Part 1

Section I

GENERAL PHARMACOLOGY

1. Introduction to Pharmacology
2. Routes of Drug Administration
3. Pharmacokinetics
4. Clinical Pharmacokinetics
5. Pharmacodynamics
6. Quantitative Aspects of Pharmacodynamics and Assay of Drugs
7. Drug Interactions
8. Adverse Drug Reactions
9. Development and Evaluation of New Drugs

1 Introduction to Pharmacology

INTRODUCTION

Pharmacology is a branch of medical science which deals with drugs. It is derived from two Greek words, viz. *pharmacon* (means drug) and *logos* (means studies). It contains the knowledge of history, source, physical and chemical properties, compounding, biochemical and physiological effects, mechanism of action, absorption, distribution, biotransformation and excretion and therapeutic and other uses of drugs. The first book of pharmacology was written by Samuel Dale in 1693. Oswald Schmiedeberg (1838–1921) is known as the Father of Modern Pharmacology.

Drug and Medicine

A drug is a chemical substance that affects processes in living organism and used for the treatment, prophylaxis (prevention) or diagnosis of the disease. It is derived from a French word *Drogue* (means dry herb). According to WHO (World Health Organization), a drug is a chemical substance or biological product that is used or intended to be used to modify or explore physiological systems or pathological states for the benefit of the recipient. A drug cannot create a new function but can modify (increase or decrease) an already existing function. When a drug is used in proper dosage form for safe administration in a recipient, then it is called a medicine. All medicines are drugs but all drugs are not medicines. There are thousands of drugs, but all drugs are not essential.

Essential Drug Concept

It was introduced by WHO in 1977 to avoid the complications of drug use faced by the physician. A list containing essential drugs is available for the physicians in clinical practice. Essential drugs are those drugs which satisfy the healthcare needs of the majority of the population and they should, therefore, be available at all times in adequate amounts, in appropriate dosage forms and at reasonable cost. Drugs which do not fulfill these criteria are not essential drugs. List of essential drugs is given in the last pages of this book.

- Orphan drugs are those drugs which are used for the treatment, prevention or diagnosis of rare diseases like kala-azar, cancers, viral diseases, etc. and in digoxin, heavy metal or other drug, e.g. digoxin specific fab antibodies, T_3, BAL, etc. poisoning. Though they may be life-saving for some patients, but they are less produced commercially due to high cost of manufacture and small number of patients requiring the drug.

Drug Nomenclature

A drug has more than one name, for example:

- **Acetylsalicylic acid (chemical name)**
  – Aspirin (official name)

- Dispirin (proprietary/brand name)
- Salicylate (generic name).

- **Chlorphenothiazine propyldiamine (chemical name)**
  - Chlorpromazine (official name)
  - Largactil (brand name/trade name)
  - Phenothiazine (generic name).

Drug Therapy

It is of three types:

1. **Rational therapy:** It is the logical treatment of diseases by drugs based on pharmacological effects of drugs correlated with pathological aspects of diseases, e.g. digoxin in congestive cardiac failure, ferrous sulphate in iron deficiency anaemia, antibiotics in bacterial infections, etc.
2. **Empirical therapy:** It is the treatment of diseases based on experience, belief or guess and without any pharmacological explanation, i.e. uses of homeopathic drugs for curing diseases.
3. **Accessory therapy (alternative medicine):** It is the treatment of diseases by other means besides drugs, e.g. use of physiotherapy in arthritis, administration of suitable foods in malnutrition, etc.

Branches of Pharmacology

These are described as follows:

1. **Pharmacokinetics (*Kinesia* meaning movement):** It deals with the absorption, distribution, metabolism and excretion of drugs. It is what the body does to the drug.
2. **Pharmacodynamics (*Dynamics* meaning power):** It deals with the biochemical and physiological effects of drugs and their mechanisms of action. It is what the drug does to the body.
3. **Pharmacotherapeutics (therapeutics):** It is the use of drugs in the prevention and treatment of diseases.
4. **Toxicology:** It deals with adverse reactions of drugs and their treatment.
5. **Clinical pharmacology:** It deals with the study of drug effects in human beings (normal and patients).
6. **Experimental pharmacology:** It deals with the study of drug effects in laboratory animals (rats, guinea pigs, rabbits, etc.).
7. **Pharmacy:** It is the collection, compounding and dispensing of drugs for use in man or animal.
8. **Posology:** It deals with the dosage of drugs.

Pharmacopoeia

It is an official book published by the authorised body in a country containing description of commonly used drugs with their sources, properties, uses, doses and tests of identity, purity and potency, e.g. Indian pharmacopoeia (I.P.), British Pharmacopoeia (B.P.), United States Pharmacopoeia (U.S.P.). Extra pharmacopoeia (Martindale), etc. It is revised every five years to contain newly developed and essential drugs. Harmful drugs that have better substitutes are omitted in the new edition. Drugs contained in pharmacopoeia are called official drugs.

Sources of Drugs

Drugs are obtained from various sources. According to sources they are as follows:

1. **Natural drugs:** These are obtained from:
   a. Plants, for example:
      - Morphine from poppy capsules
      - Atropine from belladonna leaves
      - Quinine from cinchona barks
      - Castor oil from castor seeds.
   b. Microorganisms, for example:
      - Penicillin from *Penicillium notatum* (a fungus).
      - Streptomycin from *Streptomyces griseus* (a soil dwelling organism)
      - Bacitracin from *Bacillus subtilis* (a bacteria)
      - Diastase from *Aspergillus oryzae* (a fungus)
   c. Animals, for example:
      - Insulin from pig or ox pancreas
      - Thyroxine from pig or ox thyroid gland

- Heparin from pig or ox liver
- Cod liver oil from cod fish liver.

d. Minerals, for example:

- Calcium, magnesium, aluminium, sodium, potassium and iron salts.
- Liquid paraffin from petroleum.

2. **Synthetic drugs:** These are prepared by chemical synthesis in pharmaceutical laboratories, e.g. sulphonamides, quinolones, salicylates, barbiturates, benzodiazepines, etc.
3. **Semisynthetic drugs:** These are prepared by chemical modification of natural drugs in pharmaceutical laboratories, e.g. ampicillin from penicillin-G, cephalexin from cephalosporin-C, dehydroemetine from emetine, dihydroergotamine from ergotamine.
4. **Biosynthetic drugs:** These are prepared by cloning of human DNA into the bacteria like *E. coli*. Cloning means production of identical subjects like the parent. The technique is called recombinant DNA technology or genetic engineering, for example:
   - Human insulins (insulin-S, insulin-I)
   - Human growth hormones (somatrem, somatropin)
   - Human interferons (interferon-α, interferon-β)
   - Tissue plasminogen activator (alteplase)
   - Human BCG vaccine
   - Human hepatitis B vaccine, etc.

Gene-based Therapy (Gene Therapy)

It is the introduction of functional genetic material (DNA) into the target cells to replace or supplement the defective genes. It can impart new functions to cells. By it many diseases which are now only palliated can be cured, e.g. cancers, Alzheimer's disease, Parkinson's disease, diabetes mellitus, hypertension, hyperlipidaemia, haemophilia, cystic fibrosis, muscular dystrophy, Gaucher's disease, sickle cell anaemia, dwarfism, multiple sclerosis, HIV infections, etc.

Chemical Natures of Drugs

Plant Products

The pharmacologically active substances (principles) of plants are as follows:

1. **Alkaloids:** These are organic nitrogenous substances containing cyclic nitrogen and also carbon, hydrogen and sometimes oxygen obtained from plants. These are basic substances (bases) which are insoluble in water but when combined with mineral acids, they form acidic salts, which are soluble in water. Their names end with "ine", e.g. atropine, morphine, nicotine, pilocarpine, emetine, caffeine, etc. Most alkaloids are solid and nonvolatile but some alkaloids are liquid and volatile, e.g. pilocarpine, nicotine, lobeline and amphetamine. Animal alkaloids are called amines, e.g. adrenaline, noradrenaline, dopamine, histamine and 5-hydroxytryptamine.
2. **Glycosides:** These are organic non-nitrogenous substances containing C, H, O and sometimes S obtained from plants. They are neutral or slightly acidic substances which are soluble in water. They do not combine with acids to form salts. When they are heated with mineral acids they hydrolyze and split up into two components, viz. sugar and non-sugar (aglycone or genin). Sugar component is responsible for water and lipid solubility, cell permeability, tissue fixation and potency, while non-sugar component is responsible for pharmacological actions, e.g. digoxin is obtained from leaves of digitalis lanata, sinigrin is obtained from mustard seeds, senna is obtained from senna leaves and picrotoxin is obtained from fish berries (*Anamirta cocculus*).
3. **Oils:** These may be fixed oils or volatile oils obtained from plants. Fixed oils are glycerides of oleic, palmitic and stearic acids. They are obtained from seeds of plants by expression. They are insoluble in water and cannot be distilled. Many of them are edible and have food (caloric) value. They are used for cooking, e.g.

mustard oil, sunflower oil, peanut oil and coconut oil. Some are used for pharmacological actions, e.g. castor oil is used as purgative and olive oil is used as emollient and cholagogue (to cause evacuation of gallbladder by contraction). Fixed oils of animal origin are cod liver oil, shark liver oil, halibut liver oil, butter, lard (animal fats), etc. Fats are fixed oils which remain solid due to presence of more stearin (solid) than palmitin (semi-solid) and olein (liquid). Oils contain more olein than others. Volatile oils are obtained from flowers, leaves, fruits and seeds of plants by distillation. They contain the hydrocarbon "terpene" or some polymers of it, which serves as a diluent or solvent of the active compound. They are soluble in water and impart to it their taste and smell. They are volatalized by heat and possess aromas (smell). They have no food (caloric) value. They are used as carminative, flavouring agent, antiseptic, anodyne or counter-irritant, e.g. cardamom oil, peppermint oil, thymol, clove oil or turpentine oil respectively. Some volatile oils remain solid at ordinary temperature. They are called stearoptenes, e.g. camphor, menthol and thymol. Mineral oils are mixture of hydrocarbons of the methane and related substances obtained from petroleum by fractional distillation, e.g. liquid paraffin, soft paraffin and hard paraffin. Liquid paraffin is used as emollient laxative, while soft and hard paraffins are used as ointment bases.

4. **Tannins:** These are organic non-nitrogenous substances obtained from plants. They have astringent action upon the mucous membrane and thus exert a protective action. They are soluble in water and have astringent taste. They are used as tincture, e.g. tincture of catechu is used as anti-diarrhoeal agent (releases tannic acid in intestine) and tincture of Kalmegh is bitter and is used as appetizer in hepatic dysfunction.
5. **Resins:** These are solid nonvolatile substances formed by oxidation or polymerization of volatile oils in plants. They are insoluble in water but soluble in alcohol, e.g. jalap and colocynth (previously used as drastic purgatives). Podophyllum resins (20% suspension in liquid paraffin) is used as cauterising agent in venereal warts. Oleoresin is a mixture of resin with volatile oil, e.g. male fern extract (previously used to remove tapeworms from the intestine).
6. **Antibiotics:** These are antibacterial substances derived from fungi, actinomycetes and bacteria, e.g. penicillin, streptomycin and bacitracin respectively.
7. **Vitamins:** Majority of vitamins are obtained from plants, e.g. vitamins B complex, C, E, K, etc.

The pharmacologically inert (inactive) substances of plants are:

- **Gums:** These are secretory products of plants. These are colloidal carbohydrates (polysaccharides). These are dispersible in water and form thick mucilaginous colloids, e.g. gum acacia and gum tragacanth are used as emulsifying or suspending agents for preparation of emulsions or suspensions in pharmacy.
- **Waxes:** These are waxy or plastic substances obtained from various plants (vegetable wax) or from animals, e.g. sheep wool (wool wax/lanolin) and honeycomb deposited by bees (beeswax). These are esters of long chain fatty acids with higher, usually monohydroxy alcohols. Beeswax is yellow. It is converted to white wax by bleaching with chlorine. Waxes are used in pharmacy for preparing ointments, creams, suppositories, etc.

Animal Products

These are hormones, enzymes, fixed oils, vitamins and waxes. These have been discussed in respective chapters.

2 Routes of Drug Administration

Drugs can be administered by different routes for local and systemic effects.

Factors Deciding the Route of Choice

- **Physicochemical properties** of the drug, i.e. whether the drug is solid (tablet, capsule, powder, pessary and suppository), liquid (mixture, syrup, lotion, enema and injection) or gas, soluble or insoluble, irritant or nonirritant, etc.
- **Type** of desired effect, i.e. systemic or local effect for which the drug is used.
- **Rapidity** of desired effect, e.g. oral, IM, etc. for routine treatment, IV for emergency treatment.
- **Quality** of desired effect, e.g. magnesium sulphate given orally is a purgative, but when given rectally lowers intracranial tension.
- **Condition** of the patient, i.e. whether the patient is conscious or unconscious, vomiting or not vomiting, etc.

Local Routes

These are used for localized lesions at accessible sites. Systemic absorption of the drug from these sites is minimum or absent. Thus a high concentration of the drug is attained at the desired site without exposing the rest of the body. This minimizes systemic adverse effects or toxicity of the drug. The drug is applied on the skin and various mucous membranes as ointment, cream, lotion, drops, jelly, powder, tablet, suppository, or pessary. Injections in deeper tissues include intra-articular (in joint cavity), e.g. hydrocortisone hemisuccinate; intrathecal (into subarachnoid space of L 2–3 or L 3–4), e.g. lidocaine, isoniazid, amphotericin B, etc. intramedullary (into bone marrow), e.g. hematinics; intra-arterial (into artery of limbs), e.g. anticancer drugs in limb cancer; retrobulbar (behind the eyeball), e.g. hydrocortisone hemisuccinate/acetate; intrapleural (inside pleural cavity), e.g. antitubercular drugs, anticancer drugs and intraperitoneal (inside peritoneal cavity), e.g. antitubercular drugs, inhalation have been discussed later on anticancer drugs.

Systemic Routes

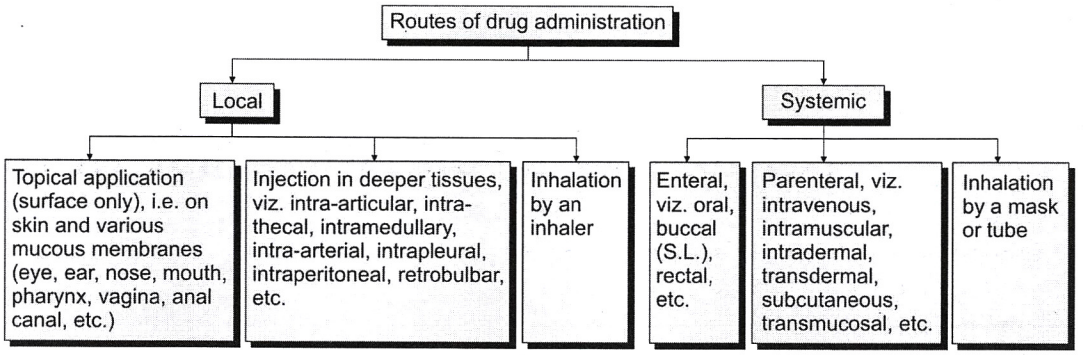
These are used for systemic effects of drugs. The drug is absorbed into blood and distributed all over the body including the site of action through circulation (Flow Chart 2.1). These routes are:

Enteral (GIT)

These are given as follows:

a. **Oral (ingestion of drug):** It is most convenient and economical. It is more safe than other routes. It requires patient's cooperation. The absorption of the drug may be variable and erratic. Drugs that are poorly soluble, slowly absorbed, unstable or extensively metabolized by the liver

Flow Chart 2.1: Routes of drug administration



have low bioavailability. The drug is administered as tablet, dragee, capsule, powder, syrup, mixture, suspension or emulsion. Majority of drugs are administered orally as self-administration is possible and adverse effects appear slowly.

b. **Buccal (sublingual):** In it, the drug (as tablet) is placed under the tongue, where from it is absorbed and reaches the systemic circulation directly without passing via the liver (thus avoiding first pass metabolism in the liver and gut wall). Only lipid-soluble and non-irritant drugs can be administered by this route, e.g. glyceryl trinitrate, isosorbide dinitrate, nifedipine, buprenorphine, clonidine, isoprenaline, methyl testosterone, etc. The drug is rapidly absorbed producing prompt effect. Quick termination of drug effect can be done by spitting the remaining portion of the drug from the mouth and so adverse effects can be avoided but on frequent use may produce ulceration locally.

c. **Rectal:** Some irritant and unpleasant drugs can be put in rectum (as suppository or enema) for systemic effects. This route is also used when the patient is having recurrent vomiting the drug is absorbed from lower rectum (by external haemorrhoidal veins), then it directly reaches systemic circulation bypassing first pass metabolism in the liver. If the drug is absorbed from upper rectum (by internal haemorrhoidal veins) then it cannot bypass liver. Drugs administered rectally are bisacodyl, glycerine, indomethacin and phenylbutazone as suppository and paraldehyde, tribromoethanol, prednisolone, soap water or barium sulphate as enema. This route is inconvenient and embarrassing and sometimes can cause rectal inflammation.

Parenteral ('Par' meaning away, 'Enteron' meaning intestine)

These include all types of injections and controlled release drug delivery systems.

Injections

i. **Intravenous (IV) injection:** It produces immediate effect, as absorption is not required. It is valuable for emergency use and in seriously ill patients. It permits titration of dose, of the drug. It is usually required for high molecular weight proteins and peptide drugs. It is suitable for large volume and for irritant drugs when diluted. It is not suitable for oily solutions or insoluble substances. It can be given as a bolus or vary slowly (as drip). With it, there is increased risk of adverse reactions. Thrombophlebitis commonly occurs. Lack of sterility in the procedure may cause viral hepatitis or AIDS. Drugs like thiopentone sodium, diazepam, frusemide, diazoxide, sodium nitroprusside, etc. are administered IV for quick effects in emergency conditions.

ii. **Intramuscular (IM) injection:** It produces rapid effect from aqueous solution and slow and sustained effects from repository (depot) preparations. It is suitable for moderate volumes, oily vehicles and some irritant substances, e.g. penicillins, aminoglycosides, iron preparations, etc. It can produce pain (from irritant substances) and abscess formation (if not properly sterilized). It should be avoided during anticoagulant medication. It may damage a nerve if injected into it producing severe pain and paresis.

iii. **Subcutaneous (SC) injection:** It produces rapid effect from aqueous solution and slow and sustained effects from repository preparations as IM injection. It is suitable for some insoluble suspensions and for implantation of solid pallets. It is not suitable for large or moderate volumes of drugs. It can produce pain or necrosis of tissue from irritant substances. Drugs like insulin, adrenaline, heparin (low M.W.), tetanus toxoid, etc. are administered subcutaneously.

Pellet implantation: The drug as solid pellet is introduced into the subcutaneous tissue with the help of a trocar and cannula. It provides a sustained release of the drug for several weeks or months, e.g. testosterone, DOCA, etc.

iv. **Intradermal injection:** In it, the drug is injected into the layers of skin by raising a bleb or multiple puncture of epidermis (by a needle), e.g. BCG or smallpox vaccination. It is also used for testing of drug sensitivity, e.g. penicillin, ATS, etc.

v. **Controlled release drug delivery systems:** These are given as follows:

a. *Transdermal (transcutaneous) drug delivery system (TDS):* It is used for percutaneous absorption of some drugs, e.g. glyceryl trinitrate, isosorbide dinitrate, scopolamine, clonidine, insulin, verapamil, timolol, digoxin, fentanyl, nicotine, prostaglandin, oestradiol, testesterone, etc. The drug is held in a reservoir of suitable materials are applied on the surface of the skin in the form of adhesive patches of various shapes and sizes (5–20 cm sq.), which deliver the incorporated drug at a constant rate into the systemic circulation *via* the skin by diffusion. A single patch can ensure continuous low grade absorption for about 7 days. Common sites of application of adhesive patches are chest, abdomen, upper arm, lower back, buttock, mastoid region and behind the ear (pinna). Its advantages are longer duration of action, decreased frequency of administration, relatively stable plasma concentration, minimum adverse effects (local irritation and oedema) and better patient compliance. The patch can be removed if adverse effects (local inflammation and oedema) appear. Other types of transdermal drug delivery system are dermojet injection, iontophoresis, inunction and implantable miniature syringe pump. Iontophoresis provides penetration of drug into deeper tissues from surface of the skin by galvanic current, e.g. salicylates in arthritis. Inunction provides absorption of drugs into blood by rubbing the drug on the surface of the skin, e.g. nitroglycerine ointment in angina pectoris. Dermojet is a type of transdermal drug delivery system in which needle is not used. A high velocity jet of drug solution is projected from a microfine orifice using a gun-like implement. The drug passes through the layers of skin and gets deposited in the subcutaneous tissue. It is painless and suitable for mass inoculation of vaccines. Implantable computerized miniature syringe pump is used for adminiztration of drugs like insulin, glyceryl trinitrate, etc.

b. *Transmucosal drug delivery system:* It is used for slow mucosal absorption of some drugs, e.g. pilocarpine, progesterone, etc. Ocular insert (ocusert) of pilocarpine is placed directly under the eyelid and delivers a small amount of the drug at a steady rate for about 7 days without causing any discomfort. It thus avoids the need of repeated eye drops application. Progestasert is an intrauterine contraceptive device which provides controlled release of small amount of progestrone within the uterus for about one year.

vi. **Inhalation:** Volatile liquid and gaseous anaesthetics and therapeutic gases (O_2, CO_2 and He) are administered by inhalation. Amylnitrate pearl is broken in handkerchief and inhaled. Other devices like dry powder inhaler spinhaler (of chromolyn sodium), metered dose aerosol inhaler (of salbutamol, beclomethasone, etc.) and compressed air-driven nebulized solution inhaler/nebulizer (of salbutamol, acetylcysteine, etc.) are also available for inhalation. In it, the irritant substances can cause inflammation of respiratory tract.

3 Pharmacokinetics

INTRODUCTION

Pharmacokinetics is the study of movements of the drug in the body. It includes absorption, distribution, metabolism and excretion (ADME) of the drug. It is what the body does to the drug **(Fig. 3.1)**.

Pharmacokinetic Processes

These are given as follows:

Absorption of Drugs

Absorption is the transfer of the drug across the biological (cell) membrane, from the site of administration into the blood. When a drug is administered orally, it passes through the mucous membrane of the gut and capillary endothelium to reach the blood. When it is given by injection, it passes only through capillary endothelium, except given intravenously, when it reaches the blood directly.

Biological (cell) membrane is a lipoprotein (lipoidal) membrane. The lipid portion of the membrane is bimolecular (bilayer) and made of phospholipid and cholesterol molecules. Protein molecules are adsorbed on the outer and inner surfaces of the lipid layer. The membrane contains many aqueous pores (water filled channels), through which filtration of small drug molecules occur.

Absorption of the drug can occur by following processes (mechanisms):

i. **Passive (simple) diffusion:** A drug which is lipid-soluble can cross the cell membrane easily by diffusion, e.g. ether, propranolol, thiopentone, diazepam, etc. cross the cell membrane by dissolving in the lipid matrix of the membrane. The rate

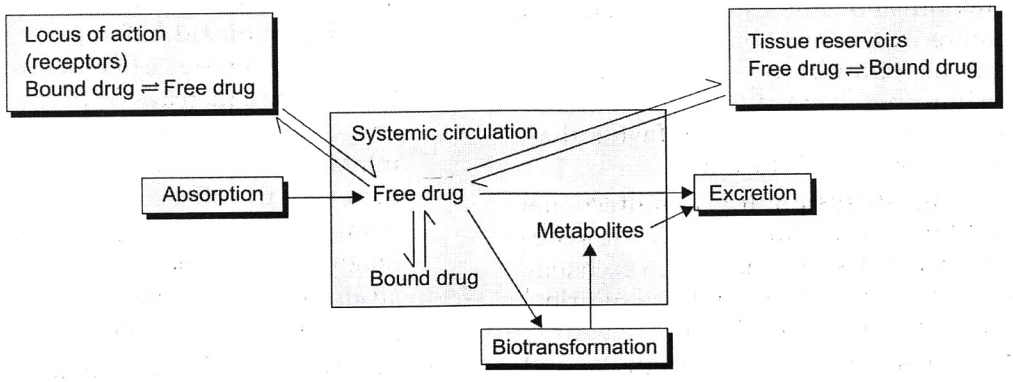


Fig. 3.1: Pharmacokinetics of the drug

of transfer of the drug is proportional to lipid/water partition coefficient of the drug. Greater the coefficient of the drug, higher the concentration of the drug in the membrane and faster the diffusion of the drug through the membrane. Ionized drugs are not lipid-soluble and cannot easily cross cell membrane by diffusion.

ii. **Filtration:** It is the passage of the drug molecule through the aqueous pores in the cell membrane or through inter-epithelial gaps (paracellular spaces). Water-soluble drugs of low molecular weights whether ionized or unionized can easily pass through the membrane pores and enter intracellular or extracellular space or excreted in urine by glomerular filtration, e.g. atenolol, heparin, alcohol, mannitol, etc.

iii. **Active transport:** It is the movement of the drug molecule across the cell membrane against the concentration gradient (uphill movement) requiring expenditure of energy (ATP). It is carrier mediated, i.e. carried by a specific carrier called a transport protein. It can be inhibited by metabolic poisons (antimetabolites). Absorption of glucose, iron and amino acids from intestine occurs by active transport process.

iv. **Facilitated diffusion:** In it, the drug molecule crosses the cell membrane by the help of a carrier protein but the movement of the drug molecule is towards the concentration gradient (i.e. downhill movement) and so does not require expenditure of energy. Absorption of vitamin B_{12}, folic acid and pyrimidines from intestine occurs by facilitated diffusion. It is faster than passive (simple) diffusion.

v. **Exchange diffusion:** It is a bidirectional facilitated diffusion, e.g. Na^+ is reabsorbed in distal renal tubules in exchange of K^+ or H^+ (which are excreted in urine) to preserve important Na^+ in the body.

vi. **Pinocytosis:** It is vesicular uptake and transport. In it, the cell engulfs (swallows) a large size drug molecule (polypeptide, lipoprotein, etc.) by an infolding process of a small portion of a cell membrane forming a vesicle and transports it. It requires expenditure of energy like active transport. It occurs mainly in liver cells.

Factors influencing the rate of absorption of drugs from GIT are as follows:

i. ***Biological factors (intrinsic factors):*** These are gut (GIT) related factors.

   a. **Local pH of gut:** Most drugs are either weak acids or weak bases and exist in aqueous solution as a mixture of ionized and unionized forms. Ionized form is lipid-insoluble, whereas unionized form is lipid-soluble. Acidic drugs like salicylates, barbiturates and sulphonamides are rapidly absorbed from the stomach as they are poorly ionized in the acid pH (less than 5) of stomach and thus remain mostly in unionized form. Basic drugs like morphine, quinine, chloroquine, amphetamine and ephedrine are not absorbed from the stomach, but absorbed from the small intestine in the alkaline pH where they remain mostly in unionized form in the alkaline pH (more than 7) of small intestine.

   b. **Presence of food and other drugs in gut:** Most drugs are better absorbed in empty stomach. Presence of food in the stomach dilutes the drug and retards absorption of the drug, e.g. ampicillin, aspirin, isoniazid, rifampicin, tetracycline, etc. Presence of other drugs in the gut may increase or decrease the absorption of the drug by drug-drug interaction, e.g. presence of vitamin C increases the absorption of iron salt from the gut, presence of calcium, magnesium or iron salt decreases the absorption of tetracyclines by forming poorly absorbed chelate complexes.

   c. **Surface area of gut:** The greater the surface area of the absorbing surface

on which the drug is spread, the more rapid is the rate of absorption. Drugs are better absorbed from the small intestine than the stomach due to the greater surface area. Decrease in surface area due to gastrectomy or enterectomy reduces absorption of drugs.

d. **Motility of gut:** Increase in gut motility as in diarrhoea, decreases absorption of drugs due to rapid elimination in faeces. Decrease in gut motility as in shock or CCF slows absorption of drugs. Vomiting also decreases absorption of drugs.

e. **Local circulation (blood flow) in gut:** Increase of blood flow in gut due to vasodilation increases absorption of drugs. Decrease of blood flow in gut due to vasoconstriction as in haemorrhagic shock decreases absorption of drugs.

f. **First pass effect:** Some drugs, e.g. glyceryl trinitrate, isosorbide dinitrate, isoprenaline, propanolol, chlorpromazine, etc. undergo first pass metabolism in gut wall and liver during passage through portal circulation which decreases their therapeutic effects. These drugs are better administered sublingually to reach the systemic circulation directly by passing gut wall and hepatic metabolism.

ii. *Pharmaceutical factors (extrinsic factors):* These are drug related factors:

a. **Physical state of drug:** Drugs given in liquid dosage forms are better and rapidly absorbed from gut than when given in solid dosage forms. Colloids are slowly absorbed than crystalloids.

b. **Water or lipid solubility of drug:** Drugs given in aqueous solution mix more readily with the aqueous phase of the absorbing surface than when given in oily solution and so rapidly absorbed from gut. At the cell surface, a lipid-soluble drug penetrates the cell membrane more easily than a water-soluble drug and so better absorbed from gut.

c. **Particle size of drug:** Solid dosage forms of drugs that contain smaller particles (microfine crystals) are better absorbed from gut, e.g. aspirin, griseofulvin, chloramphenicol, warfarin, tolbutamide, corticosteroids, etc. They should be given in smaller dose to avoid systemic toxicity. Solid dosage forms that contain larger particles, e.g. bephenium, streptomycin, neomycin, etc. are very little absorbed from gut and so used for local effects. Larger tablet breaks down more quickly than the highly compressed small tablet and so more rapidly absorbed from gut.

d. **Disintegration time of drug:** It is the time taken for a solid dosage form (e.g. tablet) of a drug to disintegrate (break down) into finer particles in the gut completely. It depends on the type of drug and the excipient (binding agent) used in it. If the disintegration time is longer, the absorption of the drug is delayed.

e. **Dissolution time of drug:** It is the time taken for a solid dosage form (e.g. tablet) of a drug to go into the solution in the gut after it has been disintegrated. Solution as a rule is absorbed faster than the solid form.

f. **Enteric coating of drug:** Some tablets or dragees are made enteric coated by means of cellulose, acetate or phthalate, which resist disintegration and dissolution of the drug in the gut by acid gastric juice but permit disintegration and dissolution of the drug by the alkaline intestinal juice. This produces an uniform and sustained blood level of the drug without requiring too frequent dosing. Some sustained release (S.R.) and time release (T.R.) capsules are now avai-

lable that release the active drug over an extended period of time. These contain drug particles covered with different coatings, that are dissolved at different time intervals in the gut. This produces uniform medication for a prolonged period.

Distribution of Drugs

After a drug is absorbed or injected into the bloodstream, it is distributed to various fluid compartments of the body such as interstitial, transcellular and intracellular fluids. Transcellular fluids are CSF, aqueous humour, endolymph, GIT fluids and joint fluids. In the initial phase of distribution of drugs, the heart, liver, kidney, brain and other highly vascular (well perfused) organs receive most of the drugs during first few minutes after absorption. In the later phase of distribution of drugs, the muscle, viscera, skin, connective tissue and adipose tissue receive a much lower proportion of the drug. Ultimately, the drug in all tissues achieve equilibrium with the drug present in the blood. It takes several minutes to several hours to attain the steady state concentration. Diffusion of drug into interstitial compartment occurs rapidly because of the highly permeable nature of capillary endothelial membrane (except in brain). Lipid-insoluble drugs that permeate membrane poorly are restricted in their distribution. Distribution is also limited to drugs that bind with plasma proteins, particularly albumin for acidic drugs and α_1-acid glycoprotein for basic drugs. A drug that is extensively and strongly bound with plasma proteins has limited excess to cellular sites of action and is metabolized and excreted slowly. Drugs may accumulate in tissues in higher concentrations than would be expected from diffusion equilibria as a result of pH gradients, binding to intracellular constituents or partitioning into lipid. Drug that has accumulated in a given tissue may act as a reservoir, which prolongs drug action. Drug reservoirs are body compartments in which a drug accumulate, e.g. plasmaproteins and cellular reservoirs.

Many drugs are bound to plasma proteins. This binding is usually reversible. Free drug + protein $\rightleftharpoons$ drug protein complex. Irreversible (covalent) binding may occur with reactive drugs like alkylating agents.

Cellular reservoirs: Many drugs accumulate in muscle, fat, liver, bone, etc. in higher concentrations than in the extracellular fluids. Accumulation of drugs in cells may be due to active transport or due to binding with cellular constituents. Tissue binding of drugs occurs with proteins, phospholipids or nucleoproteins and is generally reversible.

CNS transfer of drugs: Some drugs can be present in the blood but they cannot reach the brain as they are prevented to reach. The brain by a barrier called the blood–brain barrier (BBB). However, some drugs can cross the BBB. Endothelial cells of the brain capillaries do not possess intracellular pores and pinocytotic vesicles. Presence of tight junctions and pericapillary glial cells restrict the passage of drugs into the brain. Highly lipid-soluble drugs like thiopentone can easily cross the BBB producing rapid action (within a minute after IV injection). As there is little binding of the drug to the brain constituents, so its action is terminated rapidly by redistribution to less vascular tissues of the body (muscle, viscera, adipose tissue, etc.).

Placental transfer of drugs: This is important because some drugs (e.g. phenytoin, prednisolone, warfarin, etc.) can cause congenital anomalies (malformation of foetus) by crossing the placental barrier by simple diffusion. Some drugs (e.g. morphine, barbiturates, etc.) when administered before delivery may cause serious adverse effects on the neonate. Lipid-soluble, unionized drugs readily enter the foetal blood from the maternal circulation. Drugs that are ionized or have low lipid solubility cannot cross placental barrier.

Biotransformation of Drugs

Biotransformation of the drug within a living organism means alteration of chemical structure of the drug. In simple words, it is

the metabolism of the drug molecule in the body. The fate of a drug in the body is metabolism and then excretion mainly as metabolites. Drugs are treated by the body as foreign substances (xenobiotics), which the body tries to remove from the body by metabolism and excretion. Drugs may be lipid-soluble (lipophilic) or water-soluble (hydrophilic). Lipid-soluble drugs are nonionizable (nonpolar) and can easily cross the cell membrane. They are metabolized in the liver as they can easily enter the liver cells (hepatocytes). They are not excreted in urine, as they are reabsorbed in renal tubules. They are to be converted to water-soluble metabolites in order to eliminate them from the body. Water-soluble drugs are ionizable (polar). They are little metabolized in the liver and excreted in urine as they are not reabsorbed in renal tubules.

Sites of metabolism of drugs: These are liver, GIT, plasma, kidneys, lungs and skin. In the liver, the metabolic reactions occur mainly in the smooth endoplasmic reticulum (microsomes). The enzymes for metabolism of drugs are collectively called cytochrome P-450 (CYP). They are haeme-containing membrane proteins (haemoproteins), present in the microsomes of liver and other cells. They catalyze a wide variety of oxidative and reductive reactions. There are three types of CYP, viz. CYP_1, CYP_2 and CYP_3 each of which has four subtypes (A, B, C and D), e.g. CYP_1A, CYP_1B, CYP_2C and CYP_2D. These have different substrate specificities.

Metabolic reactions of drugs: These are of two types:

i. **Phase I reactions (non-synthetic/functionalization reactions):** These are oxidation, reduction and hydrolysis of drugs. Here, the metabolites formed are active or inactive.

ii. **Phase II reactions (synthetic/conjugation reactions):** These are conjugation (union) of drugs with endogenous water-soluble substances like glucuronic acid, acetic acid, methionine, glutathione, glycine, etc. Here, the metabolites formed are inactive (inert).

A drug may undergo following reactions to form metabolites for excretion in urine or bile **(Fig. 3.2)**.

If the metabolites formed in phase I reactions are highly ionized (polar), they will be excreted in urine or bile. If the metabolites formed in phase I reaction are poorly ionized (nonpolar) then they will undergo phase II reactions to become water-soluble products, which are then excreted in urine or bile.

Enzymes responsible for metabolic reactions are microsomal and nonmicrosomal enzymes. Microsomal (cytosolic, mitochondrial, etc.) enzymes catalyze most oxidation and reduction reactions, some hydrolysis reactions and glucuronide conjugation reaction. Nonmicrosomal enzymes catalyze some oxidation and reduction reactions, many hydrolysis reactions and all conjugation reactions except glucuronidation.

Oxidative reactions: Oxidation is the process of addition of oxygen or negatively charged radical to a drug molecule or removal of hydrogen or positively charged radical

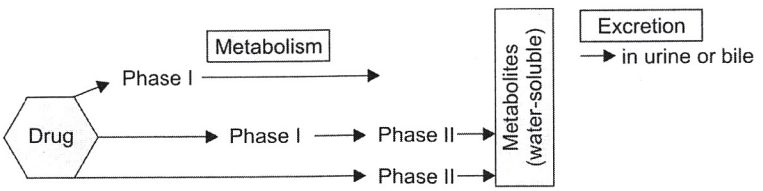


Fig. 3.2: Phases of drug metabolism

from a drug molecule. It occurs most commonly within the microsomes of liver cells. The enzyme system which oxidizes the drug is called mixed function oxidase (MFO) system or mono-oxygenase (MO) system. The system consists of cytochrome oxidase enzyme (cytochrome P-450), NADPH (a coenzyme), NADPH-cytochrome P-450 reductase and molecular oxygen. The drug (substrate) reacts with the oxidized (Fe^{3+}) form of cytochrome P-450 to form an enzyme substrate complex. The cytochrome P-450 reductase accepts an electron from NADPH, which in turn, reduces the oxidized cytochrome P-450 substrate complex. The reduced (Fe^{2+}) cytochrome P-450 substrate complex then reacts with molecular oxygen and a second electron from NADPH donated through the same flavoprotein reductase to form an activated oxygen species. In the final step, one atom of oxygen is released as H_2O and the second atom of oxygen is transferred to the substrate. Upon release of the oxidized substrate, the oxidized cytochrome P-450 enzyme is regenerated. There are several types of oxidation reactions:

1. N-Dealkylation, e.g.

Imipramine	→	Desmethylimipramine
Diazepam	→	Desmethyldiazepam.
Other drugs	:	Codeine, erythromycin, morphine, theophylline and tamoxifen.

2. O-Dealkylation, e.g.

Codeine	→	Morphine
Phenacetin	→	Paracetamol
Other drugs	:	Indomethacin and dextromethorphan.

3. Aliphatic hydroxylation, e.g.

Salicylic acid	→	Gentisic acid
Meprobamate	→	Hydroxymeprobamate
Other drugs	:	Tolbutamide, pentobarbitone, ibuprofen, midazolam and cyclosporin.

4. Aromatic hydroxylation, e.g.

Phenobarbitone	→	P-hydroxyphenobarbitone
Phenytoin	→	Hydroxyphenytoin
Other drugs	:	Propranonol, phenylbutazone and ethinylestradiol.

5. N-Oxidation, e.g.

Mephobarbitone	→	Phenobarbitone
Chlorpheneramine	→	Phenteramine
Other drugs	:	Chlorpheniramine, guanethidine, quinidine and acetaminophen.

6. S-Oxidation, e.g.

Cimetidine	→	Cimetidine sulphoxide
Other drugs	:	Chlorpromazine and thioridazine.

7. Deamination, e.g.

Amphetamine	→	Benzyl methyl ketone (phenylacetone).
Other drugs	:	Diazepam and flurazepam.

Deamination of adrenaline and noradrenaline by MAO is nonmicrosomal enzyme reaction occurring in mitochondria.

Reduction reactions: Reduction is the process opposite to that of oxidation. Here also both microsomal and nonmicrosomal enzymes are involved. There are several types of reduction reactions.

1. Azoreduction, e.g.

Prontosil → Sulphanilamide.

2. Aldehyde reduction, e.g.

Chloral hydrate → Trichloroethanol.

3. Nitroreduction, e.g.

Chloramphenicol → Arylamine.

4. Ketoreduction, e.g.

Cortisone → Hydrocortisone.

5. Chlororeduction, e.g.

Halothane → Trifluoroethanol.

6. Disulphide reduction, e.g.

Disulfiram → Diethyldithiocarbamic acid.

Hydrolysis reactions: Hydrolysis is the cleavage (division) of a drug molecule by addition of a molecule of water. It occurs with the help of the enzymes esterase, amidase and peptidase in the ester, amide and polypeptide respectively in liver and plasma. Here also both microsomal and nonmicrosomal enzymes are involved, e.g.

$$\text{Acetylcholine} + H_2O \xrightarrow[\text{(Cholinesterase)}]{} \text{Acetic acid} + \text{Choline}$$

$$\text{Procaine} + H_2O \xrightarrow[\text{(Procaine esterase)}]{} \text{PABA} + \text{Diethylaminoethanol}$$

$$\text{Procainamide} + H_2O \xrightarrow[\text{(Procaine amidase)}]{} \text{PABA} + \text{Diethylaminoethanolamine}$$

$$\text{Meperidine} + H_2O \xrightarrow[\text{(Peptidase)}]{} \text{Meperidinic acid} + \text{Ethanol}$$

Other drugs: Aspirin, clofibrate, lidocaine and indomethacin.

Conjugation reactions: Conjugation is the addition of an endogenous water-soluble molecule (group) to the parent drug or its oxidized metabolite by the help of transferase enzyme leading to termination of biological activity of the drug and elimination of it in urine. These are given as follows:

1. Glucuronide conjugation, e.g.

$$\underset{\text{(Glucuronidation)}}{\text{Morphine} + \text{Glucuronic acid}} \xrightarrow[\text{(Glucuronyl transferase)}]{} \text{Morphine-glucuronide}$$

Other drugs: Diazepam, aspirin and acetaminophen.

2. Sulphate conjugation, e.g.

$$\underset{\text{(Sulphation)}}{\text{Chloramphenicol} + H_2SO_4} \xrightarrow[\text{(Sulphotransferase)}]{} \text{Chloramphenicol ethereal sulphate}$$

Other drugs: Acetaminophen, oestrogen and other steroids and methyldopa.

3. Acetate conjugation, e.g.

$$\underset{\text{(Acetylation)}}{\text{Sulphanilamide} + \text{Acetic acid}} \xrightarrow[\text{(Acetyltransferase)}]{} \text{Acetyl sulphanilamide}$$

Other drugs: Isoniazid, dapsone and clonazepan.

4. Methyl conjugation, e.g.

$$\underset{\text{(Methylation)}}{\text{Adrenaline} + CH_3} \xrightarrow[\text{(Transmethylase)}]{} \text{Methyladrenaline}$$

Other drugs: Noradrenaline, dopamine and histamine.

5. Glycine conjugation, e.g.

$$\text{Salicylic acid} + \text{glycine} \xrightarrow[\text{(Glycinetransferase)}]{} \text{Salicyluric acid}$$

Other drugs: PAS, cholic acid and benzoic acid.

Types of metabolic product (metabolites):

These are given as follows:

1. **Formation of active metabolites** from active drugs, e.g.

Chloral hydrate ⟶ Trichloroethanol

Phenyl butazone ⟶ Oxyphenbutazone

Other drugs: Aspirin, phenacetin, codeine, imipramine, amitriptyline, diazepam, propranolol, chloroquine and spironolactone.

2. **Formation of active metabolites** from inactive drugs (prodrugs), e.g.

Levodopa ⟶ Dopamine

Acyclovir ⟶ Acycloguanosine

Other drugs: Prednisone, proguanil, enalapril, sulindac, cyclophosphamide, talampicillin, diazepam, benorylate and vitamin D.

3. **Formation of inactive** or toxic metabolites from active drugs. These are most drugs,

Morphine → Morphine glucuronide (inactive).

Isoniazid → Acetyl isoniazid (toxic).

Paracetamol → N-acetyl benzoquinone-imine (toxic).

Oestrogen → Oestrogen ethereal sulphate (inactive).

Factors influencing biotransformation of drugs are:

a. **Genetic factors**: Inter individual variations in the biotransformation of the drug within a population is due to genetically determined differences (genetic polymorphism) in the rate of metabolism (by oxidation and conjugation) in the liver, e.g. propranolol, metaprolol, isoniazid, hydralazine, primaquine, succinylcholine, etc. Phenotypic differences in the amount of drug excreted through a polymorphically controlled pathway lead to the classification of individuals as extensive (rapid) or poor (slow) metabolizers. Most deficiencies in drug metabolizing activity are inherited as autosomally recessive traits. Due to deficiency of corresponding metabolizing enzyme in some persons, the metabolism of the drug is decreased leading to increased therapeutic effect or toxicity of the drug. Pharmacogenetics deals with the genetically mediated variations in drug metabolism and responses (will be discussed with drug effects).

b. **Environmental factors:** Exposure to certain chemicals and environmental pollutants leads to increased biosynthesis of cytochrome P-450. This enzyme induction leads to an increased rate of metabolism and corresponding decrease in the availability of the parent drug. Drugs that are metabolized to reactive compounds by enzyme induction can produce increased toxicity. Some drugs can induce both the metabolism of other drugs and its own metabolism, e.g. carbamazepine and phenobarbitone. These are called **autoinducers.** Inhibition of drug metabolizing enzyme results in increased concentration of the parent drug, prolonged pharmacological effects and increased toxicity.

c. **Physiological factors:** Drugs produce greater and more prolonged effects at the extremes of age (elderly persons and infants) due to decreased rate of metabolism of drugs or lack of ability of conjugation of drugs (due to enzyme deficiency). Deficiency of nutritional factors (protein, fat, vitamins and minerals) in diet decreases the rate of drug metabolism. Drugs that are highly plasma protein bound are slowly metabolized in the body as they cannot reach the site of metabolism (especially liver) by diffusion.

d. **Pathological factors:** In liver and heart diseases, the metabolism of drugs is decreased. In hyperthyroidism, the metabolism of drugs is increased and in hypothyroidism the metabolism of drugs is decreased. In malnutrition, the metabolism of drugs is decreased.

Excretion of Drugs

Drugs are excreted from the body either unchanged or as metabolites. Excretory organs of drugs are kidneys, liver, lungs, intestine, breasts, skin, salivary and lacrimal glands. Lipid-soluble drugs are not readily excreted until they are metabolized to polar (ionized) compounds.

Renal excretion: Kidneys are most important organs for excretion of drugs and their metabolites by glomerular filtration, active tubular secretion and passive tubular reabsorption. The amount of drug entering the tubular lumen by filtration is dependent on its plasma protein binding and GFR. In the proximal renal tubules some organic anions and cations enter the glomerular filtrate by active, carrier mediated tubular secretion. Many organic acids such as penicillins and metabolites such as glucuronides are transported to tubular urine by the active transport system, that secretes uric acid. Organic bases such as tetraethyl ammonium are transported by a separate active transport system, that secretes choline, histamine and other endogenous bases. In the proximal and distal renal tubules, the unionized forms of

weak acids and bases undergo passive reabsorption. The concentration gradient for back diffusion is produced by reabsorption of water with Na^+ and other inorganic ions. As the tubular cells are less permeable to the ionized forms of weak electrolytes, so passive reabsorption of these substances is pH dependent. When tubular urine is made more alkaline, weak acids are excreted more rapidly, because they are more ionized and passive reabsorption is decreased. When the tubular urine is made more acidic, the excretion of weak acids is decreased but excretion of weak bases is increased.

Biliary and faecal excretion: Many metabolites of drugs are excreted into the intestinal tract from the liver via bile. These metabolites are either excreted in the faeces or reabsorbed into the blood and ultimately excreted in urine.

Excretion in other routes: Anaesthetic gases and vapours, alcohol, paraldehyde and occasionally small quantities of other drugs or metabolites are excreted by lungs. Some drugs are excreted through breast milk and may cause adverse effects in the nursing infants, e.g. penicillins, sulphonamides, tetracyclines, chloramphenicol, isoniazid, morphine, sedative-hypnotics, anticancer drugs, etc. Some drugs are excreted in small amounts in sweat, saliva and tears, e.g. lithium, potassium iodide, potassium thiocyanate, rifampicin and heavy metals (like arsenic and mercury) but this is quantitatively unimportant.

4 Clinical Pharmacokinetics

Commonly used pharmacokinetic parameters are:

- Bioavailability
- Volume of distribution
- Clearance
- Half-life.

BIOAVAILABILITY

Bioavailability means the availability of a biologically active drug. It is the fraction of unchanged drug that reaches the blood (systemic circulation) or the site of action following administration by any route. When a drug is administered IV, all the drugs are available for biological activity and so bioavailability is 100%. When a drug is administered IM or SC, the bioavailability of the drug is usually complete (100%) or may be incomplete (<100%) due to local tissue binding of the drug. When a drug is administered orally, then the bioavailability of the drug may vary from 0 to 100% due to nonabsorption, partial absorption or complete absorption of the drug. Conventionally, bioavailability is applied for oral bioavailability.

Importance of Bioavailability

It is necessary to know bioavailability of each drug, because it will determine whether the drug can be administered orally or not, and if it can be administered orally, then what should be the oral dose in comparison to IV dose of the drug.

Determination of Bioavailability

To determine bioavailability of a drug, the drug is first administered IV and its plasma concentrations are measured at one hourly intervals. The plasma concentration–time curves following IV and oral administration of the same dose of the drug are plotted in graph paper (as shown in **Fig. 4.1**). From these curves, the area under the curve (AUC) is measured for IV and oral dose of the drug. Bioavailability is determined by the formula:

$$BA = \frac{\text{AUC after an oral dose}}{\text{AUC after an IV dose}}$$

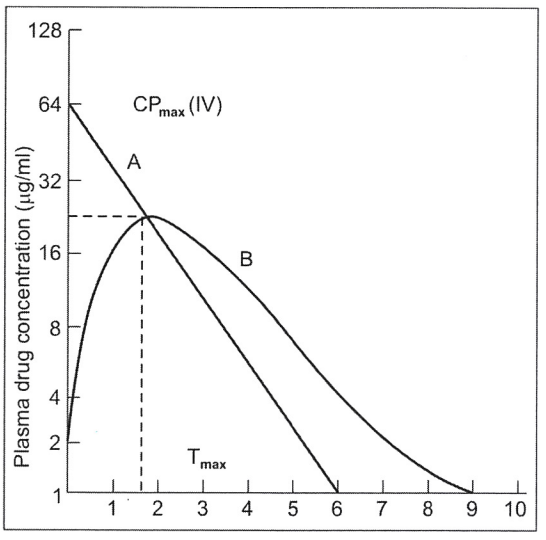


Fig. 4.1: AUC after oral and IV dose of a drug

(where BA is the bioavailability and AUC is the area under curve). It is always expressed in percentage (%). A is the plasma concentration–time curve following IV administration of a drug. B is the plasma concentration–time curve following oral administration of a drug.

CP_{max} is peak (maximum) plasma concentration following IV/oral administration of a drug. T_{max} is the time of peak plasma concentration following oral administration of a drug.

For the sake of convenience, instead of measuring plasma concentrations of the drug by repeated injections, the total urinary excretion of the drug at one hourly intervals can be measured if the drug is primarily excreted in urine.

Bioavailability determined by these methods is called absolute bioavailability. Relative bioavailability is determined by comparing the AUC of oral dose of the test preparation with the AUC of oral dose of the standard preparation of the drug without IV administration.

Factors Modifying Bioavailability

These are given as follows:

1. **Route of administration of the drug:** Bioavailability of a drug is 100% after IV administration and less than 100% after oral or other route of administration.
2. **Biological (intrinsic) factors like:**
   a. *Degradation of the drug in gut (GIT)*: Some drugs such as benzyl penicillin, insulin, adrenaline, etc. are destroyed in the gut and so they have low bioavailability after oral administration.
   b. *Route of absorption of the drug from gut*: Some drugs, e.g. streptomycin, neomycin, aluminium hydroxide, magnesium trisilicate, etc. are poorly absorbed from the gut and so they have poor bioavailability when administered orally. Water-soluble drugs are absorbed easily from GIT producing greater bioavailability than the lipid-soluble drugs.
   c. *Presence of food and other drugs in gut*: Presence of food in stomach dilutes the drug and retards the absorption of the drug. Presence of calcium, magnesium, aluminium or iron salts decreases the absorption of tetracyclines by forming poorly absorbed chelate complexes and thus reduces bioavailability. Cholestyramine resin binds with thyroxine, warfarin or other acidic drugs in gut and decreases their bioavailability.
   d. *First pass (presystemic) metabolism of the drug*: Some drugs are metabolized in the gut wall and liver by enzymes before reaching the systemic circulation, e.g. GTN, isosorbide dinitrate, isoprenaline, nifedipine, buprenorphine, etc. and their bioavailability is low after oral administration. So, they are administered sublingually for systemic action.
   e. *Enterohepatic circulation (recycling)*: Some drugs undergo enterohepatic circulation, e.g. tetracylines, erythromycin, ampicillin, rifampicin, digitoxin, oral contraceptives, etc. and so their bioavailability is decreased.
3. **Pharmaceutical (extrinsic) factors:** Pharmaceutical factors like physicochemical properties of drugs, dosage forms, particle size of drugs, type of excipient used, disintegration time and dissolution rate of drugs influence bioavailability of drugs. Drugs administered as microcrystalline form, e.g. aspirin, griseofulvin, nitrofurantoin, etc. are rapidly absorbed producing greater bioavailability than when administered as coarse particles.

Bioequivalence

It means equal therapeutic effectiveness of two or more drug products (formulations) manufactured by different pharmaceutical laboratories due to same bioavailability of the active ingredient. Differences in bioavailability (low or high) of drug formulations can reduce therapeutic effect or increase toxicity of the drug. For this reason, a clinician must be careful while changing one brand (drug formulation) to another brand (drug formulation) of the same drug. It is of great clinical significance in drugs with narrow safety margin, e.g. digoxin, quinidine, phenytoin, warfarin, salbutamol, glibenclamide, lithium, etc.

VOLUME OF DISTRIBUTION

The volume of distribution of a drug means the fluid volume that would be required to contain all the drugs in the body at the same concentration as in blood or plasma. The total volume of the fluid compartments of the body into which a drug may be distributed is about 40 litres in an adult (70 kg). These fluid compartments are extracellular fluid (20 L) including plasma water (5 L) interstitial fluid (10 L) and transcellular fluid (5 L) and intracellular fluid (20 L). The fluid volume into which a drug is supposed to be distributed is called volume of distribution (Vd). It is calculated by the formula:

$$Vd = \frac{\text{Amount of the drug in the body (D)}}{\text{Concentration (C) of the drug in the blood or plasma}}$$

where Vd is the apparent volume of distribution of the drug after IV administration. Vd calculated by this method is not real but hypothetical (imaginary) and so it is called apparent volume of distribution.

From this estimated Vd, some useful conclusions can be drawn:

i. Drugs which remain mostly confined within the blood or plasma and cannot go beyond the vascular compartment, e.g. heparin, warfarin, phenytoin, aspirin, frusemide, etc. have small Vd (5–10 L). Such drugs can be removed easily by haemodialysis (dialysis of plasma) in case of poisoning.

ii. Drugs which go beyond the vascular compartment and are distributed in the tissue fluids, e.g. ampicillin, cephalexin, sulphamethoxazole, etc. have large Vd (10–20 L).

iii. Drugs which are present not only in blood and tissue fluid, but are heavily dissolved in adipose tissue (e.g. thiopentone, pethidine, etc.) or concentrate in tissue proteins of liver or other organs, e.g. chloroquine, mepacrine, etc. have very large Vd (500–50,000 L).

iv. Lipid-soluble (nonionized) drugs which can readily cross cell membranes and distributed throughout the body fluids have large Vd and water-soluble (ionized) and highly plasma proteins bound drugs have small Vd.

v. When the Vd exceeds the total volume of body water (40 L), then there is uptake and binding of the drug within the tissue such as adipose tissue, muscle, liver, brain, heart and bone.

One compartmental vs. multicompartmental models: For some drugs, the body may be considered to consist of a single (one) homogeneous compartment made of blood and tissues. In this, all drug administration occurs directly into the body compartment and distribution of drug is instantaneous through the body fluid volume. Clearance of drug from this compartment occurs by first order kinetic, i.e. the amount of drug eliminated per unit time depends on the amount (concentration) of drug in the body compartment. In it, a plot of the logarithm of the concentration of drug in plasma against time will be straight line **(Fig. 40.2a).**

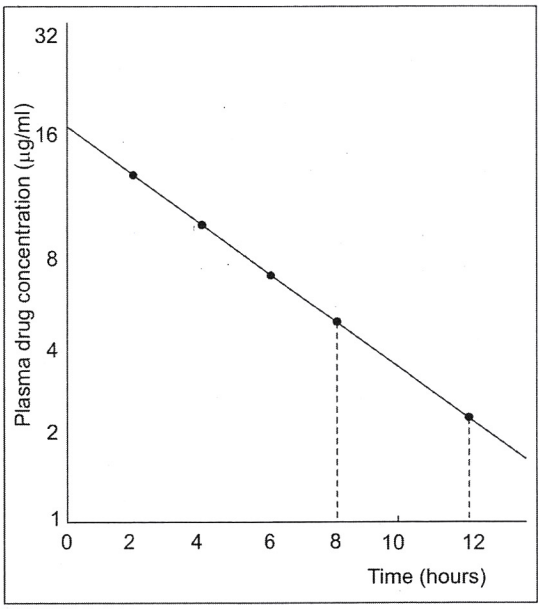


Fig. 4.2a: One compartment model after IV administration

For most of the drugs, the body is considered to be consisting of multiple (two) compartments. The first compartment is called central compartment and consists of blood and some organs like heart, brain, liver, lung and kidney, which are highly vascular and where the drug can enter very easily from the vascular compartment. The second compartment is called peripheral compartment and consists of mostly muscles and adipose tissue, where the vascularity is less (poor) in comparison to first compartment. When a drug is administered by IV bolus, it is rapidly distributed in the central compartment and after sometime it enters the peripheral compartment.

Clearance of drug from these compartments occurs by multiple exponential kinetics. In it, a plot of the logarithm of the concentration of drug in plasma against time will be a curved line. The curve shows two phases—an initial rapid decline phase of the plasma concentration of drug due to distribution to the tissue and then a slow and uniform decline phase of the plasma concentration of drug due to elimination from the body **(Figs 4.2b and c).**

Factors influencing the volume of distribution of the drug are:

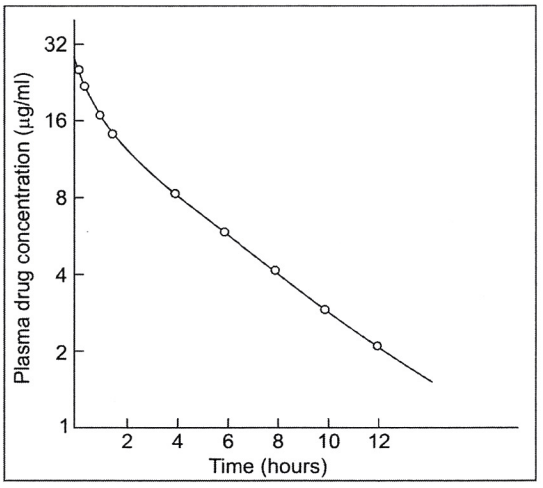


Fig. 4.2b: Multicompartment model after IV administration

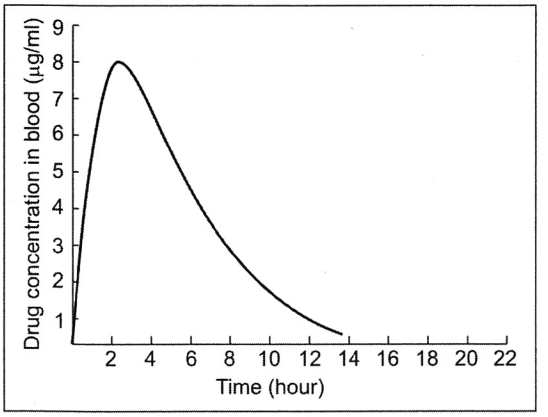


Fig. 4.2c: One compartment model after oral administration

i. **pKa of the drug:** It is the negative logarithm of dissociation (ionization) constant 'Ka', i.e. pKa = – log Ka. For an acidic drug,

$$pKa = pH + \log \frac{\text{Unionized acid}}{\text{Ionized base}}$$

and for a basic drug,

$$pKa = pH + \log \frac{\text{Ionized base}}{\text{Unionized acid}}$$

(Handerson-Hassalbalch equation)

Generally acidic drugs have a lower pKa value (2.5–6) and basic drugs have a higher pKa value (6–10). As a general rule, acidic drugs (e.g. aspirin) are more ionized, lipid-insoluble and less diffusible in a relatively alkaline medium (e.g. intestine), whereas they are more nonionized, lipid-soluble and more diffusible in relatively acidic medium (e.g. stomach). Similar is the relationship between the basic drug and the environmental pH. Thus, pKa of drug influences volume of distribution of a drug.

ii. **Degree of binding of the drug with plasma proteins or with other tissue proteins:** The greater the binding, the less the volume of distribution of the drug.

iii. **Lipid solubility of the drug:** The higher the lipid solubility of the drug, the greater is the volume of distribution of the drug.

iv. **Patient's age, gender, disease and body composition:** These can also change volume of distribution of the drug.

CLEARANCE

Clearance is the measure of body's ability to eliminate a drug. It is the rate of elimination of a drug by all routes normalized to the concentration of drug in the biological fluid (blood or plasma). It is calculated by the formula.

$$CL = \frac{\text{Rate of elimination of drug (vol/min)}}{\text{Concentration of drug in blood or plasma}}$$

where CL is the clearance of drug. It indicates the volume of biological fluid (blood or plasma) that would have to be completely freed of drug to account for the elimination. It is expressed as a volume per unit of time. It may be blood clearance (CL_p), plasma clearance (CL_p) or clearance based on the concentration of unbound (free) drug (CL_u) depending on the concentration measured (C_b, C_p or C_u). The organs of elimination can only clear drug from the blood or plasma with which they are in direct contact. Clearance by means of various organs of elimination is additive. Elimination of drug may occur as a result of processes that occur in the kidney (renal clearance), liver (hepatic clearance) or other organs. Dividing the rate of elimination of each organ by a concentration of drug (e.g. plasma concentration) will give the respective clearance by that organ. Total systemic clearance is the sum of all these separate clearances.

Steady state concentration (C_{ss}): When a drug is administered at a constant rate, then a steady state concentration will be achieved ultimately. At this point, the rate of elimination of a drug is equal to the rate of drug availability. If the drug is administered as intermittent doses (e.g. 250 mg every 8 hours), then during each interdose interval the concentration of drug rises or falls. At steady state, the entire cycle is repeated identically in each interval **(Fig. 4.3)**. For steady state concentration of a drug 4 or 5 plasma half-lives are required.

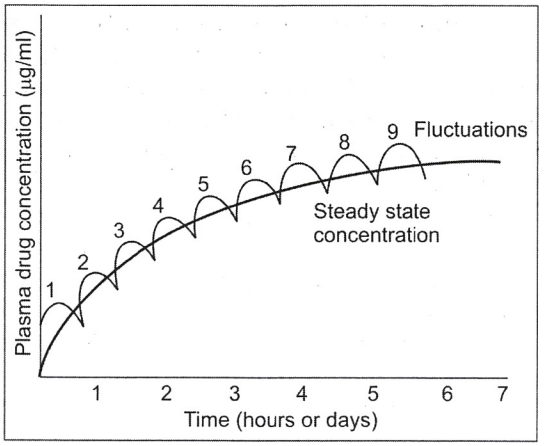


Fig. 4.3: Steady state concentration of drug

HALF-LIFE ($t^1/_2$)

Half-life of a drug is the time taken for the plasma concentration or the amount of drug in the body to be reduced to half (50%) of its original (peak) concentration or amount. It is a simple parameter, which indicates the rate of elimination of a drug from the body. After single dose of a drug, plasma concentration of the drug rises to its peak value. Then it begins to fall due to elimination of the drug by the kidneys or by any other route. The time when the concentration of the drug becomes exactly half of the peak value is the plasma half-life. It is a time and is expressed in terms of units of time (minutes/hours/days). It is not dependent upon the value of plasma concentration of the drug. For complete elimination of a drug from the body 5 plasma half-lives are required **(Table 4.1)**.

Determination of plasma half-life of a drug: A single intravenous bolus injection of the drug is given and plasma concentrations of the drug are estimated at one hour interval. From the data obtained, the log plasma concentration–time curve is plotted (as shown in **Fig. 4.4**). Plasma half-life can also be determined by the formula:

$$t½ = 0.693 \times \frac{V}{CL},$$

where V is the volume of distribution and CL is the clearance of the drug.

In one compartmental model [as shown in **Fig. 4.2(a)**], the plasma t½ can be determined readily. As it follows first order kinetics, so it is always same, irrespective of the value of its peak concentration. In multi (two) compartmental model [as shown in **Fig. 4.2(b)**] drug concentrations in plasma follow a multi-exponential pattern of decline. Initially there is rapid decline phase due to distribution of the drug to tissue and later on a slow and uniform decline phase due to elimination of the drug. Thus two half-lives, viz. α-t½ (distribution half-life) and β-t½ (elimination half-life) can be calculated from the two slopes (α and β).

Table 4.1: Plasma half-life of a drug

1. Plasma t½ means 50% drug is eliminated.
2. Plasma t½ means 75% (50 + 25) drug is eliminated.
3. Plasma t½ means 87.5% (50 + 25 + 12.5) drug is eliminated.
4. Plasma t½ means 93.75% (50 + 25 + 12.5 + 6.25) drug is eliminated.
5. Plasma t½ means 96.87% (50 + 25 + 12.5 + 6.25 + 3.12) drug is eliminated.

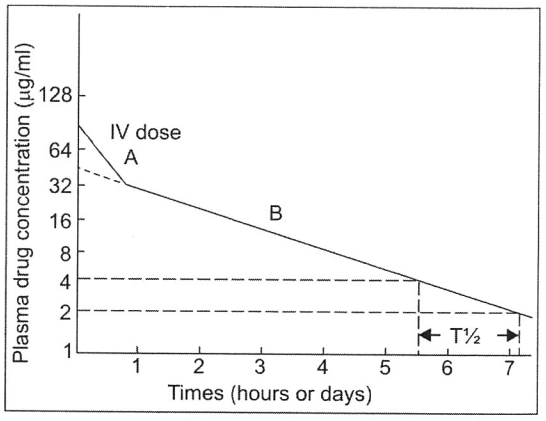


Fig. 4.4: Plasma half-life of a drug

Importance of Plasma Half-life

It is necessary:

- To know the duration of effect and frequency of administration of a drug.
- To determine the dosage schedule of a drug. A drug with short half-life requires frequent daily dosing, while a drug with long half-life requires one or more daily dosing.
- To know the steady state concentration of a drug.
- To assess the therapeutic efficacy of a drug.
- To assess the adverse effects of a drug.

Plasma Half-lives of Some Drugs

Glyceryl trinitrate (20 minutes), penicillin-G (30 minutes), insulin (40 minutes), amoxycillin (1 hour), aspirin (4 hours), tolbutamide (5 hours), doxycycline (20 hours), digoxin (40 hours), diazepam (43 hours), phenylbutazone (60 hours), sulphadoxine/digitoxin (7 days).

Factors Influencing Plasma t½

These are as follows:

- **Rate of clearance of drug:** Faster the clearance, shorter the plasma t½ of a drug.
- **Metabolic degradation of drug:** Faster the metabolism, shorter the plasma t½ of a drug.
- **Enterohepatic circulation (cycling) of drug:** It increases plasma t½ of a drug.
- **Plasma protein binding of drug:** High plasma protein binding of a drug increases the plasma t½ of a drug.
- **Distribution and storage of drug:** A drug which is widely distributed in the body and stored in tissues, has long plasma t½.

Other Terms

Biological half-life: It is the time taken for the biological activity of a drug in the body to be reduced to 50% of its original value (activity). It is measured with the help of radioactive isotope administered IV.

Biological effect of half-life: It is the time taken for the pharmacological effect of a drug

in the body to be reduced to 50% of its original value (effect). It is measured in hit and run drugs, e.g. reserpine, MAO-inhibitors, organophosphorus compounds and anticancer drugs, which have short plasma half-life but long biological effect half-life.

Kinetics of drug clearance (elimination): Drugs are eliminated from the body by first order kinetics or zero order kinetics.

i. **First order kinetics (exponential kinetics):** Here, the elimination of a drug is directly proportional to its plasma concentration. In it a constant fraction (%) of a drug present in the body is eliminated per unit time (hour). This occurs with most of the drugs including penicillins, tetracyclines, sulphonamides, digitalis glycosides, calcium channel blockers, β-blockers, etc. It is non-saturable and dose independent kinetics. Graphically, decline in plasma concentration of the drug which follows first order kinetics is shown in **Fig. 4.2(a)**. It shows a straight line.

ii. **Zero order kinetics (saturation kinetics):** Here, the elimination of a drug is not proportional to its plasma concentration. In it a fixed amount of the drug present in the body is eliminated per unit time irrespective of plasma concentration of the drug. It occurs with few drugs like phenytoin, alcohol, warfarin, aspirin, phenylbutazone, aminophylline, paraldehyde and general anaesthetics in higher doses. In these drugs, elimination occurs by first order kinetics in lower plasma concentrations but by zero order kinetics in higher plasma concentrations. This is because the reacting enzyme for the metabolism of the drug is limited and gets saturated at higher dose of the drug. These drugs have no constant plasma $t_{1/2}$, which rises with the increase of plasma concentration of the drug. Special care is needed to increase the dose of these drugs to avoid adverse effects. Graphically, decline in plasma concentration of the drug which follows zero order kinetics is shown in **Fig. 4.2(b)**. It shows a curved line.

5 Pharmacodynamics

INTRODUCTION

Pharmacodynamics is the study of biochemical and physiological effects of drugs and their mechanisms of action. It deals with the targets of drug action; types, sites and natures of drug action and factors modifying/influencing drug action. It is what the drug does to the body.

Sites (Targets) of Drug Action

These are receptors, ion channels, enzymes, carrier proteins and structural proteins (as shown in **Fig. 5.1**).

i. **Drug receptors:** These are regulatory macromolecular proteins or nucleic acids present in the cell (in different cellular constituents including cell membrane). These are specific in size, shape and structure and allow interaction with specific ligands or substrates including drugs. Only specific drugs bind with specific receptors. If the forces (i.e. chemical bonds) that bind the drugs with receptors are weak (e.g. ionic bond, hydrogen bond, hydrophobic bond and Van der Waals bond), the binding will be reversible but if the forces involved are strong (e.g. covalent bond) then the binding will be almost irreversible. Drug receptor interaction involves two steps. First, the drug binds with the receptor and then generates stimulus, which in turn produces the effect. The combining capacity of the drug with the receptor is called affinity and the power to generate the stimulus or to produce action (effect) is called efficacy or intrinsic activity. A large number of receptors have been identified in the body. These are physiological and nonphysiological receptors. Physiological receptors mediate responses to transmitters, hormones, autacoids and other endogenous regulatory ligands, e.g. cholinergic, adrenergic, histaminergic, serotonergic, prostaglandins, steroid hormones, thyroid hormones and other growth factors. Nonphysiological receptors are true drug receptors, e.g. benzodiazepine receptor, cardiac glycoside receptor, thiazide receptor, etc. Drugs can act on both physiological and nonphysiological receptors. Receptors and drug action will be discussed later on.

ii. **Ion channels:** These are minute pores present in the cell membrane. Common ion channels are of Na^+, K^+, Ca^{2+} and Cl^-. These are modulated (open or block) by drugs in different ways. Most of the ion channels are modulated by binding of drugs directly to the parts of channel protein in the cell. Some are ligand gated receptor mediated ion channels and others are modulated indirectly involving G-proteins or other intermediaries. Opening of Na^+ or Ca^{2+} channels by drugs produces depolarization of cell mem-

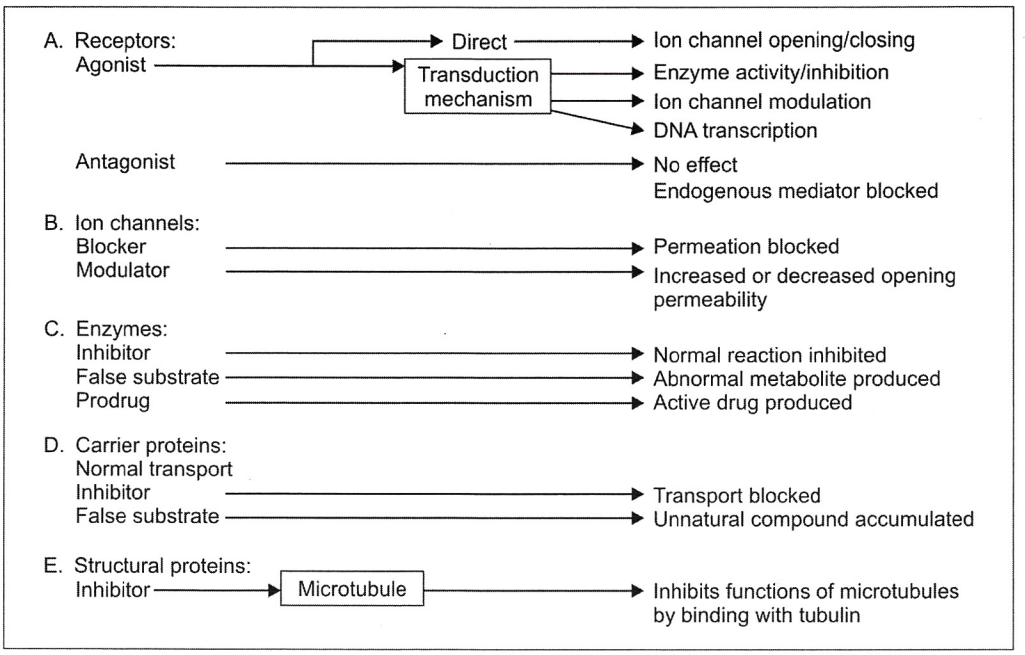


Fig. 5.1: Mechanisms of action (MoA) of drugs

brane, whereas opening of K^+ or Cl^- channels produces hyperpolarization of the cell membrane.

iii. **Enzymes:** These are biocatalysts present in the cell. These are targets for many drugs. Most commonly the drug molecule acts as a substrate analogue, which acts as a competitive inhibitor of the enzyme. Common target enzymes are cholinesterase (ChE), monoamine oxidase (MAO), cyclo-oxygenase (COX) and angiotensin converting enzyme (ACE). In some cases, the inhibition can be irreversible, e.g. organophosphorus compounds on acetylcholinesterase (AChE) and aspirin on platelet cyclo-oxygenase (COX).

iv. **Carrier molecules:** These are carrier proteins which transport ions and small organic molecules across the cell membrane. These are targets for some drugs. Common carrier molecules are of myocardial Na^+ pump (Na^+/K^+-ATPase pump), gastric proton pump (H^+/K^+- ATPase pump), neuronal noradrenaline uptake pump and apoferritin (in intestine)/ transferrin (in blood) in iron transport.

v. **Structural proteins:** A few drugs act on structural proteins of cells, e.g. 5-fluorouracil incorporates into mRNA of cells; colchicine, paclitaxel and vinca alkaloids (vinblastine and vincristine) bind with the protein 'tubulin' of microtubules of cells.

Discussion in Detail

Receptors and Drug Action

Paul Ehrlich (1901) introduced the concept of receptor for drug action. He described drug-receptor interaction as a lock and key system. Many theories have been proposed from time to time to explain drug-receptor interaction. Of these receptor theories, three are popular.

a. **Receptor occupation theory (proposed by Clark in 1933):** According to this theory, drug action is due to occupation of receptors by specific drugs. The interaction between the drug (D) and receptor (R) is governed by the law of mass action as follows:

$$D + R \rightleftharpoons DR \text{ complex} \rightarrow \begin{matrix} E \text{ (effect) or} \\ R \text{ (response)} \end{matrix}$$

The intensity of E or R is proportional to the number (fraction) of receptors occupied by the drug. Maximum response (effect) is produced when all receptors are occupied by the drug.

b. **Modified (classical) receptor theory (proposed by Ariens, Furchgott, Nickerson and Stephenson in 1957):** According to this theory, all receptors need not be occupied for a maximum response of a drug. Spare (reserve) receptors, which are majority of total receptors are responsible for maximum response (full agonist action) of the drug. They also introduced the concept of affinity and intrinsic activity (efficacy) in relation to drug action through receptors. Affinity is the ability of the drug to combine with the receptor and intrinsic activity (efficacy) is the ability of the drug to produce action (effect) by combining with the receptor. An agonist has both affinity and intrinsic activity (efficacy), while an antagonist has affinity but no intrinsic activity (efficacy).

c. **Rate theory of drug action (proposed by Paton in 1961):** According to this theory, the effect of a drug depends on the rate of drug-receptor interaction (combination) to produce drug-receptor complex and subsequently breakdown (dissociation) of the drug from the receptor and not to the number (fraction) of receptors occupied by the drug. It provides the basis for "Fade Phenomenon", i.e. the response of an agonist is initially high but decreases (fades) later on in spite of continued presence of the agonist.

Functions of Receptors

(1) Ligand binding (Latin: *Ligure* meaning binding) and (2) Message propagation, i.e. to propagate regulatory signals to the target (effector) cells either directing or indirectly through intermediary cellular molecules called transducers. The receptor, its cellular target and any intermediary molecule are called receptor-effector system or signal transduction pathway. Receptors and their associated effector and transducer proteins also act as integrators of extracellular information as they coordinate signals from multiple ligands with each other and with the metabolic activities of the cell. An important property of physiological receptors, which make them an excellent target for drugs is that they act catalytically and so they are biochemical signal amplifiers. The catalytic nature of physiological receptors is obvious when the receptor itself is an enzyme, e.g. (1) when a signal ligand molecule binds to a receptor that is an ion channel and opens it causing flow of many ions through the channel, (2) when a single steroid hormone molecule binds to its receptor and initiates transcription of many copies of specific mRNA, which in turn can give rise to multiple copies of a single protein.

Types of drug receptors: These are of three types:

a. **Pharmacoreceptors:** These are receptors with which the drug molecules first interact (combine) to produce effects.

b. **Spare (reserve) receptors:** These are receptors with which the drug molecules next interact to produce effects. These are majority of receptors (about 80%).

c. **Silent (storage) receptors:** These do not produce pharmacological effects. These are involved in binding of drugs to plasma proteins, cellular proteins or enzymes for distribution and metabolism of drugs.

Again, drug receptors may be cell surface receptors or intracellular receptors. Cell surface receptors are ligand gated receptor mediated ion channels, G-protein coupled receptors and tyrosine (protein) kinase linked receptors.

a. **Ligand gated receptor mediated ion channels:** The natural ligands which act by regulating transmembrane flow of ions are acetylcholine, GABA, glutamic acid and aspartic acid, which are synaptic transmitters. When these ligands bind to the

specific receptor channel, the gate opens and the respective ion flows along the concentration gradient and thereby alter the electric potential across the membrane, e.g. acetylcholine opens Na^+ channel, GABA opens Cl^- channel and glutamate and aspartate open K^+ channels.

b. **G-protein coupled receptors:** Many receptors in the cell membrane regulate distinct effective protein through the mediation of a group of GTP-binding proteins called G-proteins. These are present at the inner surface of the cell membrane. Receptors for biogenic amines, eicosanoids and many peptide hormones are G-protein coupled receptors. They act by facilitating the binding of GTP to specific G-protein (Gs or Gi). GTP binding activates the G-protein, which regulates the activity of specific effectors through second messengers. The effector includes enzymes such as adenylcyclase and phospholipases (A_2, C and D) and ion channels that are specific for Na^+, K^+, Ca^{2+} or Cl^- (as shown in **Fig. 5.2**). An individual cell may express multiple G-proteins. Each of these may respond to several different receptors and regulate several different effectors with a characteristic pattern of selectiveness.

c. **Thyrosine (protein) kinase-linked receptors:** These membrane bound receptors mediate the action of insulin, epidermal growth factor, platelet derived growth factor and certain lymphokines. Insulin by acting through these receptors triggers uptake of glucose and amino acids and regulates metabolism of glycogen and triglycerides in the cell.

Intracellular (cytosol and nuclear) receptors: These are receptors which regulate gene expression (DNA transcription). They have no second messenger. Receptors for steroid hormones, thyroid hormones, vitamin D and retinoids are soluble DNA binding proteins, which regulate the transcription of specific genes leading to synthesis of particular proteins and production of cellular effects.

Second messengers: These are produced by the 1st messenger, i.e. original ligand (natural hormone or drug mimicking the naturally occurring ligand), e.g. cAMP, cGMP, IP_3, DAG and Ca^{2+}, etc. These are ultimately degraded.

Response (Effect) of Drug Receptor Interaction

If a drug has affinity for the receptor and if it is in close proximity of the receptor site, then receptor occupancy takes place. This drug-receptor interaction (coupling) leads to a variety of responses (effects) depending on the nature of drug molecules, which are:

Agonists

These are drugs that resemble the natural transmitter or hormone and activate the concerned receptors leading to responses (effects). They have affinity for the receptors

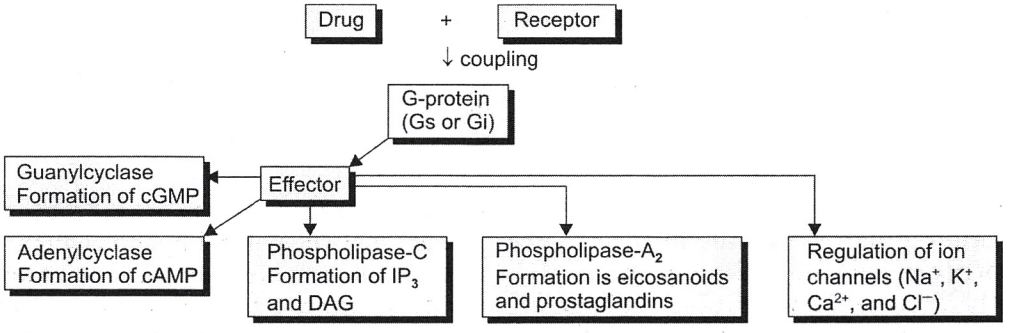


Fig. 5.2: Drug action through G-protein

and maximal efficacy/intrinsic activity (discussed before), e.g. acetylcholine, noradrenaline, histamine, 5-HT, angiotensin, prostaglandins and their chemical analogues.

Antagonists

These are drugs that antagonize or block responses (effects) of the concerned agonists. They have affinity for the receptors but no efficacy/intrinsic activity. These are three types:

i. **Reversible (competitive) antagonists:** For example, atropine for acetylcholine, phentolamine/propranolol for adrenaline/noradrenaline, mepyramine for histamine, naloxane for morphine, flumaxenil for diazepam, etc.

ii. **Irreversible (noncompetitive) antagonists:** For example, organophosphorus compounds for acetylcholine, phenoxybenzamine for adrenaline/noradrenaline, papaverine/decamethonium/α-bungarotoxin for acetylcholine, etc.

iii. **Partial agonists:** These are drugs that have both agonist and antagonist actions. They have affinity for the receptors but submaximal efficacy (intrinsic activity). They competitively antagonize the effect of a full agonists but in the absence of the agonist they can produce some responses (effects) like that of the agonist, e.g. succinylcholine for acetylcholine, nalorphine for morphine, saralasin for angiotensin II, etc.

iv. **Inverse agonists (negative antagonists):** These are drugs that have affinity for the receptors but negative efficacy (intrinsic activity). They produce effects opposite to those of agonists (e.g. β-carbolines like dimethoxyethyl-carbomethoxy (β-carboline/DMCM)) produce effects opposite to that of benzodiazepines (e.g. diazepam) by occupying benzodiazepine receptors. They produce anxiety, increased muscle tone and convulsions, while benzodiazepines produce sedation, anxiolysis, muscle relaxation and control of convulsions. Both these types of drugs act on benzodiazepine (BDZ) receptors by modulating the effects of the neurotransmitter GABA. Actions of both groups of drugs can be blocked by specific BDZ antagonist "Flumazenil".

Ion Channels and Drug Action

Some drugs act by influencing ion channels and modulating passage of ions across the cell membrane. Ion channels can be voltage dependent ligand gated (indirect) or direct. In the former type, combination of the drug with the receptor results in opening or blocking of ion channels. G-protein (Gs or Gi) are commonly involved in such interactions, e.g. opening of Na^+ channels by DDT, aconitine, veratradine, etc.; blocking of Na^+ channels by minoxidil, diazoxide, cromakaline, etc.; blocking of K^+ channels by sulphonylureas, amiodarone, sotalol, etc.; blocking of Ca^{2+} channels by calcium channel blockers (verapamil, nifedipine, diltiazem, etc.); opening of GABA-receptor chloride channel by benzodiazepines (diazepam, alprazolam, lorazepam, etc.). Some drugs can also combine with specific channel protein directly.

Enzymes and Drug Action

Enzymes are protein in nature and catalyze biological reactions. Substrates are substances that are acted upon by the enzymes. The drug (substrate) combines with an enzyme forming a complex and mediating the resultant drug effect.

The enzyme substrate complex producing effect can be reversible (competitive) or irreversible (noncompetitive) (as shown in **Table 5.1**).

Some drugs stimulate the activity of some endogenous cellular enzymes, e.g. adrenaline and noradrenaline stimulate adenylcyclase, pyridoxine stimulates decarboxylase (DC), etc. Some drugs stimulate the synthesis of microsomal enzymes and thus increase metabolism of other drugs, e.g. phenobarbitone, phenytoin, carbamazepine, rifampicin, phenyl butazone, diphenhydramine, prednisolone and other glucocorticoids, DDT, etc. These are microsomal enzyme inducers. Auto-inducers

Table 5.1: Drug and enzyme inhibition

Drug	*Enzyme*	*Effect*
Physostigmine	Cholinesterase	Inhibition (reversible)
Organophosphorus compounds (D, F, P, etc.)	Cholinesterase	Inhibition (irreversible)
Hemicholinium	Choline-acetyl transferase	Inhibition of acetylcholine synthesis by acting as false substrate (reversible)
Methyldopa	Dopa-decarboxylase	Inhibition of noradrenaline synthesis by acting as false substrate (reversible)
Allopurinol	Xanthine oxidase	Inhibition (reversible)
Sulphonamides	Folate synthetase	Inhibition of growth of bacteria (reversible)
Aspirin	Cyclo-oxygenase	Inhibition (irreversible)
Acetazolamide	Carbonic anhydrase	Inhibition (irreversible)
Digoxin	Na^+/K^+-ATPase	Inhibition (irreversible)
Theophylline	Phosphodiesterase	Inhibition (irreversible)
MAO inhibitors	Monoamino oxidase (MAO)	Inhibition (irreversible)

are drugs which stimulate their own metabolism by inducing microsomal enzymes, e.g. phenobarbitone and carbamazepine. Again some drugs inhibit the synthesis of microsomal enzymes and thus decrease the metabolism of other drugs, e.g. cimetidine, isoniazid, allopurinol, amiodarone, ciprofloxacin in metronidazole, erythromycin, choloramphenicol, alcohol, etc. These are microsomal enzyme inhibitors.

Carrier Molecules and Drug Action

Some drugs are carried to the site of action by binding with carrier protein molecules, e.g. passage of glucose across the cell membrane by glucokinase, iron transport by apoferritin and transferrin, uptake of neurotransmitters (noradrenaline, acetylcholine, etc.) or other precursor carrier proteins can act by inhibition of transport process, e.g. blockade of organic acid transport in renal tubules by probenecid causing delay in penicillin excretion and enhancement of urate excretion.

Carrier proteins have specific recognition sites, which bind to and carry the permitting molecules, e.g. digoxin blocks sodium pump (Na^+/K^+-ATPase pump), omeprazole blocks proton pump (H^+/K^+-ATPase pump), imipramine blocks noradrenaline uptake, etc.

Structural Proteins and Drug Action

Discussed before.

Types of Drug Action

Drugs may produce their effects on cell by stimulation, depression, irritation or cytotoxic action on cells or may act as replacement agent or by modification of immune status (as shown in **Table 5.2**).

Natures of Drug Action

Drugs may act by one or more of the following ways:

1. **Primary action:** It is the action of drug in unchanged (intact) form, e.g. aluminium hydroxide as a gastric antacid and magnesium sulphate as a purgative.
2. **Secondary action:** It is the action of a drug in changed form, i.e. after metabolic degradation, e.g. hexamine as an urinary antiseptic

Table 5.2: Types of drug action

Process	*Drugs*	*Site of action*
Stimulation	Adrenaline, isoprenaline, dopamine Pilocarpine and physostigmine	Cardiac muscle Exocrine glands
Depression	Barbiturates, alcohol, morphine and other opioids Quinidine and procainamide	CNS Cardiac muscle
Irritation	Purgatives (irritant)	GIT
Cytotoxic action	Antimicrobials Anticancer drugs	Parasitic cells Neoplastic (cancer) cells
Replacement Immunomodulation	Hormones Sera and vaccines (immunostimulants) Glucocorticosteroids, cyclosporine, azathioprine, cyclophosphamide (immunosuppressants)	Endocrine system Immunity system Immunity system

after being converted to formaldehyde, proguanil as an antimalarial after being converted to cycloguanil.

3. **Topical (local) action/antimicrobial action:** It is the action of a drug at the site of contact with the tissue to kill the microorganisms, e.g. silver sulphadiazine cream in skin burn, miconazole cream in fungal infection of skin and sulphacetamide drops in ocular infection.
4. **Systemic (remote) action:** It is action of a drug after absorption and distribution to the site of action, e.g. digoxin on heart, diazepam on CNS and ergometrine on uterus.
5. **Reflex action:** It is the action of a drug modified through reflex pathway, e.g. potassium iodide as reflex expectorant, ipecacuanha as reflex emetic and kalmegh (bitter) as reflex stomachic.
6. **Salt action:** It is the action of a drug produced by the physical property of the salt form of the drug, e.g. magnesium sulphate as osmotic purgative and mannitol as osmotic diuretic.
7. **Ionic action:** It is the action of a drug produced by the liberated ions (especially cations) from the drug, e.g. Ca^{2+} from calcium gluconate in tetany and Fe^{2+} from ferrous sulphate in iron deficiency anaemia.
8. **Chelating action:** It is the action of the drug produced by formation of chelate complex (ring-like structure) with metallic ions, e.g. dimercaprol in arsenic poisoning and penicillamine in copper poisoning.

Structure-Activity Relationship (SAR) and Drug Design

Both the affinity of a drug and its intrinsic activity (efficacy) are determined by its chemical structure. This relationship is called structure-activity relationship. It was first proposed by Crum Brown and TS Frazer in 1968: Change of chemical structure of the drug produces following changes in drug responses (effects):

1. Relatively minor modification in the drug molecule may result in major changes in pharmacological properties of the drug, e.g. procaine (aLA) to procainamide (a potent cardiac antiarrhythmic drug with prolonged duration of action), atropine (an anticholinergic drug) to homatropine (a mydriatic and cycloplegic with short duration of action on eye) and chlorthiazide (a diuretic) to polythiazide (a more potent diuretic).
2. Chemical modification of the structure of a drug has lead to development of congeners with less side effects and toxicity, e.g. nicotinic acid (vitamin B_3) to nicotinamide

(vitamin B_4), which does not produce flushing or itching, chlorpromazine (a tranquilizer) to trioflupromazine (a more potent tranquilizer with negligible antihistaminic and hypotensive actions) and testosterone (an androgen) to nandrolone (an anabolic steriod with negligible androgenic action).

3. Chemical modification of structure of a drug also has lead to development of competitive antagonists, e.g. morphine to nalorphine, naloxone or naltrexone, para-aminobenzoic acid (PABA) to para-aminobenzene sulphanilamide (sulphonamide) or para-aminobenzene salicylic acid (PAS).
4. The study of SAR of series of agonists and antagonists has enabled the identification of receptors and their subtypes for various neurotransmitters (ligands) and synthesis of specific drugs for each receptor subtypes, e.g. acetylcholine acts on muscarinic (M_1 to M_5) and nicotinic (N_N and N_M) receptors. Atropine, pirenzepine, etc. are muscarinic antagonists, while hexamethonium, d-tubocurarine, etc. are nicotinic antagonists. Adrenaline acts on α (α_1 and α_2) and β (β_1, β_2 and β_3) receptors.

   Phenoxybenzamine, prazosin, etc. are α-receptor antagonists, while propranolol, atonolol, etc. are β-receptor antagonists.

Stereoselectivity of Drugs

Drug molecules are not flat structures as depicted on paper, but they have three dimensional configuration. This special orientation is important for drug receptor interaction. Only a limited portion of a drug molecule interacts with the receptor depending on electrostatic binding sites. For this reason, sometimes diverse chemical substances can react with the same receptor, e.g. diethyl stilbestrol and oestradiol on the oestrogen receptor.

Many drugs have one or more asymmetric centres in their structures. These drugs exit in two nonidentical mirror image forms, viz. d-form (dextroform) and l-form (levoform), which can exhibit different biological activities, e.g. d-amphetamine is more active than l-amphetamine and l-hyoscyamine is more active than d-hyoscyamine. Similarly, S (sensitive) isomer form of warfarin is more active than R (resistant) isomer form of warfarin. In all these cases chemical structure may be the same, but due to a change in 3-dimensional configuration, the biological activities are changed.

Regulation of Receptors

In a cell the total number of functionally active receptors may be high or low. When high, the state is called upregulation and when low, the state is called downregulation of receptors. Change in the receptor population is a homeostatic mechanism of the tissue to maintain a physiological state in tissue function. Drug responsiveness changes with increase or decrease in receptor population, due to increased synthesis or degradation of receptors respectively.

1. **Upregulation of receptors:** Prolonged administration of an antagonist, e.g. propranolol (a β-blocker) leads to formation of new receptors causing increased tissue sensitivity. This phenomenon leads to hyperactivity (supersensitivity) of receptors to an agonist following sudden withdrawal of the antagonist after prolonged treatment, e.g. rebound hypertension, appearance of angina pectoris or cardiac arrhythmias following sudden withdrawal of propranolol or other β-blockers. Other drugs like clonidine (a central α_2-agonist), glucocorticoids and opioids can produce upregulation of receptors after prolonged administration and their sudden withdrawal leads to dangerous withdrawal reactions. This will be discussed with the respective drugs.
2. **Downregulation of receptors:** Prolonged administration of an agonist (ligand) leads to decrease in number of receptors, causing decreased tissue sensitivity. This phenomenon leads to hypoactivity (hyposensitivity) of receptors to an agonist after prolonged treatment. Ligand binding of receptors

induces accelerated endocytosis (internalization) of receptors, followed by degradation of those receptors. When this process occurs at a rate faster than denovosynthesis of receptors, then the total number of cell surface receptors is decreased, causing diminished responsiveness of the tissue. This produces tachyphylaxis or tolerance due to diminished drug effects after continued use of drugs like coronary dilators (e.g. nitrites and nitrates), bronchodilators (e.g. ephedrine, theophylline and salbutamol) or centrally active drugs (e.g. cocaine, morphine, phenobarbitone, etc.).

Potency and Efficacy of Drugs

Potency (strength) of a drug is the concentration (amount) of the drug in relation to its therapeutic effect. It is generally used to compare two or more drugs having similar biological activity (effect). Drugs with low potency are used in higher doses and vice versa, e.g. anti-inflammatory potency of some NSAIDs like piroxicam (daily dose 20 mg), diclofenac (daily dose 100 mg) and ibuprofen (daily dose 200 mg). So they have high, moderate and low potencies respectively.

Efficacy (therapeutic effectiveness) of a drug depends not only on its potency but also the type of response (maximum, moderate or poor/low) produced by the drug, e.g. full agonists produce maximum response and partial agonists produce moderate or low response.

Factors Modifying Drug Action and Therapeutic Outcome

These can be divided into two groups, *viz.* subject related factors and drug related factors.

Subject (Patient) Related Factors

These are given as follows:

1. **Age:** At extremes of age (infants and old people), there is increased sensitivity to the drug than in the adults. Infants are more sensitive to some drugs, e.g. chloramphenicol, gentamicin, morphine or other narcotics and thyroxine or other hormones. It is due to immaturity of drug metabolizing enzyme system, lower plasma protein binding of the drug, incomplete development of excretory system and smaller tissue mass, e.g. gray baby syndrome occurring in neonates after administration of chloramphenicol in large dosage. Thus infants require smaller amount of drugs than children. Similarly, old people require less amount of drugs than adult due to inability to metabolize the drug properly and degenerative changes in brain, liver, kidney and other organs of the body.
2. **Body weight/surface area:** The concentration of a drug at the site of action depends on the ratio between the body weight or surface area and the amount of the drug administered. Thus, the dose of a drug should be suitably adjusted for abnormally lean or obese persons and for those who are markedly dehydrated or oedematous (i.e. underweight or overweight persons).
3. **Sex:** The metabolism of a drug is slow in female due to more adipose tissues. Drugs that cause pelvic congestion, e.g. irritant purgatives like castor oil, senna, etc. should be avoided during menstruation and pregnancy. Drugs like antithyroids (e.g. carbimazole, propyl thiouracil, etc.), antimetabolites (e.g. methotrexate, mercaptopurine, etc.) and CNS depressants (e.g. phenobarbitone, morphine, etc.), should be avoided during pregnancy as they can effect the foetus.
4. **Race/species:** Indians tolerate thiacetazone (an antitubercular drug) more than Europeans. Japanese people suffer from subacute myelo-optic neuropathy (SMON) by taking diiodohydroxyquin and iodochlorohydroxyquin, but not Indian people. Negroes require higher concentrations of atropine and ephedrine to dilate pupil than Mongolians (Caucasians). These variations are due to different race. Similarly, animals like rabbits are more sensitive to d-tubocurarine than cats due to different species. Rats and mice are resistant to digoxin, but not dogs and cats.

5. **Genetic variations (genetic polymorphism):** There is interindividual variation in the rate of drug metabolism and the effect and toxicity of some drugs, e.g. propranolol, isoniazid, primaquine, phenobarbitone, halothane, succinylcholine, etc. This is mainly due to different rates of drug metabolism, as the amount of hepatic microsomal enzymes is genetically controlled. There is also differences in the target tissue/organ sensitivity. This will be discussed with the respective drugs. Pharmacogenetics deals with genetically mediated variation in drug action.
6. **Psychological/emotional state:** It can affect drug effects specially in CNS acting drugs, e.g. more general anaesthetics are required in nervous and anxious persons, higher doses of chlorpromazine (500 to 1000 mg/day) is required to produce tranquillization in schizophrenic persons than in normal individuals. Placebos (inert dosage form to please some persons) sometime produce therapeutic benefits in patients of psychosomatic disorders like angina pectoris and bronchial asthma, who are placebos reactors (easily respond to placebos).
7. **Physiological state:** Effects of some drugs vary with the physiological state, e.g. salicylates reduce body temperature only in presence of fever, uterus is more sensitive to the effect of oxytocin during pregnancy, irritant purgatives like castor oil and senna should be avoided during pregnancy as they can cause abortion. Drugs which are secreted in milk, e.g. tetracyclines should be used with caution in lactating mothers as they may affect infants. Children require smaller dosage than the adult due to many differences in the physiological functions (especially in pharmacokinetics) between the child and the adult.
8. **Pathological state:** Presence of diseases alter the effects of drugs, e.g. thiazides induce more marked diuresis in oedematous patients than in normal persons, adrenaline and digoxin induce more cardiac arrhythmias in patients of myocardial infarction, hypothyroid patients are more sensitive to the effects of digoxin, morphine and CNS depressant drugs, myasthenic patients are more sensitive to d-tubocurarine and other curaremimetic drugs. Hypnotics given in patients with severe pain may cause mental confusion and delirium. Presence of hepatic and renal diseases impair metabolism and excretion of many drugs leading to toxicity.

Drug Related Factors

These are given as follows:

1. **Dose of a drug (drug dosage):** It is the appropriate amount of a drug needed to produce a certain degree of response in a patient. It is expressed in terms of weight (g, mg, μg), volume (ml) or unit (IU). It is given in range because of individual variation. It can be prophylactic dose, therapeutic dose or toxic dose depending on the concentration of the drug. If the dose is too small, there will be no effect and if too large, toxic effects will be produced. There are some formulas for calculation of child dose (1–12 years).
   a. *According to age—*
      Young's formula:

$$\text{Child dose} = \frac{\text{Age}}{\text{Age} + 12} \times \text{Adult dose}$$

      Dilling's formula:

$$\text{Child dose} = \frac{\text{Age}}{20} \times \text{Adult dose}$$

   b. According to body weight (BW)— Clark's formula:

$$\text{Child dose} = \frac{\text{Body weight (kg)}}{70} \times \text{Adult dose}$$

   c. According to body surface area (BSA) — Duboi's formula:

$$\text{Child dose} = \frac{\text{BSA } (m^2)}{1.7} \times \text{Adult dose}$$

      Body surface area (m^2) = Body weight (kg) × Height (cm) × 0.008

Last two formulas are more accurate for calculating paediatric dose, because total body weight, extracellular fluid volume and metabolic activity are considered here.

Types of dosage of drugs

a. ***Standard dose:*** It is the dose which is given in most paetients due to wide margin of safety of the drug, e.g. penicillin, albendazole, etc.

b. ***Loading dose:*** It is the dose given at the onset of therapy with the aim of achieving the target concentration of the drug rapidly. It is required during the use of some drugs, e.g. digoxin, digitoxin, chloroquine, thiouracils, etc.

c. ***Maintenance dose:*** It is the dose given to maintain steady state concentration of drug in plasma within a given therapeutic range. It is required during the use of above drugs after giving in loading dose.

d. ***Target level dose:*** It is the dose required to produce target steady state concentration of the drug. It is adjusted by monitoring of plasma concentration of the drug. It is required during the use of antiepileptic drugs, antidepressants, lithium, digoxin, etc.

e. ***Titrated dose:*** It is the optimal dose required to produce maximum therapeutic effect with tolerable adverse effects. It is calculated by giving high initial dose and downward titration. (in critical situations) or low initial dose and upward titration (in most non-critical situations). Optimal dose is arrived at by titrating it with an acceptable level of adverse effects. It is required during the use of anticancer drugs, corticosteroids, levodopa, etc.

f. ***Regulated dose:*** It is the dose which is accurately adjusted by repeated measurement of the affected physiological parameter. It is required during use of antihypertensives, anticoagulants, hypoglycaemics, diuretics, etc.

2. **Form of a drug**: When a drug is administered in the form of injection, it is rapidly absorbed and produces rapid effect. Among the oral preparations, mixtures and powders are more rapidly absorbed than tablets and capsules and so produce rapid effects.

3. **Route of administration of a drug:** It can alter the effects of a drug, e.g. magnesium sulphate when given orally acts as a purgative, when given rectally lowers intracranial tension and when given parenterally (IV) produces CNS and cardiac depressant effects. N-acetylcysteine when given by inhalation is a mucolytic agent and when given parenterally (IV) is useful in paracetamol poisoning. Drugs like insulin and adrenaline are not effective by oral route and so administered parenterally (SC or IM) to produce effects.

4. **Time of administration of a drug:** It can alter the effect of a drug, e.g. hypnotics administered at night produce better effects than when taken during daytime, corticosteroids administered as a single morning dose cause less pituitary adrenal suppression than when taken in divided doses in the whole day.

5. **Fixed dose drug combination:** Combined use of two or more drugs in fixed dose produces **synergism** (Greek: *syn* meaning together and *ergon* meaning work). It means synergistic effect (similar effect) of two or more drugs. It can be—

   Additive effect: Here, the total effect of two or more drugs is equal to the sum of the effect of the individual drug, e.g. combination of antacids, analgesics, antihypertensives, diuretics, bronchodilators or chemotherapeutic agents.

   Supra-additive effect (Potentiation): Here, the total effect of two or more drugs is more than the sum of the effect of the individual drug, e.g. combination of levodopa and carbidopa, sulphamethoxazole and trime-

thoprim, acetylcholine and physostigmine or penicillin and probenecid. This effect occurs when one drug increases the action of another drug either by acting in different mechanism or delaying metabolism or excretion of the other drug.

Antagonism (Infra-additive effect): Here one drug decreases or inhibits the effect of another drug by opposite action, i.e. the two drugs have action in opposite direction. It can be:

a. **Physical antagonism:** It is due to physical properties of drugs, e.g. activated charcoal in alkaloidal poisoning (charcoal adsorbs alkaloid and prevent their absorption from the GIT).
b. **Chemical antagonism:** It is due to chemical interaction in solution between two drugs, e.g. acid (HCl) and alkalies ($NaHCO_3$), chelating agents (BAL) and heavy metal (arsenic), etc.
c. **Physiological antagonism:** It is due to opposite effects of two drugs on the same physical function, e.g. histamine and adrenaline on B.P. or bronchial muscle, insulin and glucagon on blood sugar level, hydrochlorothiazide and triamterene on urinary K^+ excretion, etc.
d. **Pharmacological antagonism:** It is due to opposite effects of two drugs binding to the same receptor. It can be competitive antagonism or noncompetitive antagonism.

Competitive antagonism is usually reversible and can be overcome (surmountable/equilibrium type) by increasing the dose of agonist, e.g. acetylcholine (agonist) and atropine d-tubocurarine (antagonist), adrenaline (agonist) and tolazoine/propranolol (antagonist), histamine (agonist) and mepyramine/cimetidine (antagonist), morphine (agonist) and nalorphine/naloxone (antagonist), diazepam (agonist) and flumazenil (antagonist), etc. It can be irreversible, if the binding occurs by covalent bond, e.g. adrenaline (agonist) and phenoxy benzamine (antagonist), acetylcholine (agonist) and decamethonium (antagonist). Noncompetitive antagonism is irreversible and cannot be overcome (unsurmountable/nonequilibrium type) by increasing the dose of the agonist, e.g. acetylcholine (agonist) and decamethonium/papverine/α-bungarotoxin (antagonist), diazepam (agonist) and bicuculine (antagonist), etc. Differences between competitive antagonism and noncompetitive antagonism is shown in **Table 5.3**.

6. **Drug cumulation (cumulative action):** Repeated administration of some drugs lead to accumulation of the drug in the body tissues, if the rate of administration of the drug is more than the rate of elimination. This produces cumulative toxicity, e.g. digoxin, digitoxin, emetine, chloro-

Table 5.3: Comparison between competitive and noncompetitive antagonisms

Parameter	*Competitive antagonism*	*Noncompetitive antagonism*
1. Receptor binding	Reversible (by hydrogen bond or van der Waals bond)	Irreversible (by covalent bond)
2. Agonist-antagonist interaction	Competitive	Noncompetitive
3. Dose response curves of agonist in presence of antagonist	Parallel shifting to the right side without change in slope	Not so
4. Antagonism	Surmountable (maximum response is produced by increasing the dose)	Unsurmountable (maximum response is diminished inspite of increasing the dose)
5. Duration of action	Short (depends on drug clearance)	Long (depends on new receptors synthesis)

quine, arsenic and other heavy metals, etc. To avoid cumulation, these drugs should be administrated at a maintenance dose and checking of hepatic and renal functions must be done before and during drug administration as hepatic and renal diseases delay metabolism and excretion of these drugs.

7. **Drug tolerance:** It is the gradual decrease in responsiveness of tissue (*in vivo*) to a drug on repeated administration. It may be acute tolerance (tachyphylaxis), which develops within a few minutes or hours and chronic tolerance, which develops within a few days or weeks. Tachyphylaxis can be demonstrated in experimental animals, e.g. ephedrine on B.P. of cat or dog (due to depletion of catecholamine stores in adrenergic neurons).

Types of Tolerance

These are of following types:

1. **Pharmacokinetic (drug dispositional tolerance):** This is due to changes in drug absorption, distribution, metabolism and excretion leading to decreased availability of the drug at the target tissue, e.g. barbiturates after repeated administration increase their own metabolism by stimulating the microsomal enzyme systems in the liver (enzyme induction). Decreased rate of absorption of drugs occurs in diarrhoea (apparent or pseudotolerance) leading to decreased effects of drugs.
2. **Pharmacodynamic (functional) tolerance:** This is due to changes in the functions of the larger tissue, which make them less sensitive to the drug. It is often associated with some cellular or tissue changes. With some drugs this may occur due to decrease in drug-receptors (downregulation of receptors), e.g. CNS depressants like morphine, barbiturates, alcohol, etc.
3. **Pharmacogenetic tolerance:** This is due to genetic (inherited) factors like enzyme variation in the body leading to decreased metabolism of the drug, e.g. isoniazid, halothane, succinylcholine, etc.
4. **Racial/species tolerance:** Some human races and animal species are tolerant to some drugs due to racial/species variation, e.g. Negroes are tolerant to ephedrine, atropine and other mydriatics. Rabbits can tolerate large doses of atropine due to presence of the enzyme atropine esterase in their liver and plasma.
5. **Cross-tolerance:** If a person initially develops tolerance to a drug belonging to a particular group, he also develops tolerance to other drugs belonging to the same group due to similar chemical structure. This is called cross-tolerance, e.g. between alcohol and general anaesthetics, between glyceryl trinitrate and isosorbide dinitrate, between morphine and pethedine, etc.
6. **Drug resistance** is the tolerance of microorganisms (bacteria) to some antibiotics, e.g. staphylococci may develop resistance to penicillins and tetracyclines.
7. **Drug dependence:** It is the compulsive use of some drugs. It arises due to periodic, repeated or continuous administration of some drugs like opioids, barbiturates, cocaine, alchohol, amphetamines, caffeine, nicotine, canabis indica, LSD, etc. that produce harm to the individual and to the society. It is characterized by tolerance, psychic dependence and physical dependence. Tolerance is the reduced effect of the drug due to repeated use and is due to cellular or tissue adaptation of central neurons. Psychic (psychological) dependence is a condition in which a drug produces a feeling of satisfaction and pleasure (psychic drive) by periodic or continuous administration of the drug, e.g. caffeine, amphetamines, nicotine, canabis indica, LSD, etc. produce habituation (psychic dependence). Physical (physiological) dependence is a condition in which the body (especially CNS) achieves an adaptive state that manifests itself by intense physical disturbances when the drug is suddenly withdrawn (withdrawal or abstinence syndrome). Previously the term

addiction was used to denote both psychic and physical dependence on drugs. It is now called drug abuse (detrimental use of drugs). It is characterized by an overpowering desire (intense craving) to continue use of the drug, a tendency to increase the dose of the drug and occurrence of severe withdrawal symptoms, which are not only debilitating but may sometimes prove fatal, e.g. opioids, barbiturates, alcohol, cocaine, etc. produce addiction (drug abuse).

Outline of a treatment of drug dependence (addiction):

1. Hospitalization of the patient.
2. Gradual withdrawal of the drug.
3. Substitution therapy, e.g. methadone for morphine.
4. Specific drug therapy, e.g. antabuse for alcohol.
5. Correction of nutritional deficiencies.
6. Psychotherapy and occupational therapy.
7. Rehabilitation of the patient.

6 Quantitative Aspects of Pharmacodynamics and Assay of Drugs

PHARMACODYNAMIC PARAMETERS

These are given as follows:

1. Dose response relationship of agonists.
2. Antagonism of agonist response by antagonists.
3. Therapeutic index and protective index.
4. Therapeutic efficacy and therapeutic window.

As there are wide quantitative variations in drug responses in different species and in the same species under different conditions, so different methods have been devised to study the phenomenon of biological variation in drug response and to minimize the errors of prediction in clinical use of drugs. These are useful in study of drug effects in animals and men.

Dose Response Relationship of Agonists

Two types of dose response relationship are commonly seen:

a. **Graded dose response relationship:** In it, as the dose of a drug is increased, the response (effect) of the tissue or organ is also increased. The individual units of the responding system are capable of producing progressively increasing responses with increase of the dose of a particular drug. The increase in response can be measured. With increase of dose, at first there is considerable increase in the response and there are smaller increments as the response approaches the maximum limit. After the maximum response has been reached, no further increase in the response can be obtained with further increase of dose (as shown in **Fig. 6.1a).** This phenomenon is called **ceiling response** (effect).

If the graded responses are plotted in a graph paper against the doses in arithmetic units, then a hyperbolic dose response curve is obtained (as shown in **Fig. 6.1b**). When the graded responses are plotted in a graph paper against the doses in logarithmic units, then a sigmoid (s-shaped) dose response curve is obtained with a straight line (linear shaped) in the middle (as shown in **Fig. 6.1c).**

The log dose response curve is particularly useful for the comparison of potencies of different compounds as in bioassays. The linear relationship in the middle has the advantage that a given increase in the log dose always corresponds to the same increase in the response.

b. **Quantal or all-or-none dose response relationship:** Here responses follow all-or-none phenomenon, i.e. the individual of the responding system either respond to their maximum limit or not at all to a dose of a drug and there is no gradation of response. This type of relationship is observed when the presence or absence of some drug induced phenomena such as

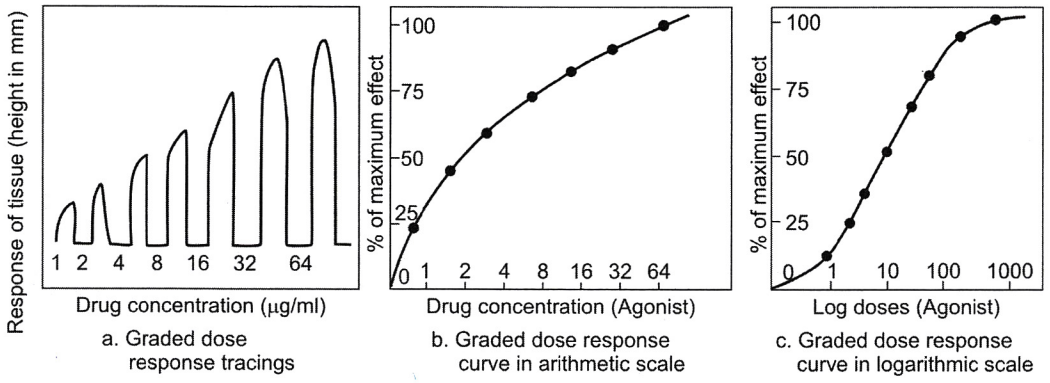


Fig. 6.1

convulsion, death, etc. is determined in a population of animals as in toxicity studies. Different doses of the drugs are used in different groups of animals and the percentage of positive responses (data) are recorded. Here also the log dose response curve is sigmoidal (s-shaped) with a straight line (linear shaped) in the middle. The Gaussian or the normal nature of the quantal log dose response curve is usually bell-shaped or symmetrical (as shown in **Fig. 6.2**). This suggests that the observed differences are due to polygenic random variations in the responsiveness of the animals as well as non-random but intercoupled events like other actions of the drug.

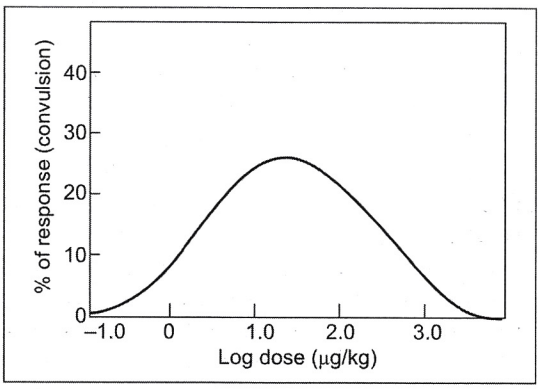


Fig. 6.2: Bimodal quantal dose response curve

Antagonism of Agonist Response by Antagonists

An agonist is a drug that has both affinity for the receptor and efficacy. The result of the agonist-receptor interaction is a drug response (effect). Antagonists are drugs that have affinity for the receptor but has no efficacy (i.e. incapable of producing biological response). Antagonists can interact directly at the same receptor site as the agonist or affect other reactions necessary for the drug response. There are two main types of pharmacological antagonism.

a. **Competitive antagonism:** If the inhibitory action of an antagonist can be overcome by increasing the concentration of the agonist, thereby achieving the same maximum response, the inhibition is called **competitive antagonism.** It can reversively bind to the same receptor site as the agonist (as shown in **Fig. 6.3**), e.g. acetylcholine and atropine, histamine and chlorpheniramine, etc. or irreversively bind to a remote receptor site that influences the affinity of the agonist for its receptor, e.g. acetylcholine and decamethonium, adrenaline and phenoxybenzamine, etc.

A competitive antagonist shifts the dose-response curve to the right but does not reduce the slope of the curve and the maximum response of the agonist (as shown in **Fig. 6.4a**).

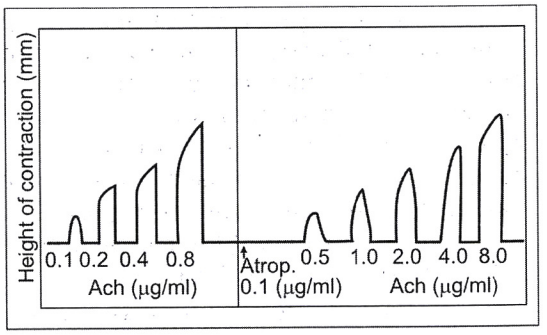


Fig. 6.3: Tracings showing competitive antagonism of acetylcholine by atropine

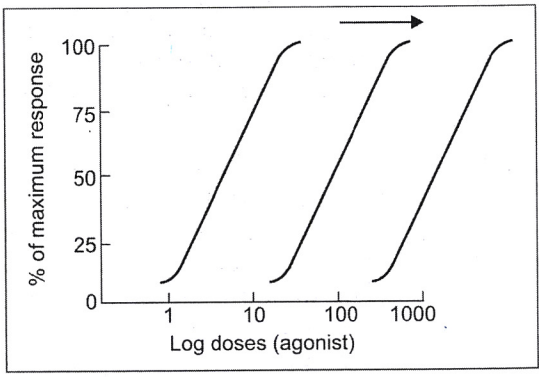


Fig. 6.4a: Curves showing competitive antagonism

b. Noncompetitive antagonism: If the inhibitory action of an antagonist cannot be overcome by increasing the concentration of the agonist, thereby not achieving the maximum response, the inhibition is called noncompetitive antagonism. A noncompetitive antagonist decreases the capacity of the agonist to combine with its receptor. This can occur by binding of the noncompetitive antagonist to either the agonist receptor site or another site that influences the capacity of the agonist that combines to its receptor, e.g. acetylcholine (agonist) and papaverine (antagonist); acetylcholine (agonist) and α-bungarotoxin (antagonist) and diazepam (agonist) and bicuculine (antagonist).

A noncompetitive antagonist does not shift the dose response curve to the right but reduces the slope of the curve and diminishes the maximum response of agonist (as shown in **Fig. 6.4b**).

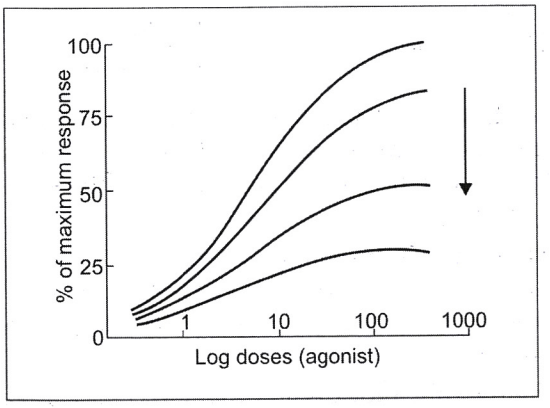


Fig. 6.4b: Curves showing noncompetitive antagonism

c. Partial agonists: Partial agonists are compounds with affinity for the receptors but with low or moderate efficacy. A partial agonist with high affinity can competitively inhibit the action of full agonist. Partial agonists thus have both agonist and antagonist properties, e.g. nalorphine, pentazocine, propiram, profadol, etc. of morphine.

A partial agonist produces characteristic dose response curve by increasing of dose (as shown in **Fig. 6.5a**).

Therapeutic Index (TI)

It is the ratio of the median lethal dose (MLD or LD_{50}) to the effective dose (MED or ED_{50})

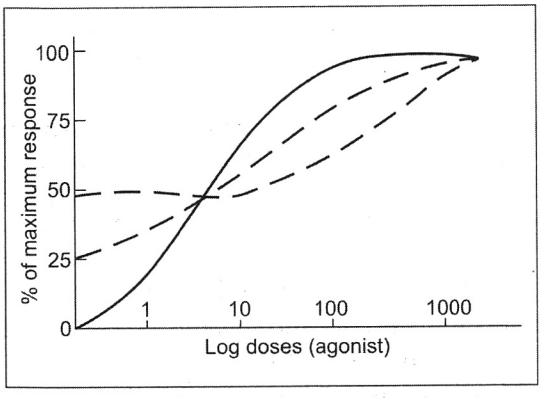


Fig. 6.5a: Curves showing effects of partial antagonism

$$\text{Therapeutic index (TI)} = \frac{LD_{50}\ (MLD)}{ED_{50}\ (MED)}$$

Median lethal dose (MLD or LD_{50}) is the dose (mg/kg), which is expected to kill one half (50%) of an unlimited population of the same species.

Median effective dose (MED or ED_{50}) is the dose (mg/kg), which produces a desired response in one half (50%) of the test population.

Therapeutic index is a number and indicative of margin of safety in animals studied. As the drug metabolism varies from species to species, so the therapeutic index would also vary in a similar fashion. It supplies reliable information when both LD_{50} and ED_{50} are determined for the same strain belonging to the same species. It is not a useful guide for the safety of a drug in humans (in clinical use), because LD_{50} is not a good guide to toxicity in the therapeutic setting and ED_{50} is often not definable, since it depends on what measure of effectiveness is used. Therapeutic index in humans can be calculated by the formula:

$$TI = \frac{\text{Maximum nontoxic dose}}{\text{Median effective dose}}$$

The greater the therapeutic index, the safer is the drug. For safe therapeutic use of a drug, its therapeutic index must be more than one, e.g. penicillin has a high therapeutic index, while digoxin has a low therapeutic index. In clinical practice, toxic symptom is more relevent than lethality. A drug may have more than one ED_{50} depending upon the measure of effectiveness, e.g. ED_{50} of aspirin for headache is much lower than ED_{50} of aspirin for anti-inflammatory action in rheumatic fever. Thus a drug may have many therapeutic indices depending upon its clinical use.

Two parameters are needed to calculate safety margin of a drug in humans, *viz.*

1. Effective dose for the specific effect in most of the humans, i.e. ED_{max}.
2. Maximum tolerated dose, which does not produce any adverse reactions, i.e. TD_0.

Physicians always prefer to use a drug which has high margin of safety for minor ailments, but in emergencies, where a drug is used for short-term or for some limited disease (e.g. CCF), drugs with low therapeutic index are also used. Care of the patient must be taken while using drugs of low margin of safety. Other measures of safety of drugs in clinical use include:

a. **Protective index (PI):** It is the ratio of ED_{50} of neurological impairment to ED_{50} of seizure (convulsion) protection of a drug in humans. A drug with a protective index of 5 (e.g. diazepam) is more promising anticonvulsant than a drug of protective index of 3 (e.g. phenobarbitone).

b. **Risk-benefit index:** It is the estimation of proportions of patients showing beneficial or harmful reactions, i.e. the number of patients who need to be treated (NNT) in order for one to show the given effect, whether beneficial or adverse, e.g. in a study of pain relief by antidepressant drugs compared with placebo, the findings were for benefit (relief of pain) NNT = 3, for minor unwanted effect NNT = 3 and for major adverse effects NNT = 22. Thus, if 100 patients treated, an average of 33 patients showed benefit from the drug, 33 patients showed minor unwanted effects and 4 or 5 patients showed major adverse effects.

Therapeutic Efficacy (Effectiveness)

It is the maximum effect that can be produced by a drug in humans, e.g. sodium depleting activity of potassium sparing diuretics (up to 5%), thiazide diuretics (up to 10%) and high ceiling diuretics (up to 20%) indicates that the therapeutic efficacy of these drugs are low, moderate and high respectively.

Therapeutic Window

It is the range of effective and safe concentrations of a drug in plasma. It is an unusual feature seen in certain drugs, e.g. tricyclic antidepressants (imipramine, amit-

riptyline, etc.) produce optimum therapeutic effects, when their plasma concentrations are maintained between 50–200 ng/ml. Clonidine produces lowering of BP when its plasma concentrations are maintained between 0.2–2 ng/ml (above which BP will rise). Therapeutic window may be narrow or wide. Drugs like digoxin, lignocaine, phenytoin ethosuximide, theophylline, imipramine, lithium and aminoglycosides (e.g. gentamycin) have narrow therapeutic window. So slight overdose of these drugs can produce toxicity. To avoid toxicity, frequent measurement of plasma or serum concentrations of these drugs is essential.

ASSAY OF DRUGS

It is the estimation of concentration or potency of drugs by chemical or biological methods. Chemical assay methods are commonly used because of convenience and reproducibility. But in some cases they are not suitable and unreliable due to presence of closely related inert chemicals. Biological assay (bioassay) methods (by measuring of biological responses produced by drugs) are highly sensitive and selective. They are used for following purposes:

1. To measure the pharmacological activity of new or chemically undefined substances.
2. To investigate the function of endogenous mediators, e.g. neurotransmitters.
3. To measure unwanted effects and toxicity of drugs.

Bioassay is essential in the development of new drugs. The activity of a new compound must be compared in various test systems with that of standard (known) compounds. The choice of suitable test systems for the preliminary bioassay is important. The test must be simple and quick. They must also be as specific as possible for the type of biological activity that is being sought.

General Principles of Bioassays

These are given as follows:

1. **Use of standards:** Bioassays are designed to measure the relative potency of the standard and the unknown (test) preparations. The standard is usually a pure substance. International standards have been developed for all drugs which are biologically standardized and their potency are described. When it is not possible to estimate accurately the exact amount of the active principle, then unit system is adopted to signify potency. One unit is a measure of fixed amount of particular biological activity, e.g. one IU of insulin is the biological activity in 0.5 mg of standard insulin preparation, one IU of heparin is the biological activity in 0.0077 mg of standard heparin sodium.
2. **Design of bioassays:** In any biological assay procedure, proper planning is absolutely necessary. The important points that should be considered are as follows:
   a. Selection of a correct reference standard preparation.
   b. Selection of a highly sensitive biological preparation (tissue or animal).
   c. A knowledge of variable factors. The response of the same organ or tissue to the same drug may vary widely due to biological variation depending upon the species, sex, age, weight, breeding, diet and environmental factors. Therefore, in the selection of animals, all the above factors must be born in mind. An ideal bioassay will be in the same animal or on the same tissue which has been treated with the standard drug as well as with the unknown drug, e.g. assay of acetylcholine on isolated frog's rectus muscle, assay of histamine on isolated guinea pig's ileum, assay of adrenaline on anaesthetized cat's BP, assay of 5-HT on isolated rat's uterus, etc.
3. **Graded or quantal responses:** An assay may be based on graded response (e.g. change of blood glucose concentration, contraction of a strip of smooth muscle, change in the time taken for a rat to run to a maze, etc.) or quantal (all-or-none) responses (e.g. death, loss of righting reflex, success in maze running within a stipulated time, etc.).

Types of Bioassays

These are of two types:

1. **Direct bioassay (matching/bracketing assay):** In it, by repeated trial and error, one dose each of standard (S) and unknown (U) samples of a drug are so identified that they produce the same response. It is difficult to determine and may give inaccurate result. It cannot be subjected to statistical analysis for estimation of margin of error, e.g. histamine bioassay on isolated guinea pig's ileum, posterior pituitary extract assay in isolated rat's uterus.
2. **Indirect bioassays:** In these bioassays, comparisons are made between standard (S) and unknown (U) samples of drug by two dose response curves (parallel line assay). The procedure is based on the following basic principles of dose response relationship.
   a. Log dose response curve in each case is linear in the middle portion (25–75% of maximum response).
   b. Log dose response curves of the standard and unknown drug having the same active principle are parallel. These are mainly of two types:
      - **4-point assay** (as shown in **Fig. 6.5b):** It is an accurate bioassay system, but it is time consuming. In it, 2 doses (low and high of standard (S) and 2 doses (low and high) of unknown (U) samples of a drug are so selected that the ratio of doses are same, i.e. S_1: S_2 = U_1: U_2 and the responses produced by these 4 doses produce fall within the middle portion of the log dose response curves.

        The sequence of 4 doses (S_1, S_2, U_1 and U_2) is usually given as Latin square design ($S_1S_2U_1U_2$, $S_2U_1U_2S_1$, $U_1U_2S_1S_2$ and $U_2S_1S_2U_1$). 16 responses (4 of each) are recorded and the data is analyzed statistically. If the log dose response curve of standard and unknown drugs are not parallel, then the responses are not produced by the same drug.

        From ED_{50} ratio of unknown and standard, the potency of unknown sample of drug is determined, i.e.

$$\text{Potency of U} = \frac{ED_{50}U}{ED_{50}S}$$

        The potency of unknown sample of drug is determined from the log dose response curve of the standard. It can also be calculated by the formula:

$$M = \frac{(S_1 - U_1) + (S_2 - U_2)}{(S_1 + U_1) - (S_2 + U_2)} \times d$$

        where "d" is the log of ratio of high to low dose, i.e. log 2 = 0.301.

        Antilog of "M" is the potency of standard sample to unknown sample of the drug.

      - **3-point assay** (as shown in **Fig. 6.5c):** It is a quicker system than 4-point assay. It is often used when the unknown sample is very small and repeated doses cannot be given. In it, 2 doses (low and high) of standard (S) sample of a drug are so selected that the responses fall within the middle portion of the dose response curve. Then the dose of unknown (U) sample of the drug is determined whose response fall within the range of responses produced by two doses of standard sample of the drug. The concentration of unknown sample of drug is deter-

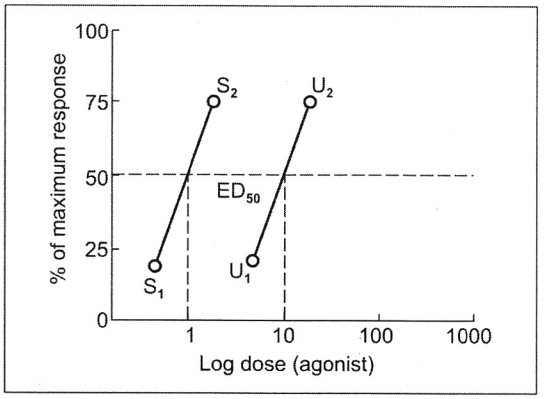


Fig. 6.5b: 4-point assay

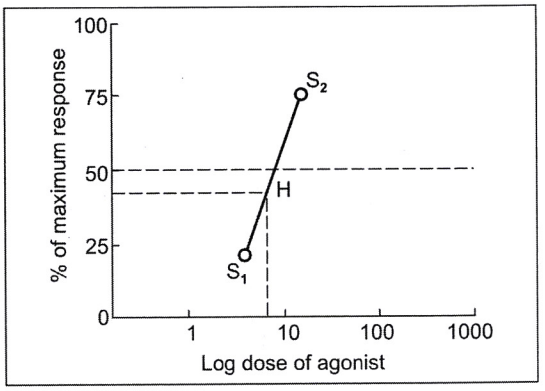


Fig. 6.5c: 3-point assay

mined from the log dose response curve of the standard.

Bioassays in man: These are done when animal tests fail to predict human responses, especially when the responses are subjective in nature and not measurable in animals. These can be done both in normal subjects and in diseased persons. These are most rational and realistic and include clinical trial and sequential trial. Clinical trial is a special type of bioassay in man to compare objectively the results of two or more therapeutic procedures. To minimize bias in clinical trial, randomization and double bind technique are adopted. For new drugs, clinical trial is carried out during phase-II of clinical development. Sequential trial is done in cases of analgesics, cough suppressants, hypotensives, etc. where the subject (patient) and the drug to be used in the same subject are alloted sequentially. This is only possible if the results of treatment can be determined after short time and the test drug or placebo can be given as a crossover design. In it, total number of subjects needed is relatively small.

7 Drug Interactions

INTRODUCTION

Drug interaction is the alteration of the drug effect when two or more drugs are administered simultaneously in the body. It is also called drug-drug interaction. Drug-food interaction can occur during the use of some drugs with some foods, e.g. MAO inhibitors like phenelzine and iproniazid with cheese, meat, yeast, wine, beer and citrous fruits containing tyramine can produce hypertensive crisis (cheese reaction).

DRUG-DRUG INTERACTION

Interaction between two drugs may produce a desired (beneficial) effect or an undesired (harmful) effect. Examples of useful drug interactions are combination of antibiotics or antihypertensives. Examples of harmful drug interactions are many and are discussed below. Drug-drug interaction that occurs *in vitro* (outside the body) is called physicochemical interaction. Drugs may be inactivated or precipitated from solution if mixed in the same syringe or added to blood or infusion fluid prior to administration, e.g. succinylcholine with thiopentone sodium mixed in the same syringe produces complex formation. Protamine zinc insulin containing excess of protamine interacts with soluble insulin if mixed in the same syringe. Ampicillin, benzyl penicillin, heparin and aminophylline are unstable with the acidic pH of dextrose solution and should not be administered with it. Noradrenaline solution cannot be infused with normal saline and can be infused with dextrose solution as it is stable at acidic pH. When noradrenaline solution is to be infused with normal saline, vitamin C should be added to the reservoir to prevent oxidation of noradrenaline. Hydrocortisone hemisuccinate or gentamicin should not be administered with ampicillin, methicillin, carbenicillin or tetracycline in the same infusion bottle, as they loss potency. No drug can be administered with blood, plasma, sodium bicarbonate, lactate or mannitol solution in the same transfusion or infusion bottle.

Drug-drug interactions that occur *in vivo* (inside the body) are of two types: pharmacokinetic interaction and pharmacodynamic interaction.

Pharmacokinetic Interaction

It occurs by altering the concentration of one drug by the other in the tissue or tissue fluid. It may occur during absorption, distribution, biotransformation or excretion of drugs. It occurs by following mechanisms:

1. Interference with the gastrointestinal absorption of one drug by another, e.g. gastric antacids containing magnesium and calcium salts decrease the absorption of tetracyclines and iron salts. Prokinetic drugs (metoclopramide, domperidone,

etc.) decrease the absorption of anticholinergics, antihistaminics, opiates and phenothiazines by increasing gastric emptying time and GIT motility. Sucralfate decreases absorption of phenytoin and liquid paraffin decreases absorption of fat-soluble vitamins (A, D, E and K).

2. Displacement of plasma protein binding of one drug by another, e.g. aspirin phenylbutazone, oxyphenbutazone, indomethacin or clofibrate can displace warfarin sodium from the binding sites leading to severe bleeding episode. Salicylates, sulphonamides and oral anticoagulants (warfarin, dicumarol, etc.) can displace oral antidiabetics (tolbutamide, chlorpropamide, etc.) from the binding sites leading to hypoglycemic coma. Salicylates and sulphonamides (long-acting) can displace bile pigments from the binding protein, especially in neonates producing kernicterus (jaundice). Similarly, digoxin is diplaced by quinidine, verapamil, nifedipine or amiodarone; phenytoin is displaced by NSAIDs; methotrexate is displaced by salicylates or sulphonamides producing severe toxicity.
3. Inhibition of adrenergic neuronal uptake of one drug by another, e.g. imipramine and chlorpromazine inhibit the neuronal uptake of guanethidine and bethanidine and, therefore, interfere with the antihypertensive activity of the latter drugs.
4. Depletion of catecholamines from adrenergic neurons, e.g. reserpine depletes catecholamines from adrenergic neurons and decreases sympathetic activity of ephedrine or metaraminol.
5. Alteration of metabolism of one drug by another by microsomal enzyme induction or inhibition. Enzyme inducers, e.g. phenobarbitone, phenytoin, carbamazepine, rifampicin, griseofulvin, etc. increase metabolism of warfarin, dicoumarol, etc. and therefore, decrease anticoagulant effect of latter drugs. Rifampicin increases metabolism of oral contraceptive drugs leading to decreased contraceptive activity and failure of contraception. Similarly, carbamazepine decreases the effect of phony ton; pyridoxine decreases the effect of levodopa; phenobarbitone, phenytoin or rifampicin decreases the effect of warfarin, digoxin or prednisolone. Enzyme inhibitors, e.g. phenylbutazone, cimetidine, metronidazole, isoniazid, chloramphenicol, etc. decrease metabolism of warfarin, tolbutamide or phenytoin leading to increased toxicity. Similarly, cimetidine increases the toxicity of diazepam, morphine, theophylline or lignocaine. Allopurinol (xanthine oxidase inhibitor) increases the toxicity of mercaptopurine or azathioprine.
6. Alteration of renal excretion of one drug by another, e.g. probenecid decreases renal excretion of penicillins leading to prolonged duration of action. Similarly, aspirin decreases uricosuric effect of sulphinpyrazone, NSAIDs decrease excretion of lithium and, therefore, increase the toxicity.

Pharmacodynamic Interaction

It occurs by modification of the pharmacological effect of one drug by another without altering the concentration of the drug in the tissue or tissue fluid. It may occur at receptor or nonreceptor site.

Receptor Site

Drugs acting on the same receptor or at different active receptors may enhance or decrease the response by additive, synergistic or antagonistic effect, e.g. d-tubocurarine with aminoglycoside antibiotics (e.g. streptomycin) may increase the block at neuromuscular junction and can produce respiratory muscle paralysis. Combined administration of morphine and barbiturates (e.g. phenobarbitone sodium) can produce marked CNS depression by additive/synergistic action, d-tubocurarine and succinylcholine at neuromuscular junction and morphine and pentazocine (partial agonist) as analgesics (by blocking μ receptors) produce antagonistic (opposite) actions. Examples of pharmacodynamic interactions are many and are described in respective chapters.

Nonreceptor Site

Drugs may interact by changing fluid and electrolyte balance or due to opposite chemotherapeutic action, e.g. diuretics causing potassium depletion can increase toxicity of digoxin by producing hypokalemia. Again hypokalemia can antagonize the antiarrhythmic activity of phenytoin, lignocaine, quinidine or procainamide. Hyperkalemia produced by potassium sparing diuretics, captopril, enalapril and NSAIDs may decrease the clinical efficacy of digoxin. Combined administration of bactericidal antibiotics (e.g. penicillins) and bacteriostatic antibiotics (e.g. tetracyclines) decrease therapeutic effect of each other.

Importance of Knowledge of Drug Interaction

Drug interaction is a vital problem in clinical practice nowadays. A physician who uses multiple drugs in a prescription must be alert about the possibility of drug interaction. He must be aware of both risky drugs and susceptible patients to avoid drug interaction. Risky drugs are those drugs that affect the vital processes in the body (e.g. warfarin, morphine and chlorpromazine), that have saturable (zero order) kinetics (e.g. phenytoin, theophylline and aspirin), that have a steep dose response curve (e.g. verapamil, levodopa and chlorpropamide), that show dose dependent toxicity (e.g. digoxin, lithium, methotrexate and aminoglycoside antibiotics), where the patient depends on the prophylactic action (e.g. oral contraceptives and cyclosporin) and where the loss of effect leads to a breakthrough of disease (e.g. quinidine, antiepileptics and glucocorticosteriods). Susceptible patients are elderly patients, patients with unstable disease (e.g. epileptics, brittle-diabetics, dementia patients and patients with cardiac arrhythmias) and patients dependent upon drug treatment for survival (e.g. transplant recipients and patients with Addison's disease).

8 Adverse Drug Reactions

INTRODUCTION

All drugs whether used systemically or topically can produce adverse drug reactions ranging from mild inconvenience to serious toxicity or even death. The incidence of adverse drug reactions (ADRs) in hospital indoor patients is about 10%.

Types of Adverse Drug Reactions

These are of two types:

1. **Predictable (Type I)** reactions, e.g. side effects, secondary effects, drug withdrawal reactions and toxic effects. These are based on pharmacological properties of the drug. These are more common and dose dependent reactions.
2. **Unpredictable (Type II)** reactions, e.g. intolerance, idiosyncrasy and allergic (hypersensitivity) reactions. These are based on the peculiarity of the patient and not on drug's own action. These are less common and dose independent reactions. These are generally more serious and often require withdrawal of the drug. Some adverse reactions are avoidable, e.g. gastric irritation produced by some drugs (e.g. iron salts, theophylline, NSAIDs, etc.) can be avoided by giving the drug after meals. Other adverse reactions are unavoidable, which are due to:
   a. Pharmacological effects in therapeutic doses, e.g. dryness of mouth and skin with anticholinergics, palpitation and tachycardia with catecholamines, etc.
   b. Sequelae of pharmacological effects in therapeutic doses, e.g. postural hypotension with prazosin, hydralazine, trimethaphan, etc. throbbing headache and syncope produced by nitrites and nitrates.
   c. Low safety margin of some drugs, e.g. digoxin, lithium carbonate, anticancer drugs, etc.

Discussion of Individual Adverse Effects or Reactions of Drugs

1. **Side effects**: These are extension of pharmacological effects of drugs in therapeutic doses, i.e. due to excess of normal pharmacodynamic effects. These are predictable and dose related effects and usually trivial in nature. These constitute about 80% of all adverse reactions, e.g. dryness of mouth and skin by anticholinergic drugs (e.g. atropine), postural hypotension by some antihypertensive drugs (e.g. prazosin), hypokalemia by loop diuretics (e.g. frusemide) and thiazide diuretics (e.g. hydrochlorothiazide), constipation by morphine and verapamil, throbbing headache by nitrites and nitrates (e.g. GTN), sedation by antihistaminics (e.g. promethazine) and systemic acidosis by carbonic anhydrase inhibitors (e.g. acetazolamide).
2. **Secondary effects:** These are indirect consequences of primary drug action, e.g. superinfection and vitamin deficiency due to suppression of normal bacterial flora in

the gut by broad spectrum/extended spectrum antibiotics, e.g. tetracyclines, chloramphenicol, ampicillin, etc.; activation of latent tuberculosis by administration of corticosteroids due to weakening of host defence mechanism.

3. **Drug withdrawal reactions:** Sudden withdrawal of certain drugs may produce worsening of the clinical condition for which the drug was being used, e.g. acute adrenal insufficiency may be precipitated by sudden withdrawal of corticosteroid. Severe hypertension and sympathetic overactivity may occur after sudden withdrawal of clonidine. Worsening of angina pectoris or myocardial infarction may occur after sudden withdrawal of β-blockers (e.g. propranolol). Frequency of seizures may increase on sudden withdrawal of antiepileptic drugs (e.g. phenytoin). These manifestations are also due to adaptive changes and can be minimized by gradual withdrawal of the drugs.
4. **Toxic effects (toxicity):** These are due to overdose of drugs producing excessive pharmacological effects (acute toxicity) or prolonged repeated use of drugs producing organ/tissue toxicity (chronic toxicity), e.g. coma produced by barbiturates and alcohol, complete heart (AV) block by digoxin, bleeding by heparin, respiratory depression by morphine and myocardial damage by emetine are due to overdose of the respective drug.

   Ototoxicity and nephrotoxicity produced by aminoglycoside antibiotics (e.g. streptomycin) and high ceiling diuretics (e.g. ethacrynic acid), oculotoxicity by chloroquine, ethambutol, methanol, etc. and neurotoxicity by isoniazid, hydralazine, vincristine, etc. are due to prolonged use of the respective drug. These are predictable and dose related effects and usually serious in nature, demanding prompt treatment.
5. **Intolerance:** It is the appearance of characteristic adverse reaction of a drug in an individual at therapeutic dose. It is due to low threshold to pharmacological action of a drug, e.g. a single dose of chloroquine may produce vomiting and abdominal pain in some patients. A single dose of trifluprομazine may produce muscular dystonias in some patients. A few doses of phenobarbitone may cause excitement and mental confusion in some patients.
6. **Idiosyncrasy:** It is a genetically determined abnormal reaction to a chemical (drug). It is due to total absence or reduced activity of some enzymes in an individual, e.g. haemolytic anaemia produced by oxidizing agents like primaquine and sulphonamides in individuals due to deficiency of the enzyme glucose-6 phosphate dehydrogenase in RBCs (glutathione present in RBCs is a highly reducing agent. Lack of glutathione leads to brittleness of RBCs). Prolonged apnoea and respiratory paralysis produced by succinylcholine in individuals whose sera contain atypical cholinesterase. Acute porphyria is produced by phenobarbitone, alcohol or chloramphenicol in individuals due to induction of the enzyme delta-amino laevulinic acid (ALA) synthetase, which forms porphyrin precursor ALA for heme synthesis. Intolerance like salicylism, iodism and cinchonism are idiosyncratic reactions. Aspirin can produce bronchial asthma in some patients. Idiosyncratic reactions are harmful and sometimes may be fatal. They may appear in low doses.
7. **Allergic (hypersensitivity) reactions (allergenicity, immunotoxicity):** Drug allergy is an immunologically mediated reaction producing serotype symptoms which are unrelated to the pharmacological effects and doses of the drug. These reactions occur only in a minority of patients (5–10%) exposed to the drug and cannot be produced in other patients at any dose. Prior sensitization of the cells is needed and a latent period of at least one to two weeks is required after the first exposure. The drug or its metabolite acts as antigen or hepten (incomplete antigen), which becomes complete antigen after binding with the endogenous protein and induces production of antibodies by sensitized

lymphocytes. Re-exposure of the body tissue to the drug produces antigen antibody reaction which may be mild to serious and reversible in most occasions. The chief target organs of drug allergy are skin, respiratory tract, gastrointestinal tract, blood and blood vessels. All drugs are not allergenic and all patients do not manifest allergy. Chemically related drugs often show cross-sensitivity. One drug can produce different types of allergic reactions in different patients, whereas widely different drugs can produce same type of reaction.

The course of drug allergy is also variable. A patient previously sensitive to one drug may subsequently tolerate it without any reaction.

Drug Poisoning and Treatment

Poison is a substance which endanger life by severely effecting one or more vital functions in the body. Drug in large dosage (either accidentally or for suicidal or homicidal purpose) can produce poisoning effects.

Treatment of a Poisoned Person

These include:

1. **Identification of the poison:** This is done by taking history from the relatives or from the patient (if conscious). Estimation of blood level of poison (e.g. barbiturate, morphine, etc.) is also important for identification of the poison.
2. **Termination of exposure of the poison:** This is done by removing the patient to fresh air (in case of inhaled poison) and washing of skin and eyes of the patient (in case of poison entering from the surface).
3. **Prevention of further absorption of the poison:** This is done by using an emetic, e.g. ipecac syrup or apomorphine injection (if the patient is conscious), gastric lavage by stomach tube (in conscious or unconscious patient) and universal antidote (suspension of activated charcoal in water or mixture of burnt toast, strong tea and milk of magnesia in the ratio 2:1:1) for removing the ingested poison from GIT. Emetics should not be used in patients of corrosive poisoning (for fear of perforation of stomach).
4. **Use of specific antidotes where possible:** These are use of receptor antagonists (e.g. naloxone in morphine poisoning, atropine and pralidoxime in organophosphorus compound poisoning), chelating agents (e.g. dimercaprol in arsenic poisoning, desferrioxamine mesylate in iron poisoning) and specific antibodies (e.g. digoxin-FAB-antibody in digoxin poisoning).
5. **General supportive measures and symptomatic treatment:**
   a. Maintenance of airway and adequate ventilation by oxygen inhalation or artificial respiration are done if required.
   b. Maintenance of BP and heartbeat by fluid infusion (normal saline or 5% dextrose saline), vasopressor agents (noradrenaline, mephentermine, etc.) and cardiac stimulants (dopamine, dobutamine, etc.) are done if required.
6. **Hastening of the elimination of the poison:** Altering the urinary pH to alkaline side (alkalinization) by using sodium bicarbonate, potassium citrate, etc. orally helps in excretion of acidic drugs like barbiturates, salicylates, etc. in urine and to acidic side (acidification) by using ammonium chloride, vitamin C, etc. orally helps in excretion of basic drugs like chloroquine, amphetamine, etc. in urine. Forced alkaline diuresis (by using frusemide or mannitol and sodium bicarbonate solution IV) is very useful in removing phenobarbitone, aspirin, etc. from the body.
7. **In serious poisoning,** peritoneal dialysis or haemodialysis can be done in a hospital for removal of the poison from the body.

Organ/Tissue Toxicity

These are chronic toxicities due to prolonged use of drugs (as shown in **Table 8.1**). Withdrawal of the respective drug helps in cure of the condition. In some cases administration of substances that prevent toxicity of drugs

Table 8.1: Organ/tissue toxicity of drugs

Type of toxicity	*Produced by drugs*
Nephrotoxicity	Aminoglycoside antibiotics, loop diuretics, sulphonamides, tetracyclines, corticosteroids, cephaloridine, amphotericin-B, heavy metals, phenacetin, phenylbutazone, phenytoin, pentamidine, hydralazine, procainamide, lithium, isoniazid, methysergide, penicillamine, NSAIDs, anticancer drugs.
Hepatotoxicity	Isoniazid, rifampicin, tetracyclines, pyrazinamide, paracetamol, pheylbutazone, chlorpromazine, chlorpropamide, erythromycin estolate, halothane, thiacetazone, PAS, sulphonamides, valproic acid, phenytoin, indomethanin, chloroform, alcohol, methyldopa, salicylates.
Haematological/ myelotoxicity (bone marrow depression)	Chloramphenicol, sulphonamides, sulphones, heavy metals, thiouracils, phenothiazines, NSAIDs, quinidine, procainamide, phenytoin, imipramine, doxapine, clozapine, radioactive isotopes, anticancer drugs.
Ototoxicity	Aminoglycoside antibiotics, loop diuretics, salicylates, quinine, chloroquine, minocycline, quinidine, anticancer drugs.
Oculotoxicity	Ethambutol, chloroquine, quinine, diodoquin, methanol, thioridazine, digoxin, mechlorethamine.
Behavioural toxicity	Reserpine, clonidine, propranolol, amphetamine, cocaine, glucocorticoids, marijuana, LSD, mescaline.
Neurotoxicity	Isoniazid, hydralazine, nalidixic acid, nitrofurantoin, vincristine.
Skin toxicity	Heavy metals, drugs producing allergic reactions (see later on).
Cardiotoxicity	Emetine, digoxin, quinidine, lithium, adrenaline, halothane, chloroform.
Pulmonary toxicity	Acetylcysteine, sodium chromoglycate, captopril, morphine.
Endocrine toxicity	Chlorpromazine, oral contraceptives, glucocorticoids.
Carcinogenicity	Anticancer drugs, radioactive isotopes, nicotine, oestrogens.
Mutagenicity	Anticancer drugs, metronidazole.

can be used, e.g. pyridoxine in isoniazid neurotoxicity.

Teratogenicity (Greek Word "Teros" Means Monster)

A teratogen is an agent which can cause physical malformation in foetus, when administered to the pregnant women during the period of organogenesis (18th–60th days of foetal life). After that period, during the remainder days of pregnancy, exposure of foetus to foetotoxic drugs may cause functional disability or in alteration in growth of organs or foetus but no physical defects. The sedative hypnotics, thalidomide prescribed in pregnant women for relief of morning sickness was found to produce various types of developmental anomalies in the newborns (1958–61). This thalidomide disaster has lead to imposement of strict teratogenicity tests on new drugs before their clinical uses **(Table 8.2)**.

Types and Mechanisms of Allergic Reactions to Drugs

These are humoral reactions and cell mediated reactions.

Humoral Reactions

These are of three types:

Type I (immediate hypersensitivity/anaphylatic) reactions: Here on re-exposure to the drug, reactions develop within a few

Table 8.2: Drugs producing teratogenicity

Drug	*Abnormality in foetus of man*
Thalidomide	Amelia (total absence of limbs), phochomelia (seal limbs or short limbs), multiple defects.
Anticancer drugs (methotrexate, etc.)	Multiple defects, foetal death.
Androgens (testosterone, etc.)	Virilization; limb, oesophageal and cardiac defects.
Progestins (progesterone, etc.)	Virilization of female foetus.
Oestrogens (stilbestrol, etc.)	Vaginal carcinoma in teenaged female offspring.
Warfarin	Nose, eye and hand defects; retardation of growth.
Corticosteroids (prednisolone, etc.)	Cleft palate and lip, cardiac defects.
Tetracyclines	Deformed and discoloured teeth, retarded bone growth.
Antithyroid drugs (carbimazole, etc.)	Foetal goitre, hypothyroidism.
Phenytoin	Hypoplastic phalanges, cleft lip or palate, microcephaly.
Carbamazepine	Neutral tube defects, other abnormalities.
Valproic acid	Spina bifida, neural tube defects.
Lithium	Foetal goitre, cardiac abnormality.
Phenobarbitone	Various malformations.
Aspirin/Indomethacin	Premature closure of ductus arteriosus.

minutes and the manifestations include urticaria, pruritus, rhinitis, bronchospasm (asthma) and anaphylactic shock. IgE antibodies are formed, which get fixed to the mast cell surface and release mediators of allergy like histamine, leukotrienes (LTs), prostaglandins (PGs) and platelet-activating factor (PAF). The most serious and life-threatening reaction is anaphylactic shock, which is manifested as urticaria, pruritus, angioedema, bronchospasm and hypotension. It is treated by prompt administration of adrenaline solution (1:1000) in the dose of 0.5 to 1 mg SC or IM and repeated after five minutes if required. It is a rapidly acting drug producing dramatic response. An antihistaminic drug and a glucocorticoid can be used IM or IV but they are slowly acting drugs. An intradermal (skin) test may forewarn type I allergic reactions.

Type II (autoallergic/cytolytic) reactions: Here the drug combines with a body protein, so that the body no longer recognizes the protein as self and treats it as a foreign protein and then form antibodies (IgG and IgM). The antigen-antibody reaction leads to activation of complement, which damages cells, including blood cells and their precursors by cytolysis leading to granulocytopenia, thrombocytopenia, aplastic anaemia and haemolytic anaemia. Collagen diseases like systemic lupus erythematosus (SLE) may be due to type II reactions.

Type III (retarded/arthus) reactions: Here antigen and antibody (IgG) form large complexes and activate complement, which precipitates on vascular endothelium giving rise to destructive inflammatory response of small blood vessels. The manifestations are serum sickness (1–3 weeks later producing fever, athralgia and lymphadenopathy), nephritis, vasculitis and pulmonary disease. These reactions usually subside after 1–2 weeks.

Cell Mediated Reactions

These are discussed as follows:

Type IV (delayed cell mediated) reactions: Here antigen specific receptors develop on T lymphocytes. On contact with antigen these T cells produce lymphokines, which attract granulocytes and then produce an inflammatory response. The manifestations are contact dermatitis, some skin rashes, pneumonitis and photosensitization of skin to UV radiation (sunray). These reactions develop after 48 hours of antigen exposure and subside gradually.

Examples of drugs that frequently producing allergic reactions: Penicillins, cefalosporins, erythromycin, tetracyclines, chloramphenicol, sulphonamides, rifampicin, isoniazid, cocaine, procaine, lidocaine, insulin, heparin, NSAIDs, sulphonylureas, phenothiazines, chloroquine, quinine, quinidine, thiouracils, methyldopa, levodopa, phenytoin, carbamazepine, hydralazine, captopril, allopurinol, halothane, streptokinase, ATS, ADS, ACS, etc.

Care should be taken in prescribing drugs for patients suffering from allergic disorders, because in them the incidence of drug allergy is more.

Treatment of Drug Allergy

The offending drug must be immediately stopped. Most mild reactions like skin rashes subside by themselves and do not require specific treatment. In some type I reactions like urticaria, pruritus, rhinitis, swelling of lips and eyelids, antihistaminics are beneficial. In case of anaphylactic shock adrenaline is the drug of choice (discussed before).

Special Adverse Drug Reactions

1. **Drugs causing unmasking or exacerbations of some diseases:** Drugs can unmask a latent condition or exacerbate an already existing disease, e.g. glucocorticoids may unmask latent diabetes or exacerbate an existing peptic ulcer or tuberculosis. Isoniazid may unmask latent epilepsy.
2. **Drugs producing new diseases (iatrogenic diseases):** Sometimes, drug themselves may produce certain diseases after prolonged use. These are called iatrogenic diseases (physician made diseases, Greek word "iatros" meaning physician), e.g. parkinsonism is produced by chlorpromazine and reserpine; peptic ulcer is produced by NSAIDs (aspirin, etc.) and corticosteroids; hypertension is produced by glucocorticoids; candidiasis is produced by broad spectrum antibiotics; glaucoma is produced by anticholinergic drugs and ocular glucocorticoids; hepatitis is produced by isoniazid, rifampicin and pyrazinamide; systemic lupus erythematosus (SLE) is produced by hydralazine and procainamide, etc.
3. **Toxicity due to drug interactions:** This can occur when two or more drugs are administered simultaneously to a patient (discussed in Drug Interactions).

Factors Influencing Adverse Drug Reactions (ADRs)

These are given as follows:

1. **Drug related factors:** Chemical and physical properties of drugs; presence of vehicles, adjuvants, binding agents or coating agents with drugs; margin of safety (low or wide) of drugs and drug-drug interactions can modify adverse drug reactions.
2. **Routes of drug administration:** The toxicity of an orally administered drug is generally greatest when it is given in an empty stomach and least when it is given after food. Drugs given by slow IV infusion produce less toxicity than when given by rapid single injection. Some drugs produce toxicity even in contact with skin by percutaneous absorption, e.g. organophosphorus compounds.
3. **Subject susceptibility (age, sex and pregnancy):** Neonates and old persons are more prone to adverse drug reactions. Women are more susceptible to adverse drug reactions than men. Teratogenic drugs should be avoided during pregnancy.

4. **Disease states:** Presence of diseases of liver or kidney, presence of congestive cardiac failure, cardiovascular shock, hypertension, bronchial asthma, diabetes mellitus, thromboembolic disorders, gastritis or peptic ulcer can precipitate adverse drug reactions by altering pharmacokinetics of drugs.
5. **Racial differences:** Persons of some races (e.g. Egyptians, Swedenese, etc.) are poor acetylators and so drugs like isoniazid, hydralazine, etc. can produce neurotoxicity in them, whereas persons of some races (e.g. Eskimos, Japanese, etc.) are rapid acetylators and so isoniazid, hydrallazine, etc. can produce hepatotoxicity in them. Again persons of some races (e.g. American Negroes) often suffer from haemolytic anaemia by using primaquine, quinine and other oxidant drugs due to deficiency of the enzyme, glucose-6-phosphate dehydrogenase in RBCs (discussed in Pharmacogenetics).

Toxicity Studies in Animals

To assess the safety of a drug, various toxicity studies are carried out in animals (mice, rats, guinea pigs, dogs and monkeys) under varying conditions of drug administration. The tests done are:

Acute Toxicity Tests

In these tests, the drug is tested for the effects of single dose by injecting graded doses in different groups of animals. Detailed observations are made on the effects of the drugs on locomotion, behaviour, respiration and production of convulsion and vomiting. This is followed by autopsy and histological examination. The studies are conducted in at least two species of animals (usually mice and rats) and two routes of administration of the drug (one by intended route of use) are used. Animals of both sexes and various age groups are subjected to such tests. These studies determine LD_{50}, ED_{50} and therapeutic index for the drug under investigation.

Subacute Toxicity Tests

In these tests, a drug is tested for the effects of daily doses for a shorter period of time (usually 14 to 21 days) to save time. Usually two species (rats and dogs) are used and the route of administration is according to the intended route of use. Evaluation of the state of health of the animals is done weekly. All animals are subjected to autopsy and histological examination of all organ systems like chronic toxicity tests.

Chronic Toxicity Tests

In these tests, a drug is tested for the effect of daily doses for a longer period of time (usually 3 months to 1 year). Usually 2 species (rats and dogs) are used and the route of administration is according to the intended route of use. Evaluation of the state of health of the animals is done weekly. All animals are subjected to autopsy and histological examination of all organ systems. In chronic toxicity studies following observations are made:

- Observation of gross changes like increase or decrease of body weight, loss of fur, behavioural effects (abnormalities), skin and eye effects and changes in mating behaviour of the animals.
- Examination of effects of the drug on the individual organs like liver, kidney, heart, adrenal and bone marrow after autopsy (by histopathology). Estimation of enzyme activities and liver function tests are carried out in animals.
- Examination of effects of the drug on the reproductive system, including teratogenicity (in animals like rats, mice or rabbits).
- Carcinogenicity and mutagenicity of the drug (in animals like rats, mice or rabbits).
- Dependence liability of the drug (in animals like cats, dogs or monkeys).

9 Development and Evaluation of New Drugs

INTRODUCTION

New drugs are continuously introduced in the market. They are developed mainly by three ways:

1. Drug designing (on the basis of chemical structures and their biological actions).
2. Modification of the chemical structure of a known drug.
3. Systemic pharmacological screening of different natural products and synthetic compounds.

Most new drugs are discovered by testing (screening) a large number of natural products or synthetic compounds for varieties of biological actions. Once a compound is found to have an effect, numerous chemical modifications are made and tested until one is found to be suitable for further evaluation. Current drug development highly focusses on prospective designing for a specific chemical effect.

Before introducing a new drug in clinical practice, the risk benefit ratio of the drug must be satisfactory. This risk benefit ratio varies from drug to drug, e.g. high risk sedative hypnotic is unacceptable, but a high risk anticancer drug is acceptable. To develop or introduce a new drug following tests are carried out:

Preclinical Tests

Preclinical tests are done in animals (like rats, mice, guinea pigs, rabbits, dogs, cats and monkeys) for following purposes:

a. ***Pharmacological studies:*** These are carried out in animals using *in vivo* or *in vitro* techniques. These studies bring out specific biological activities (effects), mechanisms of action, pharmacokinetics and effective dosage range of test compounds.
b. ***Toxicity studies:*** These should be done with compounds which have showed positive pharmacology. These include acute, subacute and chronic toxicity tests, therapeutic index, effects on reproductive system including teratogenicity; carcinogenicity and mutagenicity of drugs and mechanism of toxicity (discussed previously).

Clinical Evaluation (in Humans)

When the studies in animals predict that a new chemical compound may be a useful medicine, then it is put to clinical pharmacology (in healthy human volunteers) and clinical trial (in patients). These human studies are carried out in four phases (one after another).

Phase I (preliminary pharmacologic evaluation): In it; pharmacokinetics, safety and effects of a new drug are studied in healthy human volunteers.

Phase II (controlled clinical evaluation): In it; pharmacokinetics, safety and therapeutic efficacy of the new drug are studied in small numbers of selected patients using single blind (patient) design (procedure).

Phase III (controlled clinical trials): In it; safety and therapeutic efficacy of the new

drug are studied in large numbers (hundreds) of selected patients using double blind (patient and doctor) design (procedure) with a cross-over design. After satisfactory completion of this phase, application is made to the drug controller for permission to market the new drug.

Phase IV (postmarketing surveillance for some drugs): It begins after obtaining the approval of the drug controller to market the drug. It constitutes a vigilant postmarketing surveillance to monitor ADRs and safety of the new drug including detection of rare toxicity reported by the physicians from various clinics and hospitals. The new drug is also compared with an already used drug for efficacy and safety.

All pharmacological, clinical and toxicological data should be subjected to statistical analysis to detect the utility of the new drug.

In connection with the clinical evaluation and trials following terms should be noted:

1. **Placebo:** Placebo (Latin word, means "I please") is a dummy medication (an inert substance, i.e. without pharmacological action, but harmless) used to please a patient. It looks like the drug and when administered to patient or a healthy volunteer, the subject remains under the impression that he has received a drug. Some persons may develop temporary improvement from some diseases (angina pectoris, hypertension, diabetes, etc.) even after receiving the placebo. Placebo therapy is used during single blind and double blind studies.

   To minimize bias in clinical trials, randomization (random selection of subjects) and double blind design are adopted.
2. **Single blind design:** In it, some of the patients get drug to be tested and others get the placebo and the patient is blind about the drug or the placebo. After getting the drug or the placebo, the patients report to the doctor (investigator) about their improvement or symptoms.
3. **Double blind design:** In it, neither the patient nor the doctor (investigator) knows who has received what (drug or placebo). The drug and the placebo are supplied by a third man under a code. This avoids bias of the investigator.
4. **Cross-over design:** In it, some patients get the drug to be tested and others get the placebo by a third man and both the patients and the doctor are blind about the drug or placebo. After getting reports of the patients about their improvement or symptoms, the third man supply the drug and the placebo to the patients in alternate phases and the reports are monitored by the doctor (investigator).

Ethics in the Development of New Drugs

Majority of toxicity tests in animals, which are subjected to ethical criticism are based on studies in whole animals, because only in them it is possible to approach the complexity of organization of body systems of humans, to explore any consequence of variable drug absorption, metabolism and excretion and to reveal direct and indirect toxic effects of drugs. Use of animals for toxicity tests would be unjustified if results useful to man could not be obtained. Animals are similar to man in many respects and so they are used initially for toxicity tests. To exclude the need for tests in whole animals, *in vitro* tests are being introduced, which are scientifically highly satisfactory. The idea to exclude whole animal tests is not only ethical but also economical, because whole animals are very costly to breed in house and to keep them healthy. It is better to choose non-whole animal methods, if they are scientifically satisfactory and practically available.

In clinical trials using humans, the comfort, health and food of the subject must be satisfactory and there should not be any bias in choosing the subject.