearance

    Answer : 82.2 ml/min.

    e. the renal clearance if fraction excreted unchanged in urine is 0.8

    Answer : 65.8 ml/min.

31. A 70 Kg patient is to be given oubain by i.v. infusion. The drug has a half-life of 22 hours, apparent V_d 15.7 liters and the desired steady-state plasma concentration is 0.0002 mcg/ml. Assuming one-compartment kinetics, calculate:

a. the time required to reach 90% of C_{ss}

Answer : 73 hours.

b. the infusion rate to achieve the desired C_{ss}

Answer : 0.1 mcg/hour.

c. the loading dose to attain C_{ss} rapidly

Answer : 3.14 mcg.

d. the concentration of drug in plasma after 48 hours from the start of infusion

Answer : 0.00014 mcg/ml and when loading dose is given, 0.00018 mcg/ml.

32. The equation that best fits the pharmacokinetics of paracetamol after oral administration of 500 mg dose is:

$$C = 1.18\ (e^{-0.24t} - e^{-1.6t})$$

Assuming one-compartment kinetics, determine—

a. peak time

Answer : 1.4 hours.

b. peak plasma concentration

Answer : 0.717 mcg/ml.

c. elimination half-life of the drug

Answer : 2.88 hours.

d. apparent V_d if fraction bioavailable is 0.4

Answer : 200 liters.

e. concentration of drug in plasma after 3 hours of administration

Answer : 0.565 mcg/ml.

33. The equation that best fits the plasma level time curve of azlocillin after an i.v. bolus dose of 2000 mg (assuming one-compartment kinetics) is:

$$C = 143\ e^{-0.87t}$$

a. What is the apparent V_d?

Answer : 14 liters.

b. What is the elimination $t_{½}$ of the drug?

Answer : 0.8 Hours.

c. What will be the plasma drug concentration after 6 hours?

Answer : 0.77 mcg/ml.

d. How much of the drug will be left in the body after 6 hours?

Answer : 10.8 mg.

e. When should the next dose be administered if the drug becomes ineffective when the plasma level falls below 50 mcg/ml?

Answer : after 1.2 hours.

f. What will be the therapeutic index of the drug if the therapeutic range is 50 to 500 mcg/ml?

Answer : TI = 10.

g. How much dose should be administered to attain an instantaneous plasma concentration of 500 mcg/ml?

Answer : 7000 mg.

h. For how long will the plasma level lie in the therapeutic window if the above dose is given as i.v. bolus?

Answer : 2.6 hours.

34. The amount of drug excreted in urine after an i.v. dose of 400 mg of norfloxacin was as follows:

t (hours)	*X_u (mg)*	*dX_u/dt (mg/H)*	*t**
0	0		
2	49.05		
4	80.0		

After completing the table and using rate of excretion method, determine—

a. the elimination rate constant and half-life of the drug.

Answer : K_E = 0.2303/hour and $t_{½}$ = 3 hours.

b. urinary excretion rate constant

Answer : K_e = 0.077/hour.

c. fraction of drug excreted unchanged in urine

Answer : 33.4%

d. whether the drug can be used for urinary tract infections?

35. The half-life of propranolol in a 60 Kg patient is 4 hours and V_d is 5.5 liter/Kg.

a. Determine the total systemic clearance of the drug

Answer : Cl_T = 953 ml/min.

b. What will be its renal clearance if fraction excreted unchanged in urine is 0.047?

Answer : Cl_R = 44.8 ml/min.

c. Comment on the mechanism of renal excretion of drug.

d. If the drug is eliminated only by hepatic and renal routes, what will be the hepatic extraction ratio if blood flow to the liver is 1.5 liter/min?

Answer : ER_H = 0.6054.

e. If the blood flow rate to the liver reduces to 0.8 liter/min in situations of CCF, what will be the new hepatic and total systemic clearance values?

Answer : Cl_H = 484.4 ml/min and Cl_T = 529.2 ml/min.

f. What is the % decrease in the overall clearance of the drug?

Answer : 44.5%.

36. Following a 650 mg i.v. bolus dose of a drug to a 65 Kg subject, the plasma drug concentration was found to decline biexponentially. The equation that best described the drug kinetics was:

$$C = 67\ e^{-14t} + 33\ e^{-3t}$$

where t is in hours and C is in mcg/ml. Calculate the following:

a. The volume of the central compartment.

Answer : V_c = 6.5 liters.

b. The volume of the peripheral compartment.

Answer : V_p = 3.95 liters.

c. The apparent V_d at steady-state.

Answer : $V_{d,ss}$ = 10.45 liters.

d. Volume of distribution by area.

Answer : $V_{d,area}$ = 13.7 liters.

e. The microconstants K_{12} and K_{21}

Answer : K_{12} = 4.035/hour and K_{21} = 6.63/hour.

f. The elimination rate constant for the disposal of drug from the central compartment.

Answer : K_E = 6.33/hour.

g. The overall elimination half-life

Answer : $t_{½}$ = 0.231 hours.

h. The total systemic clearance of the drug (use $V_{d,area}$ for calculation).

Answer : Cl_T = 686.2 ml/min.

i. The plasma level of the drug after 30 minutes of i.v. dose.

Answer : 7.42 mcg/ml.

j. The infusion rate if the drug is to be given as constant rate i.v. infusion (desired C_{ss} is 20 mcg/ml).

Answer : R_o = 823 mg/hour.

k. The loading dose to attain the C_{ss} rapidly.

Answer : $X_{o,L}$ = 130 mg.

11

Nonlinear Pharmacokinetics

In most cases, at therapeutic doses, the change in the amount of drug in the body or the change in its plasma concentration due to absorption, distribution, binding, metabolism or excretion, is proportional to its dose, whether administered as a single dose or as multiple doses. In such situations, the rate processes are said to follow first-order or linear kinetics and all semilog plots of C versus t for different doses when corrected for dose administered, are superimposable. This is called as **principle of superposition**. The important pharmacokinetic parameters viz. F, K_a, K_E, V_d, Cl_R and Cl_H which describe the time-course of a drug in the body remain unaffected by the dose i.e. the pharmacokinetics is dose independent.

In some instances, the rate process of a drug's ADME are dependent upon carrier or enzymes that are substrate specific, have definite capacities, and susceptible to saturation at high drug concentration. In such cases, an essentially first-order kinetics transform into a mixture of first-order and zero-order rate processes and the pharmacokinetic parameters change with the size of the administered dose. The pharmacokinetics of such drugs are said to be **dose-dependent**. Other terms synonymous with it are **mixed-order**, **nonlinear** and **capacity-limited** kinetics. Drugs exhibiting such a kinetic profile are sources of variability in pharmacologic response.

There are several *tests to detect nonlinearity* in pharmacokinetics but the simplest ones are:

1. Determination of steady-state plasma concentration at different doses. If the steady-state concentrations are directly proportional to the dose, then linearity in the kinetics exist. Such a proportionality is not observable when there is nonlinearity.
2. Determination of some of the important pharmacokinetic parameters such as fraction bioavailable, elimination half-life or total systemic clearance at different doses of the drug. Any change in these parameters which are usually constant, is indicative of nonlinearity.

CAUSES OF NONLINEARITY

Nonlinearities can occur in drug absorption, distribution, metabolism and excretion:

Drug Absorption

Nonlinearity in drug absorption can arise from 3 important sources:

1. *When absorption is solubility or dissolution rate-limited* e.g. griseofulvin. At higher doses, a saturated solution of the drug is formed in the GIT or at any other extravascular site and the rate of absorption attains a constant value.
2. *When absorption involves carrier-mediated transport systems* e.g. riboflavin, ascorbic acid, cyanocobalamin, etc. Saturation of the transport system at higher doses of these vitamins results in nonlinearity.
3. *When presystemic gut wall or hepatic metabolism attains saturation* e.g. propranolol.

The parameters affected will be F, K_a, C_{max} and AUC. A decrease in these parameters is observed in the former two cases and an increase in the latter case. Other causes of nonlinearity in drug absorption are changes in gastric emptying and GI blood flow and other physiologic factors. Nonlinearity in drug absorption is of little consequence unless availability is drastically affected.

Drug Distribution

Nonlinearity in distribution of drugs administered at high doses may be due to:

1. *Saturation of binding sites on plasma proteins* e.g. phenylbutazone.
2. *Saturation of tissue binding sites.*

In both cases, the free plasma drug concentration increases but V_d increases only in the former case whereas it decreases in the latter. Clearance is also altered depending upon the extraction ratio of the drug. Clearance of a drug with high ER is greatly increased due to saturation of binding sites. Unbound clearance of drugs with low ER is unaffected and one can expect an increase in pharmacologic response.

Drug Metabolism

The nonlinear kinetics of most clinical importance is capacity-limited metabolism since small changes in dose administered can produce large variations in plasma concentration at steady-state. It is a major source of

large intersubject variability in pharmacologic response. Two important causes of nonlinearity in metabolism are:

1. *Capacity-limited metabolism due to enzyme and/or cofactor saturation.* Typical examples include phenytoin, alcohol, theophylline, etc.
2. *Enzyme induction* e.g. carbamazepine, where a decrease in peak plasma concentration has been observed on repetitive administration over a period of time. Autoinduction characterized in this case is also dose-dependent. Thus, enzyme induction is a common cause of both dose- and time-dependent kinetics.

Saturation of enzyme results in decreased Cl_H and therefore increased C_{ss}. Reverse is true for enzyme induction. Other causes of nonlinearity in biotransformation include saturation of binding sites, inhibitory effect of the metabolite on enzyme and pathologic situations such as hepatotoxicity and changes in hepatic blood flow.

Drug Excretion

The two active processes in renal excretion of a drug that are saturable are:

1. *Active tubular secretion* e.g. penicillin G, and
2. *Active tubular reabsorption* e.g. water soluble vitamins and glucose.

After saturation of the carrier systems, a decrease in renal clearance in the former case and an increase in the latter situation is observed. Other sources of nonlinearity in renal excretion include forced diuresis, changes in urine pH, nephrotoxicity and saturation of binding sites.

Biliary secretion, which is also an active process, is also subject to saturation e.g. tetracycline and indomethacin.

MICHAELIS MENTEN EQUATION

The kinetics of capacity-limited or saturable processes is best described by Michaelis-Menten equation:

$$-\frac{dC}{dt} = \frac{V_{max}\,C}{K_m + C} \qquad (11.1)$$

where $-dC/dt$ = rate of decline of drug concentration with time,

V_{max} = theoretical maximum rate of the process, and

K_m = Michaelis constant.

Three situations can now be considered depending upon the values of K_m and C:

1. When $K_m = C$: Under this situation, the equation 11.1. reduces to:

$$-\frac{dC}{dt} = \frac{V_{max}}{2} \qquad (11.2)$$

i.e. the rate of process is equal to one-half its maximum rate (Fig. 11.1).

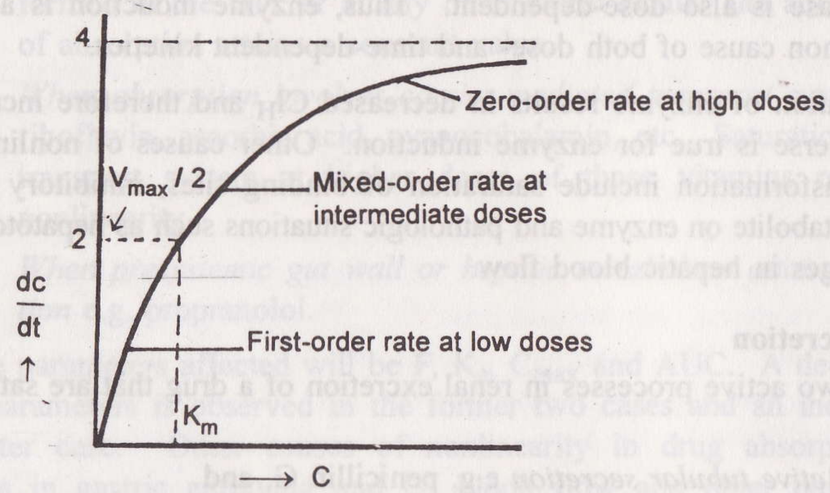


Fig. 11.1 A plot of Michaelis-Menten equation (elimination rate dC/dt versus concentration C). Initially, the rate increases linearly (first-order) with concentration, becomes mixed-order at higher concentration and then reaches maximum (V_{max}) beyond which it proceeds at a constant rate (zero-order).

2. When $K_m >> C$: Here, $K_m + C \equiv K_m$ and the equation 11.1. reduces to:

$$-\frac{dC}{dt} = \frac{V_{max}\,C}{K_m} \qquad (11.3)$$

The above equation is identical to the one that describes first-order elimination of a drug where $V_{max}/K_m = K_E$. This means that the drug concentration in the body that results from usual dosage regimens of most drugs is well below the K_m of the elimination process with certain exceptions such as phenytoin and alcohol.

3. When $K_m << C$: Under this condition, $K_m + C \equiv C$ and the equation 11.1 will become:

$$-\frac{dC}{dt} = V_{max} \qquad (11.4)$$

The above equation is identical to the one that describes a zero-order process i.e. the rate process occurs at a constant rate V_{max} and is independent of drug concentration e.g. metabolism of ethanol.

Estimation of K_m and V_{max}

The parameters of capacity-limited processes like metabolism, renal tubular secretion and biliary excretion can be easily defined by assuming one-compartment kinetics for the drug and that elimination involves only a single capacity-limited process.

The parameters K_m and V_{max} can be assessed from the plasma concentration-time data collected after i.v. bolus administration of a drug with nonlinear elimination characteristics.

Rewriting equation 11.1.

$$-\frac{dC}{dt} = \frac{V_{max}\,C}{K_m + C} \qquad (11.1)$$

Integration of above equation followed by conversion to log base 10 yields:

$$\log C = \log C_o + \frac{(C_o - C)}{2.303\,K_m} - \frac{V_{max}\,t}{2.303\,K_m} \qquad (11.5)$$

A semilog plot of C versus t yields a curve with a terminal linear portion having slope $-V_{max}/2.303K_m$ and when back extrapolated to time zero gives *Y*-intercept $\log \overleftarrow{C}_o$ (*see* Fig. 11.2). The equation that describes this line is:

$$\log C = \log \overleftarrow{C}_o - \frac{V_{max}\,t}{2.303\,K_m} \qquad (11.6)$$

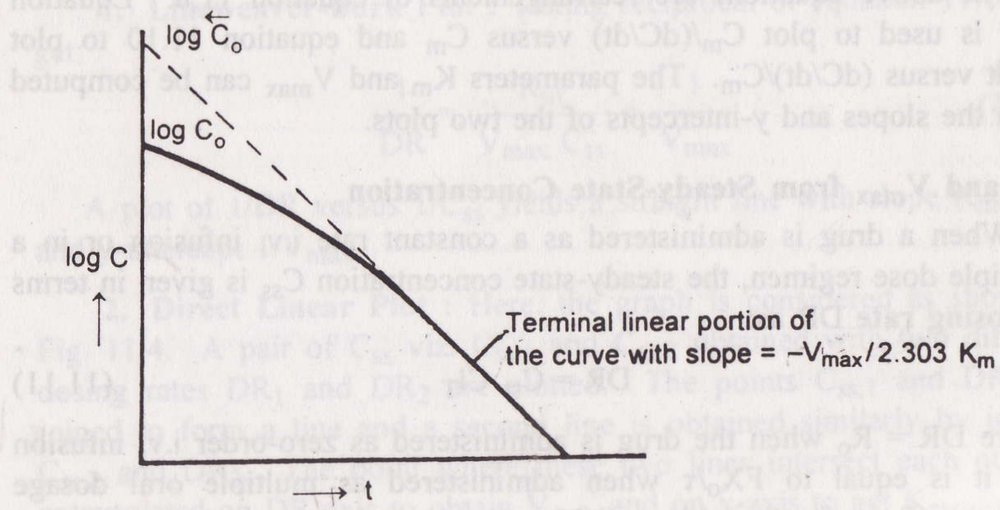


Fig. 11.2 Semilog plot of a drug given as i.v. bolus with nonlinear elimination and that fits one-compartment kinetics.

At low plasma concentrations, equations 11.5 and 11.6 are identical. Equating the two and simplifying further, we get:

$$\frac{(C_o - C)}{2.303\ K_m} = \log \frac{\overleftarrow{C_o}}{C_o} \qquad (11.7)$$

K_m can thus be obtained from above equation. V_{max} can be computed by substituting the value of K_m in the slope value.

An alternative approach of estimating V_{max} and K_m is determining the rate of change of plasma drug concentration at different times and using the reciprocal of the equation 11.1. Thus:

$$\frac{1}{dC/dt} = \frac{K_m}{V_{max}\ C_m} + \frac{1}{V_{max}} \qquad (11.8)$$

where C_m = plasma concentration at midpoint of the sampling interval. A double reciprocal plot or the **Lineweaver-Burk plot** of 1/(dC/dt) versus $1/C_m$ of the above equation yields a straight line with slope = K_m/V_{max} and y-intercept = $1/V_{max}$.

A *disadvantage* of Lineweaver-Burk plot is that the points are clustered. More reliable plots in which the points are uniformly scattered are **Hanes-Woolf plot** (equation 11.9) and **Woolf-Augustinsson-Hofstee plot** (equation 11.10).

$$\frac{C_m}{dC/dt} = \frac{K_m}{V_{max}} + \frac{C_m}{V_{max}} \qquad (11.9)$$

$$\frac{dC}{dt} = V_{max} - \frac{dC/dt\ K_m}{C_m} \qquad (11.10)$$

The above equations are rearrangements of equation 11.8. Equation 11.9 is used to plot $C_m/(dC/dt)$ versus C_m and equation 11.10 to plot dC/dt versus $(dC/dt)/C_m$. The parameters K_m and V_{max} can be computed from the slopes and y-intercepts of the two plots.

K_m and V_{max} from Steady-State Concentration

When a drug is administered as a constant rate i.v. infusion or in a multiple dose regimen, the steady-state concentration C_{ss} is given in terms of **dosing rate** DR as:

$$DR = C_{ss}\ Cl_T \qquad (11.11)$$

where DR = R_o when the drug is administered as zero-order i.v. infusion and it is equal to FX_o/τ when administered as multiple oral dosage regimen (F is fraction bioavailable, X_o is oral dose and τ is dosing interval).

At steady-state, the dosing rate equals rate of decline in plasma drug concentration and if the decline (elimination) is due to a single capacity-limited process (for e.g. metabolism), then;

$$DR = \frac{V_{max}\ C_{ss}}{K_m + C_{ss}} \qquad (11.12)$$

A plot of C_{ss} versus DR yields a typical *hockey-stick shaped curve* as shown in Fig. 11.3.

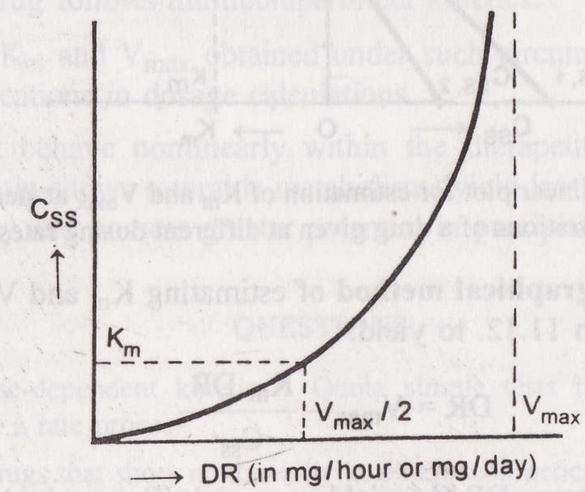


Fig. 11.3 Curve for a drug with nonlinear kinetics obtained by plotting the steady-state concentration versus dosing rates.

To define the characteristics of the curve with a reasonable degree of accuracy, several measurements must be made at steady-state during dosage with different doses.

Practically, one can graphically compute K_m and V_{max} in 3 ways:

1. Lineweaver-Burk Plot : Taking reciprocal of equation 11.12, we get:

$$\frac{1}{DR} = \frac{K_m}{V_{max}\ C_{ss}} + \frac{1}{V_{max}} \qquad (11.13)$$

A plot of 1/DR versus $1/C_{ss}$ yields a straight line with slope K_m/V_{max} and y-intercept $1/V_{max}$.

2. Direct Linear Plot : Here, the graph is considered as shown in Fig. 11.4. A pair of C_{ss} viz. $C_{ss.1}$ and $C_{ss.2}$ obtained with two different dosing rates DR_1 and DR_2 are plotted. The points $C_{ss.1}$ and DR_1 are joined to form a line and a second line is obtained similarly by joining $C_{ss.2}$ and DR_2. The point where these two lines intersect each other is extrapolated on DR axis to obtain V_{max} and on x-axis to get K_m.

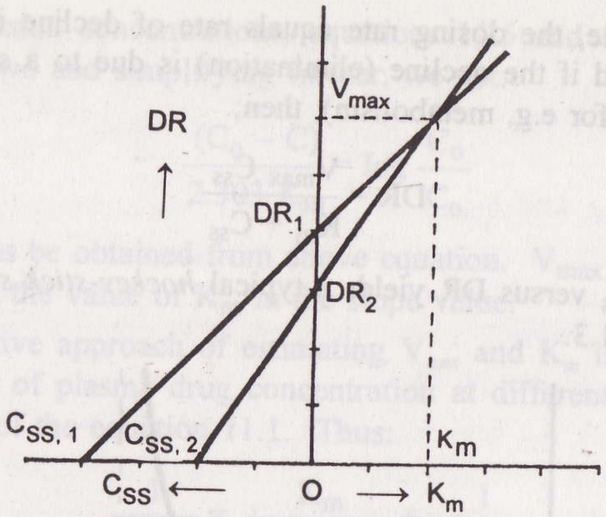


Fig. 11.4 Direct linear plot for estimation of K_m and V_{max} at steady-state concentrations of a drug given at different dosing rates.

3. The third graphical method of estimating K_m and V_{max} involves rearranging equation 11.12. to yield:

$$DR = V_{max} - \frac{K_m\, DR}{C_{ss}} \qquad (11.14)$$

A plot of DR versus DR/C_{ss} yields a straight line with slope $-K_m$ and *Y*-intercept V_{max}.

K_m and V_{max} can also be calculated numerically by setting up simultaneous equations as shown below:

$$DR_1 = \frac{V_{max}\, C_{ss,1}}{K_m + C_{ss,1}} \qquad (11.15)$$

$$DR_2 = \frac{V_{max}\, C_{ss,2}}{K_m + C_{ss,2}} \qquad (11.16)$$

Combination of the above two equations yields

$$K_m = \frac{DR_1 - DR_2}{\dfrac{DR_1}{C_{ss,1}} - \dfrac{DR_2}{C_{ss,2}}} \qquad (11.17)$$

After having computed K_m, its subsequent substitution in any one of the two simultaneous equations will yield V_{max}.

It has been observed that K_m is much less variable than V_{max}. Hence, if mean K_m for a drug is known from an earlier study, then instead of two, a single measurement of C_{ss} at any given dosing rate is sufficient to compute V_{max}.

There are several limitations of K_m and V_{max} estimated by assuming one-compartment system and a single capacity-limited process. More complex equations will result and the computed K_m and V_{max} will usually be larger when:

1. The drug is eliminated by more than one capacity-limited process.
2. The drug exhibits parallel capacity-limited and first-order elimination processes.
3. The drug follows multicompartment kinetics.

However, K_m and V_{max} obtained under such circumstances have little practical applications in dosage calculations.

Drugs that behave nonlinearly within the therapeutic range (for example, phenytoin shows saturable metabolism) yield less predictable results in drug therapy and possess greater potential in precipitating toxic effects.

QUESTIONS

1. Define dose-dependent kinetics. Quote simple tests by which it can be detected in a rate process.
2. Why are drugs that show nonlinearity in pharmacokinetics considered sources of variability in pharmacologic response?
3. What processes of drug ADME are known to show nonlinearity? Give examples.
4. When administered at high doses, how does the pharmacokinetic parameters—$t_{½}$, V_d, C_{max}, etc. change for drugs known to undergo capacity-limited elimination?
5. What are the limitations in calculating K_m and V_{max} by assuming one-compartment model and a single capacity-limited process?
6. Using the following data of a drug having K_m of 0.2 mcg/ml and V_{max} of 1.0 mcg/ml/hour, determine the concentrations at which the drug shows first-order, zero-order and mixed-order metabolic rates.

Concentration (mcg/ml)	0.02	0.2	20.0
Metabolic Rate (mcg/ml/H)	0.1	0.5	1.0

7. Theophylline was administered to a patient at dosing rates of 600 mg/day and 1200 mg/day and the respective steady-state concentrations were found to be 9.8 mg/L and 28.6 mg/l. Find K_m and V_{max}. Determine the dosing rate to achieve a C_{ss} of 15 mg/l.

   Answer : K_m = 31.14 mg/l, V_{max} = 2507 mg/day and DR = 815 mg/day.

12

Bioavailability and Bioequivalence

The therapeutic effectiveness of a drug depends upon the ability of the dosage form to deliver the medicament to its site of action at a rate and amount sufficient to elicit the desired pharmacologic response. This attribute of the dosage form is referred to as *physiologic availability*, *biologic availability* or simply *bioavailability*. For most drugs, the pharmacologic response can be related directly to the plasma levels. Thus, *the term* **bioavailability** *is defined as the rate and extent (amount) of absorption of unchanged drug from its dosage form.* It is an absolute term. The rate or rapidity with which a drug is absorbed is an important consideration when a rapid onset of action is desired as in the treatment of acute conditions such as asthma attack, pain, etc. A slower absorption rate is however desired when the aim is to prolong the duration of action or to avoid the adverse effects. On the other hand, extent of absorption is of special significance in the treatment of chronic conditions like hypertension, epilepsy, etc.

If the size of the dose to be administered is same, then bioavailability of a drug from its dosage form depends upon 3 major factors:

1. Pharmaceutic factors related to physicochemical properties of the drug and characteristics of the dosage form
2. Patient related factors
3. Route of administration

The former two factors have been dealt with comprehensively in *chapter 2 on Absorption of Drugs*. The influence of route of administration on drug's bioavailability is generally in the following order : *parenteral > oral > rectal > topical* with few exceptions. Within the parenteral route, intravenous injection of a drug results in 100% bioavailability as the absorption process is bypassed. However, for reasons of stability and convenience, most drugs are administered orally. In such cases, *the dose available to the patient, called as the* **bioavailable dose**, is often less than

the administered dose. *The amount of drug that reaches the systemic circulation (i.e. extent of absorption) is called as* **systemic availability** or simply **availability**. *The term* **bioavailable fraction F**, *refers to the fraction of administered dose that enters the systemic circulation.*

$$F = \frac{\text{Bioavailable dose}}{\text{Administered dose}} \qquad (12.1)$$

Objectives of Bioavailability Studies

Bioavailability studies are important in the—

1. Primary stages of development of a suitable dosage form for a new drug entity.
2. Determination of influence of excipients, patient related factors and possible interaction with other drugs on the efficiency of absorption.
3. Development of new formulations of the existing drugs.
4. Control of quality of a drug product during the early stages of marketing in order to determine the influence of processing factors, storage and stability on drug absorption.

CONSIDERATIONS IN BIOAVAILABILITY STUDY DESIGN

Bioavailability—Absolute versus Relative

When the systemic availability of a drug administered orally is determined in comparison to its intravenous administration, it is called as **absolute bioavailability**. It is denoted by symbol F. Its determination is used to characterize a drug's inherent absorption properties from the e.v. site. Intravenous dose is selected as a standard because the drug is administered directly into the systemic circulation (100 % bioavailability) and avoids absorption step. Intramuscular dose can also be taken as a standard if the drug is poorly water soluble. An oral solution as reference standard has also been used in certain cases. However, there are several *drawbacks of using oral solution as a standard* instead of an i.v. dose—

1. Limits the pharmacokinetic treatment to one-compartment model only; one cannot apply the most applicable two-compartment kinetics to the data and all pharmacokinetic parameters cannot be assessed.
2. Differentiation between the fraction of dose unabsorbed and that metabolized is difficult.
3. If the rate of oral absorption is not sufficiently greater than the rate of elimination, the true elimination rate constant cannot be computed.

At best, when oral solution is used in conjunction with i.v. route, one can distinguish the dissolution rate limitation in drug absorption from solid dosage forms.

When the systemic availability of a drug after oral administration is compared with that of an oral standard of the same drug (such as an aqueous or nonaqueous solution or a suspension), *it is referred to as* **relative bioavailability**. It is denoted by symbol F_r. In contrast to absolute bioavailability, it is used to characterize absorption of a drug from its formulation. F and F_r are generally expressed in percentages.

Single Dose versus Multiple Dose Studies

The dose to be administered for a bioavailability study is determined from preliminary clinical experiments. *Single dose bioavailability studies* are very common. They are easy, offer less exposure to drugs and are less tedious. However, it is difficult to predict the steady-state characteristics of a drug and intersubject variability with such studies. On the other hand, *multiple dose study* is difficult to control (poor subject compliance), exposes the subject to more drug and is highly tedious and time consuming. Nevertheless, such a study has several *advantages*—

1. More accurately reflects the manner in which the drug should be used.
2. Easy to predict the peak and valley characteristics of the drug since the bioavailability is determined at steady-state.
3. Requires collection of fewer blood samples.
4. The drug blood levels are higher due to cumulative effect which makes its determination possible even by the less sensitive analytic methods.
5. Can be ethically performed in patients because of the therapeutic benefit to the patient.
6. Small intersubject variability is observed in such a study which allows use of fewer subjects.
7. Better evaluation of the performance of a controlled release formulation is possible.
8. Nonlinearity in pharmacokinetics, if present, can be easily detected.

In multiple dose study, one must ensure that the steady-state has been reached. For this, the drug should be administered for 5 to 6 elimination half-lives before collecting the blood samples.

Human Volunteers—Healthy Subjects versus Patients

Ideally, the bioavailability study should be carried out in *patients* for whom the drug is intended to be used because of the apparent *advantages*—

1. The patient will be benefited from the study.
2. Reflects better the therapeutic efficacy of a drug.
3. Drug absorption pattern in disease states can be evaluated.
4. Avoids the ethical quandary of administering drugs to healthy subjects.

Patients are generally preferred in multiple dose bioavailability studies. The drawbacks of using patients as volunteers are equally large—disease, other drugs, physiologic changes, etc. may modify the drug absorption pattern. Stringent study conditions such as fasting state required to be followed by the subject is also difficult. In short, establishing a standard set of conditions necessary for a bioavailability study is difficult with patients as volunteers. Such studies are therefore usually performed in young (20 to 40 years), healthy, male adult volunteers (body weight within a narrow range; ± 10%), under restricted dietary and fixed activity conditions. Female volunteers are used only when drugs such as oral contraceptives are to be tested. The number of subjects to be selected depends upon the extent of intersubject variability but should be kept to a minimum required to obtain a reliable data. The consent of volunteers must be obtained and they must be informed about the importance of the study, conditions to be followed during the study and possible hazards if any, prior to starting the study. Medical examination should be performed in order to exclude subjects with any kind of abnormality or disease. The volunteers must be instructed to abstain from any medication for at least a week and to fast overnight prior to and for a minimum of 4 hours after dosing. The volume and type of fluid and the standard diet to be taken must also be specified. Drug washout period for a minimum of ten biological half-lives must be allowed for between any two studies in the same subject.

Measurement of Bioavailability

The methods useful in quantitative evaluation of bioavailability can be broadly divided into two categories—pharmacokinetic methods and pharmacodynamic methods.

I. Pharmacokinetic Methods

These are very widely used and based on the assumption that the pharmacokinetic profile reflects the therapeutic effectiveness of a drug.

Thus, these are **indirect methods**. The two major pharmacokinetic methods are:

1. Plasma level-time studies.
2. Urinary excretion studies.

II. Pharmacodynamic Methods

These methods are complementary to pharmacokinetic approaches and involve **direct** measurement of drug effect on a (patho)physiologic process as a function of time. The two pharmacodynamic methods involve determination of bioavailability from:

1. Acute pharmacologic response.
2. Therapeutic response.

Plasma Level—Time Studies

Unless determination of plasma drug concentration is difficult or impossible, it is the most reliable method and method of choice in comparison to urine data. The method is based on the assumption that two dosage forms that exhibit superimposable plasma level-time profiles in a group of subjects should result in identical therapeutic activity.

With single dose study, the method requires collection of serial blood samples for a period of 2 to 3 biological half-lives after drug administration, their analysis for drug concentration and making a plot of concentration versus corresponding time of sample collection to obtain the plasma level-time profile. With i.v. dose, sampling should start within 5 minutes of drug administration and subsequent samples taken at 15 minute intervals. To adequately describe the disposition phase, at least 3 sample points should be taken if the drug follows one-compartment kinetics and 5 to 6 points if it fits two-compartment model. For oral dose, at least 3 points should be taken on the ascending part of the curve for accurate determination of K_a. The points for disposition or descending phase of the curve must be taken in a manner similar to that for i.v. dose.

The 3 parameters of plasma level-time studies which are considered important for determining bioavailability are:

1. C_{max} : The **peak plasma concentration** that gives an indication whether the drug is sufficiently absorbed systemically to provide a therapeutic response.
2. t_{max} : The **peak time** that gives an indication of the rate of absorption.
3. AUC : The **area under the plasma level-time curve** that gives a measure of the extent of absorption or the amount of drug that reaches the systemic circulation.

The extent of bioavailability can be determined by following equations:

$$F = \frac{[AUC]_{oral}\ D_{iv}}{[AUC]_{iv}\ D_{oral}} \tag{12.2}$$

$$F_r = \frac{[AUC]_{test}\ D_{std}}{[AUC]_{std}\ D_{test}} \tag{12.3}$$

where D stands for dose administered and subscripts **iv** and **oral** indicates the route of administration. Subscripts **test** and **std** indicate the test and the standard doses of the same drug to determine relative availability. The rate of absorption can be computed from one of the several methods discussed in *chapter 10*.

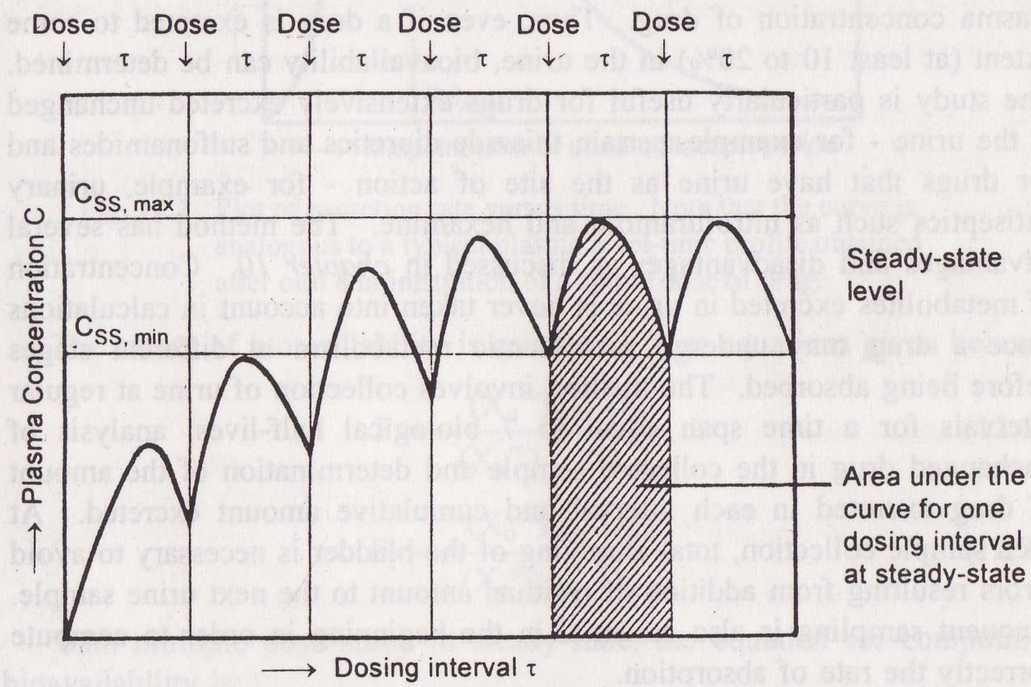


Fig. 12.1 Determination of AUC and $C_{ss,max}$ on multiple dosing upto steady-state

With multiple dose study, the method involves drug administration for at least 5 biological half-lives with a dosing interval equal to or greater than the biological half-life (i.e. administration of at least 5 doses) to reach the steady-state. A blood sample should be taken at the end of previous dosing interval and 8 to 10 samples after the administration of next dose. The extent of bioavailability is given as:

$$F_r = \frac{[AUC]_{test}\ D_{std}\ \tau_{test}}{[AUC]_{std}\ D_{test}\ \tau_{std}} \tag{12.4}$$

where [AUC] values are area under the plasma level-time curve of one dosing interval in a multiple dosage regimen, after reaching the steady-state (Fig. 12.1) and τ is the dosing interval.

Bioavailability can also be determined from the peak plasma concentration at steady-state $C_{ss,max}$ according to following equation:

$$F_r = \frac{(C_{ss,max})_{test}\ D_{std}\ \tau_{test}}{(C_{ss,max})_{std}\ D_{test}\ \tau_{std}} \qquad (12.5)$$

The rate of absorption is not important in the multiple dosing method.

Urinary Excretion Studies

This method of assessing bioavailability is based on the principle that the urinary excretion of unchanged drug is directly proportional to the plasma concentration of drug. Thus, even if a drug is excreted to some extent (at least 10 to 20%) in the urine, bioavailability can be determined. The study is particularly useful for drugs extensively excreted unchanged in the urine - for example, certain thiazide diuretics and sulfonamides and for drugs that have urine as the site of action - for example, urinary antiseptics such as nitrofurantoin and hexamine. The method has several advantages and disadvantages as discussed in *chapter 10*. Concentration of metabolites excreted in urine is never taken into account in calculations since a drug may undergo presystemic metabolism at different stages before being absorbed. The method involves collection of urine at regular intervals for a time span equal to 7 biological half-lives, analysis of unchanged drug in the collected sample and determination of the amount of drug excreted in each interval and cumulative amount excreted. At each sample collection, total emptying of the bladder is necessary to avoid errors resulting from addition of residual amount to the next urine sample. Frequent sampling is also essential in the beginning in order to compute correctly the rate of absorption.

The three major parameters examined in urinary excretion data obtained with a single dose study are:

1. $(dX_u/dt)_{max}$: The **maximum urinary excretion rate**, it is obtained from the peak of plot between rate of excretion versus midpoint time of urine collection period. It is analogous to the C_{max} derived from plasma level studies since the rate of appearance of drug in the urine is proportional to its concentration in systemic circulation. Its value increases as the rate of and/or extent of absorption increases (*see* Fig. 12.2).

2. $(t_u)_{max}$: The **time for maximum excretion rate**, it is analogous to the t_{max} of plasma level data. Its value decreases as the absorption rate increases.

3. X_u : The **cumulative amount of drug excreted in the urine**, it is related to the AUC of plasma level data and increases as the extent of absorption increases.

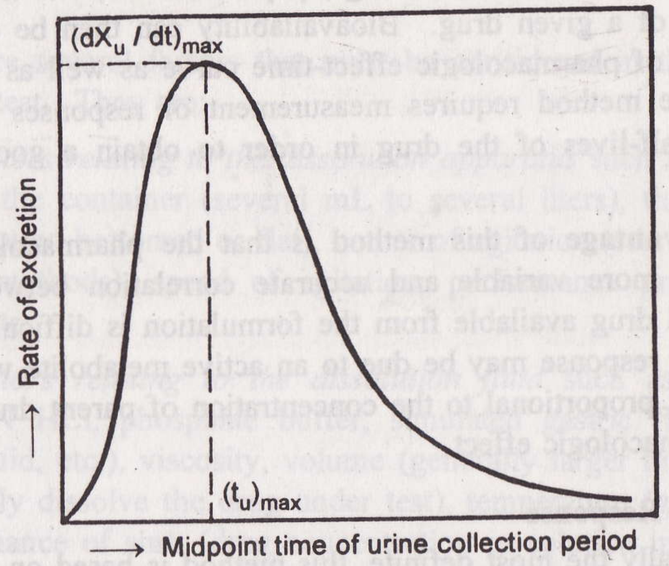


Fig. 12.2 Plot of excretion rate versus time. Note that the curve is analogous to a typical plasma level-time profile obtained after oral administration of a single dose of drug.

The extent of bioavailability is calculated from equations given below:

$$F = \frac{(X_u^{\infty})_{oral}\ D_{iv}}{(X_u^{\infty})_{iv}\ D_{oral}} \qquad (12.6)$$

$$F_r = \frac{(X_u^{\infty})_{test}\ D_{std}}{(X_u^{\infty})_{std}\ D_{test}} \qquad (12.7)$$

With multiple dose study to steady-state, the equation for computing bioavailability is:

$$F_r = \frac{(X_{u,ss})_{test}\ D_{std}\ \tau_{test}}{(X_{u,ss})_{std}\ D_{test}\ \tau_{std}} \qquad (12.8)$$

where $(X_{u,ss})$ is the amount of drug excreted unchanged during a single dosing interval at steady-state.

Bioavailability can also be determined for a few drugs by assay of biologic fluids other than plasma and urine. In case of theophylline, salivary excretion can be used whereas for cephalosporin antibiotics, appearance of drug in CSF and bile can be determined. Caution must however be exercised to account for salivary and enterohepatic cycling of the drugs.

Acute Pharmacologic Response

When bioavailability measurement by pharmacokinetic methods is difficult, inaccurate or nonreproducible, an acute pharmacologic effect such as change in ECG or EEG readings, pupil diameter, etc. is related to the time course of a given drug. Bioavailability can then be determined by construction of pharmacologic effect-time curve as well as dose-response graphs. The method requires measurement of responses for at least 3 biological half-lives of the drug in order to obtain a good estimate of AUC.

A **disadvantage** of this method is that the pharmacologic response tends to be more variable and accurate correlation between measured response and drug available from the formulation is difficult. Moreover, the observed response may be due to an active metabolite whose concentration is not proportional to the concentration of parent drug responsible for the pharmacologic effect.

Therapeutic Response

Theoretically the most definite, this method is based on observing the clinical response to a drug formulation given to patients suffering from disease for which it is intended to be used. A major *drawback* of this method is that quantitation of observed response is too improper to allow for reasonable assessment of relative bioavailability between two dosage forms of the same drug.

Drug Dissolution Rate and Bioavailability

The physicochemical property of most drugs that has greatest influence on their absorption characteristics from the GIT is dissolution rate. The best way of assessing therapeutic efficacy of drugs with a slow dissolution rate is *in vivo* determination of bioavailability which is usually done whenever a new formulation is to be introduced into the market. However, monitoring batch-to-batch consistency through use of such *in vivo* tests is extremely costly, tedious and time consuming besides exposing the healthy subjects to hazards of drugs. It would therefore be always desirable to substitute the *in vivo* bioavailability tests with inexpensive *in vitro* methods. The simple *in vitro* disintegration test is unreliable. *The best available tool today which can at least quantitatively assure about the biologic availability of a drug from its formulation is its* **in vitro dissolution test.**

In Vitro Drug Dissolution Testing Models

For an *in vitro* test to be useful, it must predict the *in vivo* behavior to such an extent that *in vivo* bioavailability test need not be performed.

Despite attempts to standardize the test performance, the *in vitro* dissolution technique is still by no means a perfect approach. The efforts are mainly aimed at mimicking the environment offered by the biological system.

There are several factors that must be considered in the design of a dissolution test. They are:

1. *Factors relating to the dissolution apparatus* such as—the design, the size of the container (several mL to several liters), the shape of the container (round bottomed or flat), nature of agitation (stirring, rotating or oscillating methods), speed of agitation, performance precision of the apparatus, etc.

2. *Factors relating to the dissolution fluid* such as—composition (water, 0.1N HCl, phosphate buffer, simulated gastric fluid, simulated intestinal fluid, etc.), viscosity, volume (generally larger than that needed to completely dissolve the drug under test), temperature (generally 37°C) and maintenance of **sink** (drug concentration in solution maintained constant at a low level) or **nonsink** conditions (gradual increase in the drug concentration in the dissolution medium).

3. *Process parameters* such as method of introduction of dosage form, sampling techniques, changing the dissolution fluid, etc.

The dissolution apparatus has evolved gradually and considerably from a simple beaker type to a highly versatile and fully automated instrument. The devices can be classified in a number of ways. Based on the absence or presence of sink conditions, there are two principal types of dissolution apparatus:

1. Closed-compartment apparatus : It is basically a limited-volume apparatus operating under nonsink conditions. The dissolution fluid is restrained to the size of the container, e.g. beaker type apparatus.

2. Open-compartment apparatus : It is the one in which the dosage form is contained in a column which is brought in continuous contact with fresh, flowing dissolution medium (perfect sink condition).

A third type called as **dialysis systems** are used for very poorly aqueous soluble drugs for which maintenance of sink conditions would otherwise require large volume of dissolution fluid. Only the official methods will be discussed here briefly.

Rotating Basket Apparatus (Apparatus 1)

It is basically a closed-compartment, beaker type apparatus comprising of a cylindrical glass vessel with hemispherical bottom of one liter capac-

ity partially immersed in a water bath to maintain the temperature at 37°C. A cylindrical basket made of 22 mesh to hold the dosage form is located centrally in the vessel at a distance of 2 cm from the bottom and rotated by a variable speed motor through a shaft (Fig. 12.3*a*). The basket should remain in motion during drawing of samples. All metal parts like basket and shaft are made of S.S. 316.

Rotating Paddle Apparatus (Apparatus 2)

The assembly is same as that for apparatus 1 except that the rotating basket is replaced with a paddle which acts as a stirrer (Fig. 12.3*b*). The dosage form is allowed to sink to the bottom of the vessel. A small, loose, wire helix may be attached to the dosage form that would otherwise float.

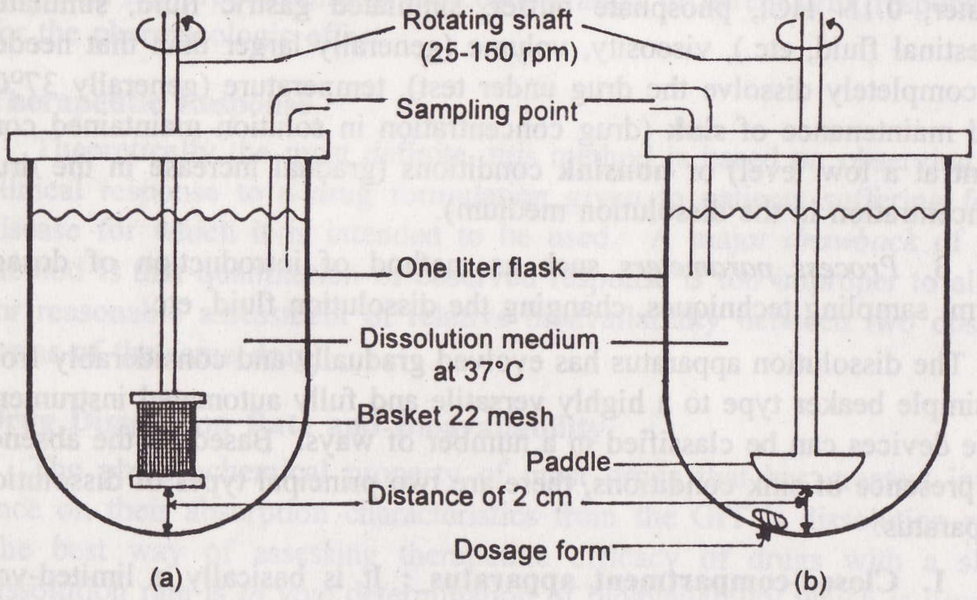


Fig. 12.3 Schematic representation of official dissolution apparatus —forced convection nonsink type (*a*) rotating basket apparatus, and (*b*) rotating paddle apparatus.

In Vitro—In Vivo Correlation

A simple *in vitro* dissolution test on the drug product will be insufficient to predict its therapeutic efficacy. Convincing correlation between *in vitro* dissolution behavior of a drug and its *in vivo* bioavailability must be experimentally demonstrated to guarantee reproducibility of biologic response. The two *major objectives* of developing such a correlation are—

1. To ensure batch-to-batch consistency in the physiologic performance of a drug product by use of such *in vitro* values.

2. To serve as a tool in the development of a new dosage form with desired *in vivo* performance.

There are two basic approaches by which a correlation between dissolution testing and bioavailability can be developed:

1. By establishing a relationship, usually linear, between the *in vitro* dissolution and the *in vivo* bioavailability parameters, and

2. By using the data from previous bioavailability studies to modify the dissolution methodology in order to arrive at meaningful *in vitro-in vivo* correlation.

Though the former approach is widely used, the latter still holds substance, since to date, there is no single dissolution rate test methodology that can be applied to all drugs.

Some of the often used quantitative linear *in vitro-in vivo* correlations are:

1. Correlations Based on the Plasma Level Data : Here linear relationships between dissolution parameters such as percent drug dissolved, rate of dissolution, rate constant for dissolution, etc. and parameters obtained from plasma level data such as percent drug absorbed, rate of absorption, C_{max}, t_{max}, K_a, etc. are developed; for example, percent drug dissolved versus percent drug absorbed plots.

2. Correlation Based on the Urinary Excretion Data : Here, dissolution parameters are correlated to the amount of drug excreted unchanged in the urine, cumulative amount of drug excreted as a function of time, etc.

3. Correlation Based on the Pharmacologic Response : An acute pharmacologic effect such as LD_{50} in animals is related to any of the dissolution parameters.

Statistical moments theory can also be used to determine the relationship such as mean dissolution time (*in vitro*) versus mean residence time (*in vivo*).

Though examples of good correlations are many, there are instances when positive correlation is difficult or impossible; for example, in case of corticosteroids, the systemic availability may not depend upon the dissolution characteristics of the drug. Several factors that limit such a correlation include variables pertaining to the drug such as dissolution methodology, physicochemical properties of the drug such as particle size, physiologic variables like presystemic metabolism, etc.

BIOEQUIVALENCE STUDIES

It is commonly observed that there are several formulations of the same drug, in the same dose, in a similar dosage form and meant to be given by the same route. Substitution of one product for another can be made provided they are equally effective therapeutically as the standard accepted. In order to ensure clinical performance of such drug products, bioequivalence studies should be performed. Some of the important terms relevant in this context will be defined.

Equivalence : It is a relative term that compares drug products with respect to a specific characteristic or function or to a defined set of standards. There are several types of equivalences.

Chemical Equivalence : It indicates that two or more drug products contain the same labeled chemical substance as an active ingredient in the same amount.

Pharmaceutic Equivalence : This term implies that two or more drug products are identical in strength, quality, purity, content uniformity and disintegration and dissolution characteristics; they may however differ in containing different excipients.

Bioequivalence : It is a relative term which denotes that the drug substance in two or more identical dosage forms, reaches the systemic circulation at the same relative rate and to the same relative extent i.e. their plasma concentration-time profiles will be identical without significant statistical differences.

When statistically significant differences are observed in the bioavailability of two or more drug products, **bioinequivalence** is indicated.

Therapeutic Equivalence : This term indicates that two or more drug products that contain the same therapeutically active ingredient, elicit identical pharmacologic effects and can control the disease to the same extent.

The *in vivo* bioequivalence study requires determination of relative bioavailability after administration of a single dose of test and reference formulations by the same route, in equal doses, but at different times. The reference product is generally a previously approved product, usually the innovator's product or some suitable reference standard. The study is performed in fasting, young, healthy, adult male volunteers to assure homogeneity in the population and to spare the patients, elderly or pregnant women from rigors of such a clinical investigation. Homogeneity in the study population permits focus on formulation factors. The volunteers

are used in complete, open-label cross-over fashion such as the **Latin square cross-over design** in which

—each formulation is administered just once to each subject and once in each study period, and

—unlike parallel design, all the subjects do not receive the same formulation at the same time; in a given study period, they are administered different formulations.

Such a randomized, balanced, cross-over study has several *advantages*—

1. It minimizes the intersubject variability in plasma drug levels.
2. Minimizes the carry-over effects which could occur when a given dosage form influences the bioavailability of a subsequently administered product (intra-subject variability).
3. Minimizes the variations due to time effect, and thus,
4. Makes it possible to focus more on the formulation variables which is the key to success for any bioequivalence study.

An example of Latin square design for a bioequivalence study in human volunteers is given in Table 12.1.

TABLE 12.1

Latin Square Cross-over Design for 6 (or 12) Subjects to Compare Three Different Formulations, X, Y and Z

Subject	*Drug Formulation*		
Number	*Study Period 1*	*Study Period 2*	*Study Period 3*
1.7	X	Y	Z
2.8	Y	Z	X
3.9	Z	X	Y
4.10	X	Z	Y
5.11	Z	Y	X
6.12	Y	X	Z

A *drawback* of such a cross-over design is that the study takes a long time since an appropriate washout period between two administrations is essential which may be very long if the drug has a long $t_{1/2}$. Moreover when the number of formulations to be tested are more, the study becomes more difficult and subject dropout rates are also high. This can be overcome by use of a balanced incomplete block design in which a subject receives no more than 2 formulations.

As for bioavailability studies, either plasma level or urinary excretion studies may be performed to assess bioequivalence between drug products. *In vitro-in vivo* correlation can also be established for the formulations.

It is always easier to establish bioequivalence between existing drug products than determination of pharmacokinetics of a new drug or bioavailability of a new dosage form since—

1. The human volunteers used for the study of both products are same and all pharmacokinetic parameters can be assumed to be same for both drug formulations and there is no need to investigate nonlinearity.
2. The study protocol for all subjects is uniform, the efficiency of drug absorption from both formulations can be considered as same and thus differences in absorption pattern can be ascribed to differences in drug release from the two dosage form.

Statistical Interpretation of Bioequivalence Data

After the data has been collected, statistical methods must be applied to determine the level of significance of any observed difference in the rate and/or extent of absorption in order to establish bioequivalence between two or more drug products. Typically, an **analysis of variance** (ANOVA) method is applied to determine statistical differences. If a statistically significant difference is observed, it is important to determine if it is clinically significant. Quite often, it is found that for e.g. a statistically significant difference of 10% in the extent of absorption between the two formulations is insignificant clinically. Currently, a simple acceptable rule is that if the relative bioavailability of the test formulation is in the range 80 to 120% of reference standard, it is considered bioequivalent. Another accepted opinion is that the difference between the bioavailabilities of the test formulations should not be greater than ± 20% of the average of reference standard.

METHODS FOR ENHANCEMENT OF BIOAVAILABILITY

As far as the definition of bioavailability is concerned, a drug with poor bioavailability is the one with -

1. Poor aqueous solubility and/or slow dissolution rate in the biologic fluids.
2. Poor stability of the dissolved drug at the physiologic pH.
3. Inadequate partition coefficient and thus poor permeation through the biomembrane.
4. Extensive presystemic metabolism.

The three major approaches in overcoming the bioavailability problems due to such causes are:

1. **The Pharmaceutic Approach** which involves modification of formulation, manufacturing process or the physicochemical properties of the drug without changing the chemical structure.
2. **The Pharmacokinetic Approach** in which the pharmacokinetics of the drug is altered by modifying its chemical structure.
3. **The Biologic Approach** whereby the route of drug administration may be changed such as changing from oral to parenteral route.

The second approach of chemical structure modification has a number of drawbacks of being very expensive and time consuming, requires repetition of clinical studies and a long time for regulatory approval. Moreover, the new chemical entity may suffer from another pharmacokinetic disorder or bear the risk of precipitating adverse effects. Only the pharmaceutic approach will be dealt herewith.

The attempts, whether optimizing the formulation, manufacturing process or physicochemical properties of the drug, are mainly aimed at enhancement of dissolution rate as it is the major rate-limiting step in the absorption of most drugs. There are several ways in which the dissolution rate of a drug can be enhanced. Some of the widely used methods, most of which are aimed at increasing the effective surface area of the drugs, will be discussed briefly.

1. Micronization : The process involves reducing the size of the solid drug particles to 1 to 10 microns commonly by spray drying or by use of air attrition methods (fluid energy mill). Examples of drugs whose bioavailability have been increased by micronization include griseofulvin and several steroidal and sulfa drugs.

2. Use of Surfactants : The surface-active agents enhance dissolution rate primarily by promoting wetting and penetration of dissolution fluid into the solid drug particles. They are generally used in concentration below their critical micelle concentration (CMC) values since above CMC, the drug entrapped in the micelle structure fails to partition in the dissolution fluid. Nonionic surfactants like polysorbates are widely used. Examples of drugs whose bioavailability have been increased by use of surfactants in the formulation include steroids like spironolactone.

3. Use of Salt Forms : Salts have improved solubility and dissolution characteristics in comparison to the original drug. Alkali metal salts of acidic drugs like penicillins and strong acid salts of basic drugs like atropine are more water-soluble than the parent drug.

4. Alteration of pH of the Drug Microenvironment : This can be achieved in two ways—*in situ* salt formation, and addition of buffers to the formulation e.g. buffered aspirin tablets.

5. Use of Metastable Polymorphs : A metastable polymorph is more soluble than the stable polymorph of a drug that exhibits polymorphism—for example, the B form of chloramphenicol palmitate is more water soluble than the A and the C forms.

6. Solute-Solvent Complexation : Solvates of drugs with organic solvents (also called as pseudopolymorphs) generally have higher aqueous solubility than their respective hydrates or the original drug. Much higher solubility can be attained by freeze drying such a solute in solution with an organic solvent with which it is known to form a solvate. Such a process results in a powder of particles of submicron size, e.g. 1:2 griseofulvin-benzene solvate. However, one should take care that the solvent is nontoxic.

7. Solvent Deposition : In this method, the poorly aqueous soluble drug such as nifedipine is dissolved in an organic solvent like alcohol and deposited on an inert, hydrophilic, solid matrix such as starch or micro-crystalline cellulose by evaporation of solvent.

8. Selective Adsorption on Insoluble Carriers : A highly active adsorbent such as the inorganic clays like bentonite can enhance the dissolution rate of poorly water soluble drugs such as griseofulvin, indomethacin and prednisone by maintaining the concentration gradient at its maximum. The two reasons suggested for the rapid release of drugs from the surface of clays are—the weak physical bonding between the adsorbate and the adsorbent, and hydration and swelling of the clay in the aqueous media.

9. Solid Solutions : The three means by which the particle size of a drug can be reduced to submicron level are—

i. use of solid solutions,
ii. use of eutectic mixtures, and
iii. use of solid dispersions.

In all these cases, the solute is frequently a poorly water soluble drug acting as the **guest** and the solvent is a highly water-soluble compound or polymer acting as a **host** or **carrier**.

A **solid solution** *is a binary system comprising of a solid solute molecularly dispersed in a solid solvent.* Since the two components crystallize together in a homogeneous one phase system, solid solutions

are also called as **molecular dispersions** or **mixed crystals**. Because of reduction in particle size to the molecular level, solid solutions show greater aqueous solubility and faster dissolution than eutectics and solid dispersions. They are generally prepared by fusion method whereby a physical mixture of solute and solvent are melted together followed by rapid solidification. *Such systems, prepared by fusion, are often called as* **melts** e.g. griseofulvin-succinic acid (Fig. 12.4). The griseofulvin from such solid solution dissolves 6 to 7 times faster than pure griseofulvin.

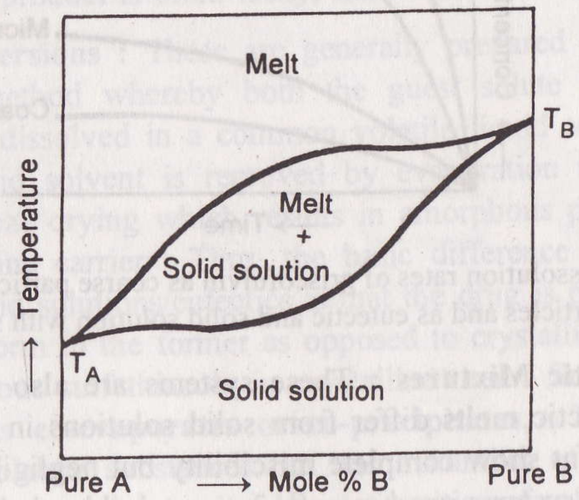


Fig. 12.4 Binary phase diagram for continuous solid solution of A and B. T_A and T_B are melting points of pure A and pure B respectively.

If the diameter of solute molecules is less than 60% of diameter of solvent molecules or its volume less than 20% of volume of solvent molecule, the solute molecule can be accommodated within the intermolecular spaces of solvent molecules e.g. digitoxin-PEG 6000 solid solution. Such systems show faster dissolution. *When the resultant solid solution is a homogeneous transparent and brittle system, it is called as* **glass solution**. Carriers that form glassy structure are citric acid, urea, PVP and PEG and sugars such as dextrose, sucrose and galactose.

The two mechanisms suggested for enhanced solubility and rapid dissolution of molecular dispersions are:

1. When the binary mixture is exposed to water, the soluble carrier dissolves rapidly leaving the insoluble drug in a state of micro-crystalline dispersion of very fine particles, and
2. When the solid solution, which is said to be in a state of randomly arranged solute and solvent molecules in the crystal lattice, is exposed to the dissolution fluid, the soluble carrier dissolves rapidly leaving the insoluble drug stranded at almost molecular level.

Fig. 12.5 shows a comparison between the dissolution rates of different forms of griseofulvin.

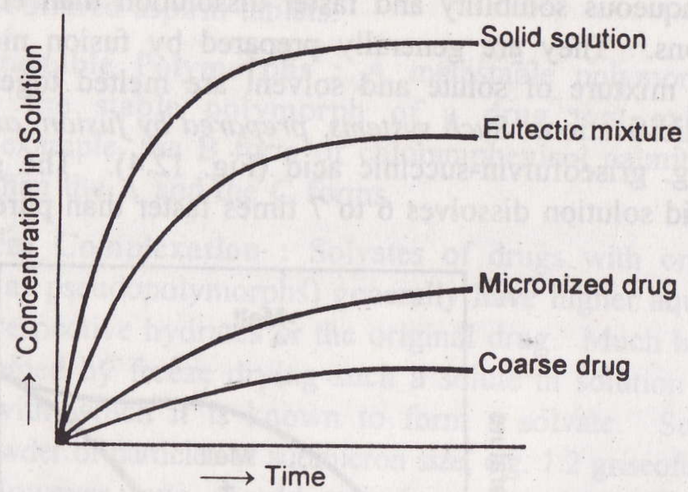


Fig. 12.5 Dissolution rates of griseofulvin as coarse particles, as micronized particles and as eutectic and solid solution with succinic acid.

10. Eutectic Mixtures : These systems are also prepared by fusion method. Eutectic melts differ from solid solutions in that the fused melt of solute-solvent show complete miscibility but negligible solid-solid solubility i.e. *such systems are basically intimately blended physical mixture of two crystalline components.* A phase diagram of two component system is shown in Fig. 12.6. When the eutectic mixture is exposed to water, the soluble carrier dissolves leaving the drug in a microcrystalline state which solubilizes rapidly.

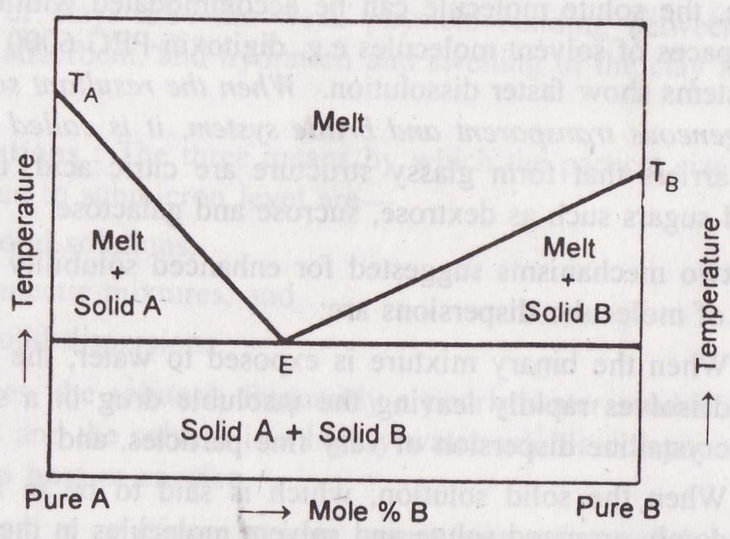


Fig. 12.6 Simple binary phase diagram showing eutectic point E. The eutectic composition at point E of substances A and B represents the one having lowest melting point. T_A and T_B are melting points of pure A and pure B respectively.

Examples of eutectics include paracetamol-urea, griseofulvin-urea, griseofulvin-succinic acid, etc. Solid solutions and eutectics, which are basically melts, are easy to prepare and economical with no solvents involved. The method however cannot be applied to:

—drugs which fail to crystallize from the mixed melt,

—thermolabile drugs, and

—carriers such as succinic acid that decompose at their melting point.

The eutectic product is often tacky, intractable or irregular crystals.

11. Solid Dispersions : These are generally prepared by **solvent** or **co-precipitation** method whereby both the guest solute and the solid carrier solvent are dissolved in a common volatile liquid solvent such as alcohol. The liquid solvent is removed by evaporation under reduced pressure or by freeze drying which results in amorphous precipitation of guest in a crystalline carrier. Thus, the basic difference between solid dispersions and solid solutions/eutectics is that the drug is precipitated out in an amorphous form in the former as opposed to crystalline form in the latter; e.g. amorphous sulfathiazole in crystalline urea. Such dispersions are often called as **co-evaporates** or **co-precipitates**. The method is suitable for thermolabile substances but has a number of disadvantages like higher cost of processing, use of large quantities of solvent, difficulty in complete removal of solvent, etc. The carriers used are same as for eutectics or solid solutions. With glassy materials, the dispersions formed are called as **glass dispersions** or **glass suspensions**. Fig. 12.7. shows comparative dissolution rates of griseofulvin from PVP dispersions.

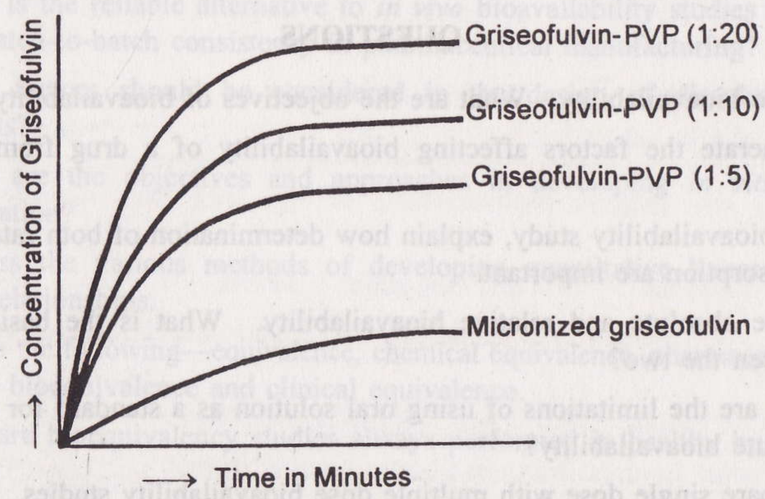


Fig. 12.7 Dissolution rate enhancement of griseofulvin by solid dispersion technique.

12. Molecular Encapsulation with Cyclodextrins : The beta- and gamma-cyclodextrins and several of their derivatives are unique in having the ability to form molecular inclusion complexes with hydrophobic drugs having poor aqueous solubility. These cyclodextrin molecules are versatile in having a hydrophobic cavity of size suitable enough to accommodate the lipophilic drugs as guests; the outside of the host molecule is relatively hydrophilic (Fig. 12.8). Thus, the molecularly encapsulated drug has greatly improved aqueous solubility and dissolution rate. There are several examples of drugs with improved bioavailability due to such a phenomena—thiazide diuretics, barbiturates, benzodiazepines and a number of NSAIDs.

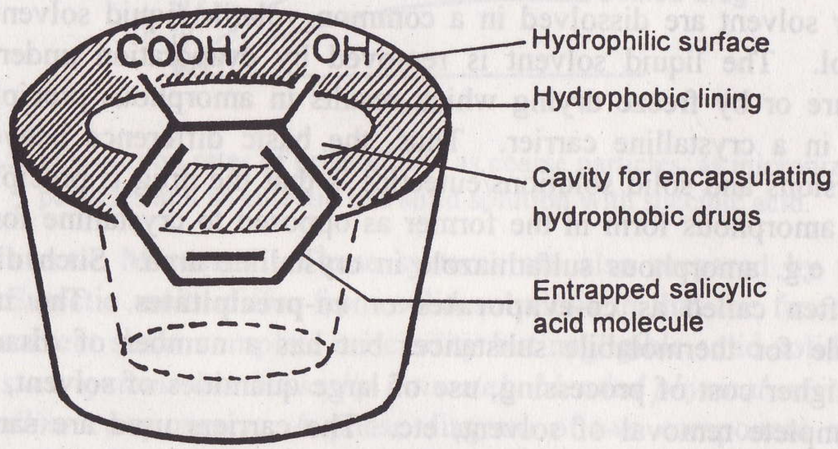


Fig. 12.8 Functional and structural feature of a cyclodextrin molecule showing an encapsulated drug.

QUESTIONS

1. Define bioavailability. What are the objectives of bioavailability studies?
2. Enumerate the factors affecting bioavailability of a drug from its dosage form.
3. In a bioavailability study, explain how determination of both rate and extent of absorption are important.
4. Define absolute and relative bioavailability. What is the basic difference between the two?
5. What are the limitations of using oral solution as a standard for determining absolute bioavailability?
6. Compare single dose with multiple dose bioavailability studies.
7. Discuss the merits and demerits of using healthy subjects and patients as volunteers for bioavailability studies.

8. What should be the duration of washout period between any two bioavailability studies in the same subject? Why?
9. Name the methods for determining bioavailability of a drug from its dosage form.
10. Which is the method of choice in bioavailability determination? On what principle is such a study based?
11. Explain with significance the parameters used in bioavailability determination by plasma level studies.
12. In multiple dose study, bioavailability determination is done at steady-state and one dosing interval. Explain.
13. Why is determination of absorption rate not considered important in the multiple dosing method?
14. What is the principle behind assessment of bioavailability using urinary excretion studies?
15. Determination of metabolites in urine is not used as a measure of bioavailability? Why?
16. Why should the volunteers be instructed to completely empty their bladders while giving urine samples?
17. Why should frequent sampling of urine/plasma be done initially after drug administration in bioavailability determination?
18. Name the parameters examined in urinary excretion data to determine bioavailability. What is their analogy with parameters of plasma level studies?
19. What are the drawbacks of using acute pharmacologic response and therapeutic response as measures of bioavailability?
20. What is the reliable alternative to *in vivo* bioavailability studies in monitoring batch-to-batch consistency in pharmaceutical manufacturing?
21. What factors should be considered in the design of dissolution testing models?
22. What are the objectives and approaches in developing *in vitro- in vivo* correlation?
23. Discuss the various methods of developing quantitative linear *in vitro-in vivo* relationships.
24. Define the following—equivalence, chemical equivalence, pharmaceutic equivalence, bioequivalence and clinical equivalence.
25. Why are bioequivalency studies always performed in healthy human volunteers?
26. What are the advantages and limitations of randomized, balanced, cross-over study in bioequivalence determination?

27. Why is it easy to establish bioequivalence between dosage forms in comparison to determination of pharmacokinetics or bioavailability of a new formulation?

28. What are the generally accepted, statistical rules for establishing bioequivalence between formulations?

29. Layout a Latin square cross-over diagram for bioequivalency study on three formulations—A, B and C, in six volunteers.

30. What are the various approaches aimed at enhancing bioavailability of a drug from its dosage form?

31. Discuss the methods aimed at enhancing bioavailability through enhancement of drug dissolution rate.

32. What are the various means by which the particle size of a drug can be reduced to submicron level?

33. What is the basic difference between solid solutions and solid dispersions? Give reasons for the faster dissolution of the former.

34. Solid solutions dissolve faster than eutectics. Why?

35. A new drug undergoes both degradation in gastric fluids as well as extensive presystemic hepatic metabolism. In order to obtain optimum oral bioavailability, which of the following formulations will you prefer : solution, suspension, tablet, enteric coated tablet, S.R. tablet or enteric coated microspheres? Why? Rank them in order of decreasing bioavailability.

36. Following data is obtained for 4 formulations of pentoxifylline in volunteers of average weight 50 Kg.

Drug Product	*Dose (mg/kg)*	*AUC (mcg.hr/l)*
i.v. solution	1.2	450
oral solution	4.0	822
oral capsule	4.0	736
oral S.R. tablet	8.0	1040

a. What is the absolute bioavailability of the drug from capsule and S.R. tablet?

Answer : Capsule = 49% and S.R.tablet = 34.6%.

b. What is the relative bioavailability of capsule and S.R.tablet against oral solution?

Answer : Capsule = 89.5% and S.R.tablet = 63.2%.

c. Which solid formulation shows better bioavailability?

d. Are the two solid formulations bioequivalent?

e. What could be the possible reasons for poor bioavailability from S.R. tablets?

37. The three pharmacokinetic parameters from urinary excretion data of a drug given as 50 mg oral formulations of two different companies, of which A is the innovator's product, are as follows:

Parameters	*Formulation*	
	A	*B*
$(dX_u/dt)_{max}$ (mg/hr)	6.0	8.0
$(t_u)_{max}$ (hour)	2.0	1.0
$X_{u,\infty}$ (mg)	39.1	35.9

a. What is the relative availability of formulation B against A?

Answer : 92.0%.

b. Are the two formulations bioequivalent?

c. If the drug is meant for the treatment of an acute condition, which one of the two formulations is better?

13

Applications of Pharmacokinetic Principles

The time course of drug concentration in the body after its administration can be defined by a number of pharmacokinetic parameters. Often, the information gained about the pharmacokinetics of one drug helps in anticipating the pharmacokinetics of another. The knowledge of pharmacokinetic behavior of a drug coupled with important pharmacodynamic parameters like therapeutic index can be put to several applications:

1. Design and development of new drugs with greatly improved therapeutic effectiveness and fewer or no toxic effects
2. Design and development of an optimum formulation for better use of the drug
3. Design and development of controlled/targeted release formulation
4. Select the appropriate route for drug administration
5. Select the right drug for a particular illness
6. Predict and explain drug-food and drug-drug interactions
7. Design an appropriate multiple dosage regimen
8. Therapeutic drug monitoring in individual patients
9. Dosage adjustments in situations of altered physiology and drug interactions.

The applications of pharmacokinetic principles are mainly aimed at achieving the therapeutic objective. *The* **therapeutic objective** *is often control or cure of the condition in shortest possible time with minimum side effects by the use of least amount of drug.* New drug development or chemical modification is frequently done to improve pharmacokinetic properties and increase efficacy. Drug product design is aimed at optimizing bioavailability or better control/cure of illness through controlled or targeted release. Proper choice of route of administration is necessary to ensure that the drug moves to the site of action at a sufficiently rapid rate

and amount. Selection of a suitable drug is based on achieving optimal therapy by balancing the desirable and undesirable effects. Co-administration of several drugs to a patient may lead to changes in the pharmacokinetic profile of a drug which is indicative of interactions between drugs. Such an understanding of interaction makes possible more rational use of drugs that have to be co-administered.

Clinically, the two most important applications of pharmacokinetic principles are:

1. Design of an optimal dosage regimen, and
2. Clinical management of individual patient and therapeutic drug monitoring.

Such applications permit the physician to use certain drugs more safely and sensibly.

DESIGN OF DOSAGE REGIMENS

Dosage regimen *is defined as the manner in which a drug is taken.* For some drugs like analgesics, hypnotics, antiemetics, etc., a single dose may provide effective treatment. However, the duration of most illnesses is longer than the therapeutic effect produced by a single dose. In such cases, drugs are required to be taken on a repetitive basis over a period of time depending upon the nature of illness. Thus, for successful therapy, design of an optimal multiple dosage regimen is necessary. *An* **optimal multiple dosage regimen** *is the one in which the drug is administered in suitable doses (by a suitable route), with sufficient frequency that ensures maintenance of plasma concentration within the therapeutic window (without excessive fluctuations and drug accumulation) for the entire duration of therapy.* For some drugs like antibiotics, a minimum effective concentration should be maintained at all times and for drugs with narrow therapeutic indices like phenytoin, attempt should be made not to exceed toxic concentration.

In designing a dosage regimen—

1. It is assumed that all pharmacokinetic parameters of the drug remain constant during the course of therapy once a dosage regimen is established; the same becomes invalid if any change is observed.
2. The calculations are based on open one-compartment model which can also be applied to two compartment model if ß is used instead of K_E, and $V_{d,ss}$ instead of V_d while calculating the regimen.

Irrespective of the route of administration and complexity of pharmacokinetic equations, the two major parameters that can be adjusted in developing a dosage regimen are—

1. The dose size—the quantity of drug administered each time, and
2. The dosing frequency—the time interval between doses.

Both parameters govern the amount of drug in the body at any given time.

Dose Size

The magnitude of both therapeutic and toxic responses depends upon dose size. Dose size calculation also requires the knowledge of amount of drug absorbed after administration of each dose. Greater the dose size, greater the fluctuations between $C_{ss,max}$ and $C_{ss,min}$ during each dosing interval and greater the chances of toxicity (Fig. 13.1).

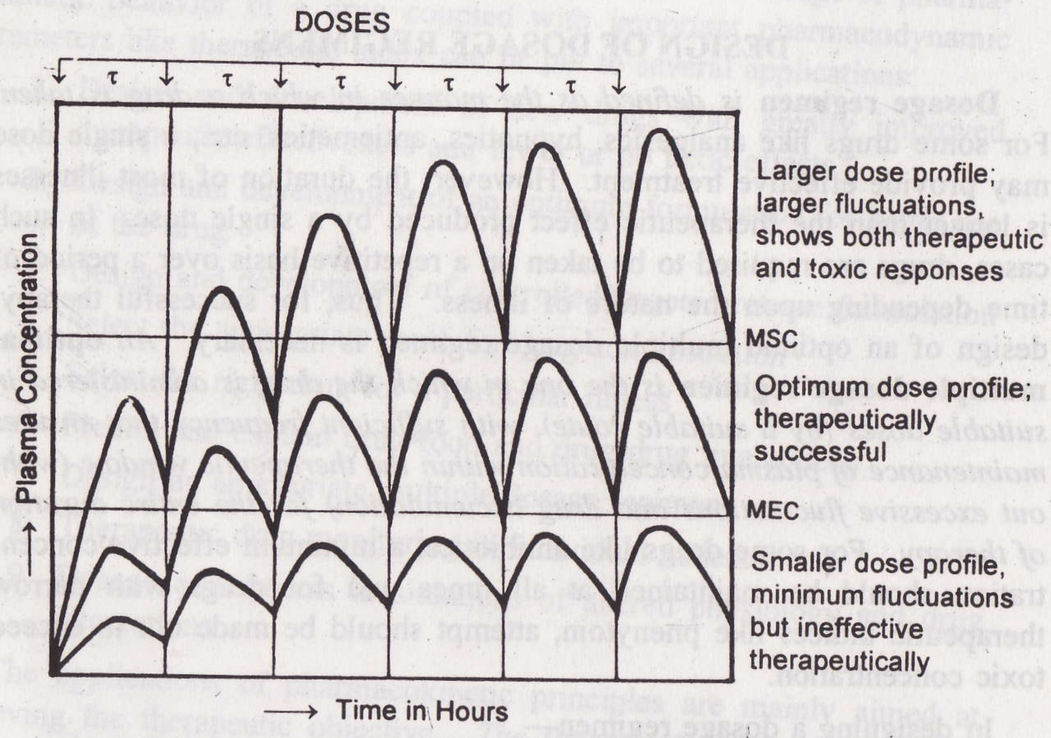


Fig. 13.1 Schematic representation of influence of dose size on plasma concentration-time profile after oral administration of a drug at fixed intervals of time.

Dosing Frequency

The dose interval (inverse of dosing frequency) is calculated on the basis of half-life of the drug. If the interval is increased and the dose is unchanged, C_{max}, C_{min} and C_{av} decrease but the ratio C_{max}/C_{min} increases. Opposite is observed when dosing interval is reduced or dosing

frequency increased. It also results in greater drug accumulation in the body and toxicity (Fig. 13.2).

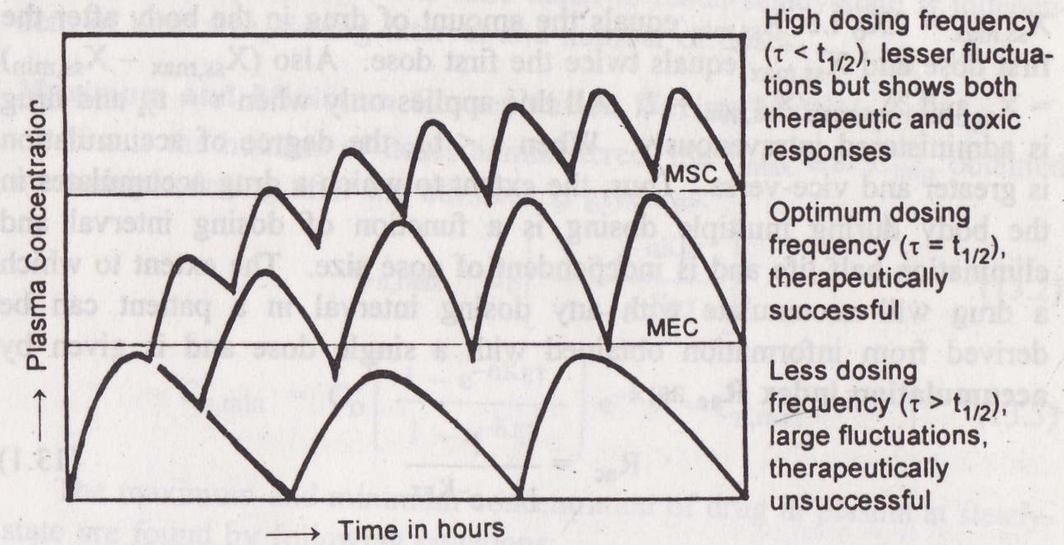


Fig. 13.2 Schematic representation of the influence of dosing frequency on plasma concentration-time profile obtained after oral administration of fixed doses of a drug.

A proper balance between both dose size and dosing frequency is often desired to attain steady-state concentration with minimum fluctuations and to ensure therapeutic efficacy and safety. The same cannot be obtained by giving larger doses less frequently. However, administering smaller doses more frequently results in smaller fluctuations. A drug with a narrow therapeutic index such as digoxin is best administered in small doses at frequent intervals, usually less than the $t_{½}$ of the drug, to obtain a profile with least fluctuations which is similar to that observed with constant rate infusion or controlled release system.

Drug Accumulation During Multiple Dosing

Consider the amount of drug in the body-time profile shown in Fig. 13.3. obtained after i.v. multiple dosing with dosing interval equal to one $t_{½}$.

After the administration of first dose X_0 at $\tau = 0$, the amount of drug in the body will be $X = 1X_0$. At the next dosing interval when $X = ½X_0$, the amount of drug remaining in the body, administration of the next i.v. dose raises the body content to $X = X_0 + ½X_0$ i.e. drug accumulation occurs in the body. Thus, *accumulation occurs because drug from previous doses has not been removed completely*. As the amount of drug in the body rises gradually due to accumulation, the rate of elimination also rises proportionally until a steady-state or plateau is reached when the rate

of drug entry into the body equals the rate of exit. The maximum and minimum values of X i.e. $X_{ss,max}$ and $X_{ss,min}$ approach respective asymptotes at plateau. It is interesting to note that at plateau, $X_{ss,min} = 1X_o$ and $X_{ss,max} = 2X_o$ i.e. $X_{ss,min}$ equals the amount of drug in the body after the first dose and $X_{ss,max}$ equals twice the first dose. Also $(X_{ss,max} - X_{ss,min}) = X_o$ and $X_{ss,max}/X_{ss,min} = 2$. All this applies only when $\tau = t_{½}$ and drug is administered intravenously. When $\tau < t_{½}$, the degree of accumulation is greater and vice-versa. Thus, the extent to which a drug accumulates in the body during multiple dosing is a function of dosing interval and elimination half-life and is independent of dose size. The extent to which a drug will accumulate with any dosing interval in a patient can be derived from information obtained with a single dose and is given by **accumulation index R_{ac}** as:

$$R_{ac} = \frac{1}{1 - e^{-K_E\tau}} \qquad (13.1)$$

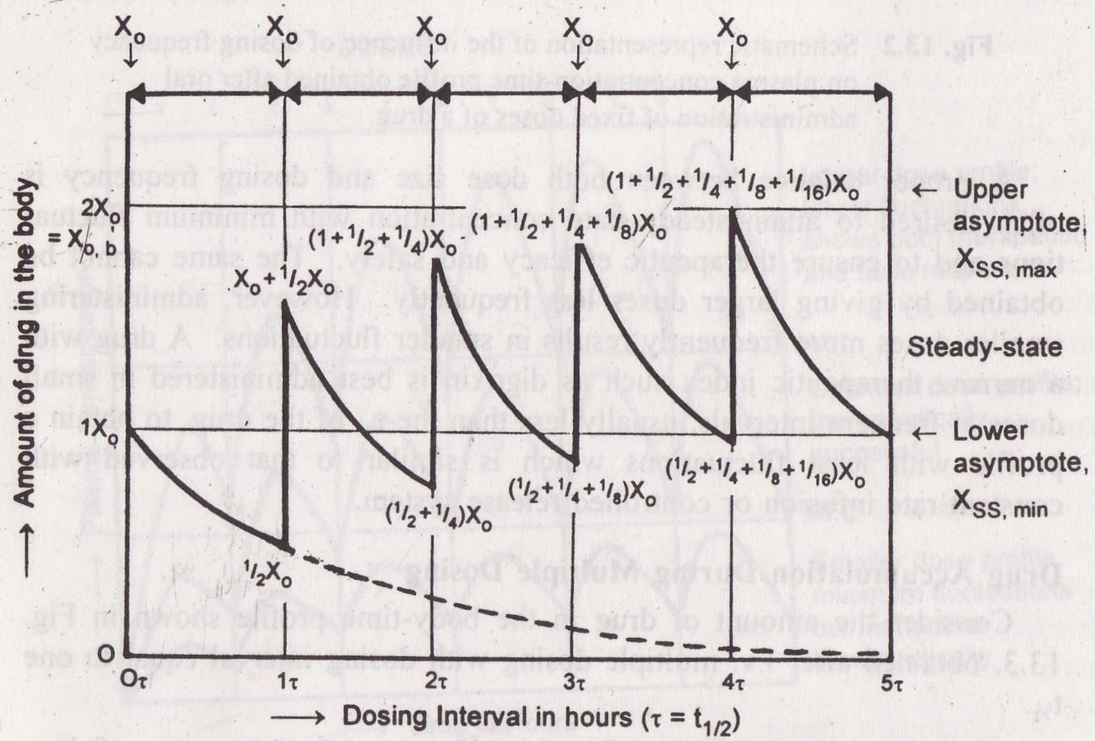


Fig.13.3 Accumulation of drug in the body during multiple dose regimen of i.v. bolus with dosing interval equal to one half-life of the drug. Approximately 5 half-lives are required for attainment of steady-state.

Time to Reach Steady-State During Multiple Dosing

The time required to reach steady-state depends primarily upon the half-life of the drug. Provided $K_a >> K_E$, the plateau is reached in approximately 5 half-lives. This is called as **plateau principle**. It also

means that the rate at which the multiple dose steady-state is reached is determined only by K_E. The time taken to reach steady-state is independent of dose size, dosing interval and number of doses.

Maximum and Minimum Concentration During Multiple Dosing

If n is the number of doses administered, the C_{max} and C_{min} obtained on multiple dosing after the nth dose is given as:

$$C_{n,max} = C_o \left[\frac{1 - e^{-nK_E\tau}}{1 - e^{-K_E\tau}} \right] \qquad (13.2)$$

$$C_{n,min} = C_o \left[\frac{1 - e^{-nK_E\tau}}{1 - e^{-K_E\tau}} \right] e^{-K_E\tau} = C_{n,max}\, e^{-K_E\tau} \qquad (13.3)$$

The maximum and minimum concentration of drug in plasma at steady-state are found by following equations:

$$C_{ss,max} = \frac{C_o}{1 - e^{-K_E\tau}} \qquad (13.4)$$

$$C_{ss,min} = \frac{C_o\, e^{-K_E\tau}}{1 - e^{-K_E\tau}} = C_{ss,max}\, e^{-K_E\tau} \qquad (13.5)$$

where C_o = concentration that would be attained from instantaneous absorption and distribution (obtained by extrapolation of elimination curve to time zero). Equations 13.2 to 13.5 can also be written in terms of amount of drug in the body. Fraction of dose absorbed, F, should be taken into account in such equations.

Fluctuation is defined as the ratio C_{max}/C_{min}. Greater the ratio, greater the fluctuation. Like accumulation, it depends upon dosing frequency and half-life of the drug. It also depends upon the rate of absorption. The greatest fluctuation is observed when the drug is given as i.v. bolus. Fluctuations are small when the drug is given extravascularly because of continuous absorption.

Average Concentration and Body Content on Multiple Dosing to Steady-State

The average drug concentration at steady-state $C_{ss,av}$ is a function of maintenance dose X_o, the fraction of dose absorbed F, the dosing interval τ and clearance Cl_T (or V_d and K_E or $t_{½}$) of the drug.

$$C_{ss,av} = \frac{F\, X_o}{Cl_T\, \tau} \qquad (13.6)$$

$$= \frac{1.44\ F\ X_o\ t_{½}}{V_d\ \tau} = \frac{\text{AUC (single dose)}}{\tau} \qquad (13.7)$$

where the coefficient 1.44 is the reciprocal of 0.693 in equation 13.7. AUC is the area under the curve following a single maintenance dose. Equation 13.7 can be used to calculate maintenance dose of a drug to achieve a desired concentration. Since $X = V_d\ C$, the body content at steady-state is given as:

$$X_{ss,av} = \frac{1.44\ F\ X_o\ t_{½}}{\tau} \qquad (13.8)$$

These average values are not arithmetic mean of $C_{ss,max}$ and $C_{ss,min}$ since the plasma drug concentration declines exponentially.

Loading and Maintenance Doses

A drug does not show therapeutic activity unless it reaches the desired steady-state. It takes about 5 half-lives to attain it and therefore the time taken will be too long if the drug has a long half-life. Plateau can be reached immediately by administering a dose that gives the desired steady-state instantaneously before the commencement of maintenance doses X_o. *Such an initial or first dose intended to be therapeutic is called as* **priming dose** or **loading dose** $X_{o,L}$. A simple equation for calculating loading dose is:

$$X_{o,L} = \frac{C_{ss,av}\ V_d}{F} \qquad (13.9)$$

After e.v. administration, C_{max} is always smaller than that after i.v. administration and hence loading dose is proportionally smaller. For drugs having low therapeutic indices, the loading dose may be divided into smaller doses to be given at various intervals before the first maintenance dose. When V_d is not known, loading dose may be calculated by the following equation:

$$\frac{X_{o,L}}{X_o} = \frac{1}{(1 - e^{-K_a\tau})(1 - e^{-K_E\tau})} \qquad (13.10)$$

The above equation applies when $K_a >> K_E$ and drug is distributed rapidly. When the drug is given i.v. or when absorption is extremely rapid, the absorption phase is neglected and the above equation reduces to:

$$\frac{X_{o,L}}{X_o} = \frac{1}{(1 - e^{-K_E\tau})} = R_{ac} \qquad (13.11)$$

The ratio of loading dose to maintenance dose $X_{o,L}/X_o$ *is called as* **dose ratio**. As a rule, when $\tau = t_{½}$, dose ratio should be equal to 2.0 but must be smaller than 2.0 when $\tau > t_{½}$ and greater when $\tau < t_{½}$. Fig. 13.4. shows that if loading dose is not optimum, either too low or too high, the steady-state is attained within approximately 5 half-lives in a manner similar to when no loading dose is given.

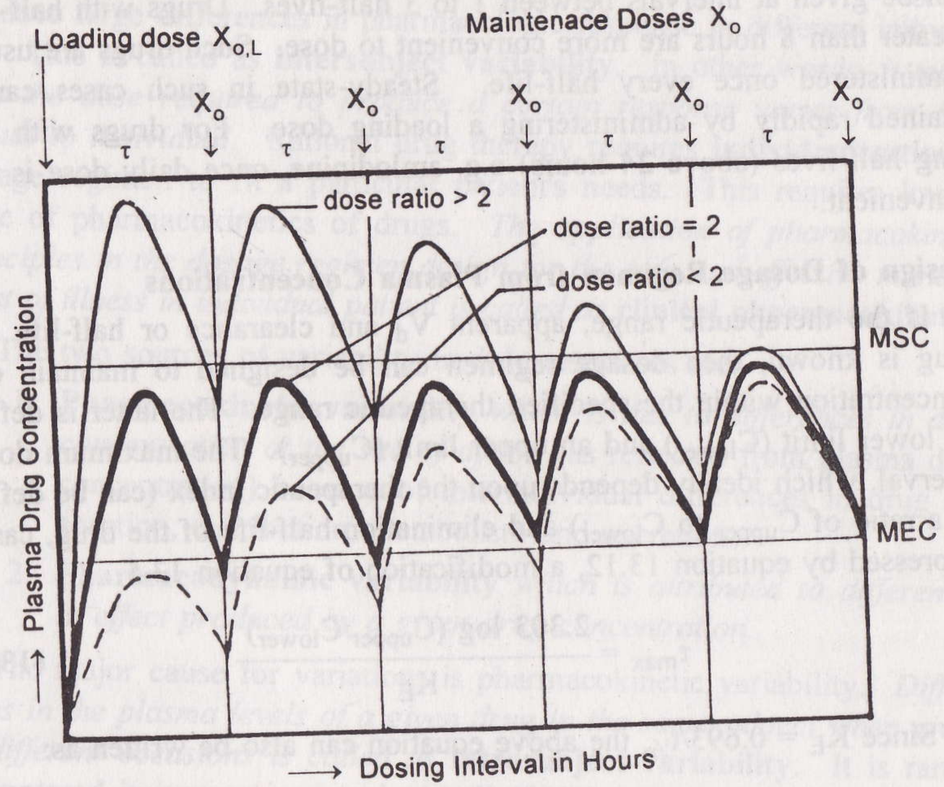


Fig. 13.4 Schematic representation of plasma concentration-time profiles that result when dose ratio is greater than 2.0, equal to 2.0 and smaller than 2.0.

The foregoing discussion and the equations expressed until now apply only to drugs that follow one-compartment kinetics with first-order disposition. Equations will be complex for multicompartment models.

Maintenance of Drug within the Therapeutic Range

The ease or difficulty in maintaining drug concentration within the therapeutic window depends upon—

1. The therapeutic index of the drug.
2. The half-life of the drug.
3. Convenience of dosing.

It is extremely difficult to maintain such a level for a drug with short half-life (less than 3 hours) and narrow therapeutic index e.g. heparin,

since the dosing frequency has to be essentially less than $t_{1/2}$. However, drugs such as penicillin ($t_{1/2}$ = 0.9 hours) with high therapeutic index may be given less frequently (every 4 to 6 hours) but the maintenance dose has to be larger so that the plasma concentration persists above the minimum inhibitory level. A drug with intermediate $t_{1/2}$ (3 to 8 hours) may be given at intervals $\tau \leq t_{1/2}$ if therapeutic index is low and those with high indices can be given at intervals between 1 to 3 half-lives. Drugs with half-lives greater than 8 hours are more convenient to dose. Such drugs are usually administered once every half-life. Steady-state in such cases can be attained rapidly by administering a loading dose. For drugs with very long half-lives (above 24 hours) e.g. amlodipine, once daily dose is very convenient.

Design of Dosage Regimen from Plasma Concentrations

If the therapeutic range, apparent V_d and clearance or half-life of a drug is known, then dosage regimen can be designed to maintain drug concentration within the specified therapeutic range. The latter is defined by lower limit (C_{lower}) and an upper limit (C_{upper}). The maximum dosing interval, which ideally depends upon the therapeutic index (can be defined as a ratio of C_{upper} to C_{lower}) and elimination half-life of the drug, can be expressed by equation 13.12, a modification of equation 13.5.

$$\tau_{max} = \frac{2.303 \log (C_{upper}/C_{lower})}{K_E} \qquad (13.12)$$

Since $K_E = 0.693/t_{1/2}$, the above equation can also be written as:

$$\tau_{max} = 3.32\, t_{1/2} \log (C_{upper}/C_{lower}) \qquad (13.13)$$

Understandably, the dosing interval selected is always smaller than τ_{max}. The maximum maintenance dose $X_{o,max}$ that can be given every τ_{max} is expressed as:

$$X_{o,max} = \frac{V_d (C_{upper} - C_{lower})}{F} \qquad (13.14)$$

After a convenient dosing interval τ, smaller than τ_{max} has been selected, the maintenance dose is given as:

$$X_o = \left[\frac{X_{o,max}}{\tau_{max}}\right] \tau \qquad (13.15)$$

Administration of X_o every τ produces an average steady-state concentration defined by equation 13.7. The value of $C_{ss,av}$ can also be calculated from the therapeutic range according to equation 13.16.

$$C_{ss,av} = \frac{(C_{upper} - C_{lower})}{2.303 \log (C_{upper}/C_{lower})} \qquad (13.16)$$

INDIVIDUALIZATION

Because of reasonable homogeneity in humans, the dosage regimens are calculated on population basis. However, same dose of a drug may produce large differences in pharmacologic response in different individuals. This is called as **intersubject variability**. In other words, *it means that the dose required to produce a certain response varies from individual to individual.* Rational drug therapy requires **individualization** of dosage regimen to fit a particular patient's needs. This requires knowledge of pharmacokinetics of drugs. *The application of pharmacokinetic principles in the dosage regimen design for the safe and effective management of illness in individual patient is called as* **clinical pharmacokinetics**.

The two sources of variability in drug responses are:

1. **Pharmacokinetic variability** *which is due to differences in drug concentration at the site of action* (as reflected from plasma drug concentration) because of interindividual differences in drug absorption, distribution, metabolism and excretion.
2. **Pharmacodynamic variability** *which is attributed to differences in effect produced by a given drug concentration.*

The major cause for variations is pharmacokinetic variability. *Differences in the plasma levels of a given drug in the same subject when given on different occasions is called as* **intrasubject variability**. It is rarely encountered in comparison to interindividual variations. The differences in variability differ for different drugs. Some drugs show greater variability than the others. The major causes of intersubject pharmacokinetic variability are genetics, disease, age, body weight and drug-drug interactions. Less important causes are pharmaceutical formulation, route of administration, environmental factors and patient noncompliance.

The main objective of individualization is aimed at optimizing the dosage regimen. An inadequate therapeutic response calls for a higher dosage whereas drug related toxicity calls for a reduction in dosage. Thus, in order to aid individualization, a drug must be made available in dosage forms of different **dose strengths**. The number of dose strengths in which a drug should be made available depends upon 2 major factors—

i. The therapeutic index of the drug, and

ii. The degree of intersubject variability. Smaller the therapeutic index and greater the variability, more the number of dose strengths required.

Based on the assumption that all patients require the same plasma drug concentration range for therapeutic effectiveness, the steps involved in the individualization of dosage regimen are:

1. Estimation of pharmacokinetic parameters in individual patient and determining their deviation from the population values to evaluate the extent of variability; greater the accountability of variations, better the chances of attaining the desired therapeutic objective.
2. Attributing the variability to some measurable characteristic such as hepatic or renal disease, age, weight, etc.
3. Designing the new dosage regimen from the collected data.

The design of new dosage regimen involves:

1. Adjustment of dosage, or
2. Adjustment of dosing interval, or
3. Adjustment of both dosage and dosing interval.

Dosing of Drugs in Obese Patients

The apparent volume of distribution of a drug is greatly affected by changes in body weight since the latter is directly related to the volume of various body fluids. The ideal body weight (IBW) for men and women can be calculated from following formulae :

$$\text{IBW (men)} = 50\ \text{Kg} \pm 1\ \text{Kg/2.5 cm above or below 150 cm in height} \quad (13.17)$$

$$\text{IBW (women)} = 45\ \text{Kg} \pm 1\ \text{Kg/2.5 cm above or below 150 cm in height} \quad (13.18)$$

Any person whose body weight is more than 25% above the IBW is considered **obese**. In such patients, the lean to adipose tissue ratio is small because of greater proportion of body fat which alters the V_d of drugs. The ECF of adipose tissue is small in comparison to lean tissue in obese patients.

Following *generalizations* can be made regarding drug distribution and dose adjustment in obese patients:

1. For drugs such as digoxin that do not significantly distribute in the excess body space, the V_d do not change and hence dose to be administered should be calculated on IBW basis.

2. For polar drugs such as antibiotics (gentamicin) which distribute in excess body space of obese patients to an extent less than that in lean tissues, the dose should be lesser on per Kg total body weight basis but more than that on IBW basis.

3. In case of drugs such as caffeine, theophylline, lidocaine and lorazepam which distribute to the same extent in both lean and adipose tissues, the V_d is larger in obese patients but same on per Kg total body weight basis; hence, dose should be administered on total body weight basis.

4. For drugs such a phenytoin, diazepam and thiopental which are lipid soluble and distribute more in adipose tissues, the V_d is larger per Kg body weight in obese patients and hence they require larger doses, more than that on total body weight basis

Changes in dose based on alteration of V_d is also attributed to modification of clearance and half-life of the drug.

Dosing of Drugs in Neonates, Infants and Children

The usual dosage regimen calculated on population basis refers to that for adults. Neonates, infants and children require different dosages than adults because of differences in body surface area, TBW and ECF on per Kg body weight basis. The dose for such patients are calculated on the basis of their body surface area and not on body weight basis because the body surface area correlates better with dosage requirement, cardiac output, renal blood flow and glomerular filtration in children. A simple formula in comparison to DuBois and DuBois for computing surface area (SA) in square meters is **Mosteller's equation**:

$$\text{SA (in m}^2) = \frac{(\text{height} \times \text{weight})^{1/2}}{60} \quad (13.19)$$

Infants and children require larger mg/Kg doses than adults because:

1. their body surface area per Kg body weight is larger, and hence
2. larger volume of distribution (particularly TBW and ECF).

The child's maintenance dose can be calculated from adult dose by using the following equation:

$$\text{Child's Dose} = \frac{\text{SA of Child in m}^2}{1.73} \times \text{Adult Dose} \quad (13.20)$$

where 1.73 is surface area in m^2 of an average 70 Kg adult. Since the surface area of a child is in proportion to the body weight according to equation 13.21,

$$\text{SA (in m}^2) = \text{Body Weight (in Kg)}^{0.7} \quad (13.21)$$

the following relationship can also be written for child's dose:

$$\text{Child's Dose} = \left[\frac{\text{Weight of Child in Kg}}{70}\right]^{0.7} \times \text{Adult Dose} \quad (13.22)$$

As the TBW in neonates is 30% more than that in adults,

1. the V_d for most water soluble drugs is larger in infants, and
2. the V_d for most lipid soluble drugs is smaller.

Accordingly, the dose should be adjusted.

Dosing of Drugs in Elderly

Drug dose should be reduced in elderly patients because of a general decline in body function with age. The lean body mass decreases and body fat increases by almost 100% in elderly persons as compared to adults. Because of smaller volume of body water, higher peak alcohol levels are observed in elderly subjects than in young adults. V_d of a water soluble drug may decrease and that of a lipid soluble drug like diazepam increases with age. Age related changes in hepatic and renal function greatly alters the clearance of drugs. Because of progressive decrease in renal function, the dosage regimen of drugs that are predominantly excreted unchanged in urine should be reduced in elderly patients.

A general equation that allows calculation of maintenance dose for a patient of any age (except neonates and infants) when maintenance of same $C_{ss,av}$ is desired is:

$$\text{Patient's Dose} = \frac{(\text{Weight in Kg})^{0.7}\,(140 - \text{Age in years})}{1660} \times \text{Adult Dose} \quad (13.23)$$

Dosing of Drugs in Hepatic Disease

Disease is a major source of variations in drug response. Both pharmacokinetics and pharmacodynamics of many drugs are altered by diseases other than the one which is being treated.

The influence of hepatic disorder on drug availability and disposition is unpredictable because of the multiple effects that liver disease produces—effects on drug metabolizing enzymes, on drug binding and on hepatic blood flow. Hence, a correlation between altered drug pharmacokinetics and hepatic function is often difficult. For example, unlike excretion, there are numerous pathways by which a drug may be metabolized and each is affected to a different extent in hepatic disease. Generally speaking, drug dosage should be reduced in patients with hepatic dysfunction since clearance is reduced and availability is increased in such a situation.

Dosing of Drugs in Renal Disease

In patients with renal failure, the half-life of a drug is increased and its clearance drastically decreased if it is predominantly eliminated by way of excretion. Hence, dosage adjustment should take into account the renal function of the patient and the fraction of unchanged drug excreted in urine. One such method was discussed in *chapter 7 on Excretion of Drugs*.

There are two additional methods for dose adjustment in renal insufficiency if the V_d change is assumed to be negligible. These methods are based on maintaining the same average steady-state concentration during renal dysfunction as that achieved with the usual multiple dosage regimen and normal renal function. The adjustments are based on equations 13.6 and 13.7.

1. Dose adjustment based on total body clearance : Rewriting equation 13.6, the parameters to be adjusted in renal insufficiency are shown below:

$$\underset{\substack{\uparrow \\ \text{to be kept} \\ \text{normal}}}{C_{ss,av}} = \underset{\substack{\uparrow \\ \text{assumed} \\ \text{constant}}}{F} \times \underset{\substack{\uparrow \\ \text{decreased} \\ \text{due to disease}}}{\frac{1}{Cl_T}} \times \underset{\substack{\uparrow \\ \text{needs} \\ \text{adjustment}}}{\left(\frac{X_o}{\tau}\right)} \quad (13.6)$$

If Cl_T', X_o' and τ' represent the values for the renal failure patient, then the equation for dose adjustment is given as:

$$C_{ss,av} = \frac{X_o}{Cl_T\,\tau} = \frac{X_o'}{Cl_T'\,\tau'} \quad (13.24)$$

Rearranging in terms of dose and dose interval to be adjusted, the equation is:

$$\frac{X_o'}{\tau'} = \frac{Cl_T'\,X_o}{Cl_T\,\tau} \quad (13.25)$$

From above equation, the regimen may be adjusted by reduction in dosage or increase in dosing interval or a combination of both.

2. Dose adjustment based on elimination rate constant or half-life : Rewriting equation 13.7, the parameters to be adjusted in renal insufficiency are:

$$C_{ss,av} = \frac{1.44\ F}{V_d} \times t_{1/2} \times \left(\frac{X_o}{\tau}\right) \quad (13.7)$$

↑	↑	↑	↑
to be kept normal	assumed constant	increased due to disease	needs adjustment

If $t_{1/2}'$, X_o' and τ' represent the values for the renal failure patient, then:

$$C_{ss,av} = \frac{t_{1/2}\ X_o}{\tau} = \frac{t_{1/2}'\ X_o'}{\tau'} \quad (13.26)$$

Rearranging the above equation in terms of dose and dose interval to be adjusted, we get:

$$\frac{X_o'}{\tau'} = \frac{t_{1/2}\ X_o}{t_{1/2}'\ \tau} \quad (13.27)$$

Because of prolongation of half-life of a drug due to reduction in renal function, the time taken to achieve the desired plateau takes longer the more severe the dysfunction. Hence, such patients sometimes need loading dose.

MONITORING DRUG THERAPY

Management of drug therapy in individual patient often requires evaluation of response of the patient to the recommended dosage regimen. This is known as **monitoring of drug therapy**. It is necessary to ensure that the therapeutic objective is being attained and failure to do so requires readjustment of dosage regimen.

Depending upon the drug and the disease to be treated, management of drug therapy in individual patient can be accomplished by:

1. Monitoring therapeutic effects—therapeutic monitoring
2. Monitoring pharmacologic actions—pharmacodynamic monitoring
3. Monitoring plasma drug concentration—pharmacokinetic monitoring.

Therapeutic Monitoring

In this approach, the management plan involves monitoring the incidence and intensity of both the desired therapeutic effects and the undesired adverse effects. A direct measure of the desired effect is considered as a **therapeutic endpoint** e.g. prevention of an anticipated attack of angina or shortening of duration of pain when attack occurs through the use of sublingual glyceryl trinitrate. In certain cases, definition of therapeutic endpoint may not be clear and onset of toxicity is used as a dosing guide

i.e. dosage regimen is titrated to a **toxic endpoint** e.g. excessive dryness of mouth with atropine when used as an antispasmodic agent.

Pharmacodynamic Monitoring

In some instances, the pharmacologic actions of a drug can be measured and used as a guide to therapeutic process. The response observed may or may not correlate exactly with the therapeutic effect e.g. blood glucose lowering with insulin, lowering of blood pressure with antihypertensives, enhancement of hemoglobin levels with hematinics, etc.

Pharmacokinetic Monitoring

This approach involves monitoring the plasma drug concentration within a target concentration range (called as **target concentration strategy**) and based on the principle that free drug at the site of action is in equilibrium with the drug in plasma. The strategy is particularly useful when:

1. Therapeutic endpoints are difficult to define or are lacking e.g. control of seizures with phenytoin.
2. The objective is to maintain the therapeutic effect in order to obtain optimum drug use.
3. The probability of therapeutic failure is high as with drugs having low therapeutic indices, erratic absorption, pharmacokinetic variability or when the drug is used in multiple drug therapy.

Successful application of this approach requires complete knowledge of pharmacokinetic parameters of the drug, the situation in which these parameters are likely to be altered and the extent to which they could be altered, and a sensitive, specific and accurate analytic method for determination of drug concentration. Examples of drugs monitored by this method include digoxin, phenytoin, gentamicin, theophylline, etc. The strategy is very useful in individualizing therapy in patients with hepatic or renal impairment when dose adjustments are necessary.

QUESTIONS

1. Enlist various applications of pharmacokinetic principles.
2. What do you understand by therapeutic objective? What are the various ways of attaining it?
3. Define optimal dosage regimen. What does it mean for a drug with narrow therapeutic index such as phenytoin and for drugs like antibiotics?
4. What assumptions are made in the design of a dosage regimen?
5. Name the two parameters that are generally adjusted in developing a dosage regimen.

6. It is safer to administer a drug with a narrow therapeutic index in small doses at frequent intervals. Explain.
7. On what parameters does extent of drug accumulation on multiple dosing depend? Does dose size have a bearing on it?
8. State the plateau principle. Which parameters govern attainment of steady-state?
9. Define fluctuation of plasma level. Why are fluctuations smaller when the drug is given e.v. whereas larger when administered as multiple i.v. boluses?
10. The $C_{ss,av}$ is not an arithmetic mean of $C_{ss,max}$ and $C_{ss,min}$. Why?
11. Define dose ratio. Why is it always smaller for extravascularly administered drugs in comparison to those given intravenously?
12. On what factors do maintenance of drug concentration within the therapeutic range depend?
13. How is dosing interval determined on the basis of half-life and therapeutic index of the drug?
14. What are the two major sources of variability in drug response?
15. Quote some of the more important causes of intersubject variability in drug response.
16. Enlist the steps involved in individualization of dosage regimen.
17. How does obesity influence V_d of a drug and hence its dose size?
18. The dose for neonates, infants and children should be calculated on body surface area basis rather than on the basis of body weight. Explain.
19. Why do neonates, infants and children require larger mg/Kg body weight doses than adults?
20. Give possible reasons for reduction in dose of a drug in elderly patients.
21. Unlike renal function, why is it difficult to establish a correlation between hepatic dysfunction and altered drug pharmacokinetics ?
22. Why does it take longer to attain the steady-state in a renal failure patient in comparison to a patient with normal renal function?
23. What do you understand by monitoring of drug therapy? When does it become necessary?
24. What are the various ways of monitoring drug therapy in individual patient?
25. What parameters are monitored in therapeutic drug monitoring?
26. On what principle is pharmacokinetic drug monitoring based? When does such a strategy becomes useful?
27. What criteria are necessary for successful pharmacokinetic drug monitoring?
28. The loading dose is calculated on the basis of apparent V_d of a drug whereas maintenance dose is determined from its $t_{1/2}$. Explain.

29. Atenolol is to be administered orally to a 50 Kg patient suffering from hypertension. The typical parameters of the drug on population basis are:

F	V_d	Cl_T	*Therapeutic Range*
0.4	1.23 l/Kg	118.4 ml/min	0.2 to 1.3 mcg/ml

Design a dosage regimen to attain and maintain the plasma concentration within the therapeutic range. Assume rapid absorption.

Answer : $C_{ss,av}$ = 0.588 mcg/ml, τ_{max} = 16.21 hours, $X_{o,max}$ = 169.12mg. It is convenient to administer the drug once every 12 hours. Therefore, X_o in such a situation should be 125.3 mg. Loading dose is 90.4 mg.

30. Diltiazem is administered in a dose of 60 mg q.i.d. The oral availability of the drug is 50%, V_d is 30 liters and elimination half-life is 4 hours.

a. Determine the maximum and minimum amounts of drug in the body after 4 doses.

Answer : $X_{4,max}$ = 45.7 mg and $X_{4,min}$ = 16.16 mg.

b. Calculate the maximum and minimum amounts of drug in the body at steady-state.

Answer : $X_{ss,max}$ = 46.4 mg and $X_{ss,min}$ = 16.4 mg.

c. What is the accumulation index?

Answer : R_{ac} = 1.55.

d. What is the average amount of drug in the body at steady-state?

Answer : $X_{ss,av}$ = 28.8 mg.

e. If the desired $C_{ss,av}$ is 0.099 mcg/ml, is there any need to administer the loading dose?

31. Netilmicin is to be administered to a 70 Kg patient at a rate of 2 mg/Kg every 12 hours by multiple i.m. injections. The drug has a half-life of 2.2 hours and V_d of 0.2 l/Kg.

a. Determine $C_{ss,max}$, $C_{ss,min}$ and $C_{ss,av}$.

Answer : $C_{ss,max}$ = 10.2 mcg/ml, $C_{ss,min}$ = 0.23 mcg/ml and $C_{ss,av}$ = 2.64 mcg/ml.

b. If the $t_{1/2}$ increases to 5 hours in renal insufficiency, what should be the new dose or the new dosing interval?

Answer : New dose (τ unchanged) = 0.88 mg/Kg, new dosing interval (dose unchanged) = 27.3 hours.

c. If the drug is to be injected once every 24 hours, in renal insufficiency, what should be the new dose?

Answer : 1.76 mg/Kg.

d. If netilmicin is to be given to a child weighing 12 Kg with normal renal function, what should be the dose?

Answer : 3.4 mg/Kg.

e. If the same drug is to be administered to an elderly patient of 85 years and 55 Kg body weight, determine the dose.

Answer : 1.4 mg/Kg.

32. Procainamide is to be administered to a 65 Kg arrhythmic patient as 500 mg tablets every 4 hours. The drug has a half-life of 3 hours, V_d of 2 liter/Kg and oral availability of 0.85.

a. Calculate the steady-state concentration of procainamide.

Answer : $C_{ss,av}$ = 3.53 mcg/ml.

b. Determine whether the patient is adequately dosed (therapeutic range is 4 to 12 mcg/ml)

c. What changes would you recommend in the dosage regimen?

Answer : Desired $C_{ss,av}$ = 7.3 mcg/ml which can be attained by increasing the maintenance dose to 1000 mg or 2 tablets every 4 hours.

33. Two aminoglycoside antibiotics alongwith some of their parameters are listed in the table below:

Drug	*$t_{1/2}$*	*F_e*	*TI*	*Route*	*Normal dose*	*Normal interval*
Streptomycin	3 Hr	0.5	1.25	i.m.	7.5 mg/Kg	12 Hrs
Gentamicin	2 Hr	0.9	20.00	i.m.	1.0 mg/Kg	8 Hrs

These drugs are to be administered to a uremic patient. Suggest the changes that should be made in the dosage regimen—keeping the dose constant and prolonging the interval or decreasing the dose and maintaining the dosage interval or changing both. Discuss the advantages and disadvantages of each approach.

14

Drug Concentration and Pharmacologic Response

The empirical approach in optimization of drug therapy was based on relating pharmacologic response to the dose administered. There are several drawbacks of such an approach—tedious, time consuming, costly, etc. Moreover, poor correlation may be observed between drug dosage and response since a given dose or a dosing rate can result in large deviations in plasma drug concentration which in most cases are attributable to formulation factors and drug's elimination characteristics. It is well understood that the response correlates better with the plasma drug concentration or with the amount of drug in the body rather than with the administered dose. Thus, it is easy and more appropriate to design dosage regimens by application of pharmacokinetic principles. The approach is based on the principle that the response produced is proportional to the concentration of drug at the site of action which in turn is reflected in the concentration of drug in plasma. However, such relationships between plasma drug concentration and pharmacologic response are difficult to establish in certain cases.

The factors that complicate development of concentration-response relationships are:

1. Delay in Drug Distribution : Most sites of action are located in the extravascular tissues and equilibration with drug takes a long time. This results in delay in observation of response. Long delays may occur when the therapeutic response is an indirect measure of drug effect—for example, the anticoagulant effect of dicoumarol is due to indirect inhibition and depletion of clotting factors. Often, the therapeutic action in several such cases outlasts the plasma drug concentration e.g. reserpine.

2. Protein Binding : Ideally, the pharmacologic response should be correlated with the concentration of unbound drug in plasma since its activity is elicited only by the free form. However, quantification of unbound drug is difficult and thus, if the relationship is based on total

plasma drug concentration, any variation in the degree of binding will obscure it.

3. Active Metabolites : The metabolites of some drugs such as imipramine and amitriptyline are active which obscure the concentration-response relationship if it is based on the concentration of parent drug. Some drugs such as propranolol form active metabolites on first-pass hepatic metabolism and thus, result in greater response when administered orally than when given intravenously.

4. Tolerance : The effectiveness of some drugs decreases with chronic use due to development of acquired tolerance. Tolerance may be pharmacokinetic i.e. through enhanced metabolism e.g. carbamazepine or pharmacodynamic i.e. through diminished response after some period of chronic use e.g. nitroglycerine.

5. Racemates : Several drugs are administered as racemic mixture of two optically active enantiomers of which usually one is active; for example, the S(+) isomer of ibuprofen. Hence, a difference in the ratio of active to inactive isomer can lead to large differences in pharmacologic response.

Therapeutic Concentration Range

The therapeutic effectiveness of a drug depends upon its plasma concentration. There is an optimum concentration range in which therapeutic success is most likely and concentrations both above and below it are more harmful than useful. This concentration range is called as **therapeutic window** or **therapeutic range**. *Such a range is thus based on the difference between pharmacologic effectiveness and toxicity.* The wider it is, the more is the ease in establishing a safe and effective dosage regimen. The therapeutic range of several drugs have been developed. For many, the range is narrow and for others, it is wide (*see* Table 14.1).

TABLE 14.1 **Therapeutic Range for Some Drugs**

Drug	*Disease*	*Therapeutic Range*
Digoxin	Congestive cardiac failure	0.5 to 2.0 ng/ml
Gentamicin	Infections	1.0 to 10.0 mcg/ml
Lidocaine	Arrhythmias	1.0 to 6.0 mcg/ml
Phenytoin	Epilepsy	10.0 to 20.0 mcg/ml
Propranolol	Angina	0.02 to 0.2 mcg/ml
Salicylic acid	Aches and pains	20.0 to 100.0 mcg/ml
	Rheumatoid arthritis	100.0 to 300.0 mcg/ml
Theophylline	Asthma	6.0 to 20.0 mcg/ml

Some drugs can be used to treat several diseases and will have different ranges for different conditions e.g. salicylic acid is useful both in common aches and pains as well as in rheumatoid arthritis. The upper limit of the therapeutic range may express loss of effectiveness with no toxicity e.g. tricyclic antidepressants or reflect toxicity which may be related to pharmacologic effect of the drug e.g. hemorrhagic tendency with warfarin or may be totally unrelated e.g. ototoxicity with aminoglycosides.

Concentration-Response Relationships

With most drugs, the response produced is reversible i.e. a reduction in concentration at the site of action reverses the effect. The response produced by a drug can be classified as *graded* or *quantal*. A majority of drugs produce **graded response** i.e. *the intensity of effect increases with the dose or concentration of drug.* The response can be measured on a continual basis in such cases and establishing a linear relationship between drug concentration and intensity of response is easy. However, for certain other drugs, the responses are not observed on a continuous basis. Such drugs may either show their effect or not at all — for example, prevention of seizures by phenytoin. Such responses are called as **quantal** or **all-or-none**. Thus, establishing a concentration-response relationship in such circumstances is difficult but can be developed in terms of the *frequency* with which a particular event occurs at a given drug concentration.

Mathematical models that relate pharmacologic effect to a measured drug concentration in plasma or at the effector site can be used to develop quantitative relationships. Such models are often called as **pharmacodynamic models**. Some of the commonly used relationships or models are discussed below.

1. Linear Model : When the pharmacologic effect (E) is directly proportional to the drug concentration (C), the relationship may be written as:

$$E = P\,C + E_o \qquad (14.1)$$

where P is the slope of the line obtained from a plot of E versus C and E_o is the extrapolated y-intercept called as **baseline effect** in the absence of drug.

2. Logarithmic Model : If the concentration-effect relationship does not conform to a simple linear function, a logarithmic transformation of the data is needed.

$$E = P \log C + I \qquad (14.2)$$

where I is empiric constant. This transformation is popular because it expands the initial part of the curve where response is changing markedly with a small change in concentration and contracts the latter part where a large change in concentration produces only a slight change in response. An important feature of this transformation is the linear relationships between drug concentration and response at concentrations producing effects of between 20 to 80% of the maximum effect (Fig. 14.1). Beyond this range, a larger dose produces a larger concentration of drug in the body.

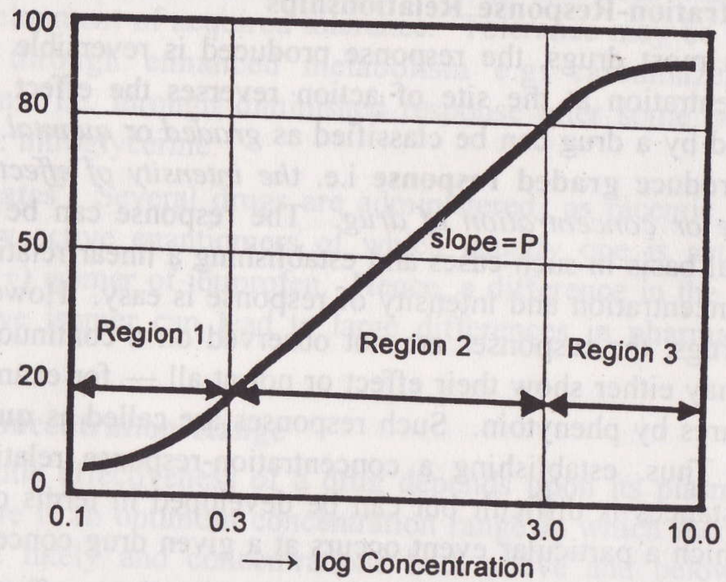


Fig. 14.1 A typical sigmoidal shape log drug concentration-effect relationship

3. E_{max} Models : Unlike earlier models, these models describe nonlinear concentration-effect relationships i.e. the response increases with an increase in drug concentration at low concentrations and tends to approach

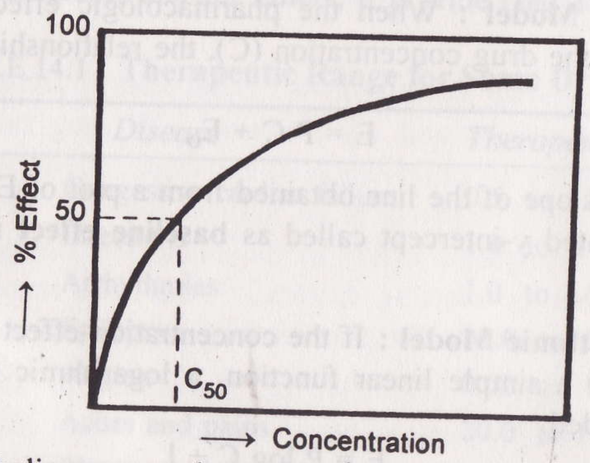


Fig. 14.2 A hyperbolic concentration-response relationship based on E_{max} model

maximum (asymptote) at high concentrations (Fig. 14.2). Such a plot is characteristic of most concentration-response curves. None of the preceding models can account for the maximal drug effect as the E_{max} models.

Michaelis-Menten equation for a saturable process (saturation of receptor sites by the drug molecules) is used to describe such a model.

$$E = \frac{E_{max}\, C}{C_{50} + C} \qquad (14.3)$$

where E_{max} = maximum effect, and

C_{50} = the concentration at which 50% of the effect is produced.

When $C << C_{50}$, the equation reduces to a linear relationship. In the range 20 to 80%, the E_{max} model approximates equation 14.2.

In certain cases, the concentration-response relationship is steeper or shallower than that predicted from equation 14.3. A better fit may otherwise be obtained by considering the **shape factor 'n'** to account for deviations from a perfect hyperbola.

$$E = \frac{E_{max}\, C^n}{C_{50}^n + C^n} \qquad (14.4)$$

If n = 1, a normal plot is obtained. Larger the value of n, steeper the linear portion of the curve and greater its slope; such a plot is often sigmoidal and the model may be called as **sigmoid-E_{max} model** (Fig. 14.3).

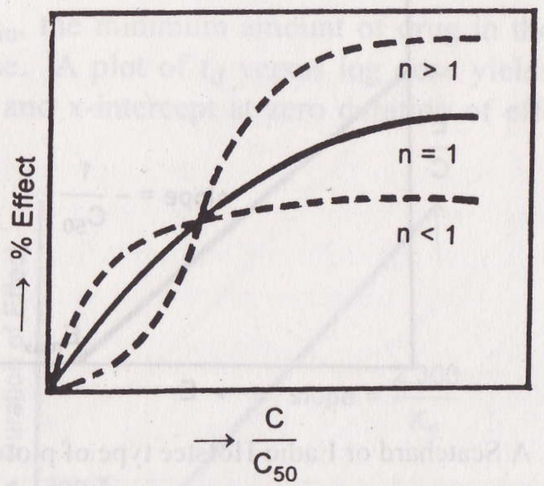


Fig. 14.3 Effect of shape factor n on the concentration-response curves

Equation 14.3 can be linearized in several ways. One linearization is obtained by taking the reciprocal and constructing a Lineweaver-Burk plot (equation 14.5 and Fig. 14.4).

$$\frac{1}{E} = \frac{C_{50}}{E_{max}\,C} + \frac{1}{E_{max}} \qquad (14.5)$$

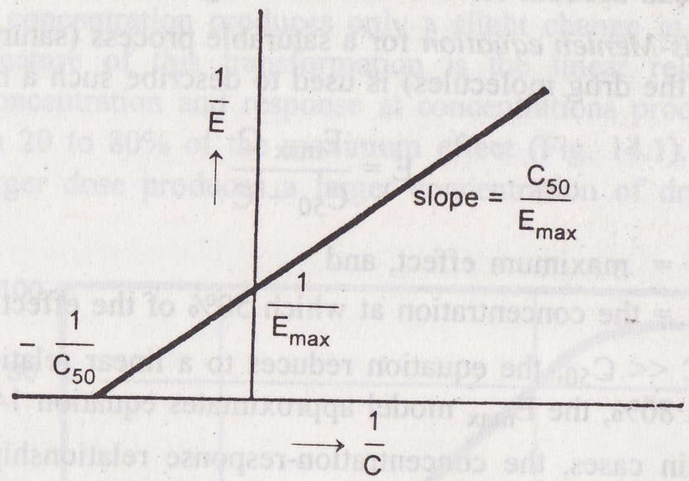


Fig. 14.4 Lineweaver-Burk plot of equation 14.5, a linearized form of the hyperbolic plot

Another linear plot is similar to Scatchard or Eadie-Hofstee plots and based on equation 14.6.

$$\frac{E}{C} = -\left(\frac{1}{C_{50}}\right)\frac{E + E_{max}}{C_{50}} \qquad (14.6)$$

A plot of E/C versus E yields a line with slope $-1/C_{50}$ and x-intercept E_{max} (Fig. 14.5).

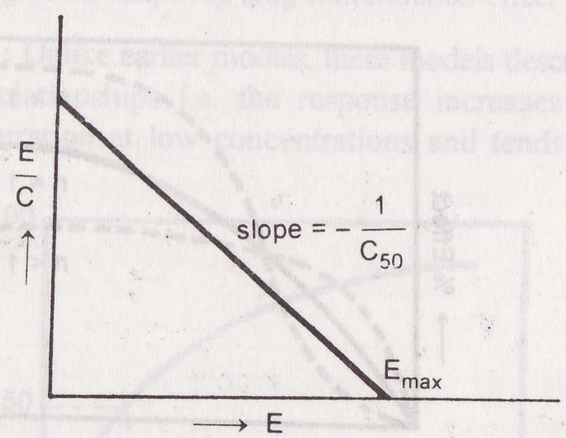


Fig. 14.5 A Scatchard or Eadie-Hofstee type of plot of equation 14.6

Onset/Duration of Effect-Concentration Relationships

The onset of action of a drug that produces quantal response occurs when a minimum effective level of drug in the plasma C_{min} is reached. The duration of action of such a drug will depend upon how long the plasma concentration remains above the C_{min} level.

The factors which influence duration of action of a drug are:

1. The dose size, and
2. The rate of drug removal from the site of action which in turn depends upon the redistribution of drug to poorly perfused tissues and elimination processes.

An increase in dose promotes rapid onset of action by reducing the time required to reach the C_{min} and prolongs the duration of effect. The influence of dose on duration of action can be explained as follows. Consider a drug that distributes rapidly (one-compartment kinetics) and administered as i.v. bolus dose. The plasma drug concentration is given by the equation:

$$\log C = \log\left(\frac{\text{Dose}}{V_d}\right) - \frac{K_E\,t}{2.303} \qquad (14.7)$$

The plasma concentration falls eventually to a level C_{min} below which the drug does not show any response. At this time, $t = t_d$, the duration of effect of a drug. The above equation thus becomes:

$$\log C_{min} = \log\left(\frac{\text{Dose}}{V_d}\right) - \frac{K_E\,t_d}{2.303} \qquad (14.8)$$

Rearranging to define duration of effect, the equation is:

$$t_d = \frac{2.303}{K_E}\left[\log \text{Dose} - \log (C_{min}\,V_d)\right] \qquad (14.9)$$

where $C_{min}V_d = X_{min}$, the minimum amount of drug in the body required to produce a response. A plot of t_d versus log dose yields a straight line with slope $2.303/K_E$ and x-intercept at zero duration of effect of log X_{min} (Fig. 14.6).

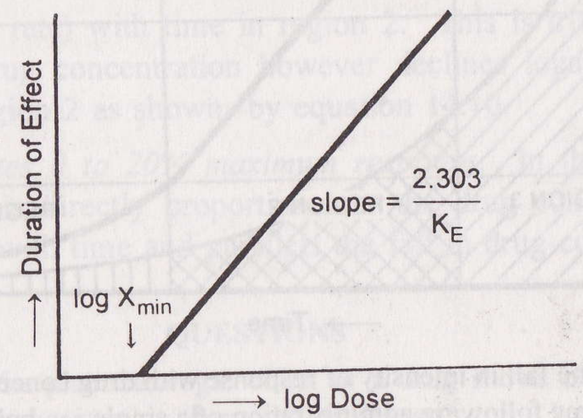


Fig. 14.6 Relationship between dose of drug and duration of action

Equation 14.9 shows that the duration of effect is also a function of $t_{½}$ ($0.693/K_E$). With each doubling of dose the duration of effect increases by one half-life. This can be explained by considering that a dose X_o produces a duration of effect t_d; so when double the dose i.e. $2X_o$ is administered, the dose remaining after one half-life will be X_o which can produce a duration of effect equal to t_d. Thus, the total duration of effect produced by $2X_o$ will be $t_{½} + t_d$. However, the approach of extending the duration of action by increasing the dose is harmful if the drug has a narrow therapeutic index. An alternative approach is to administer the same dose when the drug level has fallen to X_{min}. Thus, after the second dose, the drug in the body will be (dose + X_{min}). If X_{min} is small in relation to dose, very little increase in duration of action will be observed.

Intensity of Effect-Concentration Relationships

In case of a drug that produces **quantal response**, *the pharmacodynamic parameter that correlates better with its concentration is* **duration of action**. *The parameter* **intensity of response** *is more useful for correlation with the concentration of a drug that shows* **graded effect**. Like duration of action, intensity of action also depends upon the dose and rate of removal of drug from the site of action. The intensity of action also depends upon the region of the concentration-response curve (*refer* Fig. 14.1). If a drug with rapid distribution characteristics is given as i.v. bolus dose large enough to elicit a maximum response, the log concentration-response plot obtained will be as shown in Fig. 14.1. The relationship between dose, intensity of effect and time can be established by considering the plots depicted in Fig. 14.7. which also shows 3 regions.

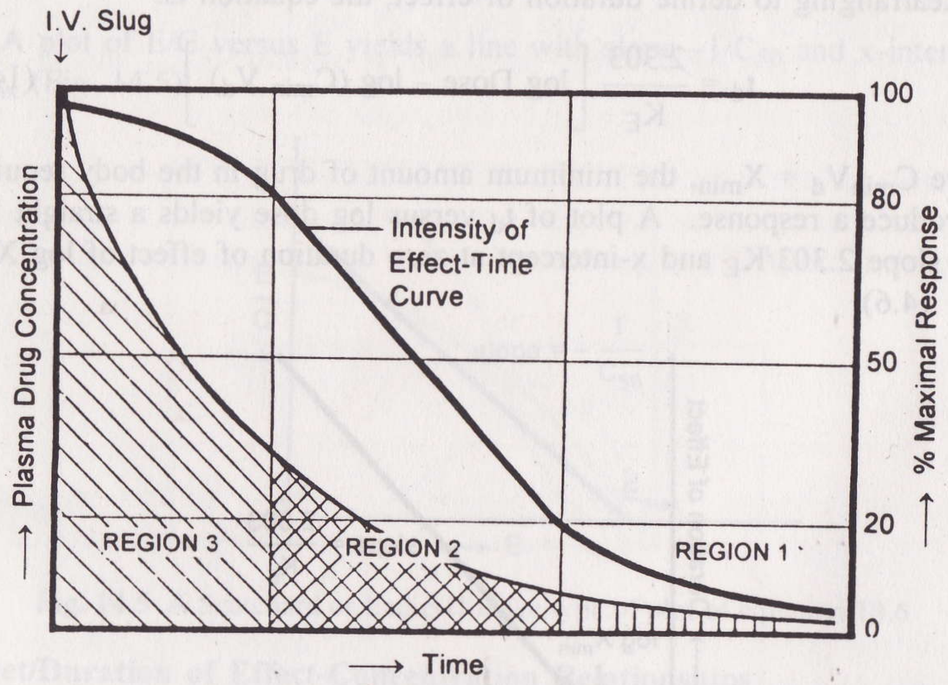


Fig. 14.7 The fall in intensity of response with drug concentration and with time following administration of a single i.v. bolus dose (Adapted from Rowland,M. and Tozer,T.N.: *Clinical Pharmacokinetics: Concepts and Applications*, 2nd edition, Philadelphia, Lea & Febiger, 1989).

Region 3 *indicates 80 to 100% maximum response.* The initial concentration of drug after i.v. bolus dose lies in this region if the dose injected is sufficient to elicit maximal response. The drug concentration falls rapidly in this region but intensity of response remains maximal and almost constant with time.

Region 2 *denotes 20 to 80% maximum response.* In this region, the intensity of response is proportional to log of drug concentration and expressed by equation 14.2.

$$\text{Intensity of Effect} = P \log C + I \qquad (14.2)$$

Since the decline in drug concentration is a first-order process, log C can be expressed as:

$$\log C = \log C_o - \frac{K_E\, t}{2.303} \qquad (14.10)$$

Substituting 14.10 in equation 14.2. and rearranging we get:

$$\text{Intensity of Effect} = (P \log C_o + I) - \frac{P\, K_E\, t}{2.303} \qquad (14.11)$$

If E_o is the intensity of response when concentration is C_o, then:

$$\text{Intensity of Effect} = E_o - \frac{P\, K_E\, t}{2.303} \qquad (14.12)$$

Equation 14.12 shows that the intensity of response falls linearly (at a constant zero-order rate) with time in region 2. This is true for most of the drugs. The drug concentration however declines logarithmically or exponentially in region 2 as shown by equation 14.10.

Region 1 *denotes 0 to 20% maximum response.* In this region, the intensity of effect is directly proportional to the drug concentration but falls exponentially with time and parallels the fall in drug concentration.

QUESTIONS

1. Why is it that the pharmacologic effect of a drug shows better correlation with its plasma concentration than with its dosage?
2. What are some of the more important causes of poor correlation between plasma drug concentration and pharmacologic response?

3. Define therapeutic range. What does plasma drug concentration beyond this range signify?
4. Define: (a) Quantal response, and (b) Graded response.
5. What are pharmacodynamic models? Discuss some of the more important models used to quantify plasma concentration-response relationships.
6. When does onset of action occur for a drug that shows graded response and for the one that elicits quantal response?
7. On what factors do duration and intensity of drug action depend?
8. Explain how with each doubling of dose, the duration of effect increases by one half-life for a drug that elicits graded response.
9. When and why is it not advisable to extend the duration of action by increasing the dose? What is the alternative in such cases?
10. How does intensity of drug action change with its plasma concentration?
11. The C_{50} of a drug showing graded response is 5 mcg/ml and has a shape factor n of 2.0. Calculate the concentration range if the drug is found to be effective when the response is between 20 to 80% of the maximal value. (*Hint*: Take reciprocal of equation 14.4 and express intensity of effect as 0.2 E_{max} and solve to obtain C_{20}. Similarly, take it next as 0.8 E_{max} and solve to determine C_{80})

    Answer : C_{20} = 2.5 mcg/ml and C_{80} = 10.0 mcg/ml.
12. Mezlocillin has a MEC of 5 mcg/ml for a particular infection when given i.v. in a bolus dose of 2.5 mg/Kg. What will be the duration of effect if a dose of 50 mg/Kg is administered? The drug has a half-life of 0.8 hours. What will be the duration of effect if the dose is doubled? Is the increase in duration of action equal to one half-life?

    Answer : 3.5 hours and 4.3 hours.
13. A newly developed antihypertensive drug shows linear relationship between intensity of effect and log drug concentration. After i.v. administration of 0.15 mg/Kg dose, the observed reduction in blood pressure with time is shown in the table below:

Time (hours)	0.5	3.0
% Reduction in B.P.	45.0	40.0

Given : P = 20.0%

a. What is the half-life of the drug? (*Hint*: Use equation 14.12 for a plot of intensity of effect versus time and compute values from slope)

   Answer : $t_{½}$ = 3 hours.

b. What will be the % intensity of response immediately after injection of drug?

   Answer : 46.0%.

c. How long is the reduction in B.P. expected to remain above 20.0% of maximum response?

15 Controlled Release Medication

An **ideal dosage regimen** *in the drug therapy of any disease is the one which immediately attains the desired therapeutic concentration of drug in plasma (or at the site of action) and maintains it constant for the entire duration of treatment.* This is possible through administration of a conventional dosage form in a particular dose and at a particular frequency. The frequency of administration or the dosing interval of any drug depends upon its half-life or mean residence time (MRT) and its therapeutic index. In most cases, the dosing interval is much shorter than the half-life of the drug resulting in a number of *limitations* associated with such a conventional dosage form:

1. Poor patient compliance; increased chances of missing the dose of a drug with short half-life for which frequent administration is necessary.
2. A typical **peak-valley** plasma concentration-time profile is obtained which makes attainment of steady-state condition difficult (*see* Fig. 15.1).
3. The unavoidable fluctuations in the drug concentration may lead to **undermedication** or **overmedication** as the C_{ss} values fall or rise beyond the therapeutic range.
4. The fluctuating drug levels may lead to precipitation of adverse effects especially of a drug with small therapeutic index whenever overmedication occurs.

There are two ways to overcome such a situation:

1. Development of new, better and safer drugs with long half-lives and large therapeutic indices, and
2. Effective and safer use of existing drugs through concepts and techniques of controlled and targeted delivery systems.

The first approach has many disadvantages which therefore resulted in increased interest in the second approach. *An* **ideal controlled drug delivery system** *is the one which delivers the drug at a predetermined rate, locally or systemically, for a specified period of time.* Thus, unlike conventional immediate release systems, the rate of appearance of drug in the body with such a system is not controlled by absorption process. Following absorption of drug from such a system, there is no control over its fate. *An* **ideal targeted drug delivery system** *is the one which delivers the drug only to its site of action and not to the nontarget organs or tissues.* With this approach, control of kinetics of drug release is difficult. This chapter will deal only with controlled drug delivery systems.

There are several terms used interchangeably viz. *controlled release, programmed release, sustained release, prolonged release, timed release, slow release, extended release* and other such dosage forms. However, controlled release will imply as defined earlier. It differs from sustained release systems which simply prolong the drug release and hence plasma drug levels for an extended period of time (i.e. not necessarily at a predetermined rate). Thus, the chief objective of most products should be controlled delivery to reduce dosing frequency to an extent that once daily dose is sufficient for therapeutic management through a uniform plasma concentration at steady-state.

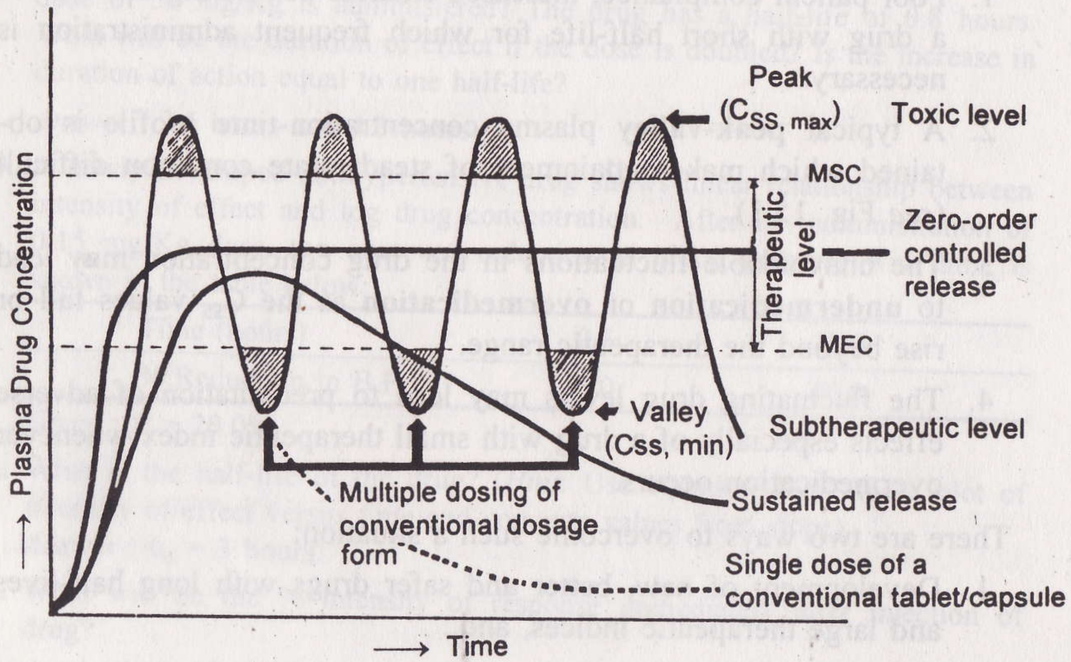


Fig. 15.1 A hypothetical plasma concentration-time profile from conventional multiple dosing and single doses of sustained and controlled delivery formulations

The several **advantages** of a controlled drug delivery system over a conventional dosage form are—

1. Improved patient convenience and compliance due to less frequent drug administration.
2. Reduction in fluctuation in steady-state levels and therefore better control of disease condition and reduced intensity of local or systemic side effects (Fig. 15.1).
3. Increased safety margin of high potency drugs due to better control of plasma levels.
4. Maximum utilization of drug enabling reduction in total amount of dose administered.
5. Reduction in health care costs through improved therapy, shorter treatment period, less frequency of dosing and reduction in personnel time to dispense, administer and monitor patients.

Disadvantages of controlled release dosage forms include—

1. Decreased systemic availability in comparison to immediate release conventional dosage forms; this may be due to incomplete release, increased first-pass metabolism, increased instability, insufficient residence time for complete release, site-specific absorption, pH-dependent solubility, etc.
2. Poor *in vitro-in vivo* correlation.
3. Possibility of dose dumping due to food, physiologic or formulation variables or chewing or grinding of oral formulations by the patient and thus, increased risk of toxicity.
4. Retrieval of drug is difficult in case of toxicity, poisoning or hypersensitivity reactions.
5. Reduced potential for dosage adjustment of drugs normally administered in varying strengths.
6. Higher cost of formulation.

DESIGN OF CONTROLLED DRUG DELIVERY SYSTEMS

The basic *rationale* of a controlled drug delivery system is to optimize the biopharmaceutic, pharmacokinetic and pharmacodynamic properties of a drug in such a way that its utility is maximized through reduction in side effects and cure or control of condition in the shortest possible time by using smallest quantity of drug, administered by the most suitable route.

Biopharmaceutic Characteristics of the Drug

The performance of a drug presented as a controlled release system depends upon its:

1. Release from the formulation.
2. Movement within the body during its passage to the site of action.

The former depends upon the fabrication of the formulation and the physicochemical properties of the drug while the latter element is dependent upon pharmacokinetics of drug. In comparison to conventional dosage form where the rate-limiting step in drug availability is usually absorption through the biomembrane, the rate-determining step in the availability of a drug from controlled delivery system is the rate of release of drug from the dosage form which is much smaller than the intrinsic absorption rate for the drug (Fig. 15.2).

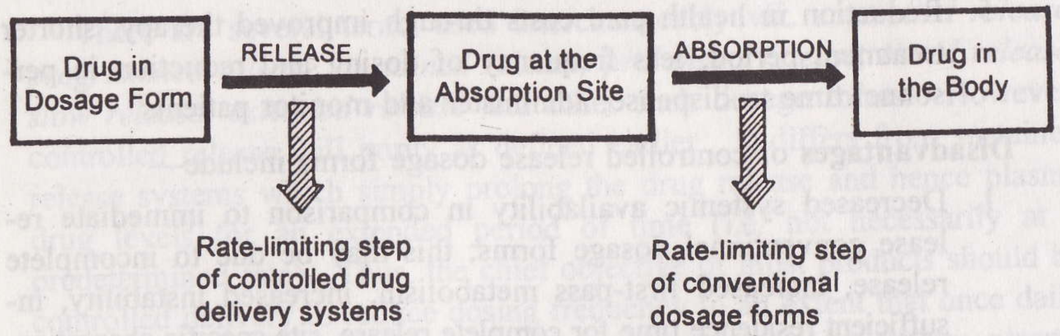


Fig. 15.2 Scheme representing the rate-limiting step in the design of controlled drug delivery system

The type of delivery system and the route of administration of the drug presented in controlled release dosage form depends upon the physicochemical properties of the drug and its biopharmaceutic characteristics. The desired biopharmaceutic properties of a drug to be used in a controlled delivery system are discussed below.

1. Molecular Weight of the Drug : The lower the molecular weight, the faster and more complete the absorption. For drugs absorbed by pore transport mechanism, the molecular size threshold is 150 daltons for spherical compounds and 400 daltons for linear compounds. However, more than 95% of drugs are absorbed by passive diffusion. **Diffusivity**, *defined as the ability of a drug to diffuse through the membranes*, is inversely related to molecular size. The upper limit of drug molecular size for passive diffusion is 600 daltons. Drugs with large molecular size are poor candidates for oral controlled release systems e.g. peptides and proteins.

2. Aqueous Solubility of the Drug : A drug with good aqueous solubility, especially if pH-independent, serves as a good candidate for controlled release dosage forms e.g. pentoxifylline. Drugs with pH-dependent aqueous solubility e.g. phenytoin, or drugs with solubility in nonaqueous solvents e.g. steroids, are suitable for parenteral (e.g. i.m depots) controlled release dosage forms; the drug precipitates at the injection site and thus, its release is slowed down due to change in pH or contact with aqueous body fluids. Absorption of poorly soluble drugs is dissolution rate-limited which means that the controlled release device does not control the absorption process; hence, they are poor candidates for such systems.

3. Apparent Partition Coefficient of the Drug : Greater the apparent partition coefficient of a drug, greater is its rate and extent of absorption. Such drugs have increased tendency to cross even the more selective barriers like BBB. The apparent volume of distribution of such drugs also increases due to increased partitioning into the fatty tissues and since the blood flow rate to such tissues is always lower than that to an aqueous tissue like liver, they may exhibit characteristics of models having two or more compartments. The parameter is also important in determining the release rate of a drug from lipophilic matrix or device.

4. Drug pK_a and Ionization at Physiologic pH : The pK_a range for acidic drugs whose ionization is pH-sensitive is 3.0 to 7.5 and that for basic drugs is 7.0 to 11.0. For optimum passive absorption, the drugs should be nonionized at that site at least to an extent 0.1 to 5%. Drugs existing largely in ionized forms are poor candidates for controlled delivery e.g. hexamethonium.

5. Drug Stability : Drugs unstable in GI environment cannot be administered as oral controlled release formulation because of bioavailability problems e.g. nitroglycerine. A different route of administration should then be selected such as the transdermal route.

6. Mechanism and Site of Absorption : Drugs absorbed by carrier-mediated transport processes and those absorbed through a *window* are poor candidates for controlled release systems e.g. several B vitamins.

7. Biopharmaceutic Aspects of Route of Administration : Oral and parenteral (i.m.) routes are the most popular followed by transdermal application. Routes of minor importance in controlled drug delivery are buccal/sublingual, rectal, nasal, ocular, pulmonary, vaginal and intrauterinal. The features desirable for a drug to be given by a particular route are discussed below.

(a) **Oral Route** : For a drug to be successful as oral controlled release formulation, it must get absorbed through the entire length of GIT. Since the main limitation of this route is the transit time (a mean of 14 hours), the duration of action can be extended for 12 to 24 hours. The route is suitable for drugs given in dose as high as 1000 mg. A drug whose absorption is pH dependent, destabilized by GI fluids/enzymes, undergoes extensive presystemic metabolism (e.g. nitroglycerine), influenced by gut motility, has an absorption window and/or absorbed actively (e.g. riboflavin), is a poor candidate for oral controlled release formulations.

(b) **Intramuscular/Subcutaneous Routes** : These routes are suitable when the duration of action is to be prolonged from 24 hours to 12 months. Only a small amount of drug, about 2 ml or 2 grams, can be administered by these routes. Factors important in drug release by such routes are solubility of drug in the surrounding tissues, molecular weight, partition coefficient and pK_a of the drug and contact surface between the drug and the surrounding tissues.

(c) **Transdermal Route** : Low dose drugs like nitroglycerine can be administered by this route. The route is best suited for drugs showing extensive first-pass metabolism upon oral administration. Important factors to be considered for percutaneous drug absorption are partition coefficient of drug, contact area, skin condition, skin permeability of drug, skin perfusion rate, etc.

In short, the main determinants in deciding a route for administration of a controlled release system are physicochemical properties of the drug, dose size, absorption efficiency and desired duration of action.

Pharmacokinetic Characteristics of the Drug

A detailed knowledge of the ADME characteristics of a drug is essential in the design of a controlled release product. An optimum range of a given pharmacokinetic parameter of a drug is necessary beyond which controlled delivery is difficult or impossible.

1. Absorption Rate : For a drug to be administered as controlled release formulation, its absorption must be efficient since the desired rate-limiting step is rate of drug release K_r i.e. $K_r << K_a$. A drug with slow absorption is a poor candidate for such dosage forms since continuous release will result in a pool of unabsorbed drug e.g. iron. Aqueous soluble but poorly absorbed potent drugs like decamethonium are also unsuitable candidates since a slight variation in the absorption may precipitate potential toxicity.

2. Elimination Half-Life : Smaller the $t_{1/2}$, larger the amount of drug to be incorporated in the controlled release dosage form. For drugs with $t_{1/2}$ less than 2 hours, a very large dose may be required to maintain the high release rate. Drugs with half-life in the range 2 to 4 hours make good candidates for such a system e.g. propranolol. Drugs with long half-life need not be presented in such a formulation e.g. amlodipine. For some drugs e.g. MAO inhibitors, the duration of action is longer than that predicted by their half-lives. A candidate drug must have $t_{1/2}$ that can be correlated with its pharmacologic response. In terms of MRT, a drug administered as controlled release dosage form should have MRT significantly longer than that from conventional dosage forms.

3. Rate of Metabolism : A drug which is extensively metabolized is suitable for controlled release system as long as the rate of metabolism is not too rapid. The extent of metabolism should be identical and predictable when the drug is administered by different routes. A drug capable of inducing or inhibiting metabolism is a poor candidate for such a product since steady-state blood levels would be difficult to maintain.

4. Dosage Form Index (DI) : *It is defined as the ratio of* $C_{ss,max}$ *to* $C_{ss,min}$. Since the goal of controlled release formulation is to improve therapy by reducing the dosage form index while maintaining the plasma drug levels within the therapeutic window, ideally its value should be as close to *one* as possible.

Pharmacodynamic Characteristics of the Drug

1. Therapeutic Range : A candidate drug for controlled delivery system should have a therapeutic range wide enough such that variations in the release rate do not result in a concentration beyond this level.

2. Therapeutic Index (TI) : The release rate of a drug with narrow therapeutic index should be such that the plasma concentration attained is within the therapeutically safe and effective range. This is necessary because such drugs have toxic concentration nearer to their therapeutic range. Precise control of release rate of a potent drug with narrow margin of safety is difficult. A drug with short half-life and narrow therapeutic index should be administered more frequently than twice a day. One must also consider the activity of drug metabolites since controlled delivery system controls only the release of parent drug but not its metabolism.

3. Plasma Concentration-Response Relationship : Drugs such as reserpine whose pharmacologic activity is independent of its concentration are poor candidates for controlled release systems.

PHARMACOKINETIC PRINCIPLES IN THE DESIGN AND FABRICATION OF CONTROLLED DRUG DELIVERY SYSTEMS

The controlled release dosage forms are so designed that they release the medicament over a prolonged period of time usually longer than the typical dosing interval for a conventional formulation. The drug release rate should be so monitored that a steady plasma concentration is attained by reducing the ratio $C_{ss,max}/C_{ss,min}$ while maintaining the drug levels within the therapeutic window. The rate-controlling step in the drug input should be determined not by the absorption rate but by the rate of release from the formulation which ideally should be slower than the rate of absorption. In most cases, the release rate is so slow that if the drug exhibits two-compartment kinetics with delayed distribution under normal circumstances, it will be slower than the rate of distribution and one can, thus, *collapse* the plasma concentration-time profile in such instances into a one-compartment model i.e. *a one-compartment model is suitable and applicable for the design of controlled drug delivery systems.* Assuming that the KADME of a drug are first-order processes, to achieve a steady, nonfluctuating plasma concentration, the rate of release and hence rate of input of drug from the controlled release dosage form should be identical to that from constant rate intravenous infusion. In other words, the rate of drug release from such a system should ideally be zero-order or near zero-order. One can thus treat the desired release rate R_o of controlled drug delivery system according to constant rate i.v. infusion. In order to maintain the desired steady-state concentration C_{ss}, the rate of drug input, which is zero-order release rate (R_o), must be equal to the rate of output (assumed to be first-order elimination process). Thus:

$$R_o = R_{output} \tag{15.1}$$

The rate of drug output is given as the product of maintenance dose D_M and first-order elimination rate constant K_E.

$$R_{output} = D_M K_E \tag{15.2}$$

For a zero-order constant rate infusion, the rate of output is also given as:

$$R_{output} = K_E C_{ss} V_d \tag{15.3}$$

Since $Cl_T = K_E V_d$, the above equation can also be written as:

$$R_{output} = C_{ss} Cl_T \tag{15.4a}$$

or

$$R_o = C_{ss} Cl_T \tag{15.4b}$$

The bioavailability of a drug from controlled release dosage form cannot be 100% as the total release may not be 100% and the drug may

also undergo presystemic metabolism. Hence, if F is the fraction bioavailable, then:

$$R_o = \frac{C_{ss} Cl_T}{F} \tag{15.5}$$

and

$$R_o = \frac{D_M}{\tau} \tag{15.6}$$

Substituting equation 15.6 in equation 15.5 and rearranging, we get:

$$D_M = \frac{C_{ss} Cl_T \tau}{F} \tag{15.7}$$

where τ = dosing interval. From above equation, one can calculate the dose of drug that must be released in a given period of time in order to achieve the desired target steady-state concentration. It also shows that total systemic clearance is an important parameter in such a computation.

Since attainment of steady-state levels with a zero-order controlled drug release system would require a time period of about 5 biological half-lives, an immediate release dose, D_I, called as 'loading dose', may be incorporated in such a system in addition to the controlled release components. The total dose, D_T needed to maintain therapeutic concentration in the body would then be:

$$D_T = D_I + D_M \tag{15.8}$$

The immediate release dose is meant to provide the desired steady-state rapidly and can be calculated by equation:

$$D_I = \frac{C_{ss} V_d}{F} = \frac{R_o}{K_E} \tag{15.9}$$

The above equation ignores the possible additive effect from the immediate and controlled release components. For many controlled release products, there is no built-in loading dose.

The dosing interval for a drug following one-compartment kinetics with linear disposition is related to elimination half-life and therapeutic index TI according to equation:

$$\tau < t_{1/2} \ln (TI/2) \tag{15.10}$$

DRUG RELEASE PATTERNS OF CONTROLLED DELIVERY DOSAGE FORMS

If one assumes that—

1. drug disposition follows first-order kinetics.

2. the rate-limiting step in the absorption is rate of drug release from the controlled release formulation (i.e. $K_r < K_a$), and
3. the released drug is rapidly and completely absorbed,

then, the four models for drug input based on the drug release pattern can be defined:

1. Slow zero-order release.
2. Slow first-order release.
3. Initial rapid release of loading dose followed by slow zero-order release.
4. Initial rapid release of loading dose followed by slow first-order release.

The resulting profiles are depicted in Fig. 15.3.

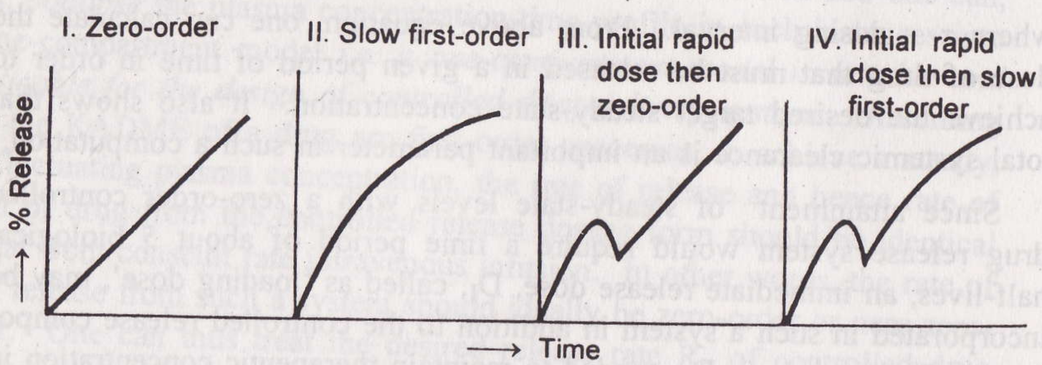


Fig. 15.3 Schematic representation of four major types of drug release characteristics from controlled release formulations

Zero-Order Release Systems

If the drug released from controlled release formulations is stable in fluids at the absorption site, has similar absorption efficiency from all absorption sites and is absorbed rapidly and completely after its release, then, its rate of appearance in plasma will be governed by its rate of release from the controlled release formulation. Thus, when the drug release follows zero-order kinetics, absorption will also be a zero-order process and concentration of drug in plasma at any given time can be given by equation:

$$C = \frac{F\,K_o\,(1 - e^{-K_E t})}{K_E\,V_d} \qquad (15.11)$$

where K_o = zero-order release rate constant, also written as R_o, the zero-order release rate.

The above equation is similar to the one that expresses the concentration-time course of a drug that shows one-compartment kinetics following

constant rate i.v. infusion. The time to reach steady-state depends upon the elimination half-life of the drug. It is usually not possible with a single oral controlled release dose to attain the plateau even with a drug having short half-life such as 3 hours since the mean GI residence time is around 12 hours. It takes 4.3 half-lives for attainment of 95% steady-state values. Thus, if $t_{1/2}$ is 3 hours, about 13 hours will be required for the drug to reach plateau. Slower the elimination, (longer the $t_{1/2}$), more the time required to reach steady-state. Once the desired steady-state is reached with repeated dosing of zero-order controlled release formulation, minimal fluctuations will be observed. Zero-order release systems are thus ideal controlled delivery formulations.

Slow First-Order Release Systems

Such systems are easier to design but are inferior to zero-order systems especially when they are meant for oral use. This is because with first-order release characteristics, smaller and smaller amounts of drug are released as time passes and secondly, as the formulation advances along the GIT, the absorption efficiency generally decreases due to a number of reasons like decreased intestinal surface area, increased viscosity and decreased mixing. Thus, larger amounts are needed to be released at a later stage when in fact the opposite happens with first-order systems.

The concentration of drug in plasma following administration of a controlled release formulation with slow first-order release is given by equation:

$$C = \frac{D_M\,K_r\,F}{V_d\,(K_r - K_E)}\,(e^{-K_E t} - e^{-K_r t}) \qquad (15.12)$$

When $K_r < K_E$, flip-flop phenomena is observed which is a common feature for such controlled release formulations. With repeated dosing of slow first-order release formulations, one generally observes a lower C_{max}, higher C_{min} and longer t_{max} in comparison to conventional release formulations.

Zero-Order Release Systems with a Rapid Release Component

With such formulations, an initial dose is rapidly released (**burst-effect**) for immediate first-order availability while the remaining amount is released and absorbed at a slow zero-order rate. The equation for concentration-time course of such a formulation contains two portions, one each to denote rapid first-order release and slow zero-order release.

$$C = \frac{D_I\,K_a\,F}{V_d\,(K_a - K_E)}\,(e^{-K_E t} - e^{-K_a t}) + \frac{K_o\,F}{K_E\,V_d}\,(1 - e^{-K_E t}) \qquad (15.13)$$

Such a formulation is ideally suited for drugs with long $t_{1/2}$ in which cases attainment of plateau would otherwise take a long time. The slow release component should ideally begin releasing the drug when the drug levels from the fast component are at a peak. However, the approach suffers from a big disadvantage when the formulation is meant for repetitive dosing—the blood level profile shows a peak-trough pattern (which normally does not result when all of the drug is released at a slow zero-order rate); this may cause a momentous rise in peak concentration immediately after each dose triggering toxic reactions (*see* Fig. 15.4). It is for this reason that such a design is unpopular.

The transient fluctuations in the peak concentration with these formulations can however be overcome by:

1. Decreasing the loading dose in the subsequent dosage forms (which appears to be impractical),
2. Increasing the dosing interval (this also seems to be tedious), or
3. Administering an immediate release conventional dosage form prior to repetitive dosing of zero-order controlled release formulation instead of incorporating it in the latter.

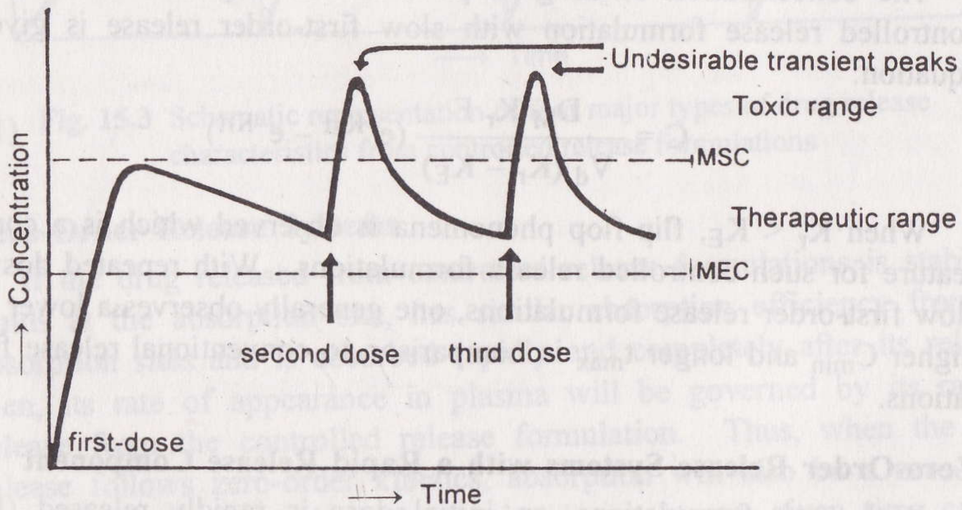


Fig. 15.4 Plasma concentration-time profile which results from repeated dosing of a controlled release formulation with zero-order release and an initial fast release component.

Slow First-Order Release Systems with a Rapid Release Component

The equation describing the time course of plasma drug concentration with this type of formulation will also contain two portions—one to describe rapid first-order absorption and the other for slow first-order absorption from controlled release portion.

$$C = \frac{D_I K_a F}{V_d (K_a - K_E)} (e^{-K_E t} - e^{-K_a t}) + \frac{D_M K_r F}{V_d (K_r - K_E)} (e^{-K_E t} - e^{-K_r t}) \quad (15.14)$$

As in the previous case, to obtain the desired plateau, the slow release component, D_M should start releasing the drug:

1. When the peak has been attained with rapid release dose, D_I; this requires $D_M >> D_I$ which results in wastage of drug since the absorption efficiency reduces as time passes and dosage form descends down the GIT, or
2. When all of the D_I has been released; this requires relatively small D_M and therefore less drug wastage and better sustained levels despite fluctuations in drug levels (Fig. 15.5).

The problems that result from repeated dosing of this type of formulation are similar to that described for the third type of release pattern and can be handled in a similar manner.

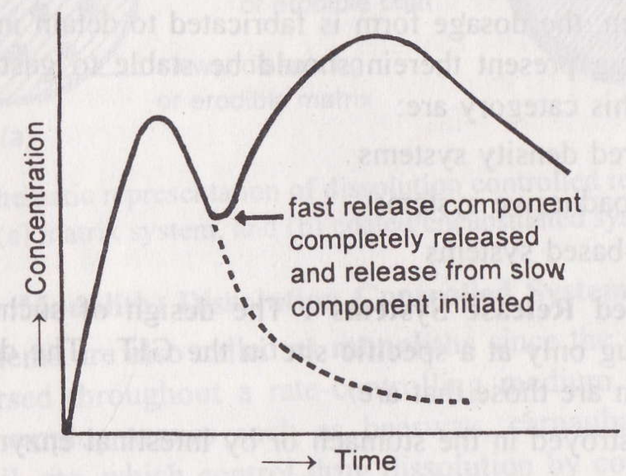


Fig. 15.5 Plasma concentration-time profile which results after a single oral dose of controlled delivery system containing a rapid release dose and a slow first-order release component

ORAL CONTROLLED RELEASE SYSTEMS

Oral route has been the most popular and successfully used for controlled delivery of drugs because of convenience and ease of administration, greater flexibility in dosage form design (possible because of versatility of GI anatomy and physiology) and ease of production and low cost of such a system.

The controlled release systems for oral use are mostly solids and based on dissolution, diffusion or a combination of both mechanisms in the

control of release rate of drug. Depending upon the manner of drug release, these systems are *classified* as follows:

A. Continuous Release Systems : These systems release the drug for a prolonged period of time along the entire length of GIT (especially upto the terminal region of small intestine) with normal transit of the dosage form. The various systems under this category are:

1. Dissolution controlled release systems
2. Diffusion controlled release systems
3. Dissolution and diffusion controlled release systems
4. Ion-exchange resin-drug complexes
5. Slow dissolving salts and complexes
6. pH-dependent formulations
7. Osmotic pressure controlled systems
8. Hydrodynamic pressure controlled systems

B. Delayed Transit and Continuous Release Systems : These systems are designed to prolong their residence in the GIT along with their release. Often, the dosage form is fabricated to detain in the stomach and hence the drug present therein should be stable to gastric pH. Systems included in this category are:

1. Altered density systems
2. Mucoadhesive systems
3. Size-based systems

C. Delayed Release Systems : The design of such systems involve release of drug only at a specific site in the GIT. The drugs contained in such a system are those that are:

i. destroyed in the stomach or by intestinal enzymes
ii. known to cause gastric distress
iii. absorbed from a specific intestinal site, or
iv. meant to exert local effect at a specific GI site.

The two types of delayed release systems are:

1. Intestinal release systems
2. Colonic release systems

Dissolution Controlled Release Systems

Such systems are easiest to design. The drug present in such a system may be the one:

i. with inherently slow dissolution rate e.g. griseofulvin and digoxin; such drugs act as natural prolonged release products
ii. that produce slow dissolving forms when it comes in contact with GI fluids e.g. ferrous sulfate, or
iii. having high aqueous solubility and dissolution rate e.g. pentoxifylline.

Drugs belonging to the last category present challenge in controlling their dissolution rate. The techniques employed are:

1. Embedment in slowly dissolving or erodible matrix, and
2. Encapsulation or coating with slowly dissolving or erodible substances (Fig. 15.6.).

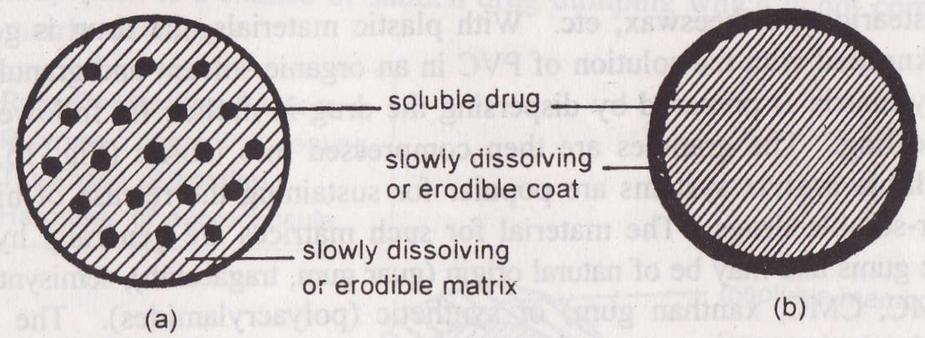


Fig. 15.6 Schematic representation of dissolution controlled release systems —(a) matrix system, and (b) coated/encapsulated system

Matrix (or Monolith) Dissolution Controlled Systems

Matrix systems are also called as **monoliths** since the drug is homogeneously dispersed throughout a rate-controlling medium. They are very common and employ waxes such as beeswax, carnauba wax, hydrogenated castor oil, etc. which control drug dissolution by controlling the rate of dissolution fluid penetration into the matrix by altering the porosity of tablet, decreasing its wettability or by itself getting dissolved at a slower rate. The wax embedded drug is generally prepared by dispersing the drug in molten wax and congealing and granulating the same. The drug release is often first-order from such matrices.

Encapsulation/Coating Dissolution Controlled Systems (Reservoir Devices)

Here, the drug particles are coated or encapsulated by one of the several microencapsulation techniques with slowly dissolving materials like cellulose, PEGs, polymethacrylates, waxes, etc. The resulting pellets may be filled as such in hard gelatin capsules (popularly called as **spansules**) or compressed into tablets. The dissolution rate of coat depends upon the

solubility and thickness of the coating which may range from 1 to 200 microns.

Diffusion Controlled Release Systems

In these types of systems, the rate-controlling step is not the dissolution rate but the diffusion of dissolved drug through a polymeric barrier. The drug release rate is never zero-order since the diffusional path length increases with time as the insoluble matrix is gradually depleted of drug. The two types of diffusion controlled systems are—matrix systems and reservoir devices.

Matrix Diffusion Controlled Systems

Here, the drug is dispersed in an insoluble matrix of rigid nonswellable hydrophobic materials or swellable hydrophilic substances. Materials used for **rigid matrix** are insoluble plastics such as PVC and fatty materials like stearic acid, beeswax, etc. With plastic materials, the drug is generally kneaded with the solution of PVC in an organic solvent and granulated. Waxy matrix is prepared by dispersing the drug in molten fat followed by congealing. The granules are then compressed into tablets (Fig. 15.7.a). **Swellable matrix** systems are popular for sustaining the release of highly water-soluble drugs. The material for such matrices are generally hydrophilic gums and may be of natural origin (guar gum, tragacanth), semisynthetic (HPMC, CMC, xanthan gum) or synthetic (polyacrylamides). The drug and the gum are granulated together with a solvent such as alcohol and compressed into tablets. The release of drug from such initially dehydrated hydrogels involves simultaneous absorption of water (resulting in hydration, gelling and swelling of gum) and desorption of drug via a swelling controlled diffusion mechanism. As the gum swells and the drug

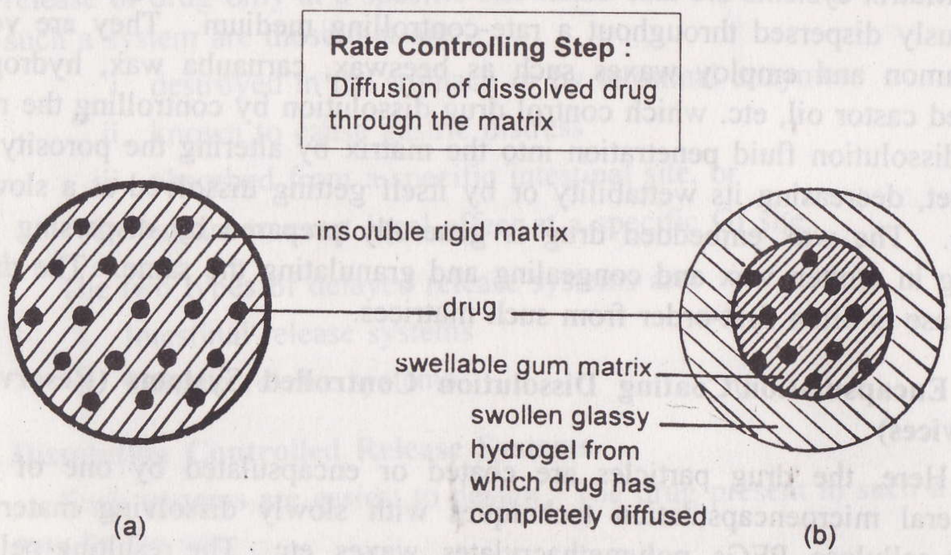


Fig. 15.7 Diffusion controlled devices—(a) rigid matrix, and (b) swellable matrix

diffuses out of it, the swollen mass, devoid of drug appears transparent or glasslike and therefore the system is sometimes called as **glassy hydrogel** (Fig. 15.7*b*). The drug release follows Fickian first-order diffusion under equilibrium conditions. However, during the swelling process, such an equilibrium may not exist and the diffusion may be non-Fickian or anomalous diffusion.

Reservoir Devices (or Laminated Matrix Devices)

These systems are hollow containing an inner core of drug surrounded in a water insoluble polymer membrane. The polymer can be applied by coating or microencapsulation techniques. The drug release mechanism across the membrane involves its partitioning into the membrane with subsequent release into the surrounding fluid by diffusion (Fig. 15.8). The polymers commonly used in such devices are HPC, ethyl cellulose and polyvinyl acetate. A disadvantage of all such microencapsulated drug release systems is a chance of sudden drug dumping which is not common with matrix devices.

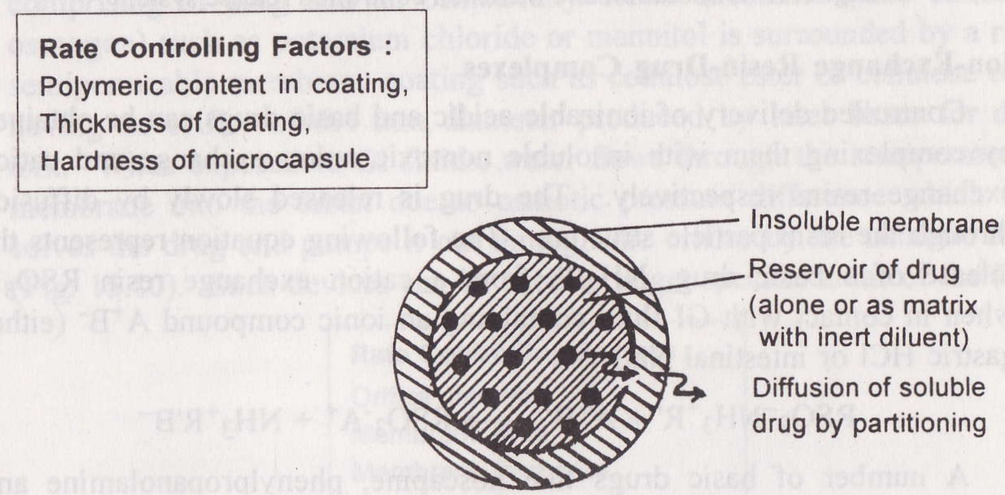


Fig. 15.8 Drug release by diffusion across the insoluble membrane of reservoir device

Dissolution and Diffusion Controlled Release Systems

In such systems, the drug core is encased in a partially soluble membrane. Pores are thus created due to dissolution of parts of the membrane which:

—permit entry of aqueous medium into the core and hence drug dissolution, and

—allow diffusion of dissolved drug out of the system (Fig. 15.9).

An example of obtaining such a coating is using a mixture of ethyl cellulose with PVP or methyl cellulose; the latter dissolves in water and creates pores in the insoluble ethyl cellulose membrane.

Rate Controlling Factor :
Fraction of soluble polymer in the coat

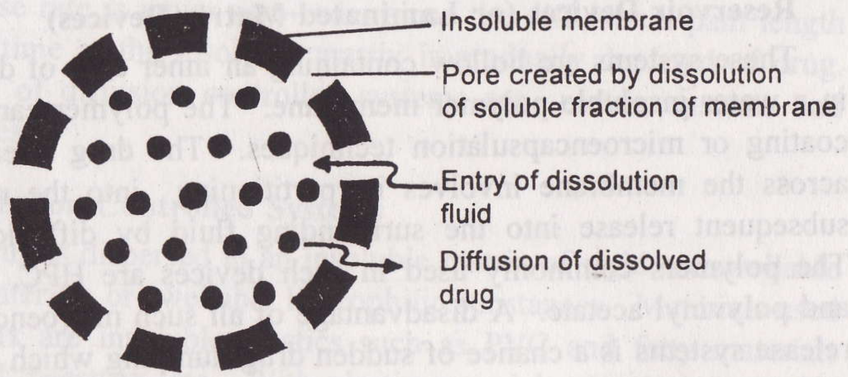


Fig. 15.9 Dissolution and diffusion controlled release system

Ion-Exchange Resin-Drug Complexes

Controlled delivery of ionizable acidic and basic drugs can be obtained by complexing them with insoluble nontoxic anion exchange and cation exchange resins respectively. The drug is released slowly by diffusion through the resin particle structure. The following equation represents the release of a basic drug, NH_2R', from a cation exchange resin RSO_3H when in contact with GI fluid containing an ionic compound A^+B^- (either gastric HCl or intestinal NaCl):

$$RSO_3^-NH_3^+R' + A^+B^- \longrightarrow RSO_3^-A^+ + NH_3^+R'B^-$$

A number of basic drugs like noscapine, phenylpropanolamine and phentermine have been retarded by such an approach. The complex can be prepared by incubating the drug-resin solution or passing the drug solution through a column containing ion-exchange resin. The drug-resin complex can be coated with cellulose or hard paraffin and formulated as ion free suspension for pediatric use.

Slow Dissolving Salts and Complexes

Salts or complexes of drugs which are slowly soluble in the GI fluids can be used for controlled release of the active principle. Amine drugs can be reacted with tannic acid to form poorly soluble complexes that can be formulated as long acting tablets. Penicillin G has been complexed with N,N'-dibenzyl ethylenediamine to give benzathine penicillin G that can be formulated as oral suspension. Such complexes can be obtained by simple acid-base reaction on mixing together solutions of individual compounds.

pH-Independent Formulations

Such systems are designed to eliminate the influence of changing GI pH on dissolution and absorption of drugs by formulating them with sufficient amount of buffering agents (salts of phosphoric, citric or tartaric acids) that adjust the pH to the desired value as the dosage form passes along the GIT and permit drug dissolution and release at a constant rate independent of GI pH. The dosage form containing drug and buffer is coated with a permeable substance that allows entry of aqueous medium but prevents dispersion of tablet.

Osmotic Pressure Controlled Systems

Unlike the solution-diffusion mechanism for most systems, an **oral osmotic pump**, popularly called as **oros**, works on the principle of osmotic pressure to release the drug at a constant zero-order rate. A core comprising of drug and an osmotically active substance (also called as **osmogen**) such as potassium chloride or mannitol is surrounded by a rigid semipermeable membrane coating such as cellulose ester or cellulose ether having an orifice of 0.4 mm diameter produced by laser beam for drug exit. When exposed to GI fluids, water flows through the semipermeable membrane into the tablet due to osmotic pressure difference which dissolves the drug and pumps it out through the orifice by the osmotic force (Fig. 15.10). Such devices can be used to target specific areas of the GIT.

Rate Controlling Factors :
Orifice diameter
Membrane area
Membrane thickness
Membrane permeability
Osmotic properties of the core
Drug solubility

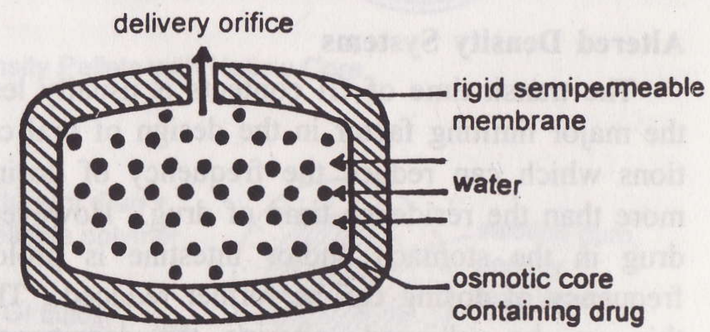


Fig. 15.10 Oral osmotic pump (oros)

The oros principle can be used to design *multiunit dosage forms* consisting of drug core particles coated with a water permeable membrane

in which the delivery orifice is made by using a channeling agent such as PVP and the coated particles filled in a capsule.

Hydrodynamic Pressure Controlled Systems

The hydrodynamic pressure generated by swelling of a hydrophilic gum can also be used to activate the delivery of drugs. The device comprises of a rigid, shape retaining housing enclosing a collapsible, impermeable compartment containing liquid drug (Fig. 15.11). The space between the external housing and the drug compartment contains a layer of swellable, hydrophilic gum such as polyhydroxyalkyl methacrylate. In the GIT, the gum imbibes water through the opening present at the lower side of external housing and swells creating a hydrodynamic pressure. The pressure thus created squeezes the collapsible drug reservoir to release the medicament through the delivery orifice at a zero-order rate. Such systems are also called as **push-pull osmotic pumps**.

Rate Controlling Factors :
Fluid permeability
Surface area of wall with openings
Hydrodynamic pressure gradient

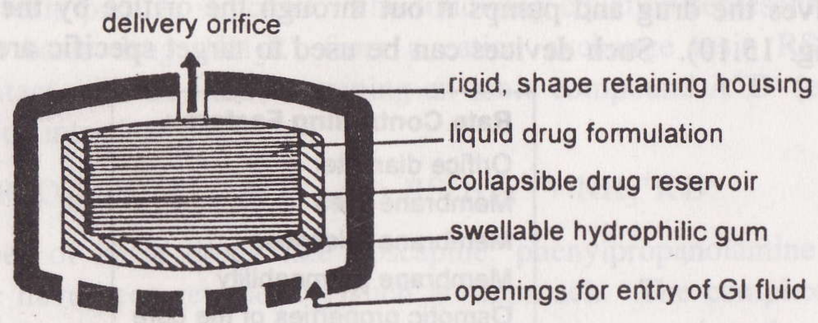


Fig. 15.11 Hydrodynamic pressure controlled system (push-pull osmotic pump)

Altered Density Systems

The transit time of GI contents is usually less than 24 hours. This is the major limiting factor in the design of oral controlled release formulations which can reduce the frequency of dosing to a time period little more than the residence time of drug. However, if the residence time of drug in the stomach and/or intestine is prolonged in some way, the frequency of dosing can be further reduced. There are 3 ways by which this can be achieved—altering the density of drug particles, use of mucoadhesive polymers and altering the size of the dosage form. The altered density approach involves use of either high or low density pellets.

High Density Pellets

The density of GI fluids is around 1.4 g/cc. Use of drug pellets having density greater than this value, preferably above 1.6 g/cc, results in prolonged GI residence that is unaffected by food. Iron oxide, titanium dioxide and barium sulfate have been used to increase the density of drug pellets. The drug is coated on the heavy core and then covered by a diffusion controlled membrane (Fig. 15.12*a*).

Low Density Pellets

Also called as **hydrodynamically balanced systems**, such pellets, having density less than that of GI fluids, float on the gastric juice for an extended period of time while slowly releasing the drug. Globular shells such as that of poprice and celluloses have been used to lower the density of system (Fig. 15.12*b*). A swellable gum like HPMC can be used for a similar purpose (Fig. 15.12*c*).

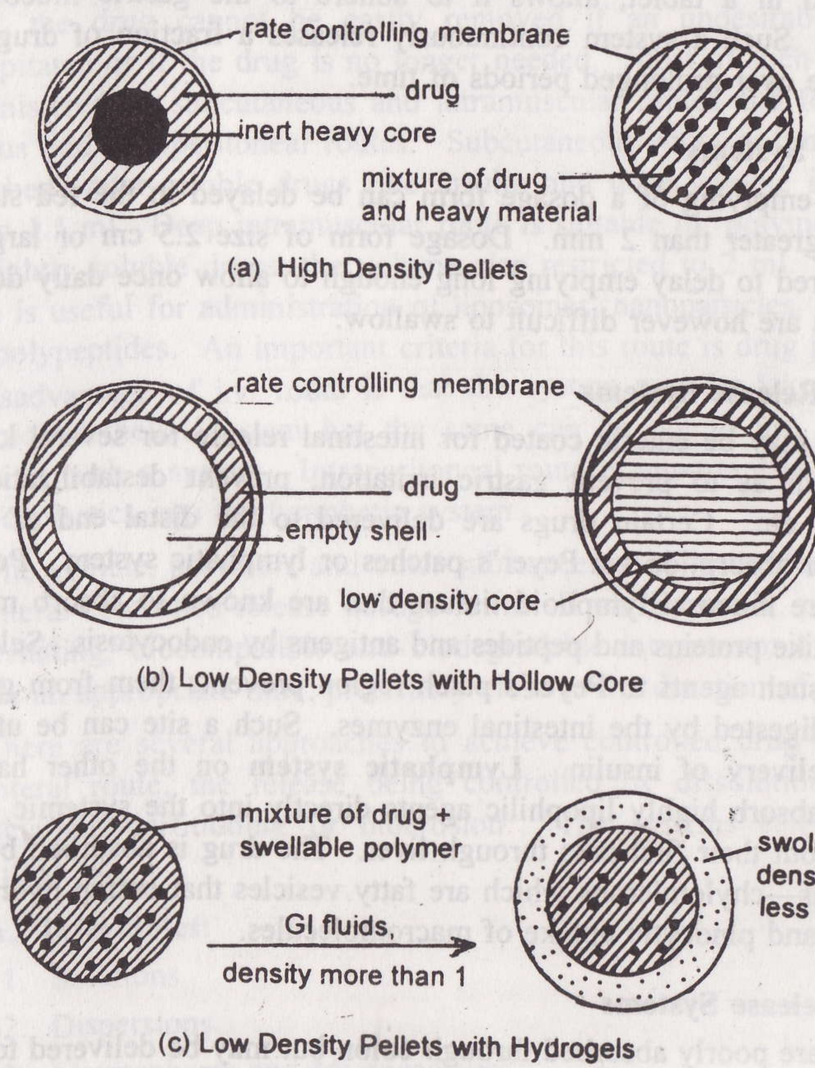


Fig. 15.12 Altered density systems

Floating or buoyant tablets/capsules can be formulated by granulating a drug with 20 to 80% of hydrogel such as HPMC, HEC and HPC. On contact with GI fluids, the tablet swells and forms a diffusible gel barrier that lowers the density of system to less than 1 allowing it to float. Lipophilic polymers such as silicone elastomer can also be modified to have swelling properties. This is achieved by impregnating a water miscible liquid such as glycerol or a water-soluble salt such as sodium chloride in the lipophilic matrix. On contact with aqueous medium, the modified lipophilic polymer swells due to absorption of water by the hydrophilic additives in the matrix. Alternatively, a gas filled flotation chamber can be attached to a membrane coated tablet for making it buoyant.

Mucoadhesive Systems

A bioadhesive polymer such as cross-linked polyacrylic acid, when incorporated in a tablet, allows it to adhere to the gastric mucosa or epithelium. Such a system continuously releases a fraction of drug into the intestine over prolonged periods of time.

Size-Based Systems

Gastric emptying of a dosage form can be delayed in the fed state if its size is greater than 2 mm. Dosage form of size 2.5 cm or larger is often required to delay emptying long enough to allow once daily dosing. Such forms are however difficult to swallow.

Intestinal Release Systems

A drug may be enteric coated for intestinal release for several known reasons such as to prevent gastric irritation, prevent destabilization in gastric pH, etc. Certain drugs are delivered to the distal end of small intestine for absorption via Peyer's patches or lymphatic system. **Peyer's patches** are mucosal lymphoid tissues that are known to absorb macromolecules like proteins and peptides and antigens by endocytosis. Selective release of such agents to Peyer's patch region prevents them from getting destroyed/digested by the intestinal enzymes. Such a site can be utilized for oral delivery of insulin. **Lymphatic system** on the other hand is known to absorb highly lipophilic agents directly into the systemic circulation without their first-pass through liver. The drug is absorbed by two mechanisms—chylomicrons which are fatty vesicles that entrap hydrophobic drugs, and pinocytic uptake of macromolecules.

Colonic Release Systems

Drugs are poorly absorbed through colon but may be delivered to such a site for two reasons—

i. Local action as in the treatment of ulcerative colitis with mesalamine, and
ii. Systemic absorption of protein and peptide drugs like insulin and vasopressin.

Advantage is taken of the fact that pH-sensitive bioerodible polymers like polymethacrylates release the medicament only at the alkaline pH of colon or use of divinylbenzene cross-linked polymers that can be cleaved only by the azoreductase of colonic bacteria to release free drug for local effect or systemic absorption.

PARENTERAL CONTROLLED RELEASE SYSTEMS

One of the major **advantages** of parenteral controlled drug delivery systems is that the duration of action can be extended for days or months and sometimes upto a year. The prime *drawback* is that, once administered, the drug cannot be easily removed if an undesirable action is precipitated or if the drug is no longer needed. Most of such systems are administered by subcutaneous and intramuscular routes and few by intravenous and intraperitoneal routes. Subcutaneous route is limited to well absorbed water-soluble drugs like insulin and dose volume is limited to 0.5 to 1.5 ml. Deep intramuscular route is suitable for polymeric systems or slightly soluble drugs, the volume size restricted to 2 ml. Intravenous route is useful for administration of liposomes, nanoparticles, erythrocytes and polypeptides. An important criteria for this route is drug particle size. A disadvantage of i.v. route is that the system may be taken up by the reticuloendothelial system but the same can be put to use in targeting drugs to such a system. Intraperitoneal route is important in targeting of antineoplastics into the lymphatic system.

The vehicle, polymers and other substances used in the formulation of parenteral controlled release dosage forms should be sterile, pyrogen free, nonirritating, biocompatible and biodegradable into nontoxic compounds within an appropriate time, preferably close to the duration of drug action.

There are several approaches to achieve controlled drug delivery via parenteral route, the release being controlled by dissolution, diffusion, dissociation, partitioning or bioerosion. The systems can be broadly classified as:

A. Injectables:

1. Solutions
2. Dispersions
3. Microspheres and Microcapsules
4. Nanoparticles and Niosomes

5. Liposomes
6. Resealed Erythrocytes

B. Implants

C. Infusion Devices:

1. Osmotic Pumps
2. Vapor Pressure Powered Pumps
3. Battery Powered Pumps

Solutions

Both aqueous as well as oil solutions may be used for controlled drug release. With *aqueous solutions* (given intramuscularly), the drug release may be controlled in three ways:

i. By increasing the viscosity of vehicle by use of MC, CMC or PVP and thus, decreasing molecular diffusion and localizing the injected drug.

ii. By forming a complex with macromolecules like MC, CMC or PVP from which the drug dissociates at a controlled rate (only free drug will get absorbed).

iii. By forming complexes that control drug release not by dissociation but by reducing the solubility of parent drug e.g. protamine zinc insulin and cyanocobalamin zinc tannate.

Oil solutions control the release by partitioning the drug out of the oil in the surrounding aqueous biofluids. Vegetable oils like arachis oil, cottonseed oil, etc. are used for such a purpose. The method is applicable only to those drugs which are oil soluble and have optimum partition coefficient.

Dispersions

Dispersed systems like emulsions and suspensions can be administered by i.m., s.c. or i.v. routes. Among *emulsions*, the o/w systems have not been used successfully since absorption of drug incorporated in the oil phase is rapid due to large interfacial area and rapid partitioning. Similarly, few w/o emulsions of water-soluble drugs have been tried for controlled release. **Multiple emulsions** of w/o/w and o/w/o types (more correctly, **double emulsions**) are becoming popular since an additional reservoir is presented to the drug for partitioning which can effectively retard its release rate (Fig. 15.13).

Control of drug release from *suspensions* is easier and predictable. Drug dissolution and subsequent diffusion are the main rate controlling

steps. Release of water-soluble drugs can be retarded by presenting it as oil suspension and *vice versa* for oil soluble drugs. Factors to be considered in the formulation of such a system include -

i. Solid content : should be ideally in the range 0.5 to 5.0%

ii. Particle size : this factor is very important since larger the particle size, slower the dissolution; however, larger particles have their own disadvantages like causing irritation at the injection site (size should therefore be below 10 microns), poor syringeability and injectability and rapid sedimentation. The latter problem can be overcome by use of viscosity builders which also retard drug diffusion.

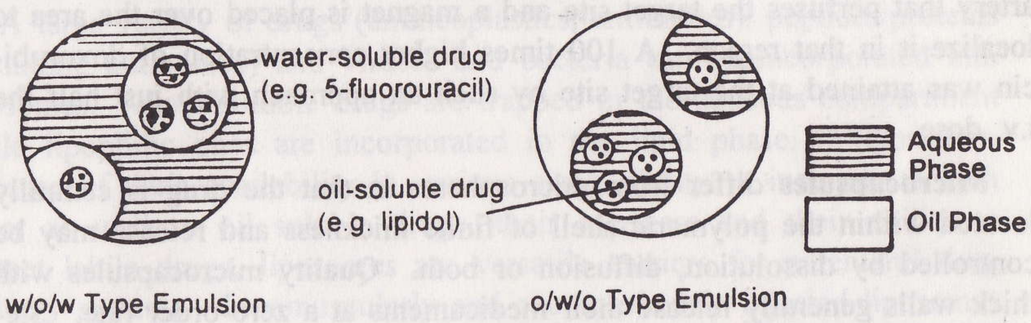


Fig. 15.13 Multiple emulsions for parenteral controlled release systems

Aqueous suspensions can be given by i.m. or s.c. routes. Generally crystalline and stable polymorphic forms of the drug are chosen rather than amorphous forms to delay release. Solubility can be further reduced by salt or complex formation e.g. crystalline zinc insulin shows more prolonged action than amorphous zinc insulin complex. *Oil suspensions*, generally given i.m., prolong drug action much more in comparison to oil solution and aqueous suspension since drug release involves two rate-limiting steps viz. dissolution of drug particles, and partitioning of the dissolved drug from oil to the aqueous biofluids.

Microspheres and Microcapsules

Microspheres are free flowing powders consisting of spherical particles of size ideally less than 125 microns that can be suspended in a suitable aqueous vehicle and injected by an 18 or 20 number needle. Each particle is basically a matrix of drug dispersed in a polymer from which release occurs by a first-order process. The polymers used are biocompatible and biodegradable e.g. polylactic acid, polylactide coglycolide, etc. Drug release is controlled by dissolution/degradation of matrix. Small matrices release drug at a faster rate and thus, by using particles of different sizes, various degrees of controlled release can be achieved. The

system is ideally suited for controlled release of peptide/protein drugs such as LHRH which have short half-lives and otherwise need to be injected once or more, daily, as conventional parenteral formulations. In comparison to peptides, proteins are difficult to formulate because of their higher molecular weight, lower solubility and the need to preserve their conformational structure during manufacture.

In order to overcome uptake of intravenously administered microspheres by the reticuloendothelial system and promote drug targeting to tumors with good perfusion, **magnetic microspheres** were developed. They are prepared from albumin and magnetite (Fe_2O_3) and have a size of 1.0 micron to permit intravascular injection. The system is infused into an artery that perfuses the target site and a magnet is placed over the area to localize it in that region. A 100 times higher concentration of doxorubicin was attained at the target site by such an approach with just half the i.v. dose.

Microcapsules differ from microspheres in that the drug is centrally located within the polymeric shell of finite thickness and release may be controlled by dissolution, diffusion or both. Quality microcapsules with thick walls generally release their medicaments at a zero-order rate. Steroids, peptides and antineoplastics have been successfully administered parenterally by use of controlled release microcapsules.

Nanoparticles and Niosomes

Nanoparticles are also called as **nanospheres** or **nanocapsules** depending upon whether the drug is in a polymer matrix or encapsulated in a shell. They differ from microspheres in having submicron particles in the nanometer size range—10 to 1000 nm. The polymers used are the usual biodegradable ones. The main advantage of this system is that it can be stored for upto 1 year and can be used for selective targeting via reticuloendothelial system to liver and to cells that are active phagocytically.

Like nanoparticles, **niosomes** are inexpensive alternatives to liposomes. They are closed vesicles formed in aqueous media from nonionic surfactants with or without the presence of cholesterol or other lipids.

Liposomes

The term **liposomes** (meaning *lipid body*) was derived on the basis of names of subcellular particles like lysosome and ribosome. It is defined as a spherule/vesicle of lipid bilayers enclosing an aqueous compartment. The lipid most commonly used is phospholipid. Sphingolipids, glycolipids and sterols have also been used to prepare liposomes. Their size ranges from 25 to 5000 nm. Depending upon their structure, liposomes are classified as:

i. MLV (*multilamellar vesicles*) : These liposomes are made of series of concentric bilayers of lipids enclosing a small internal volume.

ii. OLV (*oligolamellar vesicles*) : These are made of 2 to 10 bilayers of lipids surrounding a large internal volume.

iii. ULV (*unilamellar vesicles*) : These are made of single bilayer of lipids. They may be SUV (*small unilamellar vesicles*) of size 20 to 40 nm, MUV (*medium unilamellar vesicles*) of size 40 to 80 nm, LUV (*large unilamellar vesicles*) of size 100 to 1000 nm or GUV (*giant unilamellar vesicles*) of size greater than 1000 nm.

A large variety of drugs (antineoplastics, antibiotics), peptides/proteins (including antibodies) and viruses and bacteria can be incorporated into liposomes. Water-soluble drugs are trapped in the aqueous compartment while lipophilic ones are incorporated in the lipid phase of liposomes. Because of their availability in various sizes, ability to incorporate both water as well as oil soluble drugs, their inertness and their ability to protect labile drugs, liposomes are versatile carriers for parenteral drug delivery systems. Intramuscularly and subcutaneously injected liposomes deliver drug at a controlled rate while intravenous administration selectively targets them to reticuloendothelial system and phagocytic cells. A simple method by which liposomes can be produced involves drying an organic solvent solution of lipids onto the wall of a flask/beaker followed by hydration and dispersion of lipid by addition of buffer and mixing (Fig. 15.14).

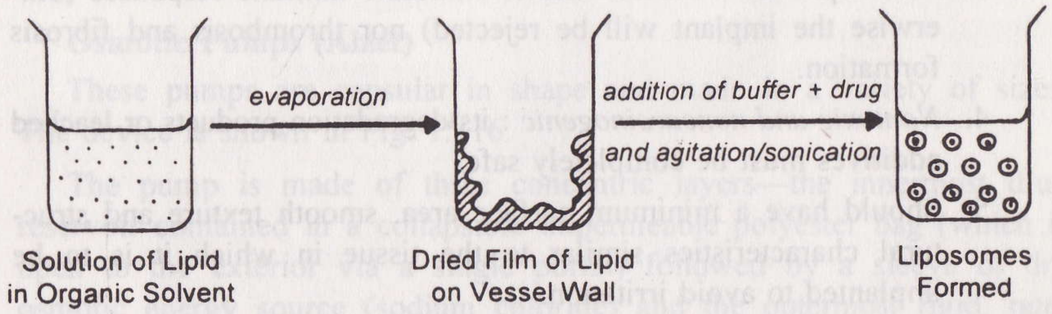


Fig. 15.14 Production procedure for liposomes.

Resealed Erythrocytes

Drug loading in body's own erythrocytes when used to serve as controlled delivery systems have several **advantages**. They are fully biodegradable and biocompatible, nonimmunogenic, can circulate intravascularly for days (act as circulatory drug depots) and allow large amounts of drug to be carried. The drug need not be chemically modified and is

protected from immunological detection and enzymatic inactivation. Drug loading can be done by immersing the cells in buffered hypotonic solution of drug which causes them to rupture and release hemoglobin and trap the medicament. On restoration of isotonicity and incubation at 37° C, the cells reseal and are ready for use (Fig. 15.15).

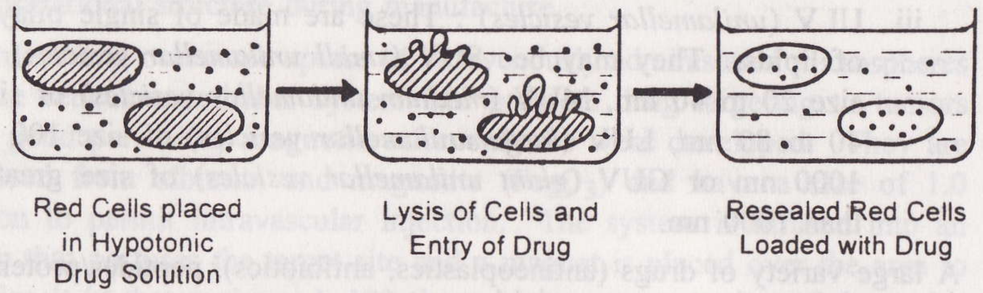


Fig. 15.15 Drug loading in erythrocytes.

Damaged erythrocytes are removed by the liver and spleen. These organs can thus be specifically targeted by drug loaded erythrocytes.

Implants

An *ideal* implantable parenteral system should possess following properties—

1. *Environmentally stable* : should not breakdown under the influence of heat, light, air and moisture.
2. *Biostable* : should not undergo physicochemical degradation when in contact with biofluids (or drugs).
3. *Biocompatible* : should neither stimulate immune responses (otherwise the implant will be rejected) nor thrombosis and fibrosis formation.
4. *Nontoxic and noncarcinogenic* : its degradation products or leached additives must be completely safe.
5. Should have a minimum surface area, smooth texture and structural characteristics similar to the tissue in which it is to be implanted to avoid irritation.
6. Should be *removable* when required.
7. Should release the medicament at a constant predetermined rate for a predetermined period of time.

Some of the important **advantages** of implants over injectable controlled release formulations are—

1. More effective and more prolonged action (for over a year).
2. A significantly small dose is sufficient.

A major **disadvantage** of such systems is that a microsurgery is required for implantation of device. Some devices can be easily implanted by use of a specially designed implanter syringe. The devices are generally implanted subcutaneously or intramuscularly. Subcutaneous tissue is an ideal location because of its easy access to implantation, poor perfusion, slow drug absorption and low reactivity towards foreign materials.

The drug may be dissolved, dispersed or embedded in a matrix of polymers that control release by dissolution, diffusion or both, bioerosion, biodegradation or an activation process such as osmosis or hydrolysis. The system is generally prepared as implantable flexible/rigid moulded or extruded rods, spherical pellets or compressed tablets. Polymers used are silicone elastomers, polymethacrylates, polycaprolactone, polylactide/glycolide, etc. Drugs generally presented in such systems are steroids like contraceptives (megestrol acetate, norgestrone, etc.), morphine antagonists like naltrexone for opiod-dependent addicts, etc.

Infusion Devices

These are also implantable devices but are versatile in the sense that they are intrinsically powered to release the medicament at a zero-order rate and the drug reservoir can be replenished from time to time. Depending upon the mechanism by which these implantable pumps are powered to release the contents, they are classified into following types:

1. Osmotic pressure activated drug delivery systems
2. Vapor pressure activated drug delivery systems
3. Battery powered drug delivery systems

Osmotic Pumps (Alzet)

These pumps are capsular in shape and made in a variety of sizes. The device is shown in Fig. 15.16.

The pump is made of three concentric layers—the innermost drug reservoir contained in a collapsible impermeable polyester bag (which is open to the exterior via a single portal) followed by a sleeve of dry osmotic energy source (sodium chloride) and the outermost rigid, rate-controlling semipermeable membrane fabricated from substituted cellulosic polymers. A rigid polymeric plug is used to form a leakproof seal between the drug reservoir and the semipermeable housing. An additional component, the flow modulator, comprising of a cap and a tube made of stainless steel is inserted into the body of osmotic pump after filling. After implantation, water from the surrounding tissue fluids is imbibed through the semipermeable membrane at a controlled rate that dissolves the osmogen creating an osmotic pressure differential across the mem-

brane. The osmotic sleeve thus expands and since the outer wall is rigid, it squeezes the inner flexible drug reservoir and drug solution is expelled in a constant volume per unit time fashion. The drug delivery continues until the reservoir is completely collapsed. Ionized drugs, macromolecules, steroids and peptides (insulin) can be delivered by such a device.

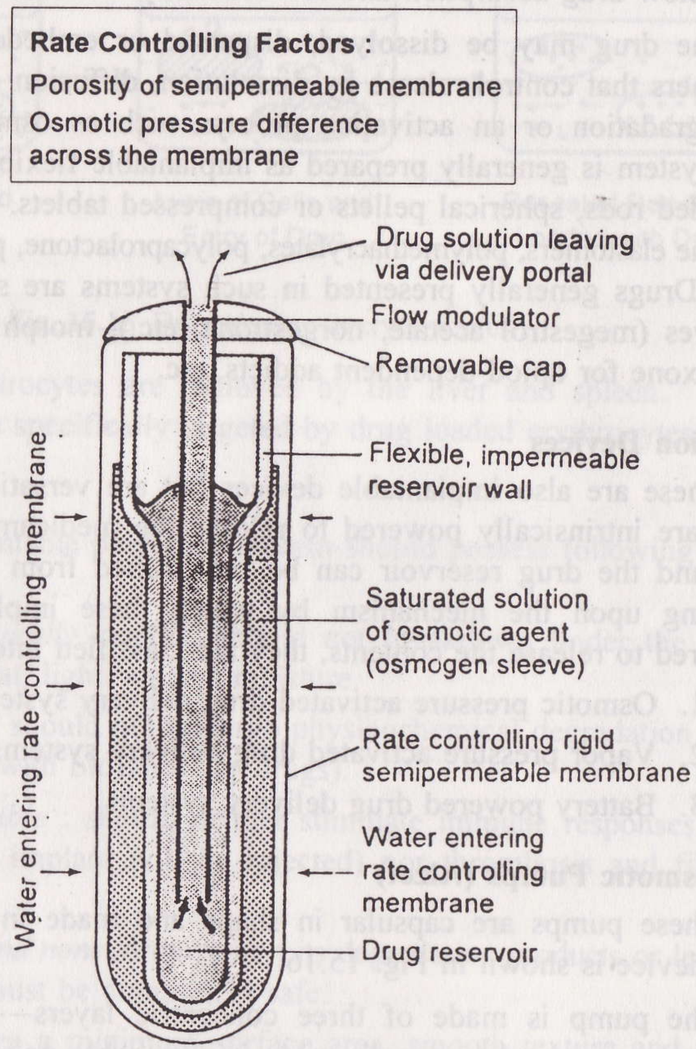


Fig. 15.16 Cross section of osmotic pump

Vapor Pressure Powered Pump (Infusaid)

This device is based on the principle that at a given temperature, a liquid in equilibrium with its vapor phase exerts a constant pressure that is independent of enclosing volume. The device is shown in Fig. 15.17.

The disc shaped device consists of two chambers—an infusate chamber containing the drug solution which is separated by a freely movable flexible bellow from the vapor chamber containing inexhaustible vaporizable fluid such as fluorocarbons. After implantation, the volatile liquid

vaporizes at the body temperature and creates a vapor pressure that compresses the bellows and expels the infusate through a series of flow regulators at a constant rate. Insulin for diabetics and morphine for terminally ill cancer patients have been successfully delivered by such a device.

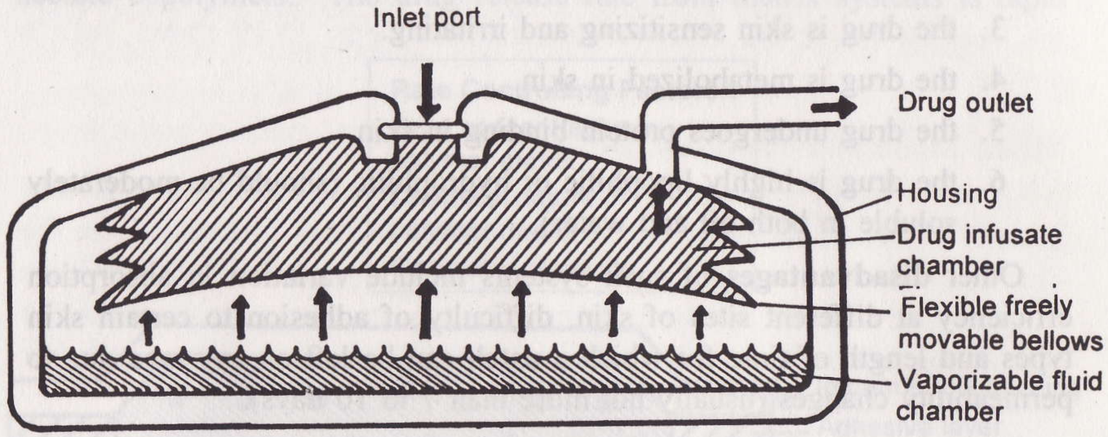


Fig. 15.17 Cross section of vapor pressure driven device

Battery Powered Pumps

Two types of battery powered implantable programmable pumps used successfully to deliver insulin are—peristaltic pump and solenoid driven reciprocating pump, both with electronic controls. The systems can be programmed to deliver drug at desired rates. Their design is such that the drug moves towards the exit and there is no backflow of the infusate.

TRANSDERMAL DRUG DELIVERY SYSTEMS

Transdermal delivery systems are topically administered medicaments in the form of patches that deliver drugs for systemic effects at a predetermined and controlled rate. Some of the **advantages** of these systems over other controlled release formulations are:

1. Drugs with very short half-lives e.g. nitroglycerine when administered as transdermal patches, release medicaments at a constant rate for a time period more than that obtainable with oral formulations.
2. Drugs with narrow therapeutic indices can be safely administered since better control of release is possible.
3. The noninvasive nature of these systems permits easy removal and termination of drug action in situations of toxicity.
4. Problems encountered with oral administration like degradation, gastric irritation, first-pass effect, etc. are avoided.

The route is **unsuitable** when —

1. the drug dose is large.
2. the drug has large molecular size (makes absorption difficult; should ideally be below 800-1000).
3. the drug is skin sensitizing and irritating.
4. the drug is metabolized in skin.
5. the drug undergoes protein binding in skin.
6. the drug is highly lipophilic or hydrophilic (should be moderately soluble in both oil and water).

Other **disadvantages** of such systems include variation in absorption efficiency at different sites of skin, difficulty of adhesion to certain skin types and length of time for which a patch can be left on any area due to permeability changes (usually not more than 7 to 10 days).

Several *types* of transdermal drug delivery devices are available but they can be basically divided into two broad categories based on the mechanism by which drug release is controlled:

1. Monolithic (or matrix) systems.
2. Reservoir (or membrane) systems.

All such devices are fabricated as multilayer laminate structures in which the drug-polymer matrix or a drug reservoir is sandwiched between two polymeric layers. The outer layer, called as *backing layer*, is impermeable and meant to prevent drug loss through it. It is generally composed of metallized plastic. The other layer which contacts the device with the skin is *adhesive layer*. It is permeable and in some cases, may act as rate-limiting membrane. It is generally made of *pressure sensitive adhesive materials* like acrylic copolymers, polyisobutylene and polysiloxane or *contact adhesives*.

The **choice** of monolithic or reservoir type of system for controlling drug release depends upon the major rate-limiting step in the absorption of drug from such devices. The two rate-limiting steps are:

1. Rate of drug diffusion from the device, R_1, and
2. Rate of drug permeation through the stratum corneum, R_2.

The overall rate of drug transport is proportional to the sum $(R_1 + R_2)$.

Monolithic (or Matrix) Devices

These devices are used when **R_2 is the rate-controlling step ($R_2 < R_1$) and the drug has a large therapeutic index** so that overdosing does not precipitate toxic reactions. The two categories of matrix devices are—one in which the drug is dissolved (usually below saturation levels)

in the polymer matrix and the other in which the drug is dispersed (generally much above saturation levels). The polymers employed for matrix systems may be hydrophilic or lipophilic and includes PVC, PVP, polysaccharides, polyesters, microporous polypropylene and ethylene vinyl acetate copolymers. The drug release rate from matrix systems is rapid

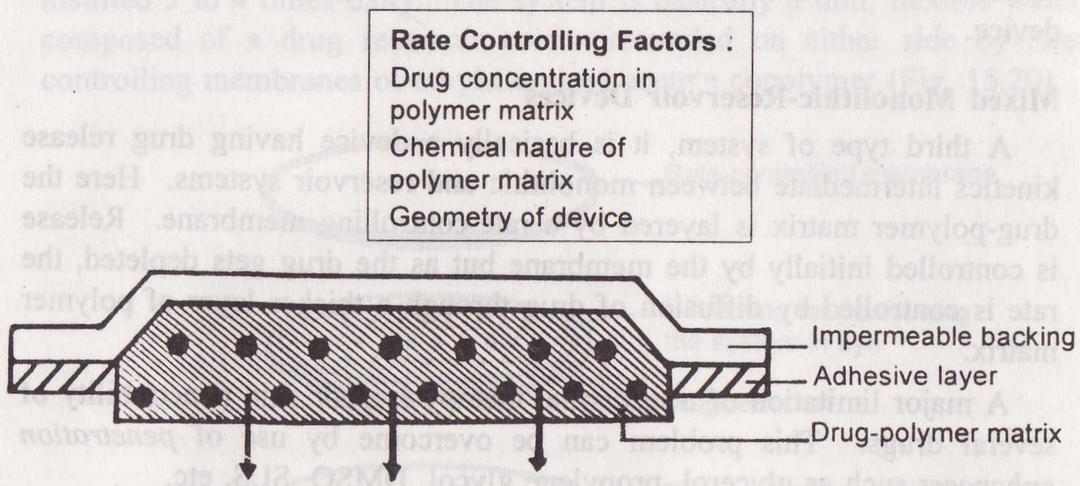


Fig. 15.18 Schematic representation of a monolith (matrix) transdermal drug delivery system

initially and falls as the matrix gets depleted of drug. The rate is thus proportional to the square root of time. Fig. 15.18. shows a typical matrix system.

Reservoir (or Membrane) Devices

These devices are used when drug permeation rate is rapid and absorption should therefore be controlled by controlling drug release ($R_1 < R_2$). It is also suitable for potent drugs with low therapeutic indices where monitoring drug levels in a narrow range is essential. The drug is usually

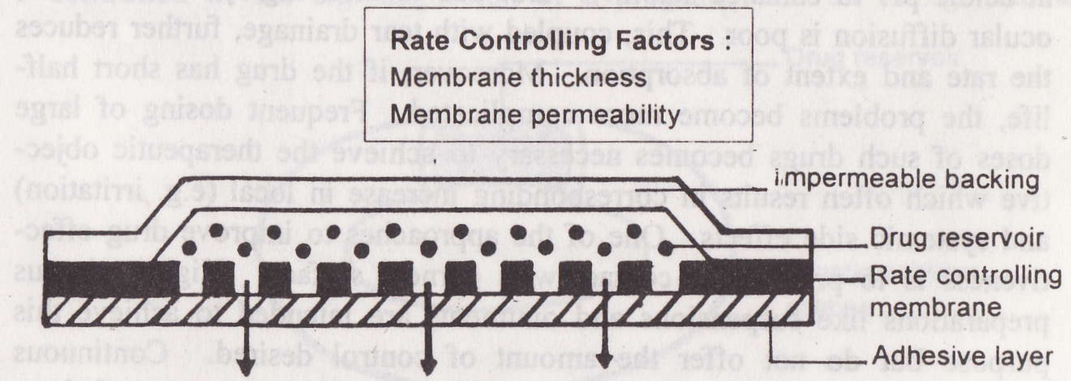


Fig. 15.19 Schematic representation of reservoir (membrane) type of transdermal drug delivery system

contained within the reservoir as a suspension in a liquid (such as silicone) or gel carrier. The rate-controlling thin polymeric membrane is made of olefinic polymers and copolymers, cellulosic esters, polyamides or PVC. When applied on skin, the device shows a rapid release at first (initial burst effect) followed by a constant zero-order release as long as the solution inside the reservoir is saturated. Fig. 15.19 shows such a device.

Mixed Monolithic-Reservoir Devices

A third type of system, it is basically a device having drug release kinetics intermediate between monolithic and reservoir systems. Here the drug-polymer matrix is layered by a rate-controlling membrane. Release is controlled initially by the membrane but as the drug gets depleted, the rate is controlled by diffusion of drug through a thicker layer of polymer matrix.

A major limitation of transdermal therapy is poor skin penetrability of several drugs. This problem can be overcome by use of *penetration enhancers* such as glycerol, propylene glycol, DMSO, SLS, etc.

Drugs commonly presented in such systems are nitroglycerine, clonidine, scopolamine and estradiol.

OPHTHALMIC DRUG DELIVERY SYSTEMS

Absorption of ophthalmic drugs across the corneal membrane is a diffusion process and depends to a large extent on:

1. Physicochemical properties of the permeating molecule, and
2. Drainage and output of tears.

Most drugs for ophthalmic use like pilocarpine, epinephrine, local anesthetics, atropine, etc. are weak bases which are generally formulated at acidic pH to enhance stability. But due to their highly ionized form, ocular diffusion is poor. This, coupled with tear drainage, further reduces the rate and extent of absorption. Moreover, if the drug has short half-life, the problems become more complicated. Frequent dosing of large doses of such drugs becomes necessary to achieve the therapeutic objective which often results in corresponding increase in local (e.g. irritation) and systemic side effects. One of the approaches to improve drug effectiveness is to prolong its contact with corneal surface. Highly viscous preparations like suspensions and ointments are intended to achieve this purpose but do not offer the amount of control desired. Continuous delivery of drugs in a controlled manner can overcome most of these problems. A number of ocular drug delivery systems have been developed for providing zero-order input. The best known system is **ocular insert** or **ocusert** developed to deliver pilocarpine in the treatment of glaucoma. Available in two release forms—20 and 40 mcg/hour, the system provides relief for 7 days (following insertion in the *cul-de-sac*, just below the cornea) in contrast to eyedrops which are required to be instilled 3 to 4 times daily. The system is basically a thin, flexible wafer, composed of a drug reservoir core surrounded on either side by rate-controlling membranes of ethylene-vinyl acetate copolymer (Fig. 15.20).

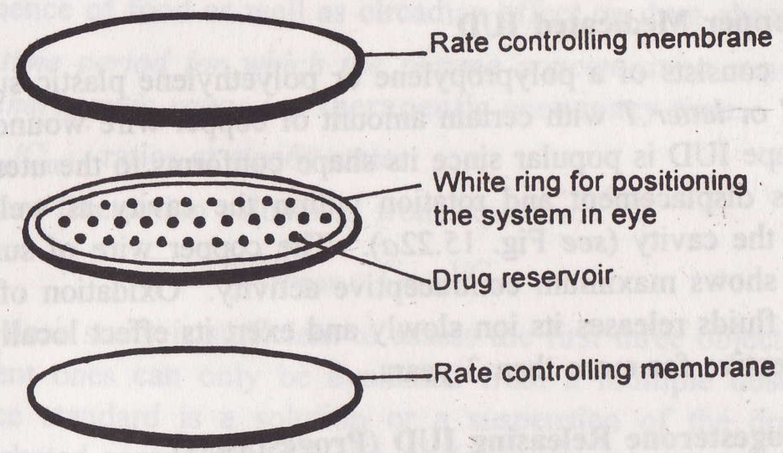


Fig. 15.20 Schematic representation of ocusert

INTRAVAGINAL & INTRAUTERINE DRUG DELIVERY SYSTEMS

Controlled release intravaginal systems are used for delivery of contraceptive steroid hormones. The advantage of this route includes—no first-pass effect, improved bioavailability and lesser drug dose in comparison to that required by oral route. Two types of devices have been developed—a matrix diffusion controlled device e.g. medroxy progesterone acetate dispersed in viscous silicone elastomer, and the other, dissolution controlled device e.g. dispersion of a progestin and an estrogen in an

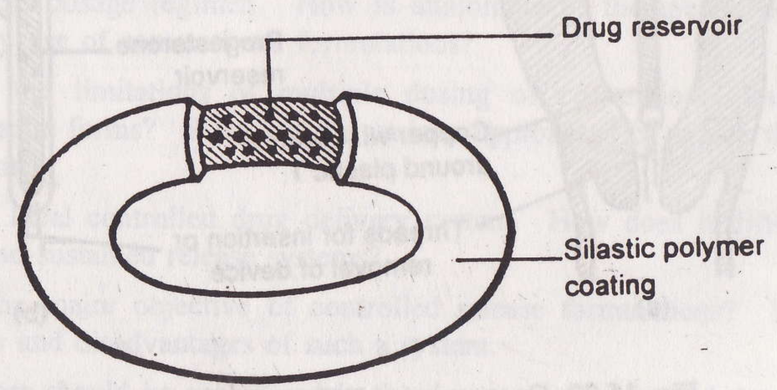


Fig. 15.21 Schematic representation of a vaginal ring

aqueous solution of PEG 400 to form microscopic drug reservoir in a mixture of silicone elastomers. The device is generally prepared by extrusion of the resultant composition into a doughnut shaped vaginal ring (Fig. 15.21). The system releases the medicament for 21 days to achieve a cyclic intravaginal contraception.

Intrauterine route is also used for fertility control. Two types of medicated intrauterine devices (IUDs) have been developed to produce effective contraception for 12 months or more.

1. Copper Medicated IUD

It consists of a polypropylene or polyethylene plastic support of *number 7* or *letter T* with certain amount of copper wire wound around them. **T** shape IUD is popular since its shape conforms to the uterine cavity and resists displacement and rotation within the cavity as well as expulsion from the cavity (*see* Fig. 15.22*a*). The copper wire of surface area 200 mm^2 shows maximum contraceptive activity. Oxidation of copper in the body fluids releases its ion slowly and exert its effect locally. The device is effective for more than 3 years.

2. Progesterone Releasing IUD (Progestasert)

It is also a **T** shaped polyethylene device with a progesterone reservoir dispersed in a silicone polymer placed in the vertical arm which is enclosed in a sleeve of rate-controlling membrane of ethylene-vinyl acetate copolymer (Fig. 15.22*b*). The device releases progesterone at a rate of 65 mcg/day for a period of one year.

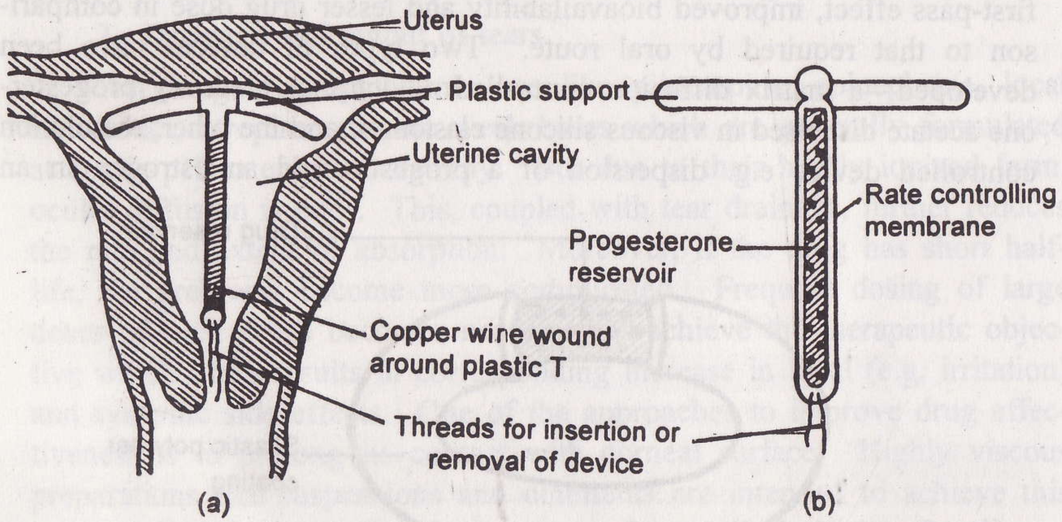


Fig. 15.22 Contraceptive IUDs—(*a*) Copper T located in the uterus, and (*b*) Progestasert

BIOAVAILABILITY TESTING OF CONTROLLED RELEASE FORMULATIONS

The purpose of *in vivo* bioavailability study on a controlled release formulation is to determine—

1. the fraction of drug absorbed (should ideally be ≥ 80% of conventional release dosage form).
2. occurrence of dose dumping.
3. influence of food as well as circadian effect on drug absorption.
4. *the time period for which the plasma concentration stays within the therapeutic range* i.e. **therapeutic occupancy time**.
5. C_{max}/C_{min} ratios at steady-state.
6. percent fluctuation calculated from equation:

$$100(C_{max} - C_{min})/C_{ss} \quad (15.15)$$

A single dose study is sufficient to assess the first three objectives but the subsequent ones can only be evaluated from a multiple dose study. The reference standard is a solution or a suspension of the drug or a currently marketed controlled release formulation.

For *in vitro* bioequivalence testing, the dissolution test should be designed to account for the major variables to which a controlled release formulation is exposed *in vivo* before getting absorbed e.g. pHs from 1 to 8, influence of food/fasting state, solubilizing influence of bile and pancreatic secretions, etc. when considering oral controlled release formulations. Establishing *in vivo-in vitro* correlation for controlled drug delivery is often difficult due to complexity of variables involved.

QUESTIONS

1. Define ideal dosage regimen. How is attainment of therapeutic objective possible by use of conventional formulations?
2. What are the limitations of multiple dosing of conventional immediate release dosage forms? What are the various approaches by which they can be overcome?
3. Define an ideal controlled drug delivery system. How does it differ from targeted and sustained release systems?
4. What is the major objective of controlled release formulations? List the advantages and disadvantages of such a system.
5. What factors should be considered in the design of a controlled drug delivery system?

6. Unlike conventional immediate release dosage forms, what is the rate-limiting step in the availability of a drug from controlled release formulation?
7. List the physicochemical and biopharmaceutic properties of the drug and state what pharmacokinetic and pharmacodynamic parameters are important in the design of controlled release formulation.
8. What criteria are necessary for the selection of a drug as candidate for formulation of a controlled release dosage form? Explain giving optimum ranges for the biopharmaceutic and pharmacokinetic properties/parameters of the drug.
9. What are the main determinants in deciding a route for administration of a controlled release system?
10. Zero-order release systems are considered ideal controlled delivery formulations. Why?
11. Even if a drug follows two-compartment kinetics, one-compartment model is considered suitable for the design of controlled release dosage forms. Why?
12. What assumptions are made in order to define the release pattern of a drug from controlled release formulation by a suitable model?
13. Name the four models commonly used to express drug input rate from controlled release formulations.
14. Give reasons for the observance of flip-flop phenomenon in the pharmacokinetics of a slow drug release formulation?
15. When is it advisable to incorporate a loading dose in the controlled release formulation? What limitations are encountered in the multiple dosing of such systems? How can such drawbacks be circumvented?
16. What are the causes of poor drug availability from oral controlled release formulations in comparison to immediate release systems?
17. Controlled drug delivery systems with slow first-order release are inferior in comparison to zero-order systems for oral use. Explain.
18. Classify the oral controlled release formulations.
19. Why is it easy to design a dissolution controlled release system?
20. The drug release from diffusion controlled matrix can never be a zero-order process. Explain.
21. Why are reservoir devices susceptible to dose dumping?
22. Name and distinguish the two categories of intrinsically powered oral controlled delivery formulations for zero-order drug release.
23. What are the various approaches by which the gastric transit of a dosage form can be delayed? To what category of drugs are such approaches applicable for controlled delivery?
24. If the mean residence time of a dosage form in the GIT is 14 hours and the $t_{1/2}$ of a drug is 6 hours, for how long can the drug effect be prolonged?

What would be the dosing frequency if the drug can be formulated as oral controlled release system?

25. Which principle is utilized in the oral delivery of protein and peptide drugs?
26. What are the various ways by which controlled drug release through injectable solutions can be attained?
27. Among injectable dispersed systems for controlled drug release, multiple emulsions and suspensions are superior in comparison to the simple emulsions and aqueous suspensions respectively. Explain.
28. How are liposomes classified? Why are they considered versatile carriers for parenteral drug delivery?
29. What are the advantages and disadvantages of transdermal drug delivery systems? What criteria are necessary for a drug to be given by such a route?
30. Enumerate the properties of an ideal implantable parenteral system. Why is subcutaneous tissue considered an ideal site for implants?
31. How are infusion devices for controlled drug delivery classified? Give the principle behind activation of such devices to effect zero-order drug release.
32. What are the advantages of using resealed RBCs as drug delivery systems?
33. What are the two rate-limiting steps in the absorption of a drug from transdermal device? How do they govern the selection of a particular device for controlled drug delivery?
34. What are the objectives of *in vivo* bioavailability studies on controlled release formulations? Why is correlation of such studies with *in vitro* release difficult?
35. The pharmacokinetic parameters of two NSAIDs are given in the table below:

Parameters	*Diclofenac*	*Ibuprofen*
$t_{1/2}$ (hours)	2.0	2.0
V_d (liters)	7.0	8.4
F	0.6	0.9
Desired C_{ss} (mcg/ml)	2.0	10.0

Determine the following for each drug:

a. The dose needed to maintain the therapeutic concentration for 12 hours.
Answer : Diclofenac 97 mg and Ibuprofen 388 mg.

b. The zero-order release rate to maintain the desired concentration
Answer : Diclofenac 8.085 mg/H and Ibuprofen 32.34 mg/H.

c. The time in hours to reach 90% of C_{ss} values.
Answer : 6.64 hours for both drugs.

d. The loading dose if C_{ss} is to be attained rapidly.

Answer : Diclofenac 23.3 mg and Ibuprofen 93.3 mg.

e. The plasma drug concentration at the end of 5 hours if the release is zero-order.

Answer : Diclofenac 1.64 mcg/ml and Ibuprofen 8.23 mcg/ml.

f. The possibility to formulate once daily formulation for both drugs.

36. An oral controlled release tablet of pentoxifylline having a loading dose of 170 mg to attain the desired C_{ss} of 0.3 mcg/ml is to be formulated. If the V_d is 170 liters and $t_{½}$ and F after rapid release are 1 hour and 0.35 respectively, calculate:

a. The amount of drug to be placed in the zero-order controlled release core to maintain the desired level for 12 hours.

Answer : 1212 mg.

b. If it is observed that oral availability from CR drug reduces to 0.3 and $t_{½}$ increases to 3.5 hours, what should be the 12 hour maintenance dose?

Answer : 404 mg.

c. What will be the plasma drug concentration after 4 hours of administration of such a formulation if K_a is 2.2/H and F and $t_{½}$ for zero-order doses are 0.3 and 3.5 hours respectively?

Answer : 0.192 mcg/ml.

37. The following information is available about two beta-blockers:

	Propranolol	*Sotalol*
Molecular weight	259.3	277.4
pK_a (base)	9.5	9.8
Aqueous solubility	Soluble	Freely soluble
Octanol/water partition coefficient	3.65	0.8
Oral availability	0.5	1.0
t_{max} (hours)	1.0	2.0
Therapeutic index	10.0	8.0
Elimination half-life (hours)	4.0	12.0
Apparent volume of distribution (L)	275.0	105.0
Minimum effective concentration (mcg/ml)	0.02	0.56

a. Evaluate from the data the suitability of both drugs as candidates for oral controlled release system.

b. Out of the two, which drug is a better candidate? Why?

c. Determine the desired C_{ss} for both drugs (*Hint:* C_{ss} = MEC x TI/2)

Answer : Propranolol 0.1 mcg/ml and Sotalol 2.24 mcg/ml.

d. What should be the zero-order release rate for both drugs?

Answer : Propranolol 9.52 mg/H and Sotalol 13.58 mg/H.

e. How much drug should be incorporated to maintain the desired level for 24 hours?

Answer : Propranolol 228.5 mg and Sotalol 326 mg.

f. How much time will be required to attain 90% of desired C_{ss} value?

Answer : Propranolol 13.3 hours and Sotalol 40 hours.

g. What should be the loading dose if the desired C_{ss} is to be attained rapidly?

Answer : Propranolol 55 mg and Sotalol 235.2 mg.

h. If both the drugs are formulated as slow first-order release systems with dose/unit 240 mg and 320 mg and release rate constant, K_r 1.0/H and 0.5/H for propranolol and sotalol respectively, determine the plasma drug concentration after 5 hours from administration.

Answer : Propranolol 0.22 mcg/ml and Sotalol 2.3 mcg/ml.

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SUBJECT INDEX

Page numbers followed by *f* refer to figures; by *t* indicate tables.

Absolute bioavailability, 283
Absolute solubility, defined, 19
Absolute surface area, 25
Absorption, (*See also* Availability; Bioavailability)
  active transport in, 13
  adsorption and, 61
  barrier, 7
  buccal, 64
  capacity-limited, 12
  carrier-mediated transport processes in, 10
  cell membrane - structure and physiology in, 6, 7*f*
  defined, 1, 5
  dissolution rate-limited, 19
  dosage form characteristics and pharmaceutical ingredients affecting, 18*t*, 39
  endocytosis and, 15
  facilitated diffusion in, 12
  factors influencing, 16, 18*t*
  from noninvasive routes, 64*f*
  from non *per os* routes, 63, 71*t*
  gastrointestinal, 6
  interactions affecting, 206, 208*t*
  intramuscular, 67
  mechanisms of, 7, 71*t*, 16*f*
  nature and type of dosage form affecting, 18*t*, 45
  nonlinearities in, 274
  passive. *See* Passive diffusion/transport
  patient related factors affecting, 18*t*, 50
  percutaneous, 66
  permeability rate-limited, 19
  physicochemical factors affecting, 18*t*, 19
  pulmonary, 69
  rate-determining processes in, 19
  rectal, 65
  subcutaneous, 68
  sublingual, 64
  vaginal, 70
Absorption half-life, 250
Absorption interactions, 208*t*
Absorption rate constant, 248
  determination of,
    by method of residuals, 248
    by Wagner-Nelson method, 250
    from urinary excretion data, 259
Absorption window, defined, 12
Accumulation during multiple dosing, 309
Accumulation index, 310
Acetylation, 144
  polymorphism, 145
α_1-Acid glycoprotein (AAG, AGP), 92*t*
  drug binding to, 94
  influence of disease on levels of, and binding to, 102*t*
Acidification of urine, 184
Acidosis and urine pH, 182
Active metabolites, 113*t*
  formation of, and concentration-response relationship, 326
Active transport,
  in biliary excretion of drugs, 195
  in drug absorption, 13, 16*f*
Active tubular reabsorption of nutrients, 180
Active tubular secretion, 178, 180
  mechanism of, 180
Acute pharmacologic response, bioavailability determination from, 290
Addition interactions, defined, 205
ADEPT technique in cancer chemotherapy, 175*f*
Adipose tissue,
  drug localization in, 97
Administration. (*See also* particular routes of administration)
  routes of, 6
  transmucosal noninvasive routes of, 64*f*

Age
differences in metabolism due to, 152
influence on
drug absorption, 53
drug distribution, 85
drug metabolism, 152
protein-drug binding, 101
renal function and drug excretion, 189
of product and drug dissolution, 50
of skin and drug absorption, 67
Agglomerative phase of communition (APOC), effect on dissolution, 40
Albumin
drug binding to, 92*t*, 93, 94*f*
in disease states, 102*t*
Alicyclic hydroxylation. *See* Oxidation of alicyclic C-atoms
Aliphatic hydroxylation. *See* Oxidation of aliphatic C-atoms
Alkalinization of urine, 182
Alkalosis and urine pH, 182
All-or-none response(s), 327
(*See also* Quantal response)
Allosteric changes in protein molecule, 101
Allosteric effector, 101
Altered density systems
for prolonged GI residence, 351
Amount remaining to be absorbed (ARA), 251
Amount remaining to be excreted (ARE), 257
Amorphism, 27
Antagonism, defined, 205
Antibody directed enzyme prodrug therapy. *See* ADEPT
Antifrictional agents. *See* Lubricants
Apparent partition coefficient of drug in design of controlled release systems, 339
Apparent volume of distribution, 86, 234
(*See also* Volume of distribution)
assessment of, 234, 244, 252
at steady-state, 265
defined, 86
protein binding of drugs and, 103
Aqueous suspensions
for parenteral controlled release, 359
Aqueous unstirred diffusion layer, 38
Area under the curve (AUC), 214
as a measure of bioavailability, 214, 286
assessment of, 225
Area under the first moment curve (AUMC), 224
Area under the zero moment curve (AUC), 224
Aromatic hydroxylation. *See* Oxidation of aromatic C-atoms
Artificial kidney (*See also* Hemodialyzer, 193
Asynthetic reactions. *See* Phase I reactions
Autoinduction, 148
Availability (*See also* Absorption; Bioavailability)
defined, 283
relative, 284
Average concentration on multiple dosing to steady-state, 311
Azapropazone binding site
warfarin and, 93
Barrier. *See* Physiologic barriers
Bile salts, absorption interactions with drugs, 61
Biliary clearance, defined, 197
Biliary excretion, 195
assessment of, 198
drug interactions involving, 62, 198, 207
factors influencing, 195
Biliary excretion rate, 197
Bilirubin
impaired binding to HSA by drugs, 105
precipitation of kernicterus in neonates by, 101, 105
Binders
granulating agents and, 42
influence on dissolution and absorption, 42
Binding (*See also* Protein binding of drugs) 91

apparent volume of distribution and, 103
drug interactions involving. *See* Displacement interactions
factors affecting, 97
irreversible, 92
kinetics of, 106
significance of, 103
to blood components, 92
Bioactivation, defined, 154
(*See also* Toxicological activation)
Bioavailable dose, defined, 282
Bioavailable fraction, defined, 283
Bioavailability
absolute, 283
defined, 283
considerations in study design, 283
correlation with *in vitro* dissolution, 290, 292
defined, 1, 17, 282
factors influencing 16, 18*t*
methods for enhancement of, 296
methods for measurement of, 284
multiple dose studies, 284
objectives of, 283
problems, and approaches to overcome, 297
relative, defined, 284
determination of bioequivalence from, 294
single-dose studies, 284
Bioequivalence
defined, 294
Latin square study design for, 295
statistical interpretation of data, 296
Bioinequivalence, defined, 294
Biological approach to overcome bioavailability problems, 297
Biological availability. *See* Bioavailability
Biopharmaceutics, defined, 1
Biopharmaceutic characteristics of drug in design of controlled release systems, 338
Biopharmaceutic considerations in dosage form design. *See* Factors influencing absorption and bioavailability
Bioprecursors, defined (*See also* Prodrugs), 161
Bioreductions. *See* Reductive reactions
Bioreversible derivatives. *See* Prodrugs
Biotransformation. *See also* Metabolism
chemical pathways of, 116*t*
defined, 2, 111
factors affecting, 147
Blood, binding of drugs to components of, 92
Blood-brain barrier (BBB), 80*f*
altered permeability of, in disease states, 86
approaches to promote crossing of, 81
Blood cells, drug binding to, 95
Blood-cerebrospinal fluid barrier, 81*f*
Blood flow
hepatic, determination of, 238
to GIT and absorption, 59
to kidneys, perfusion rate-limited excretion, 189
Blood flow rate-limited models. *See* Physiologic models
Blood proteins
and drug binding to, 92*t*
Blood-testis barrier, 83
Body surface area
dose estimation for children using, 317
ideal. *See* Ideal body weight
Body water, compartments of, 87*t*
Body weight, relationship with surface area, 317
Bolus dose
drug concentration after multiple i.v. injections, 309
in one-compartment open model, 231
in two-compartment open model, 260
Bones, drug binding to, 97
Brain
redox system for drug delivery to, 81, 172
Brodie *et al* hypothesis. *See* pH-partition hypothesis
Buccal administration, 64
absorption mechanism after, 71*t*
factors in absorption after, 65

Buffers
influence on drug dissolution, 44
Buoyant tablet/capsule. *See* Floating tablet/capsule
Burst effect, in controlled release formulations, 345

Calcium, complexation with tetracycline, 42, 61, 208*t*
Cancer
ADEPT technique in chemotherapy of, 175
site-specific delivery in, 174
Capacity-limited kinetics. *See* Nonlinear pharmacokinetics
Capillary endothelial barrier, 79
Capsule(s)
factors in absorption of drug from hard gelatin, 41, 47
soft elastic, 48
Carbon glucuronides, 141
Carbon-nitrogen systems, oxidation of, 122
Carbon-oxygen systems, oxidation of, 128
Carbon-sulfur systems, oxidation of, 127
Carbonic anhydrase, drug binding to, 95
Cardiovascular disease
influence on bioavailability, 58
Carrier
of drug molecules, 11
of prodrugs, 161
Carrier-linked prodrugs, 161
Carrier-mediated transport, 10-12
Cascade latentiation. *See* Double prodrugs
Caternary model, 223
Cation radical, 155
Celiac disease, 58
Cell membrane
of RBC, drug binding to, 95
structure and physiology of, 6, 7*f*
Cell membrane barrier, 79*f*
Central compartment
apparent volume of, 262
defined, 221, 260
drug concentration in, 262
Cerebrospinal fluid
drug entry into, 82
Ceruloplasmin, 95
Chemical equivalence, defined, 294
Chemical factors, affecting biotransformation of drugs, 148
Chemical formulation, defined, 162
Chemical pathways of biotransformation, 114, 116*t*
Chemical stability, enhancement of, by prodrug design, 165
Children, dosing of drugs in, 317
Child's dose
equation for calculating, 317, 318
Cholestasis
influence on biliary excretion, 196
Chronokinetics, defined, 154
Chronopharmacology, defined, 154
Chylomicrons, 94, 356
Circadian rhythm
influence on biotransformation, 154
Clearance
apparent V_d and, 236, 252
biliary. *See* Biliary clearance
creatinine. *See* Creatinine clearance
defined, 186, 235
dialysis. *See* Dialysis clearance
estimation of,
after constant rate i.v. infusion, 244
after e.v. administration, 236, 252
after i.v. bolus dose, 236
half-life and, 236
hepatic. *See* Hepatic clearance
in renal impairment, 191
intrinsic. *See* Intrinsic capacity clearance
nonrenal, 236
organ, 235, 237
plasma, 235
renal. *See* Renal clearance
total body, 185
total systemic, 185
unbound, 235
Clinical equivalence. *See* Therapeutic equivalence
Clinical pharmacokinetics, defined, 2, 212, 315
Coated tablets
bioavailability from, 49

enteric,
effect on drug absorption, 49
factors influencing drug release from, 49
Coatings, effect on drug dissolution, 43
Coevaporation, 301
Colonic release systems, 356
Colorants, effect on dissolution rate, 45
Compartment(s)
central, 221
defined, 221
models, (*See also* particular models), 221
peripheral, 221
reference, 230
tissue, 221
Compartmental analysis, 221
Compartmental modeling, 230
advantages and disadvantages of, 223
Competition for active transport
therapeutic use of, 180
Competitive displacement interactions, 209*t*
Competitive inhibition, 149
Complexation
influence on absorption, 61
to enhance bioavailability, 298
Complexes
poorly soluble,
in controlled drug release, 352
resin-drug,
in controlled release, 352
Complexing agents, 45
Compression force
influence on tablet dissolution rate, 41*f*
Concentration. (*See also* Plasma concentration)
and pharmacologic response, 325
Concentration-effect relationship(s) 327
Concentration gradient, defined, 8
Concentration-response curve(s)
effect of shape factor on, 329
Concentration-response relationship(s), 327
factors complicating, 325
quantitative models for, 327
Concentration-time profile
absorption rate and, 245*f*
after constant rate i.v. infusion,
with one-compartment open model, 241*f*
after e.v. administration, 245*f*, 249*f*, 267
after i.v. bolus administration,
with one-compartment open model, 233
with two-compartment open model, 261
area under the (AUC), 214
first moment of, 224
post distribution phase of,
zero moment of, 224
Conjugation
of cyanide, 146
reactions (*See also* Phase II reactions), 116*t*
with alpha-amino acids, 142
with glucuronic acid, 139
with glutathione (GSH), 143
with ribose, 147
with sulfate moieties, 141
with taurine, 147
Constant rate processes. *See* Zero-order kinetics/processes
Contraceptive(s)
controlled release intrauterine devices (IUDs), 370
controlled release intravaginal systems, 369
implants, 363
oral, enterohepatic circulation of, 62
Controlled release medication, 335
advantages of, 335
bioavailability testing of, 371
drug release patterns of, 343
Controlled release/delivery systems
biopharmaceutic characteristics in the design of, 338
classification of, 348
defined, 336
design of, 337
ideal. *See* Ideal controlled drug delivery system

intravaginal and intrauterine, 369
ophthalmic, 368
oral, 347
parenteral, 357
pharmacodynamic characteristics in the design of, 341
pharmacokinetic characteristics in the design of, 340
pharmacokinetic principles in the design and fabrication of, 342
transdermal, 365
Conventional dosage form(s), limitations of, 335
Coprecipitation, 301
Corpuscular transport. *See* Endocytosis
Correlations, *in vitro-in vivo*, 292
Corticosteroid binding globulin (CBG), 95
Creatinine
serum, 191
age related changes in, 191
determination of renal function from, 192
use in the determination of GFR, 179
Creatinine clearance,
formula(e) to calculate, 191
methods(s) for determining, 192
renal function and, 192
Crohn's disease, effect on absorption, 58
Crystal growth inhibitors
influence on drug in suspension, 45
Crystalline form(s)
polymorphism and, 27
Cytochrome b_5-reductase. *See* Cytochrome P-450 reductase
Cytochrome P-450
oxidation-reduction cycle, 117, 118*f*
Cytochrome P-450 reductase, 117
Cubic root law of dissolution
Hixson and Crowell's, 23

Danckwert's model
for drug dissolution, 20, 23
Dehydrogenation. *See* Oxidative aromatization
Delayed distribution models. *See* Multicompartment models
Delayed transit and continuous release systems, 348
Dermal excretion. *See* Skin excretion
Desulfuration, 128
Detoxication, 115
Dialysance. *See* Dialysis clearance
Dialysate, 194
Dialysis, 192
defined, 192. (*See also* Hemodialysis)
extracorporeal, 193
hemoperfusion and, 192
peritoneal, 193
systems for drug dissolution, 291
Dialysis clearance, 194
Dialyzing fluid, composition of, 194
Diazepam binding site, 93, 94*t*
Diet, influence on metabolism, 152
Diffusion
facilitated, 12
passive. *See* Passive diffusion/transport
Diffusion coefficient, 21
Diffusion controlled release systems, 350
Diffusion layer, 20
aqueous unstirred, 38*f*
pH of,
drug dissolution and, 38
model, 20
Diffusivity. *See* Diffusion coefficient
Digitoxin binding site, 93, 94*f*
Diluents, influence on dissolution and absorption, 42
Direct linear plot
for estimation of K_m and V_{max}, 279
Direct pharmacodynamic interactions, defined, 204
Discontinuous variation
in drug metabolism, 151
Disease states. (*See also* specific diseases)
affecting drug absorption, 58
affecting drug distribution, 86
affecting drug metabolism, 153
affecting protein-drug binding, 102*t*
Disintegrants
influence on absorption, 43
Disintegration test, 39

Disintegration time,
influence on dissolution and absorption, 39
Dispersions, solid. *See* Solid dispersions
Dispersions for parenteral controlled release, 358
Displaced drug, defined, 99
Displacement interaction(s), 99, 209*t*
and toxicity, 105
clinically significant, 99
defined, 99
Displacer, defined, 99
Disposition. (*See also* Distribution; Elimination)
defined, 75
monoexponential,
in one-compartment model, 232
Disposition curve, biexponential,
for two-compartment kinetics, 261
Dissociation constant of drug
and pH-partition hypothesis, 18*t*, 32
and urinary excretion, 183
Dissolution, defined, 20
rate-determining step in absorption, 19
theories of, 20
Danckwert's model, 23
diffusion layer model, 20
interfacial barrier model, 24
Dissolution and diffusion controlled release systems, 351
Dissolution controlled release systems, 348
Dissolution rate
and bioavailability, 290
apparatus. *See* Dissolution testing models
defined, 19
factors affecting, 25
methods for enhancement of, 297
in vitro,
correlation with *in vivo* absorption, 292
Dissolution test
factors in the design of, 291
Dissolution testing models, 290
closed-compartment apparatus, 291
dialysis system, 291
open-compartment apparatus, 291
rotating basket apparatus, 291
rotating paddle apparatus, 292
Distribution
apparent volume of. *See* Apparent volume of distribution
defined, 1, 75, 76
exponent, 262
factors affecting, 76
binding of drugs to tissue components, 85
blood flow to the organ/tissue, 83
miscellaneous, 85
tissue permeability of drugs, 76
interactions affecting, 206, 209*t*
nonlinearity in, 274
rate-limiting steps in, 76
rate of penetrability, 76
rate of perfusion, 83
Distribution half-life, 84
Distribution rate constant, 84
Distributive phase, 261
Diuresis, forced, to treat overdose, 185
Dizygotic twins, 151
Dosage form(s)
characteristics affecting absorption, 18*t*, 39
nature and type of,
affecting bioavailability, 18*t*, 45
Dosage form index, defined, 341
Dosage regimen(s)
defined, 4, 307
design of, 307
from plasma concentration, 314
ideal. *See* Ideal dosage regimen
individualization of, 315
optimal multiple, defined, 307
Dose
loading. *See* Loading dose
maintenance. *See* Maintenance dose
Dose adjustment
in renal disease, 192
based on elimination rate constant and half-life, 319
based on total body clearance, 319
Dose-dependent kinetics. *See* Nonlinear pharmacokinetics
Dose ratio, defined, 313

Dose size
in the design of dosage regimen, 308
influence of,
on plasma concentration-time profile, 308
Dosing frequency, 308
Dosing interval, 308
Dosing of drugs
in elderly, 318
in hepatic diseases, 318
in neonates, infants and children, 317
in obese patients, 316
in renal disease, 319
Double barrier model. *See* Interfacial barrier model
Double prodrug, 161
Double reciprocal plot. (*See also* Lineweaver Burk plot)
for protein-drug binding, 109
Downhill transport, 9, 12, 14
Drug administration and therapy
four phases of, 2
Drug-drug interactions in the GIT, 61
Drug interactions, 204
ADME, 206
defined, 204
mechanisms of, 204
Drug latentiation, defined, 160
Drug release patterns
of controlled delivery systems, 343
Drug transport, defined, 7
(*See also* Mechanisms of absorption)
Duration of action,
defined, 215
prodrugs to prolong, 167

Eadie-Hofstee plot, 330*f*
Effect(s). *See* Response
Effective surface area of particles, 25
Elderly, dosing of drugs in, 318
Electrical gradient, 14
Electrochemical diffusion, defined, 14
(*See also* Ionic diffusion)
Electrochemical gradient. *See* Concentration gradient
Electron transfer chain
components of, 117
Electrophiles
tissue toxicity due to, 154
Elimination, defined, 2, 75, 111
(*See also* Metabolism; Excretion)
Elimination half-life,
as a secondary parameter, 234
clearance and, 234
defined, 233
dose adjustment based on, 319
estimation of,
from plasma data, 232
from urinary excretion data, 253
Elimination phase, 231, 261
of plasma concentration-time profile, 214, 245*f*
Elimination rate constant,
estimation of,
from plasma data, 232, 244, 248, 265
from urinary excretion data, 255, 256
Emulsions
factors in the absorption of drug from, 47
multiple. *See* Multiple emulsions
Enantiotropic polymorphs, 27
Encephalitis
permeability of BBB in, 86
Endocytosis, 15, 16*f*, 71*t*
Endogenous agents
as natural soft drugs, 113, 160
Enteral route, 6
Enteric coated tablets. *See* coated tablets
Enterohepatic cycling. (*See also* Biliary excretion), 195, 197*f*, 198
Environmental chemicals
influence on metabolism, 150
Enzyme(s)
bacterial, effect on orally administered drugs, 63
drug metabolizing, 113
gut wall, effect on orally administered drugs, 63
hepatic, effect on orally administered drugs, 63
lumenal, effect on orally administered drugs, 63

Enzyme induction, defined, 148
Enzyme induction interactions, 209*t*
Enzyme inhibition, defined, 149
Enzyme inhibition interactions, 210*t*
Epidermis, barrier to percutaneous absorption, 66
Equivalence, defined, 294
Erythrocytes, drug loading in, 362*f*
Esophageal transit, defined, 56
Ethnic variations in metabolism, defined, 157
Eutectic mixtures, 300
Excipients, influence on absorption/bioavailability, 18*t*, 41
Excretion, 178
(*See also* Clearance; Elimination)
biliary. *See* Biliary excretion,
defined, 2, 178
gastrointestinal. *See* Gastrointestinal excretion
genital. *See* Genital excretion
interactions, 210*t*
mammary. *See* Mammary excretion
nonlinearity in, 275
pulmonary. *See* Pulmonary excretion
ratio. *See* Renal clearance ratio
renal. *See* Renal excretion
salivary. *See* Salivary excretion
skin. *See* Skin excretion
Exogenous compounds. *See* Xenobiotics
Exponential kinetics, 218
Extracellular fluid volume, 87*t*
Extraction ratio, defined, 237
hepatic, 238
Extrahepatic metabolism, defined, 113
Extrarenal routes of excretion. *See* Nonrenal routes of excretion
Extravascular administration,
one-compartment kinetics after, 245
two-compartment kinetics after, 266
Eye(s). (*See also* Intraocular administration)
controlled release medication for, 268
drug binding to melanin of, 96
prodrugs for selective targeting to, 166, 170
topical application to, 70

False nutrients
carrier-mediated transport of, 11
Fats, drug accumulation in, 97
Feathering technique. *See* Method of residuals
Fetus
drug danger to, 83
drug transport to, 82
(*See also* Placental barrier)
Fick's first law of diffusion, 8, 21
Fick's second law of diffusion, 21
Fillers. *See* Diluents
Film theory for dissolution. *See* Diffusion layer model
First moment curve, 224
First-order absorption, 246
First-order absorption model, 246
First-order half-life, 219
First-order kinetics. (*See also* Linear kinetics), 218
First-order process(es), 218
First-order rate constants, 218
First pass effect. *See* Presystemic metabolism
Flip-flop phenomena, defined, 250
in controlled release formulations, 345
Floating tablets/capsules, 356
Fluctuation, defined, 311
Fluid compartments, 87*t*
Fluid volume
influence on GI absorption, 60
Food. (*See also* Diet)
interactions due to, 59
Food-drug interactions
effect on absorption, 59*t*
Formulation factors, absorption and, 41
Fraction excreted unchanged, 233
Fraction metabolized, 233
Fraction unbound, 104
Fraternal twins. *See* Dizygotic twins
Free radicals, and tissue toxicity, 154
Functionalization reactions. *See* Phase I reactions

Gastric emptying
as a rate-limiting step in absorption, 53

defined, 53
delayed, 54
factors influencing, 54
Gastric emptying rate, 54
Gastric emptying t½, 54
Gastric emptying time, 54
Gastrointestinal contents
influence on absorption, 59
Gastrointestinal diseases
influence on absorption, 58
Gastrointestinal enzymes
presystemic metabolism by, 62
Gastrointestinal excretion, 201
Gastrointestinal pH
influence on absorption, 57
Gastrointestinal tract (GIT), 50
blood flow to the,
influence on absorption, 59
presystemic metabolism in, 62
Genital excretion, 201
Glass dispersion, 301
Glass solution, 299
Glass suspension, 301
Glassy hydrogels, defined, 351
Globulins, binding of drugs to, 95
Glomerular filtration, 178, 179*f*
Glomerular filtration rate (GFR), 179
creatinine clearance and,
determination of, 179
Glucopyranosiduronic acid conjugate, 140
Glucosiduronic acid conjugate, 140
Glucuronic acid, 139
Glucuronidation. (*See also* Conjugation with glucuronic acid), 139
reasons for importance of, 139
steps involved in, 140
Glucuronide(s)
carbon, 141
formation of. *See* Glucuronidation
nitrogen, 141
oxygen, 140
sulfur, 140
Graded response, defined, 327
Granulating agents. *See* Binders
Gut motility. *See* Gastric emptying
Hairs, drug binding to, 97
Half-life
absorption. *See* Absorption half-life
biological. *See* Elimination half-life
distribution. *See* Distribution half-life
elimination half-life. *See* Elimination half-life
Hanes-Woolf plot, 278
Hard drugs, defined, 159
Hemodialysis, factors governing removal of substances by, 193
Hemodialyzer, 193*f*
Hemoglobin, drug binding to, 95
Hemoperfusion, 194
Henderson-Hasselbach equation(s)
to determine degree of ionization, 33
Hepatic and biliary malfunction
marker used to determine, 197
Hepatic blood flow, 238
Hepatic clearance, 238
flow dependent, 238
intrinsic capacity-limited, 239
Hepatic diseases
dosing of drugs in, 318
influence on bioavailability, 59
Hepatic enzymes
in first-pass metabolism, 63
Hepatic extraction ratio of drugs, 239*t*
Hepatic function, determination of, 143
Hepatotoxicity of paracetamol, 126, 155
High density lipoproteins (HDL), 94
High density pellets
for prolonged GI residence, 355
Highly perfused tissues, 84*t*, 221, 260
Hixson and Crowell's
cubic root law of dissolution, 23
Homing device, in ADEPT system, 175
Hormonal imbalance
influence on metabolism, 153
Human serum albumin (HSA), 92*t*
(*See also* Albumin)
binding sites on, 93
drug binding to, 93
Hybrid first-order constants, 262
Hydrates, 29
(*See also* Pseudopolymor-phism)

Hydration of skin, 67
Hydrodynamic pressure controlled systems
for oral controlled release, 354
Hydrolysis
of amides, 136
of ethers and esters, 134
Hydrolytic cleavage of nonaromatic heterocycles, 137
Hydrolytic dehalogenation, 138
Hydrolytic reactions, 116*t*, 134
miscellaneous, 138
Hydrophilic diluents
to reduce hydrophobicity, 42
Hydrophilic-lipophilic balance (HLB)
for optimum bioavailability, 35
Hydroxylases. *See* Mixed function oxidases
Hyperlipoproteinemia
effect on binding, 102
Hypoalbuminemia
effect on binding, 102

Ideal body weight, 316
Ideal controlled drug delivery system, 336
Ideal dosage regimen, 335
Ideal implantable parenteral system, properties of, 362
Ideal prodrug, properties of, 162
Ideal targeted drug delivery system, defined, 336
Identical twins, drug metabolism in, 151
Immediate release dose. *See* Loading dose
Implants, as parenteral controlled release systems, 362
Incompatibility. *See* Pharmaceutical interactions
Indirect pharmacodynamic interactions, defined, 205
Individualization
of dosage regimen, 315
Indocyanine green
as marker for determination of plasma volume, 87, 88*t*
clearance of, as indicator of hepatic blood flow rate, 238
Inducers, of drug metabolism, 148*t*
categories of, 148
Infant(s)
differences in P-D binding in, 101
dosing of drugs in, 317
Infusion
advantages of, 240
constant rate, 241
equilibrium, 240
plus loading dose, 243
plasma concentration-time profile after, 243*f*
Infusion devices, as parenteral controlled release systems, 363
battery powered systems, 365
osmotic pressure activated systems, 363
vapor pressure activated systems, 364
Inhalation route. *See* Pulmonary administration
Inhibition of enzymes, 149
Intensity of action, defined, 215
Intensity of effect/response
concentration relationships, 332*f*
Interactions. *See* Drug interactions
Interfacial barrier model for dissolution, 24
Intersubject variability, 315
in drug binding, 102
Intestinal release systems, to bypass presystemic metabolism, 356
Intestinal transit, 56,
Intracellular fluid volume
determination of, 88*t*
Intramuscular administration, 67
factors in absorption after, 68
Intranasal administration/absorption
molecular weight and, 69
Intraocular administration/absorption, 70
Intraocular penetration
prodrugs for, 166, 170
Intrasubject variability, defined, 315
Intrauterine devices (IUDs), 369
copper medicated, 370
progesterone releasing (progestasert), 370
Intravaginal controlled release systems, 369

vaginal ring, 369
Intravenous bolus administration,
one-compartment kinetics after, 231
two-compartment kinetics after, 260
Intravenous infusion,
constant rate, 239
Intrinsic capacity clearance, 239
Intrinsic dissolution rate constant, defined, 21
Inulin clearance, 191
Ion-exchange resin-drug complex
for oral controlled release, 352
Ion-pair transport, 15, 16*f*
Ionic diffusion, 14
Ionization and drug absorption, 33
Ionized drugs, absorption of, 37

Jejunum, 50, 56*t*

KADME. *See* Pharmacokinetics
Kernicterus, in neonates, 101, 105
Kidney(s)
blood flow to, 179, 189
drug binding to, 96
failure. *See* Renal failure, 190
selective drug delivery to, 169

Lag time. *See* Time lag
Latentiated drugs. *See* Prodrugs
Latin square design
for bioequivalence study, 295*t*
Limited solvation theory. *See* Interfacial barrier model
Linear kinetics, 218. (*See also* First-order kinetics/processes)
defined, 218
Linear model, for concentration-response relationships, 327
Lineweaver-Burk plot, for
concentration-response relationships,
Michaelis-Menten kinetics, 278, 279
P-D binding, 109
Lipophilicity. (*See also* pH-partition hypothesis)
and drug absorption, 35
prodrugs for enhancement of, 166
Lipoproteins, 92*t*
binding of drugs to, 94
classes of, 94
Liposomes
classification of, 361
defined, 360
production of, 362*f*
Liver
as major drug metabolizing organ, 113
binding of drugs to, 96
blood flow to. *See* Hepatic blood flow
clearance by. *See* Hepatic clearance
diseases. *See* Hepatic diseases
Loading dose
and maintenance dose, 312
calculation of, 312
attainment of desired steady-state with, 243, 312
defined, 312
incorporation in controlled release systems, 343
infusion plus, 243
Logarithmic kinetics. *See* Exponential kinetics
Logarithmic model, for concentration-response relationships, 327
Loo-Riegelman method
for estimation of K_a, 267
Loop of Henle. *See* Nephron
Lubricants, influence on dissolution and absorption, 43
Lung(s)
drug accumulation in, 96
drug delivery to. *See* Pulmonary administration
excretion by. *See* Pulmonary excretion
Lung cancer
by benzo(a)pyrene, 156*t*

Magnetic microspheres
for drug targeting to tumors, 360
Maintenance dose
in controlled release systems, 342
loading dose and, 312

Maintenance of drug
within the therapeutic range, 312
Malabsorption
influence on absorption, 58
Mammary excretion, 200
Mammillary model, 221, 222*f*
Manufacturing processes
influence on dissolution, 40
Mathematical models, for concentration-response relationships, 327
Matrix
diffusion controlled, 350
dissolution controlled, 349
transdermal devices, 360
Maximum dosing interval, 314
Maximum maintenance dose, 314
Maximum plasma concentration. *See* Peak plasma concentration
Maximum safe concentration (MSC), defined, 214
Maximum urinary excretion rate, 288
time for, 288
Mean residence time (MRT)
in noncompartmental analysis, 224
of controlled release dosage forms, 341
Mechanisms of renal clearance, 186*t*
Medium unilamellar vesicles, 361
Melts, defined, 299
Membrane cell, *See* Cell membrane
Meningitis, permeability of BBB in, 86
Mercapturic acid formation
conjugation with glutathione and, 143
Metabolic precursors. *See* Bioprecursors
Metabolic stabilization, 159
Metabolic switching, 160
Metabolism, 111
(*See also* Biotransformation)
nonlinearity in, 274
defined, 111
species differences in, 150
strain differences in, 151
Metabolism interactions, 209*t*
Metabolites
active. *See* Active metabolites
Metabolizing enzymes, 113
microsomal. *See* Microsomal enzymes
nonmicrosomal. *See* Nonmicrosomal enzymes
Metabolizing organs, 113
Metallothionin
heavy metals binding to, 96
Metastable polymorph(s)
aqueous solubility of, 28
to enhance bioavailability, 298
Method of granulation, influence on dissolution and absorption, 40
Method of residuals
for calculation of K_a, 248
Methylation reactions, 145
Michaelis constant (K_m)
estimation of, 277
Michaelis-Menten equation, 275
Michaelis-Menten kinetics. (*See also* Nonlinear pharmacokinetics), 12, 220
Microcapsules, microspheres and,
for parenteral controlled release, 359
Microclimate pH, 37
Microconstants, 262
Micronization
a method of enhancing bioavailability, 297
decrease in effective surface area due to, 26
reduction of particle size by, 26
Microsomal enzyme(s)
important characteristics of, 114
Microspheres and microcapsules,
for parenteral controlled release, 359
Milk, drug excretion in., 201*t*
(*See also* Mammary excretion)
Minimum effective concentration (MEC), defined, 214
Minimum inhibitory concentration (MIC), defined, 214
Minimum toxic concentration (MTC), defined, 214
Mixed crystals. *See* Molecular dispersions
Mixed function oxidases, 117
Mixed-order kinetics, defined, 12, 219
(*See also* Nonlinear pharmacokinetics)
Molecular dispersions, 299

Molecular encapsulation
with cyclodextrins, 302
Molecular inclusion complexes, 302
Molecular weight
in the design of controlled delivery system, 339
for passive absorption, 9
for pore transport, 10
increase in,
after conjugation, 139
influence of,
on excretion pattern, 139, 196*t*
influence on biliary excretion, 195
Monitoring
drug therapy, defined, 320
pharmacodynamic, 321
pharmacokinetic, 321
therapeutic, 320
Monolithic devices. *See* Matrix devices/systems
Monomorphism
in drug metabolism, 151
Monooxygenases. *See* Mixed function oxidases
Monotropic polymorph(s), defined, 27
Monozygotic twins. *See* Identical twins
Mosteller's equation, 317
Mucin
endogenous ion, ion-pair transport, 15
interaction with drugs, 60
Mucoadhesive systems
for oral controlled release, 356
Multi compartment models, 259
Multilamellar vesicles, 361
Multiple dose study, for determining bioavailability, 284, 289
Multiple dosing
average concentration and body content on, 311
drug accumulation during, 309
maximum and minimum concentration during, 311
time to reach steady-state during, 310
Multiple emulsions
for parenteral controlled release, 359
Mutual prodrugs, 161
N-acetyl cysteine
to treat poisoning by drugs, 156
N-dealkylation, 122
N-hydroxylation, 126
N-oxide formation, 125
Nanocapsules. *See* Nanoparticles
Nanoparticles
for parenteral controlled release, 360
Nanospheres. *See* Nanoparticles
Nasal mucosa, drug transport across, 69
Nasal route of administration. *See* Intranasal administration
Nature of biotransformation process
influence on biliary excretion, 196
Neonates
dosing of drugs in, 317
drug binding in, 101
Nephron
functional unit of kidneys, 178
Niosomes
for parenteral controlled release, 360
Noncompartmental analysis, 224
Noncompetitive inhibition
of metabolizing enzymes, 149
Nonionic diffusion. *See* Passive diffusion
Nonlinear kinetics. *See* Nonlinear pharmacokinetics
Nonlinear pharmacokinetics, 12, 273
Nonlinearities, causes of, 274
Nonlinearity, tests to detect, 273
Nonmicrosomal enzymes, 114
Nonrenal clearance, defined,
Nonrenal excretion, defined, 178
Nonrenal routes of excretion, 194
(*See also* specific routes)
Nonstoichiometric complex, 28*f*
Noyes-Whitney's equation, 21, 25
modified, 21
Nucleic acids, drug binding to, 97
Nucleophiles
affinity for electrophiles, 143
Nucleophilic addition, a mechanism of GSH conjugation, 144
Nucleophilic substitution, a mechanism of GSH conjugation, 144

Obese patients, dosing of drugs in, 316
Obesity, 85
Object drug, in drug interactions, defined, 204
Ocular insert. *See* Ocusert
Ocusert, ocular controlled delivery systems, 368
Odor, improvement of
by prodrug design, 163
Oil suspensions
for parenteral controlled release, 359
Onset of action/effect
concentration relationships, 330
defined, 215
Onset time, defined, 215
Ophthalmic drug delivery systems, 368
Optimal dosage regimen, defined, 307
Oral administration, 6. (*See also* Extravascular administration)
typical plasma concentration-time profile after, 213
Oral controlled release systems, 347
classification of, 348
Oral mucosal delivery
factors to be considered in, 65
sites for, 64
Oral osmotic pump, 353
Order(s) of reaction, 215
defined, 216
Organ(s), volume of, blood flow and perfusion rate to, 84*t*
Oros. *See* Oral osmotic pump
Orosomucoid. *See* α_1-Acid glycoprotein
Osmogen, 353
Osmotic pressure controlled systems, 353
Osmotic pump(s)
for parenteral controlled release, 363
Oxidation
of alcohol, carbonyl and acid functions, 129
of alicyclic carbon atoms, 122
of aliphatic carbon atoms, 121
of aromatic carbon atoms, 119
of benzylic, allylic C-atoms and C-atoms alpha to carbonyl and imines, 120-121
of carbon-heteroatom systems, 122
of carbon-nitrogen systems, 122
of carbon-oxygen systems, 128
of carbon-sulfur systems, 127
of olefins, 120
steps in, of xenobiotics, 118
Oxidative aromatization, 130
Oxidative deamination, 124
Oxidative reactions, 116*t*, 117
o/w/o emulsions. *See* multiple emulsions
Pain on injection, reduction of,
by prodrug design, 164
Parameters of plasma level data
for determining bioavailability, 286
Parameters of urinary excretion data
for determining bioavailability, 288
Parenteral controlled release systems, 357
infusion devices, 363
injectables, 357
implants, 362
routes for administration of, 357
Parenteral route. (*See also* particular parenteral routes), 6
Particle size
and effective surface area,
influence on dissolution and absorption, 25
techniques for reduction to microlevel, 27
Passive diffusion/transport, 7, 8-10, 16*f*, 71*t*
characteristics of, 9
Passive tubular reabsorption, 181
Patient related variables, affecting absorption and bioavailability, 18*t*, 50
Peak, defined, 213
Peak plasma concentration, defined, 213
Peak response. *See* Intensity of action
Peeling technique. *See* Method of residuals
Percutaneous absorption
factors influencing, 67
Percutaneous delivery, defined, 66
Perfusion rate. (*See also* Blood flow)
a rate-limiting step in distribution, 83
defined, 83

of various tissues, 84*t*
tissues classified on the basis of, 84*t*
Perfusion rate-limited models. *See* Physiologic models
Peripheral compartment
apparent volume of, 265
perfusion of, 221
Permeability
defined, 10
of tissue membranes. *See* Physiologic barriers to distribution through the biomembrane,
a rate-limiting step in absorption, 19
Permeation enhancers
in topical formulations, 67
to promote crossing of BBB, 81
pH
gastrointestinal. *See* Gastrointestinal pH
effect on gastric emptying, 55
influence on absorption, 32
urine. *See* Urine pH
pH-absorption curve, 37*f*
pH-independent formulations
for oral controlled release, 353
pH of microenvironment
alteration of,
to enhance bioavailability, 298
pH-partition hypothesis, 18*t*, 32
assumptions of, 32
generalizations based on,
for absorption of drugs, 33
limitations of, 36
statement of, 32
pH-partition theory. *See* pH-partition hypothesis
pH-shift, 37, 38
Phagocytosis, 15
Pharmaceutic applications of prodrug design, 162
Pharmaceutic approach, in overcoming bioavailability problems, 297
Pharmaceutic equivalence, defined, 294
Pharmaceutical interactions, 205
Pharmaceutic process, of drug therapy, 2, 3*f*
Pharmacodynamic characteristics, in controlled delivery system design, 341
Pharmacodynamic methods
for evaluating bioavailability, 286
acute pharmacologic response method, 290
therapeutic response method, 290
Pharmacodynamic interactions, 204
Pharmacodynamic models
E-max model, 328
linear model, 327
logarithmic model, 327
Pharmacodynamic monitoring, 321
Pharmacodynamic process, of drug therapy, 2, 3*f*
Pharmacodynamic variability, 315
Pharmacodynamics, defined, 2
Pharmacogenetics, defined, 151
Pharmacokinetic analysis
three approaches to, 221
Pharmacokinetic applications of prodrug design, 162
Pharmacokinetic approach, in overcoming bioavailability problems, 297
Pharmacokinetic interactions, 204, 205*f*, 208*t*
Pharmacokinetic methods
for bioavailability measurement, 285
plasma level-time studies, 286
urinary excretion studies, 288
Pharmacokinetic models, 220
Pharmacokinetic monitoring, 321
Pharmacokinetic parameters. (*See also* particular parameters)
estimation/assessment of,
for one-compartment open model, 231, 244, 247
for two-compartment open model, 264, 267
primary, defined, 234
secondary, 234
Pharmacokinetic principles
applications of, 306
Pharmacokinetic process, of drug therapy, 2, 3*f*
Pharmacokinetic variability, 315

Pharmacokinetics
basic considerations, 212
clinical. *See* Clinical pharmacokinetics
defined, 2, 212, 215
Pharmacologic activation, 113*t*
Pharmacologic inactivation, 112*t*
Pharmacophore, defined, 134
Phase I reaction(s), 114, 116*t*, 117
Phase II reactions, 115, 116*t*, 138
Phase III reactions, 115
Phosphatidyl choline, 117
Physical form of the drug, change of, by prodrug design, 163
Physicochemical factors in drug absorption, 18*t*, 19
Physiologic availability. *See* Bioavailability
Physiologic barriers to distribution of drugs, 79
(*See also* individual barriers)
Physiologic fluid compartments, 87*t*
Physiologic models, 226*f*
Pinocytosis, 15
pK_a
and drug absorption. *See* pH-partition hypothesis
and tubular reabsorption 182, 183*t*, 184*f*
for candidates of controlled delivery systems, 339
Placental barrier, 82*f*
Plasma clearance. *See* Clearance
Plasma concentration-time curve/profile 213*f*
phases of, 214
Plasma level-time studies
for bioavailability determination, 286
Plasma protein(s), 92
(*See also* particular proteins)
binding of drugs to. *See* Plasma protein-drug binding
Plasma protein-drug binding, 92
Plasma volume, 87*t*
determination of, 87, 88*t*
Plateau. *See* Steady-state
Plateau principle, 310
Polarity of drug
influence on biliary excretion, 195
Polymorphism, 27
defined, 27
in acetylation of isoniazid, 151
variations in metabolism, 151
Polymorph(s)
defined, 27
types of, 27, 28*f*
Poorly perfused tissues,
Pore transport, 10, 16*f*, 71*t*
Post absorptive phase, 246
Potentiation. *See* Synergism
Powders, factors in absorption from, 47
Precipitant drug, defined, 204
Pregnancy
danger of drugs during, 83
influence on distribution, 85
influence on metabolism, 152
Presystemic metabolism, 6, 62
effect on absorption/bioavailability, 62
prodrugs to prevent, 167
systems which affect, 62
Primary parameters. *See* Pharmacokinetic parameters
Priming dose. *See* Loading dose
Principle of superposition, 273
Proagents. *See* Prodrugs
Processing variables
influence on dissolution. *See* Manufacturing processes
Prodrug(s),
bioprecursors, defined, 161
carrier-linked, defined, 161
defined, 160
mixed type, 161
properties of an ideal. *See* Ideal prodrug
Prodrug design
applications of, 162
limitations of, 176
Prodrug-soft drugs, 168
Product inhibition, defined, 149
Progestasert. *See* Progesterone releasing IUD

Progesterone releasing IUD, 370
Pro-prodrugs. *See* Double prodrugs
Protein. (*See also* particular proteins)
  binding to. *See* Protein-drug binding
Protein-drug binding, 91*f*
  defined, 91
  factors affecting, 97
  significance of, 103
Pseudodistribution equilibrium, 261
Pseudopolymorphism, defined, 29
Pseudopolymorphs, defined, 29
Pulmonary administration, 69
Pulmonary excretion, 198

Quantal response, 327
Quaternary ammonium compounds
  absorption of, 15

R. T. William's chemical pathways of metabolism, 114, 116*t*
Racemates
  differences in pharmacologic response due to, 326
Radicals, free. *See* Free radicals
Rate, defined, 215
Rate constant(s), 215
  (*See also* particular rate constants)
Rate-controlling step(s), in enhancement of duration of action, 167
Rate-determining step (RDS). *See also* Rate-limiting steps
  defined, 17
  in absorption, 19
  in availability of drug from controlled delivery systems, 338
Rate-limiting step(s)
  in distribution, 76
  in hepatic metabolism, 238
  in oxidation of xenobiotics, 118
Rate of conversion
  of prodrug, into active drug, 167*f*
Rate of creatinine excretion, 191
Rate of elimination, 237
Rate of excretion method
  to determine K_E, 255
Rate of exit, 237
Rate of extraction, 237
Rate of filtration, 179
Rate of input (availability), 231
Rate of penetrability
  rate-limiting step in distribution, 76
Rate of presentation, 231, 237
Rate of reabsorption, 179
Rate of reaction, defined, 215
Rate of release
  of prodrug, from site of application, 167
  limiting step of controlled delivery systems, 338*f*
Rate of secretion, 179
Reabsorption
  in renal tubules. *See* Tubular reabsorption
  forced diuresis and, 185
  influence of urine pH and drug pK_a and lipid solubility on, 183
Real detoxication pathways, 138
Real volume of distribution
  relationship with body water, 87*t*
Rectal administration, 65
Redox system
  for drug delivery to brain, 172
Reduction
  of alcohols and carbon-carbon double bonds, 132
  of carbonyls (aldehydes and ketones), 131
  of N-compounds, 132
  of sulfur containing functional groups, 133
  of toxicity,
    prodrugs for, 168
Reductive dehalogenation, 133
Reductive reactions, 116*t*, 130
  miscellaneous, 133
Regimen(s). *See* Dosage regimen(s)
Relative bioavailability, defined. (*See also* Bioavailability), 284
Renal clearance
  defined, 186
  factors affecting, 187
  perfusion rate-limited, 189
  values, range of, 186*t*

Renal clearance ratio
  defined, 187
  values of, 186*t*
Renal disease, dosing of drugs in, 319
Renal dysfunction. *See* Renal impairment
Renal excretion, 178
  (*See also* Renal clearance; Urinary excretion of drugs)
  factors affecting, 187
Renal extraction ratio, 239*t*
Renal failure. (*See also* Renal impairment; Renal disease)
  causes of, 190
  dose adjustment in, 192
Renal function (RF), 190
  calculation of dose on the basis of, 192
  determination of, 190
  equation for calculating, 192
Renal impairment, 190
Renal plasma flow rate
  determination of, 180
Repression, defined, 149
Resealed erythrocytes
  for parenteral controlled release, 361
Residence time, 38
Response
  all-or-none. *See* Quantal response
  concentration and relationships, 327
  factors that complicate correlation, 325
  graded. *See* Graded response

S-dealkylation, 127
S-oxidation, 128
Saliva/plasma concentration ratio, 199
Salivary excretion, 199
Salivary cycling, 199*f*
Salt form(s)
  factor influencing solubility, 18*t*, 29
  use of, to enhance bioavailability, 297
Salt formation, *in situ*, 31
Saturation kinetics. *See* Nonlinear pharmacokinetics
Scatchard plot
  for concentration-response relationship, 330*f*
  for P-D binding, 108
Secretion
  active tubular. *See* Active tubular secretion
  bile. *See* Biliary excretion
Selective adsorptionon, on insoluble carriers, to enhance dissolution/bioavailability, 298
Selective uptake systems, 169
Self induction. *See* Autoinduction
Serum creatinine, for determining creatinine clearance, 191
Shape factor, effect of,
  on concentration-response curves, 329*f*
Shore *et al* equations, for GIT/plasma concentration ratios, 33
Sigma minus method, to determine K_E from urinary excretion data, 256
Sigmoid-E-max model, 329
Single dose study
  for bioavailability determination, 284
Sink condition(s)
  attainment of, *in vitro*, 22
  defined, 291
  dissolution rate under, 23*f*
Site-specific delivery, 169
  in cancer therapy, 174
Size of counter ion
  influence on solubility of salts, 31
Skin excretion, 200
Small intestine, components of, 52*f*
Soft drug(s), defined, 113, 160
Solid dispersions
  to enhance bioavailability, 27, 301
Solid solutions. (*See also* Molecular dispersions)
  defined, 298
  to enhance bioavailability, 298
Solubility
  absolute, defined, 19
  and dissolution rate, 19
    enhancement of, by prodrug design, 164
Solute-solvent complexation, to enhance solubility/bioavailability, 298

Solution(s)
factors influencing bioavailability from, 47
solid. *See* Solid solutions
Solvates, defined. (*See also* Pseudopolymorphism), 29
Solvent deposition
to enhance solubility/bioavailability, 298
Specialized transport mechanisms. *See* Carrier-mediated transport
Stable polymorphs, 28
Stability, problems
Influence on bioavailability, 39
Stagnant layer, defined, 20
(*See also* Diffusion layer)
Statistical interpretation
of bioequivalence data, 296
Statistical moments theory
in noncompartmental analysis, 224
to determine *in vitro-in vivo* correlation, 293
Steady-state
after constant rate i.v. infusion, 240
bioavailability studies at, 287
defined, 240
desired, 342
design of controlled release systems on the basis of, 342
design of dosage regimen on the basis of, 314
percent of, attained, 242*t*
equation for calculating, 242
Stoichiometric complexes, 28*f*
Stomach, drug absorption from, 50, 51*f*
Storage conditions, influence on dissolution and absorption, 50
Stratum corneum
barrier to entry of xenobiotics, 66
Stripping technique. *See* Method of residuals
Subcutaneous administration
ways to enhance absorption after, 68
Subcutaneous route, for controlled release medication, 357, 363
Subcutaneous site
ways to increase absorption from, 68
Subcutaneous tissue
ideal location for implants, 363
Sublingual administration, 64
(*See also* Buccal administration)
Sulfation, 141.
(*See also* Conjugation with sulfate moieties)
steps in, 141
Sulfoconjugates, tissue reactive, 142
Sulfur glucuronides, 141
Summation. *See* Addition interactions
Surface area
absolute. *See* Absolute surface area
body,
equation for calculating, 317
effective. *See* Effective surface area
Surface renewal theory. *See* Danckwert's model
Surfactants
influence on absorption, 44
Suspending agents
influence on absorption, 43
Suspension(s)
factors in bioavailability from, 47
solid. *See* Solid suspensions
Sustained release products
bioavailability from, 49
Sustained release systems
distinction from controlled release systems, 336*f*
Synergism, defined, 205
Systemic availability. *See* Availability; Bioavailability
Systemic clearance. *See* Clearance
total. *See* Clearance

Tablet(s)
coated. *See* Coated tablets
enteric coated. *See* coated tablets
factors in bioavailability from, 48*f*, 49
Tamoxifen binding site, 93, 94*f*
Target concentration strategy, defined, 321
Targeted drug delivery system
ideal. *See* Ideal targeted drug delivery system

Targeting drug. *See* Site-specific drug delivery
Taste, improvement of,
by prodrug design, 162
Temporal factors
affecting biotransformation, 154
Teratogenesis
caused by reactive metabolites, 156*t*
Termination of drug action
processes in, 111
Therapeutic concentration range, 326
(*See also* Therapeutic range)
defined, 212
for some drugs, 326*t*
Therapeutic efficacy
approaches to improve, 159
Therapeutic endpoint, 320
Therapeutic equivalence, defined, 294
Therapeutic index (TI)
a characteristic in the design of controlled delivery systems, 341
defined, 212
dosing frequency and, 313
Therapeutic monitoring, 320
Therapeutic objective, defined, 306
Therapeutic occupancy time, defined, 371
Therapeutic process, of drug therapy, 2, 3*f*
Therapeutic range
defined, 215
in the design of controlled delivery systems, 341
maintenance of drug within the, 313
Therapeutic window. *See* Therapeutic range
Therapeutics, optimization of,
approaches for, 159
Time lag, defined, 250
Time of peak concentration (tmax), defined, 214
Tissue(s), perfusion rate of, 84*t*
Tissue binding of drugs, 96
Tissue compartment. *See* Peripheral compartment
Tissue distribution half-life. *See* Distribution half-life
Tissue-drug binding
examples of, 96
Tissue localization of drugs. *See* Tissue binding of drugs
Tissue permeability
rate-limiting step in distribution, 77
Tolerance
concentration-response correlation and, 326
Topical administration, 66
Topical route, 6
Total body clearance. *See* Clearance
Total body water (TBW)
composition of, 87*t*
determination of, 88*t*
Total systemic clearance. (*See also* Clearance)
defined, 185
equations for calculating, 235
Toxic endpoint, 321
Toxicologic activation, 112*t*, 154
Transcortin. *See* Corticosteroid binding globulin
Transcytosis, defined, 16
Transdermal drug delivery systems, 365
advantages of, 365
types of, 366
mixed monolithic-reservoir devices, 368
monolithic devices, 366
rate-limiting steps in drug release from, 366
reservoir devices, 367
Transfer constants. *See* Microconstants
Transferrin, binding to, 95
Transmembrane rate-limited. *See* Permeability rate-limited
Transport moiety. *See* Carrier
Trapezoidal rule
for calculating AUC and AUMC, 225
Tubular reabsorption, 181
active. *See* Active tubular reabsorption
passive. *See* Passive tubular reabsorption
Tubular secretion. *See* Active tubular secretion

Uphill transport. *See* Active transport
Uremia, 190
Urinary excretion. (*See* Renal excretion)
principal processes of, 178
Urinary excretion data, 253
advantages of, 253
criteria for obtaining valid, 254
determination of K_a from, 259
determination of K_E from, 255
rate of excretion method, 255
sigma-minus method, 256
Urinary excretion studies, assessment of bioavailability from, 288
Urine flow rate,
influence on tubular reabsorption, 184
Urine pH
influence on tubular reabsorption, 182
Urine pH control
to treat toxicity due to overdose, 185
Urine/plasma concentration ratio
equations for calculating, 182

Vaginal administration, 70
Vaginal ring, 369*f*
Vapor pressure powered pump, 364
Variability
in drug response,
sources of, 315
intersubject. *See* Intersubject variability
pharmacodynamic.
See Pharmacodynamic variability
pharmacokinetic. *See* Pharmacokinetic variability
Vascular fluid volume, 87*t*
Vesicular transport. *See* Endocytosis
Virtual membrane pH, influence on ionization and absorption, 37
Viscosity imparters. *See* Suspending agents
Volume of central compartment, 265
Volume of distribution. (*See also* Apparent volume of distribution), 86
real. *See* Real volume of distribution
Volume of peripheral compartment, 265

Wagner-Nelson method
for estimation of K_a, 250
Warfarin
and azapropazone binding site, 93
Water, total body. *See* Total body water
Water compartments. *See* Body water, compartments of
Water flux, 10
Water loading, to promote diuresis, 254
Weight. *See* Body weight
Wet granulation
tablet manufactured by,
limitations of, 40
Woolf-Augustinsson-Hofstee plot, 278

Xenobiotics
defined, 111
enzymes that biotransform. *See* Metabolizing enzymes
site for metabolism of. *See* Metabolizing organs

Zero-moment curve, 224
Zero-order absorption model, 246
Zero-order half-life, 217
Zero-order kinetics (*See also* Constant rate processes/kinetics), 216
Zero-order release
from controlled release formulation, 344
with a rapid release component, 345

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