1

Chemical Introduction: Sources, Classification and Chemical Properties of Drugs

1.1 Introduction

Pharmacology can be divided into two major areas, pharmacodynamics (PD) – the study of what a drug does to the body and pharmacokinetics (PK) – the study of what the body does to the drug. Drug disposition is a collective term used to describe drug absorption, distribution, metabolism and excretion whilst pharmacokinetics is the study of the rates of these processes. By subjecting the observed changes, for example, in plasma concentrations as a function of time, to mathematical equations (models), pharmacokinetic parameters such as elimination half-life ($t_{1/2}$), volume of distribution (V) and plasma clearance (CL) can be derived. Pharmacokinetic modelling is important for the:

- Selection of the right drug for pharmaceutical development
- Evaluation of drug delivery systems
- Design of drug dosage regimens
- Appropriate choice and use of drugs in the clinic.

These points will be expanded in subsequent chapters.

A drug is a substance that is taken, or administered, to produce an effect, usually a desirable one. These effects are assessed as physiological, biochemical or behavioural changes. There are two major groups of chemicals studied and used as drugs. First, there is a group of pharmacologically interesting endogenous substances, for example acetylcholine, histamine and noradrenaline. Second, there are the non-endogenous, or 'foreign' chemicals (xenobiotics), which are mostly products of the laboratories of the pharmaceutical industry.

There are numerous ways in which drugs interact with physiological and biochemical process to elicit their responses. Many of these interactions are with macromolecules, frequently proteins and nucleic acids. *Receptors* are transmembrane proteins, with endogenous ligands typified by acetylcholine and noradrenaline (norepinephrine). Although substances may be present naturally in the body, they are considered drugs when they are administered, such as when adrenaline is injected to alleviate anaphylactic shock. Drugs can either mimic (agonists) or inhibit (antagonists) endogenous neurotransmitters. Salbutamol is a selective β_2-agonist whereas propranolol is a non-selective β-blocker. Some receptors are *ligand-gated ion channels*, for example the cholinergic nicotinic receptor, which is competitively antagonized by (+)-tubocurarine. *Enzymes*, either membrane bound or soluble, can be inhibited – for example neostigmine inhibits acetylcholinesterase

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and aspirin inhibits cyclooxygenase. Other proteins that may be affected are *voltage-gated (regulated) ion-channels* – a typical one being voltage-gated sodium channels which are blocked by local anaesthetics such as lidocaine (lignocaine). Antimalarials, chloroquine, for example, intercalate in DNA. Some drugs work because of their physical presence – often affecting pH or osmolarity – for example antacids to reduce gastric acidity or sodium bicarbonate to increase urinary pH and thereby increase salicylate excretion (Section 3.3.1.5).

1.1.1 Source of drugs

Primitive therapeutics relied heavily on a variety of mixtures prepared from botanical and inorganic materials. The botanical materials included some extremely potent plant extracts, with actions for example on the brain, heart and gastrointestinal tract, and also some innocuous potions, which probably had little effect. The inorganic materials were generally alkalis, which did little more than partially neutralize gastric acidity. Potassium carbonate (potash, from wood fires) was chewed with coca leaves to hasten the release of cocaine. Inevitably, the relative importance of these materials has declined, but it should be recognized that about a dozen important drugs are still obtained, as purified chemical constituents, from botanical sources and that alkalis still have a very definite value in certain conditions. Amongst the botanical drugs, are the alkaloids: morphine is still obtained from opium, cocaine is still obtained from coca leaves, and atropine is still obtained from the deadly nightshade (belladonna). Although the pure compounds have been prepared synthetically in the laboratory, the most economical source is still the botanical material. Similarly, glycosides such as digoxin and digitoxin are still obtained from plants. These naturally occurring molecules often form the basis of semisynthetic derivatives – it being more cost-effective than synthesis *de novo*.

Similar considerations apply with some of the drugs of zoological origin. For instance, while the consumption of raw liver (an obviously zoological material) was once of great importance in the treatment of anaemia, modern treatment relies on cyanocobalamin, which occurs in raw liver, and on hydroxycobalamin, a semisynthetic analogue. Another zoological example is insulin, which was obtained from the pancreatic glands of pigs (porcine insulin) but can now be genetically engineered using a laboratory strain of *Escherichia coli* to give human insulin.

Most other naturally occurring drugs, including antibiotics (antimicrobial drugs of biological origin) and vitamins, are generally nowadays of known chemical structure, and although their synthesis in the laboratory is in most cases a chemical possibility, it is often more convenient and economical to extract them from natural sources. For the simpler molecules the converse may be true, for example chloramphenicol, first extracted from the bacterium, *Streptomyces venezuelae*, is totally synthesized in the laboratory. For some antibiotics, penicillins and cephalosporins for example, the basic nucleus is of natural origin, but the modern drugs are semisynthetic modifications of the natural product.

Amongst the naturally occurring drugs are the large relative molecular mass (M_r) molecules, such as peptides, proteins (including enzymes), polysaccharides and antibodies or antibody fragments. Some of these macromolecules, snake venoms and toxins such as botulinum toxin ($M_r \sim 150{,}000$) have long been known. The anticoagulant, heparin, is a heavily sulfated polysaccharide ($M_r \sim 3{,}000$–$50{,}000$). Peptide hormones used as drugs include insulin and human growth hormone. Streptokinase, urokinase and tissue plasminogen activator (tPA) are enzymes used as thrombolytic agents. Other therapeutic enzymes are the pancreatic enzymes given to sufferers of cystic fibrosis. Antibodies are a recent addition to macromolecular drugs. Digoxin-specific antibodies, or light-chain fragments (F_{ab}) containing the specific binding site, are used to treat poisoning by cardiac glycosides. Advances in molecular biology have led to the introduction of a number of monoclonal antibodies with a range of targets: various cancers, viruses, bacteria, muscular dystrophy, the cardiovascular and immune systems, to name but some. Furthermore, the antibodies can be

modified to carry toxins, cytokines, enzymes and radioisotopes to their specific targets. Monoclonal antibodies have the suffix *-mab* and the infix indicates the source of the antibody and the intended target; *-u-* indicates human and *-tu(m)-* that the target is a tumour. Thus, trastuzumab is a monoclonal antibody directed at (breast) cancer that has been 'humanized', *-zu-*; that is over 95% of the amino acid sequence is human. The prefix is unique to the drug.

With only minor exceptions, drugs are chemicals with known structures. Some of them are simple, some complex. Some of them are purely synthetic; some are obtained from crude natural products and purified before use. Most are organic chemicals, a few are inorganic chemicals. With all drugs, the emphasis is nowadays on a pure active constituent, with carefully controlled properties, rather than on a mysterious concoction of unknown potency and constitution.

1.2 Drug nomenclature and classification

Drug names can lead to confusion. Generally a drug will have at least three names, a full chemical name, a proprietary name, i.e. a trade name registered to a pharmaceutical company, and a non-proprietary name (INN) and/or an approved name. Names that may be encountered include the British Approved Name (BAN), the European Pharmacopoeia (EuP) name, the United States Adopted Name (USAN), the United States Pharmacopoeia (USP) name and the Japanese Approved Name (JAN). The WHO has been introducing a system of recommended INNs (rINN) and it is hoped that this will become the norm for naming drugs, replacing alternative systems. For example, lidocaine is classed as a rINN, USAN and JAN, replacing the name lignocaine that was once a BAN. Often 'ph' is replaced by 'f', as in cefadroxil, even though the group name is cephalosporins. We have elected to use amphetamine rather than amfetamine. Generally, the alternatives obviously refer to the same drug, such as ciclosporin, cyclosporin and cyclosporine. There are some notable exceptions, pethidine is known as meperidine in the United States and paracetamol as acetaminophen. Even a simple molecule like paracetamol may have several chemical names but the number of proprietary names or products containing paracetamol is even greater, including Panadol, Calpol, Tylenol, Anadin Extra. Spelling can also lead to apparent anomolies. For example, cefadroxine is a cephalosporin (Table 1.2). Therefore it is necessary to use an unequivocal approved name whenever possible.

A rigid system for the classification of drugs will never be devised. Increasingly, it is found that drugs possess actions which would permit their categorization in several groups in any one particular classification system. This is shown most strikingly by the use of lidocaine for both local anaesthetic and cardiac effects. Additionally, with constant changes in drug usage, it is not uncommon to find drugs of several different types in use for the same purpose. The number of examples within each type is of course very large. However, drugs are commonly grouped according to one of two major systems. These are on the basis of action or effect, and on the basis of chemistry. It is not possible to include all drugs in either of these groupings, and so a hybrid classification is necessary if all possibilities are to be considered. Table 1.1 shows an abbreviated pharmacological listing. The interpretation of this is quite straightforward, and it is presented as a general aid to the reader of later chapters of this book. Most of the examples quoted in later chapters are mentioned. Not so straightforward is the chemical listing shown in Table 1.2. It will be immediately noticed that while all of the groups of drugs in Table 1.2 are represented in Table 1.1, all of the types in Table 1.1 are not represented in Table 1.2, as a great many drugs are of chemical types of which there is only a single example, and Table 1.1 is only concerned with those chemical groups of drugs which are commonly known by their chemical names. Commonly encountered chemical groups are exemplified in Table 1.3.

1.3 Properties of molecules

Drug molecules may be converted to other molecules either by spontaneous change (i.e. decomposition) or by enzymatic transformation. Enzymes are such efficient catalysts that the rate of a reaction may be increased

Table 1.1 Abbreviated listing of drug groups categorized on the basis of pharmacological use or clinical effect, with examples, or cross-referenced to the chemical types of Table 1.2

THE CENTRAL NERVOUS SYSTEM

General anaesthetics

I Gases – e.g. nitrous oxide
II Volatile liquids – e.g. halothane
III Intravenous anaesthetics, including some barbiturates

Hypnotics including some barbiturates and some benzodiazepines, and newer examples such as zolpidem

Sedatives including certain barbiturates, phenothiazines and benzodiazepines

Tranquillizers

I Major, including certain phenothiazines and butyrophenones
II Minor, including certain benzodiazepines
III Other, newer, examples, such as olanzepine

Antidepressants

I Dibenzazepines – e.g. nortriptyline
II Monoamine oxidase inhibitors – e.g. tranylcypromine
III Lithium
IV Other newer examples, such as fluoxetine

Central nervous system stimulants

I Amphetamine-related compounds – e.g methylphenidate and amphetamine
II Hallucinogens – e.g. lysergic acid diethylamide
III Xanthines – e.g. caffeine

Analgesics

I Narcotics – e.g. morphine and pethidine
II Mild analgesics, including salicylates

Miscellaneous centrally acting drugs, including respiratory stimulants (analeptics), anticonvulsants, certain muscle relaxants, drugs for Parkinson's disease, antiemetics, emetics and antitussives

CHEMOTHERAPY

Drugs used in the chemotherapy of parasitic diseases, including arsenicals
Drugs used in the chemotherapy of microbial diseases, including penicillins, cephalosporins and sulfonamides
Drugs used in the treatment of viral diseases, such as aciclovir
Drugs used in the treatment of fungal diseases, e.g. miconazole
Drugs used in the treatment of cancer, such as alkylating agents, antimetabolites, anthracycline derivatives, trastuzumab, hormone antagonists

PERIPHERAL SYSTEMS

Drugs acting at synapses and nerve endings

I Acetylcholine and analogues (parasympathomimetic agents)
II Anticholinesterase drugs – e.g. physostigmine
III Inhibitors of acetylcholine at parasympathomimetic nerve endings – e.g. atropine
IV Drugs acting at ganglia – e.g. nicotine
V Drugs acting at adrenergic nerve endings, including catecholamines and imidazolines
VI Neuromuscular blocking drugs – e.g. suxamethonium

Drugs acting on the respiratory system

I Bronchodilators – e.g. salbutamol
II Drugs affecting allergic responses – e.g. disodium cromoglycate
III Oral antiasthmatics e.g. montelukast

Autacoids and their antagonists

I Histamine and 5-hydroxytryptarnine
II Antihistamines – e.g. diphenhydramine

Drugs for the treatment of gastrointestinal acidity

e.g. ranitidine and omeprazole

Cardiovascular drugs

I Digitalis and digoxin
II Antiarrhythmic drugs – e.g. quinidine
III Antihypertensive drugs, including angiotensin-converting enzyme (ACE) inhibitors ('prils')
IV Vasodilators – e.g. glyceryl trinitrate
V Anticoagulants, including heparin and coumarins.
VI Diuretics, including thiadiazines
VII Lipid lowering drugs (e.g. 'statins')
VIII Thrombolytics (e.g. tissue plasminogen activator)

Local anaesthetics – e.g. lidocaine (lignocaine)

Locally acting drugs

e.g. gastric antacids and cathartics

Endocrinology

Hormones, hormone analogues and hormone antagonists, including steroids, sulfonylureas and biguanides (e.g. glipizide, thyroxine and insulin)

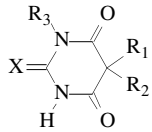
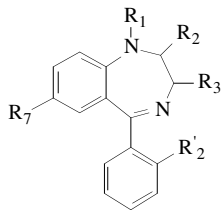
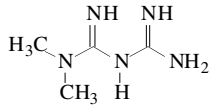
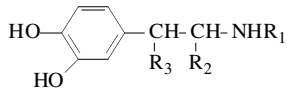
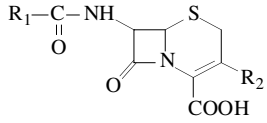
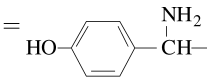
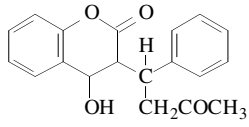
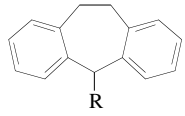
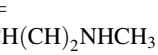
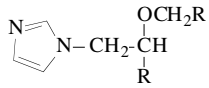
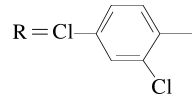
Biological response modifiers

e.g. interferon, adalimumab

Immunosuppressants

e.g. ciclosporin

Table 1.2 Some groups of drugs classified on chemical structure rather than pharmacological properties or uses

Group	Parent structure	Chemical example	Uses and examples
Barbiturates		Phenobarbital $R_1 = C_2H_5$ $R_2 = C_6H_5$ $R_3 = H$ $X = O$	As hypnotics and sedatives (pentobarbital) As anticonvulsants (phenobarbital) As general anaesthetics (thiopental)
Benzodiazepines		Lorazepam $R_1 = H$ $R_2 = O$ $R_3 = OH$ $R_7 = Cl$ $R'_2 = Cl$	As anxiolytics (diazepam) As hypnotics (temazepam)
Biguanides		Metformin (as drawn)	As oral hypoglycaemics
Catecholamines		Adrenaline $R_1 = CH_3$ $R_2 = H$ $R_3 = OH$	Sympathomimetic amines
Cephalosporins		Cefadroxil $R_1 =$  $R_1 = CH_3$	As antimicrobial drugs
Coumarins		Warfarin (as drawn)	As anticoagulants
Dibenazazepines		Nortriptyline R = 	As antidepressants
Imidazoles		Miconazole R = 	As antifungal drugs

(*continued*)

Table 1.2 (*Continued*)

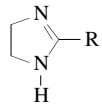
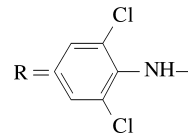
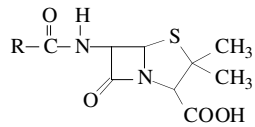
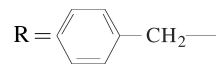
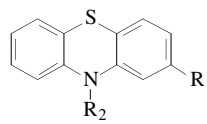
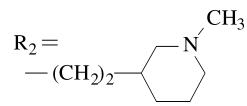
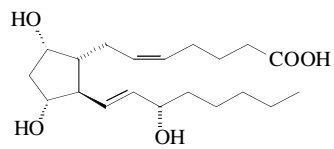
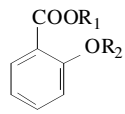
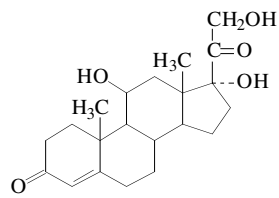
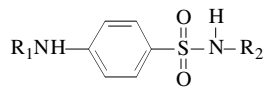
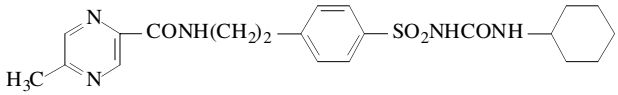
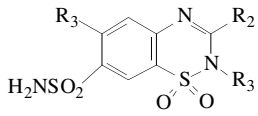
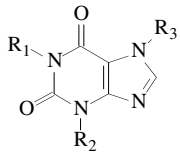
Group	Parent structure	Chemical example	Uses and examples
Imidazolines		Clonidine 	As antihypertensive drugs
Macromolecules: Polysaccharides, peptides, proteins, enzymes, antibodies		Heparin, insulin, trastuzumab	In control of blood clotting, diabetes, cancer, rheumatoid arthritis and other conditions
Penicillins		Penicillin G 	As antimicrobial drugs
Phenothiazines		Thioridazine $R_1 = SCH_3$ 	As antihistamines (promethazine) As antipsychotics (thioridazine) As antiemetics (trifluoperazine)
Prostaglandins		$PGF_{2\alpha}$ (as drawn)	As uterine stimulants and other procedures
Salicylates		Aspirin $R_1 = H$ $R_2 = COCH_3$	As antipyretic, anti-inflammatory and antipyretic drugs
Steroids		Hydrocortisone (as drawn)	Anti-inflammatory drugs
Sulfonamides		Sulfacetamide $R_1 = H$ $R_2 = COCH_3$	As antimicrobial drugs

Table 1.2 (*Continued*)

Group	Parent structure	Chemical example	Uses and examples
Sulfonylureas	Glipizide (As drawn) 		As oral hypoglycaemic drugs
Thiadiazines		Chlorthiazide $R_1 = H$ $R_2 = H$ $R_3 = Cl$	As diuretics
Xanthines		Theophylline $R_1 = CH_3$ $R_2 = CH_3$ $R_3 = H$	As respiratory stimulants and bronchodilators

by the order of 10^{13} times – in other words some reactions would not, for all practical purposes, proceed but for the presence of enzymes. The role of enzymes in the metabolism of drugs is considered in Section 3.2.

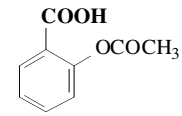
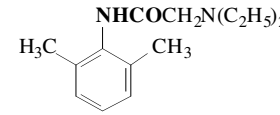
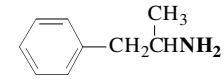
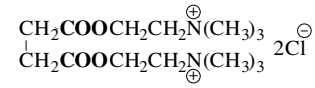
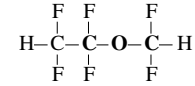
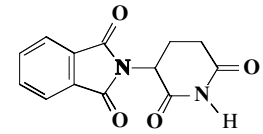
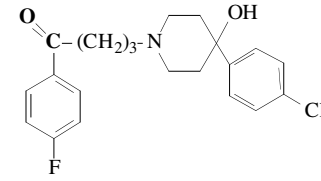
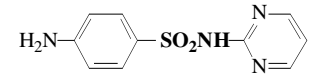
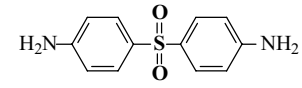
1.3.1 Decomposition of drugs

Spontaneous decomposition needs to taken into consideration during manufacture, storage and use of drugs as well as during bioanalysis, when the products may be mistakenly thought to be metabolites. Although the same compounds may be produced by metabolism, there are occasions, for example, when substances identified in biological fluids arise from decomposition rather than metabolism. Decomposition may result in visible changes and odours when drugs are stored. The reactions tend to be accelerated by the presence of one or more of the following: catalysts, light, heat and moisture.

1.3.1.1 Hydrolysis

Esters and, to a lesser extent, amides are hydrolysed, particularly if catalysed by the presence of acids or bases. Aspirin (acetylsalicylic acid) is hydrolysed to salicylic acid and acetic acid, giving bottles of aspirin a smell of vinegar. Procaine is hydrolysed *p*-aminobenzoic acid and *N*-dimethyl-2-aminoethanol, whilst cocaine is hydrolysed to benzoylecgonine.

Table 1.3 Some important functional groups found in drug molecules

Type of compound	Functional group	Specific example	
		Name	Formula
Acids e.g. carboxylic acids	−COOH	Aspirin (also an ester)	
Alcohols	−OH	Choral hydrate	$CCl_3C(\mathbf{OH})_2$
Amides	−CONH−	Lidocaine (also an amine)	
Bases e.g. amines	−NRR′	Amphetamine	
Esters	−COO−	Suxamethonium chloride (also a quaternary ammonium compound)	
Ethers	−C−O−C−	Enflurane	
Imides	−CONHCO−	Thalidomide	
Ketones	−CO−	Haloperidol (also an amine and an alcohol)	
Sulfonamides	$-SO_2NH-$	Sulfadiazine (also an amine)	
Sulfones	$-SO_2-$	Dapsone (also an amine)	
Small neutral molecules		Nitrous oxide	N_2O
Inorganic salts		Sodium bicarbonate	$NaHCO_3$
		Lithium carbonate	Li_2CO_3

1.3.1.2 Oxidation

Several drugs are readily oxidized, including phenothiazines, which form the corresponding 5-sulfoxides, via coloured semiquinone free radials. Phenothiazines with an electron-withdrawing group in the 2-position, tend to be more stable, thus promethazine (2-H) is more readily oxidized (to a blue product) than chlorpromazine (2-Cl) which gives a red semiquinone radical. Methylene blue is a phenothiazine (Figure 1.1)

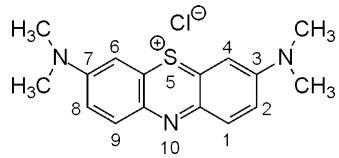
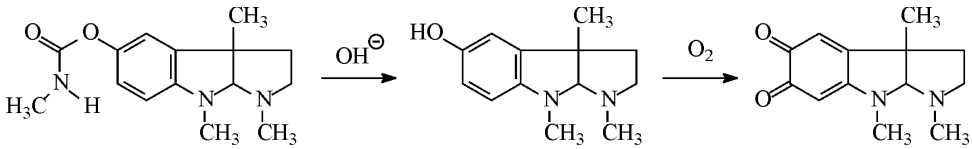


Figure 1.1 Formula of methylene blue (methylthioninium chloride). Other therapeutic phenothiazines usually have 10-substitents and often substituents at position 2.

that is used in medicine as a contrast agent, and as a treatment for methaemoglobinaemia, which is both a congenital and a drug-induced disorder. In this book it appears as an example of tri-exponential plasma concentration decay after intravenous doses (Figure 5.2), and as a valuable tool for laboratory modelling of drug disposition.

Physostigmine is oxidized to rubreserine, which is a deep brown-red colour. The first stage is probably hydrolysis to eseroline:



Physostigmine Eseroline Rubreserine

Adrenaline oxidizes in a similar manner to a brown–red material, adrenochrome.

1.3.1.3 Photodecomposition

Most compounds are photosensitive if irradiated with intense light of the appropriate wavelength. Some drugs are unstable in natural light, notably the 1,4-dihydropyridine calcium channel blocking drugs such as nifedipine. These drugs have to be formulated and handled in a darkroom under sodium light. Similarly, it is recommended that blood samples taken for nitrazepam or clonazepam analysis are protected from light to avoid photodecomposition.

1.3.1.4 Racemization

Optically active drugs (Section 1.8.2) may undergo racemization. For example during extraction from belladonna (−)-hyoscyamine may be converted to atropine [(±)-hyoscyamine].

1.4 Physicochemical interactions between drugs and other chemicals

In the present context we are principally concerned with interactions between relatively small drug molecules and relatively large endogenous molecules such as proteins e.g. enzymes, receptors and ion

channels. The majority of drug–receptor interactions are reversible although some covalent reactions are known, for example non-competitive antagonism.

1.4.1 Chemical bonding and interactions between molecules

The interaction between atoms and molecules is basically electrostatic. The positively charged nuclei of atoms would repel each other if it were not for electrons sharing the space between them such that an electron from one atom is attracted to the nucleus of another. *Ionic bonds* occur when one atom completely donates one or more electrons to another atom, such as in sodium chloride. In the solid the ions are arranged so that the structure is held together by the electrostatic attraction of the oppositely charged ions. Bonds are described as (*non-polar*) *covalent* when atoms of similar electronegativities share electrons more or less equally, or *polar covalent* when the electronegativity of one atom is appreciably greater than the other. The nature of these bonds is somewhere between that of covalent and ionic bonds. The electrons in polar covalent bonds are attracted to the more electronegative atom creating a dipole, i.e. an asymmetric electric charge. The electron-withdrawing effect of oxygen in water molecules results in a strong dipole whereas in methane, CH_4, the electron density is evenly distributed so there is no dipole (Figure 1.2).

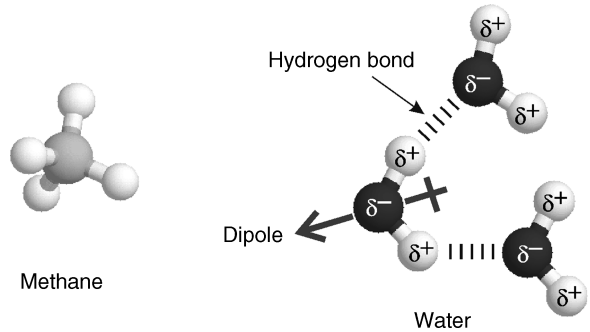


Figure 1.2 Comparison of non-polar methane (CH_4) and water (H_2O) where differences in charge densities between the more electronegative oxygen and hydrogen produces a dipole. Hydrogen bonding occurs between the slightly negatively charged oxygen and the slightly positively charged hydrogen.

Dipoles are responsible for the attractions between molecules. Molecules can align dipole to dipole, the more negative end of one being attracted to the more positive end of another; or a dipole in one molecule can induce a complementary one in an adjacent molecule. The differences in valance electron densities in the more electronegative elements, nitrogen, oxygen and fluorine, leads to *hydrogen bonding* (Figure 1.2) which in some instances can be as strong as some covalent bonds. *Van der Waals* forces arise because the density of the valence electron cloud around an atom can fluctuate causing a temporary dipole which may then induce a dipole in a neighboring atom. These are the weakest but most common forms of attractions between atoms. Another form of bonding is the *hydrophobic bonding* seen in proteins, where hydrophobic regions come together with the exclusion of water.

All the forms of bonding described above are encountered in pharmacology. Covalent binding of groups to enzymes and receptors occurs, such as acetylation of the serine groups in cyclooxgenase by aspirin. Ionic interaction is responsible for the binding of acidic drugs to albumin and of bases to α_1-acid glycoprotein. Hydrophobic binding is also important for binding of molecules to proteins. Similarly, these interactions occur when drugs bind to the active sites of enzymes and to receptors.

1.4.2 Solubility

The physicochemical interactions described above affect the solubility of molecules (solutes) in solvents. The dipole moment and hydrogen bonding in water make this a polar solvent and polar solutes readily dissolve in water, including salts. These solutes are described as hydrophilic – water loving. Organic solvents such as heptane are apolar because they have neither dipoles nor hydrogen bonding. Apolar solvents are very poorly soluble in water and vice versa, water being essentially insoluble in the organic solvent. The two liquids are said to be *immiscible*. Non-polar, non-ionized molecules tend to dissolve readily in organic solvents and lipids, and are referred to as hydrophobic or lipophilic – lipid loving.

To be transferred across lipid membranes drugs must be soluble in the barrier layer of fluid bathing the membrane. Consequently, drugs with low aqueous solubility may be poorly absorbed from the gastrointestinal tract. This can be exploited. An example is sulfasalazine, which is minimally absorbed after oral administration, and is used to treat ulcerative colitis.

1.5 Law of mass action

The Law of Mass Action states: 'the rate at which a chemical reaction proceeds is proportional to the active masses (usually molar concentrations) of the reacting substances'. This means that a non-reversible reaction proceeds at an ever-decreasing rate as the quantity of the reacting substances declines. The Law of Mass Action is easily understood if the assumption is made that, for the reaction to occur, collision between the reacting molecules must take place. It follows that the rate of reaction will be proportional to the number of collisions. The number of collisions will be proportional to the molar concentrations of the reacting molecules.

If a single substance X is in process of transformation into another substance Y, and if at any moment the active mass of X is represented by [X] (usually expressed in moles per litre) then we have:

$$\mathrm{X} \rightarrow \mathrm{Y}$$

and the rate of reaction at any time point $= k[\mathrm{X}]$ where k is the velocity, or rate, constant. This constant varies with temperature and the nature of the reacting substance.

If two substances A and B are reacting to form two other substances C and D, and if the concentrations of the reactants at any particular moment are [A] and [B] then:

$$\mathrm{A} + \mathrm{B} \rightarrow \mathrm{C} + \mathrm{D}$$

and the rate of reaction $= k[\mathrm{A}][\mathrm{B}]$.

1.5.1 Reversible reactions and equilibrium constants

Consider the reaction:

$$\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{C} + \mathrm{D}$$

The rate of the forward reaction is:

$$\text{forward rate} = k_1[\mathrm{A}][\mathrm{B}] \tag{1.1}$$

whilst the backward rate is:

$$\text{backward rate} = k_{-1}[\mathrm{C}][\mathrm{D}] \tag{1.2}$$

where k_1 and k_{-1}, are the rate constants of the forward and backward reactions, respectively. As the reaction proceeds, the concentrations of the original substances A and B diminish and the rate of the forward reaction decreases. At the same time, the substances C and D are produced in ever-increasing quantities so that the rate at which they form A and B increases. Eventually equilibrium is reached when the forward and backward rates are equal:

$$k_1[\mathrm{A}][\mathrm{B}] = k_{-1}[\mathrm{C}][\mathrm{D}] \tag{1.3}$$

The equilibrium constant, K, is the ratio of the forward and backward rate constants, so rearranging Equation 1.3 gives:

$$K = \frac{k_1}{k_{-1}} = \frac{[\mathrm{C}][\mathrm{D}]}{[\mathrm{A}][\mathrm{B}]} \tag{1.4}$$

The term *dissociation constant* is used when describing the equilibrium of a substance which dissociates into smaller units, as in the case, for example, of an acid (Section 4.6). The term is also applied to the binding of a drug, D, to a macromolecule such as a receptor, R, or plasma protein. The complex DR dissociates:

$$\mathrm{DR} \rightleftharpoons \mathrm{D} + \mathrm{R}$$

So:

$$K = \frac{[\mathrm{D}][\mathrm{R}]}{[\mathrm{DR}]} \tag{1.5}$$

An association constant is the inverse of a dissociation constant.

1.5.1.1 Sequential reactions

When a product, D, arises as a result of several, sequential reactions:

$$\mathrm{A} \xrightarrow{k_1} \mathrm{B} \xrightarrow{k_2} \mathrm{C} \xrightarrow{k_3} \mathrm{D}$$

it cannot be formed any faster than the rate of at which its precursor, C, is formed, which in turn cannot be formed any faster than its precursor, B. The rates of each of these steps are determined by the rate constants, k_1, k_2 and k_3. Therefore, the rate at which D is formed will be the rate of the slowest step, i.e. the reaction with the lowest value of rate constant. Say for example, k_2 is the lowest rate constant, then the rate of formation of D is determined by k_2 and the reaction B $\rightarrow$ C is said to be the *rate-limiting* step.

1.5.2 Reaction order and molecularity

The order of a reaction is the number, n, of concentration terms affecting the rate of the reaction, whereas molecularity is the number of molecules taking part in the reaction. The order of a reaction is measured experimentally and because it is often close to an integer, 0, 1, or 2, reactions are referred to as zero-, first- or second-order, respectively. The reaction

$$\mathrm{X} \rightarrow \mathrm{Y}$$

is clearly monomolecular, and may be either zero- or first-order depending on whether the rate is proportional to X^0 or X^1. The reactions

$$2X \rightarrow Y$$

and

$$A + B \rightarrow C + D$$

are both bimolecular and second-order providing the rate is proportional to $[X]^2$, in the first case, and to [A][B], in the second. Note how the total reaction order is the sum of the indices of each reactant: rate $\propto [A]^1[B]^1$, so $n = 2$. However, if one of the reactants, say A, is present in such a large excess that there is no detectable change in its concentration, then the rate will be dependent only on the concentration of the other reactant, B, that is, the rate is proportional to $[A]^0[B]^1$. The reaction is first-order (rate $\propto$ [B]) but still described as 'bimolecular'. Hydrolysis of an ester in dilute aqueous solution is a commonly encountered example of a bimolecular reaction which is first-order with respect to the concentration of ester and zero-order with respect to the concentration of water, giving an overall reaction order of 1.

Enzyme-catalysed reactions have reaction orders between 1 and 0 with respect to the drug concentration. This is because the Michaelis–Menten equation (Section 3.2.5) limits to zero-order when the substrate is in excess and the enzyme is saturated so that increasing the drug concentration will have no effect on the reaction rate. When the concentration of enzyme is in vast excess compared with the substrate concentration, the enzyme concentration is not rate limiting and the reaction is first-order. Thus, the reaction order of an enzyme-catalysed reaction changes as the reaction proceeds and substrate is consumed.

1.5.3 Decay curves and half-lives

As discussed above the rate of a chemical reaction is determined by the concentrations of the reactants and from the foregoing it is clear that a general equation relating rate of decline in concentration ($-dC/dt$), rate constant (λ), and concentration (C) can be written:

$$-\frac{dC}{dt} = \lambda C^n \tag{1.6}$$

Note the use of λ, to denote the rate constant when it refers to decay; the symbol is used for radioactive decay, when it is known as the decay constant. Use of λ for elimination rate constants is now the standard in pharmacokinetic equations.

1.5.3.1 First-order decay

Because first-order kinetics are of prime importance in pharmacokinetics, we shall deal with these first. For a first-order reaction, substituting $n = 1$ in Equation 1.6 gives:

$$-\frac{dC}{dt} = \lambda C \tag{1.7}$$

that is the rate of the reaction is directly proportional to the concentration of substance present. As the reaction proceeds and the concentration of the substance falls, the rate of the reaction decreases. This is exponential decay, analogous to radioactive decay where the probability of disintegration is proportional to

the number of unstable nuclei present. The first-order rate constant has units of reciprocal time (e.g. h^{-1}). Integrating Equation 1.7 gives:

$$C = C_0 \exp(-\lambda t) \tag{1.8}$$

which is the equation of a curve that asymptotes to 0 from the initial concentration, C_0 [Figure 1.3(a)]. Taking natural logarithms of Equation 1.8:

$$\ln C = \ln C_0 - \lambda t \tag{1.9}$$

gives the equation of a straight line of slope, $-\lambda$ [Figure 1.3(b)]. If common logarithms are used (log C versus t) the slope is $-\lambda/2.303$. Another way of presenting the data is to plot C on a logarithmic scale (using 'semilog.' graph paper — not shown). This approach was often used when computers were not readily available and is still frequently used to present data, for example Figure 2.10. Such plots allow computation of C_0 (read directly from the intercept) and the elimination half-life. However, a common misconception is to believe that the slope of this plot is $-\lambda/2.303$. The slope is the same as that of a C versus t plot, but it only appears to be linear — the graph is of the type shown in Figure 1.3(a), which could be viewed as a series of slopes of ever-decreasing magnitude. The slope that matters, permitting calculation of the rate constant and the half-life using linear regression, is that of a graph of ln C versus t [Figure 1.3(b)].

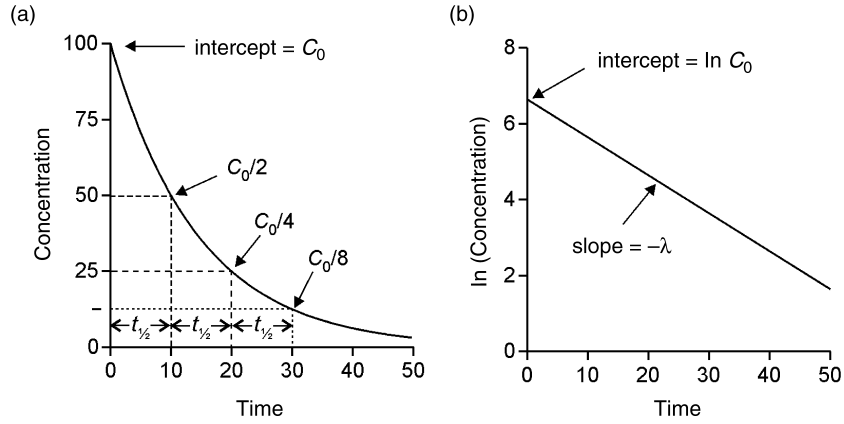


Figure 1.3 Curves for first-order decay plotted as (a) C versus t and (b) ln C versus t.

The half-life ($t_{1/2}$) is the time for the initial concentration (C_0) to fall to $C_0/2$, and substitution in Equation 1.9 gives:

$$t_{½} = \frac{\ln 2}{\lambda} = \frac{0.693}{\lambda} \tag{1.10}$$

as $\ln 2 = 0.693$. This important relationship, where $t_{1/2}$ is constant (independent of the initial concentration) and inversely proportional to λ, is *unique* to first-order reactions. Because $t_{1/2}$ is constant, 50% is eliminated in $1 \times t_{1/2}$, 75% in $2 \times t_{1/2}$, and so on. Thus, when five half-lives have elapsed less than 95% of the analyte remains, and after seven half-lives less than 99% remains.

1.5.3.2 Zero-order decay

For a zero-order reaction, $n = 0$, and:

$$-\frac{dC}{dt} = \lambda C^0 = \lambda \tag{1.11}$$

Thus, a zero-order reaction proceeds at a *constant rate*, and the zero-order rate constant must have units of rate (e.g. $g\,L^{-1}\,h^{-1}$). Integrating Equation 1.11:

$$C = C_0 - \lambda t \tag{1.12}$$

gives the equation of a straight line of slope, $-\lambda$, when concentration is plotted against time [Figure 1.4(a)]. The half-life can be obtained as before, substituting $t = t_{1/2}$ and $C = C_0/2$, gives:

$$t_{1/2} = \frac{C_0}{2\lambda} \tag{1.13}$$

The zero-order half-life is inversely proportional to λ, but $t_{1/2}$ is also directly proportional to the initial concentration. In other words, the greater the amount of drug present initially, the longer the time taken to reduce the amount present by 50% [Figure 1.4(b)]. The term 'dose dependent half-life' has been applied to this situation as well as to Michaelis–Menten kinetics cases.

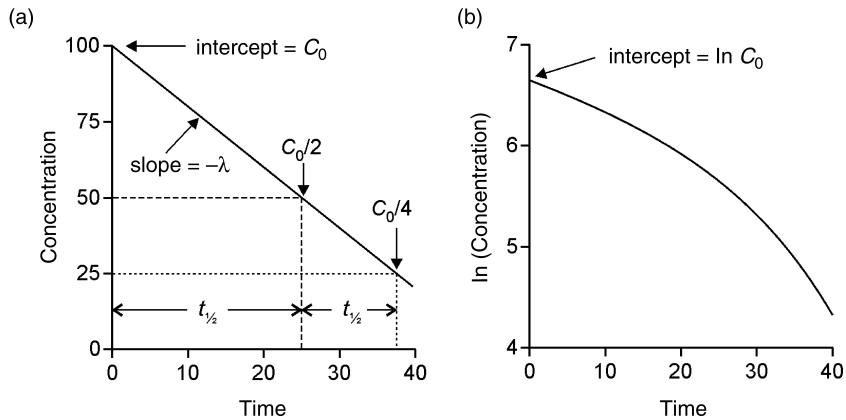


Figure 1.4 Curves for zero-order decay plotted as (a) C versus t and (b) ln C versus t.

1.5.3.3 Second-order decay

When $n \geq 2$, the integral of Equation 1.6 has a general solution, which when written in terms of λ is:

$$\lambda = \frac{1}{(n-1)t}\left(\frac{1}{C^{(n-1)}} - \frac{1}{C_0^{(n-1)}}\right) \tag{1.14}$$

So, when $n = 2$,

$$\lambda = \frac{1}{t}\left(\frac{1}{C} - \frac{1}{C_0}\right) \tag{1.15}$$

Substituting $C = C_0/2$ when $t = t_{1/2}$ and rearranging gives:

$$t_{1/2} = \frac{1}{\lambda C_0} \tag{1.16}$$

This is to be expected of second-order reactions because the probability of molecules colliding and reacting is much greater at higher concentrations.

Equations such as Equations 1.8 and 1.12 are referred to as *linear* equations. Note that in this context it is important not to confuse 'linear' with 'straight-line'. While it is true that the equation of a straight-line is a linear equation, exponential equations are also linear. On the other hand, nonlinear equations are those where the variable to be solved for cannot be written as a linear combination of independent variables. The Michaelis–Menten equation is such an example.

Despite the importance of the elimination half-life of a drug in pharmacokinetics, it is, in fact, dependent on two other pharmacokinetic parameters, apparent volume of distribution (V) and clearance (CL). The apparent volume of distribution, as its name implies, is a quantitative measure of the extent to which a drug is distributed in the body (Section 2.4.1.1) whilst clearance can be thought of as an indicator of how efficiently the body's eliminating organs remove the drug. Therefore the larger the value of CL, the shorter will be $t_{1/2}$. Changes in half-life are a result of changes in either V or CL or both (Section 4.2.1).

1.6 Ionization

Ionization is a property of all electrolytes, whether weak and strong. For example, sodium chloride (NaCl) is essentially completely ionized in aqueous solutions (forming sodium and chloride ions, Na^+ and Cl^-). Amines and carboxylic acids are only partially ionized in aqueous solutions. Their ionization reactions can be represented as follows:

$$R{-}NH_2 + H^+ \rightleftharpoons R{-}NH_3^+$$

$$R{-}COOH \rightleftharpoons R{-}COO^- + H^+$$

It will be noted that these ionization reactions are reversible, and the extent to which ionization takes place is determined by the pK_a of the compound and the pH of the aqueous solution. The pK_a of the compound is a measure of its inherent acidity or alkalinity, and it is determined by the molecular arrangement of the constituent atoms. It is the pH of the aqueous solution in which the compound is 50% ionized.

According to the Brønsted–Lowry theory, an acid is a species that tends to lose protons, and a base is a species that tends to accept protons. Acids and bases ionize in solution; acids donating hydrogen ions and bases accepting them. Thus in the examples above, R-NH_3^+ and R-COOH are acids, while R-NH_2 and R$-COO^-$ are bases. Also, R-NH_3^+ and R-NH_2, and R-COOH and R-COO^-, are termed 'conjugate acid–base pairs'.

The term *strength* when applied to an acid or base refers to its tendency to ionize. If an acid, AH, is dissolved in water, the following equilibrium occurs:

$$AH \rightleftharpoons H^+ + A^-$$

The acid dissociation constant is:

$$K_a = \frac{[H^+][A^-]}{[AH]} \tag{1.17}$$

Clearly the more the equilibrium is to the right, the greater is the hydrogen ion concentration, with a subsequent reduction in the concentration of non-ionized acid, so the larger will be the value of K_a. Taking logarithms (see Appendix 1 for details) of Equation 1.14, gives:

$$\log K_a = \log[H^+] + \log[A^-] - \log[AH] \tag{1.18}$$

and on rearrangement:

$$-\log[H^+] = -\log K_a + \log\frac{[A^-]}{[AH]} \tag{1.19}$$

Because, $-\log[H^+]$ is the pH of the solution:

$$pH = pK_a + \log\frac{[A^-]}{[AH]} = pK_a + \log\frac{[base]}{[acid]} \tag{1.20}$$

where $pK_a = -\log K_a$, by analogy with pH. Note that when $[A^-] = [AH]$ the ratio is 1 and because $\log(1) = 0$, the $pK_a = pH$, as stated earlier.

It is possible to calculate the equilibrium constant, K_b, for a base, B, ionizing in water:

$$B + H_2O \rightleftharpoons BH^+ + OH^-$$

However, one can consider the ionization of the conjugate acid, BH^+ and derive a pK_a for it as above.

$$K_a = \frac{[H^+][B]}{[BH^+]} \tag{1.21}$$

Note how for a strong base, the concentration of BH^+ is high (high tendency to ionize) and so K_a is small.

$$pH = pK_a + \log\frac{[B]}{[BH^+]} = pK_a + \log\frac{[base]}{[acid]} \tag{1.22}$$

The use of terms such as weak and strong is fraught with danger. However, because the term 'weak electrolyte' is used for all partially ionized materials, the term weak should probably be applied to all acids and bases used as drugs. However, it should not be forgotten that high concentrations of organic acids and bases, in spite of the compounds being weak electrolytes, can appear 'strong' in the sense of being corrosive, removing rust, precipitating protein etc. These compounds, however, are obviously distinct from most inorganic acids (nitric, hydrochloric and perchloric acids) which have pK_a values in the range -1 to -7, and are effectively 100% dissociated at any pH. There are, also, certain weak electrolyte inorganic acids; carbonic acid ($pK_{a_1} = 6.35$, $pK_{a_2} = 10.25$) for example. The bicarbonate to CO_2 ratio is of major significance for buffering the pH of blood.

It should be noted that it is not possible from knowledge of the pK_a alone to say whether a substance is an acid or a base. It is necessary to know how the molecule ionizes. Thiopental, $pK_a = 7.8$, forms sodium salts and so must be an acid, albeit a rather weak one. Diazepam, $pK_a = 3.3$, must be a base as it can be extracted from organic solvents into hydrochloric acid. Molecules can have more than one ionizable group; salicylic acid for example, has a carboxylic acid ($pK_a = 3.0$) and a weaker acidic phenol group ($pK_a = 13.4$). Morphine is amphoteric, that is it is both basic (tertiary amine, $pK_{a_1} = 8.0$) and acidic (phenol $pK_{a_2} = 9.9$) (Figure 1.5).

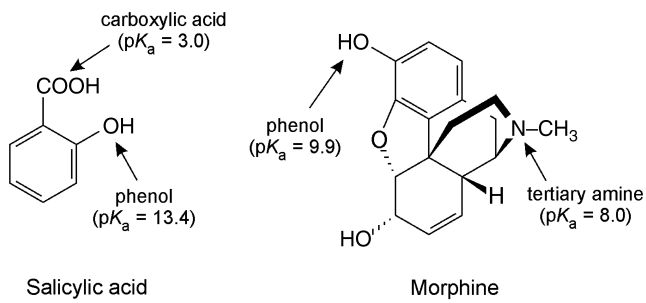


Figure 1.5 Ionizable groups of salicylic acid and morphine.

1.6.1 Henderson–Hasselbalch equation

Equation 1.20 is a form of the Henderson–Hasselbalch equation, which is important in determining the degree of ionization of weak electrolytes and calculating the pH of buffer solutions. If the degree of ionization is α, then the degree non-ionized is $(1-\alpha)$ and, for an acid:

$$\mathrm{pH} = \mathrm{p}K_\mathrm{a} + \log\frac{\alpha}{1-\alpha} \tag{1.23}$$

or

$$\log\frac{\alpha}{1-\alpha} = \mathrm{pH} - \mathrm{p}K_\mathrm{a} \tag{1.24}$$

taking antilogarithms gives:

$$\frac{\alpha}{1-\alpha} = 10^{(\mathrm{pH}-\mathrm{p}K_\mathrm{a})} \tag{1.25}$$

on rearrangement:

$$\alpha = \frac{10^{(\mathrm{pH}-\mathrm{p}K_\mathrm{a})}}{1+10^{(\mathrm{pH}-\mathrm{p}K_\mathrm{a})}} \tag{1.26}$$

The equivalent equation for a base is:

$$\alpha = \frac{10^{(\mathrm{p}K_\mathrm{a}-\mathrm{pH})}}{1+10^{(\mathrm{p}K_\mathrm{a}-\mathrm{pH})}} \tag{1.27}$$

Although Equations 1.26 and 1.27 look complex, they are easy to use. Using the ionization of aspirin as an example: the pK_a of aspirin is ~3.4, so at the pH of plasma (7.4),

$$\mathrm{pH}-\mathrm{p}K_\mathrm{a} = 7.4 - 3.4 = 4$$

$$\alpha = \frac{10^4}{1+10^4} = \frac{10000}{10001} = 0.9999$$

In other words aspirin is 99.99% ionized at the pH of plasma, or the ratio of ionized to non-ionized is 10,000 : 1. In gastric contents, pH 1.4, aspirin will be largely non-ionized; $1.4 - 3.4 = -2$, so the ratio of ionized to non-ionized is $1 : 10^{-2}$, i.e. there are 100 non-ionized molecules for every ionized one.

1.7 Partition coefficients

When an aqueous solution of a substance, such as drug, is shaken with an immiscible solvent (e.g. diethyl ether) the substance is extracted into the solvent until equilibrium between the concentration in the organic phase and the aqueous phase is established. Usually equilibration only takes a few seconds. For dilute

solutions the ratio of concentrations is known as the distribution, or partition coefficient, P:

$$P = \frac{\text{concentration in organic phase}}{\text{concentration in aqueous phase}} \tag{1.28}$$

Organic molecules with large numbers of paraffin chains, aromatic rings and halogens tend to have large values of P, whilst the introduction of polar groups such as hydroxyl or carbonyl groups generally reduces the partition coefficient. Drugs with high partition coefficients are lipophilic or hydrophobic, whereas those that are very water soluble and are poorly extracted by organic solvents are hydrophilic. Lipophilicity can have a major influence on how a drug is distributed in the body, its tendency to bind to macromolecules such as proteins and, as a consequence, drug activity. A relationship between partition coefficient and pharmacological activity was demonstrated as early as 1901, but it was Corwin Hansch in the 1960s who used regression analysis to correlate biological activity with partition coefficient. He chose *n*-octanol as the organic phase and this has become the standard for such studies (Figure 1.6). Because P can vary between <1 (poorly extracted by the organic phase) to several hundred thousand, values are usually converted to log P, to encompass the large range (see Appendix).

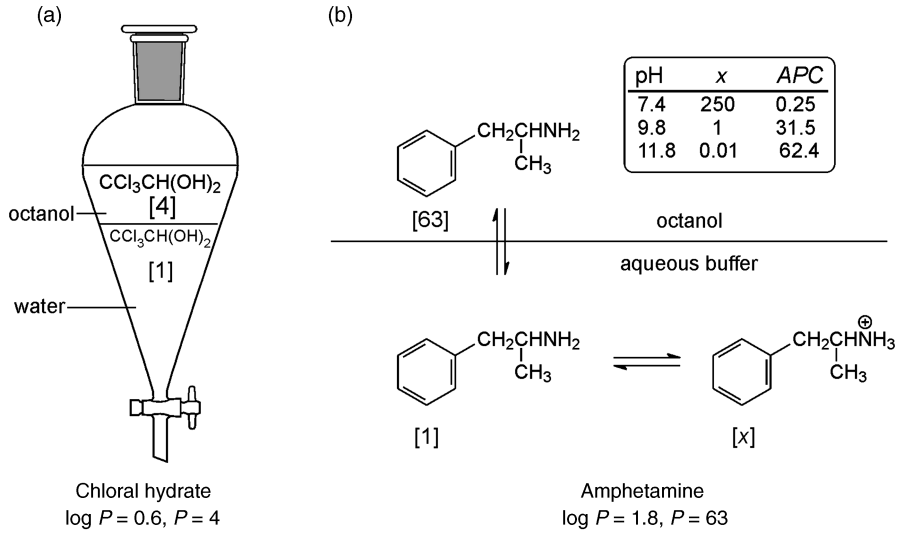


Figure 1.6 (a) Partitioning of chloral hydrate is unaffected by buffer pH. (b) Partitioning of non-ionized amphetamine remains constant, 63 : 1. However the ratio of ionized to non-ionized is affected by buffer pH and as a consequent affects the apparent partition coefficient (*APC*) and the proportion extracted (inset).

1.7.1 Effect of ionization on partitioning

Generally, ionized molecules cannot be extracted into organic solvents, or at least not appreciably. The most notable exception to this is the extraction of ion-pairs into solvents such as chloroform. Thus, for weak electrolytes the amount extracted will usually be dependent on the degree of ionization, which of course is a function of the pH of the aqueous solution and the pK_a of the ionizing group as discussed above (Section 1.5.1), and the partition coefficient. If the total concentration (ionized + non-ionized) of solute in

the aqueous phase is measured and used to calculate an apparent partition coefficient, D, then the partition coefficient, P, can be calculated. For an acid:

$$P = D[1 + 10^{(\mathrm{pH}-\mathrm{p}K_a)}] \tag{1.29}$$

and for a base:

$$P = D[1 + 10^{(\mathrm{p}K_a-\mathrm{pH})}] \tag{1.30}$$

When the pH = pK_a then, because $10^0 = 1$, $P = 2D$. When the pH is very much less than the pK_a, in the case of acids, or very much larger than the pK_a, in the case of bases, there will be no appreciable ionization and then D will be a good estimate of P [Figure 1.6(b)].

Unless stated otherwise, log P is taken to represent the logarithm of the true partition coefficient, i.e. when there is no ionization of the drug. However, for some weak electrolytes, biological activity may correlate better with the partition coefficient between octanol and pH 7.4 buffer solution. These values are referred to as log D.

Differences in the pH of different physiological environments, e.g. plasma and gastric contents can have a major influence on the way drugs are absorbed and distributed. (Section 2.2.1.1).

1.8 Stereochemistry

Compounds with the same molecular formula, but with a different arrangement of atoms are isomers. Structural isomers have different arrangements of atoms, for example, ethanol (CH_3CH_2OH) and diethyl ether (CH_3OCH_3) are structural isomers and have distinct chemical properties. Stereoisomers have the same bond structure but the geometrical positioning of atoms in space differs.

1.8.1 Cis–trans isomerism

Cis–trans isomerism is most commonly encountered when chemical groups are substituted about a double C=C bond. Because the bond is not free to rotate, structures with the substituents on the same side of the bond (*cis*-isomers) are distinct from those with substituents on opposite sides of the bond (*trans*-isomers) [Figure 1.7(a)]. This kind of isomerism can also occur in alicyclic compounds when the ring structure prevents free rotation of C–C bonds. In the *E*/*Z* system of nomenclature, *Z*, from the German *zusammen*

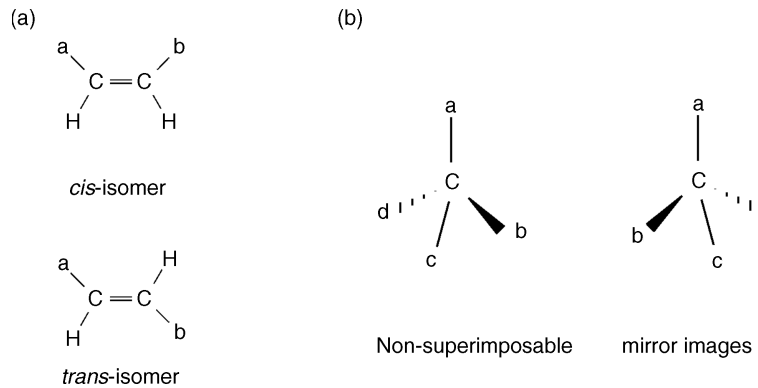


Figure 1.7 (a) *Cis–trans* isomerism occurs because the C=C bond cannot rotate and so the molecules depicted are different. (b) Asymmetric substitution produces isomers that are mirror images. The wedge represents a bond coming out of the page, the broken line represents a bond that recedes behind the page and the carbon atom and other two bonds are in the plane of the page.

meaning together, corresponds to *cis*; whilst *E*, from the German *entgegen* meaning opposite, to *trans*. The *E*/*Z* system must be used when there are more than two different substituents. The groups are assigned a rank according to the Cahn–Ingold–Prelog (CIP) rules (Section 1.8.2). If substituents of higher priority are on the same side, the isomer is designated *Z*; if they are on opposite sides, it is the *E*-isomer. *Cis–trans* isomerism is encountered in pharmacology. For example, clopenthixol is a mixture of *cis*/*trans* isomers whereas zuclopenthixol is the purified *Z*-isomer.

1.8.2 Optical isomerism

When a molecule and its mirror image cannot be superimposed, the substance is said to be *chiral*, from the Greek, *cheir*, meaning hand. The distinctive feature of such molecules is that they rotate the plane of plane-polarized light. Asymmetric substitution about carbon produces optical isomers [Figure 1.7(b)]. Other elements that show optical isomerism include sulfur, phosphorus and nitrogen. Individual isomers are referred to as enantiomers. These can be identified by whether they rotate the light to the right (dextrorotatory) or to the left (laevorotatory). The symbols *d*- and *l*- may be used to indicate the direction of rotation but (+)- and (−)- are preferred. A racemic mixture, or racemate, a 50 : 50 mixture of each enantiomer, is identified by *dl*-, (±)-, or *rac*-. Sometimes it is clear when a drug is an enantiomer from its name, for example the cough suppressant, dextrorphan, and its enantiomer, the analgesic, levorphanol. Often there is no indication, particularly with naturally occurring drugs. Morphine, hyoscine, cocaine and physostigmine are enantiomers. Similarly, many synthetic drugs are marketed as racemates without any indication, but there are examples of deliberate marketing of single isomers, sometimes for reasons connected with patents.

1.8.2.1 Absolute configuration

Although the rotation of plane-polarized light unequivocally defines a compound as one enantiomer or the other, it gives no indication of the spatial arrangement of the groups around the chiral centre – the *configuration*. Until the advent of X-ray crystallography, the absolute configurations of enantiomers were unknown. D-(+)-glyceraldehyde was arbitrarily defined as the D-configuration and all compounds derived from it were designated D-, irrespective of the optical activity, provided that the bonds to the asymmetric carbon remained intact. The naturally-occurring mammalian amino acids in proteins can be related to L-(−)-glyceraldehyde and form an L-series.

The use of D- or L- to define absolute configuration, is not readily applicable to all chiral molecules, and the CIP convention is generally used. The groups are assigned to a priority order according to sequence rules. Simply, the order is determined by the atomic numbers of the atoms attached to the chiral centre, priority being given to the higher numbers; for example O > N > C > H. When groups are attached by the same atom, then the next atom is considered, and so on in sequence until the order has been determined. The arrangement of groups around the chiral centre is 'viewed' with the group of least priority to the rear. Then the spatial arrangement of the groups is determined in decreasing priority order. If the direction is clockwise (i.e. to the right) the configuration is designated *R* (from the Latin, *rectus*, right). If the direction is anticlockwise the configuration is *S* (Latin, *sinister*, left). It must be noted that the D/L and *R*/*S* notations are not interchangeable. Using the CIP system, all L-amino acids are *S*-, with the exception of cysteine and cystine which, because they contain sulfur atoms (higher priority) that are connected to the chiral carbon, are designated *R*-.

1.8.3 Importance of stereochemistry in pharmacology

Obviously, the physical shape of a drug is important for it to bind to its receptors and so elicit a response. Because receptors are proteins, comprised of chiral amino acids, the spatial arrangement of the atoms in the

interacting drug will be crucial. There are numerous examples where stereoisomers show marked differences in their pharmacology. *R*-Thalidomide is sedative whereas the *S*-isomer inhibits angiogenesis, which is probably part of the mechanism of its teratogenic effect. However, because enzymes and transport systems are proteins, stereochemical differences may be shown in the way in which isomers are metabolized or distributed. With the advent of more convenient methods of measuring enantiomers, such as chiral high performance liquid chromatography phases, we are beginning to understand the full extent of differences in the pharmacokinetics of stereoisomers. The interaction between warfarin and phenylbutazone was not fully understood until it was shown that phenylbutazone selectively inhibits the metabolism of the more active *S*-isomer of warfarin.

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2

Drug Administration and Distribution

2.1 Introduction

In order to achieve its effect, a drug must first be presented in a suitable form at an appropriate site of *administration*. It must then be *absorbed* from the site of administration and *distributed* through the body to its site of action. For the effect to wear off the drug must nearly always be *metabolized* and/or *excreted*. These processes are often given the acronym, ADME, and occasionally, LADME, where L stands for liberation of drug (from its dosage form). Finally, drug residues are *removed* from the body (Figure 2.1). Removal refers to loss of material, unchanged drug and/or metabolic products, in urine and/or faeces, once this material has been excreted into the bladder or bowel by the kidneys and liver. Absorption and distribution comprise the *disposition* (placement around the body) of a compound. Metabolism and excretion comprise the *fate* of a compound. It should however be noted that pharmacokineticists sometimes use the word disposition in a slightly different context, invoking concepts of 'disposal'.

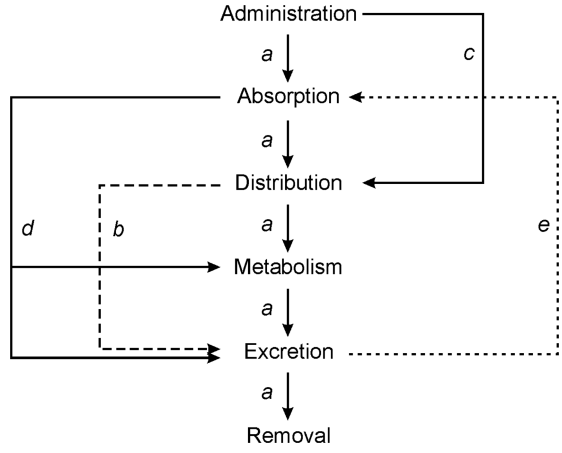

Figure 2.1 General scheme showing the relationship between the various events involved in drug disposition and fate. See text for explanation of letters *a*–*e*.

The most common pathway for an orally administered drug as indicated in Figure 2.1 is by route *a*. This pathway involves metabolism, and excretion of both unchanged drug and metabolites. A drug that is excreted in its unmetabolized form will by-pass metabolism (pathway *b*). An intravenously administered drug undergoes no absorption (pathway *c*). An oral dose can be rapidly converted to its metabolites in the intestinal

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mucosa and/or the portal circulation and then excreted in bile before distribution through the body can occur (pathway *d*) ('pre-systemic metabolism or elimination' or the so-called 'first-pass' effect). Excretion products in the intestine can be reabsorbed (pathway *e*).

2.2 Drug transfer across biological membranes

Absorption, distribution and excretion of drugs involves transfer of drug molecules across various membranes, such as the gastrointestinal epithelium, the renal tubular epithelium, the blood-brain barrier and the placental membrane. Transfer of substances across biological membranes can occur by one or more of five possible mechanisms:

- *Passive diffusion*. Through the membrane, down a concentration gradient.
- *Filtration*. Through pores in the membrane.
- *Active transport*. Involving carrier molecules (transporters), requiring energy and occurring against a concentration gradient.
- *Facilitated diffusion*. A carrier-mediated process that does not require energy and where the net flow is down a concentration gradient.
- *Pinocytosis*. Microscopic invaginations of the cell wall engulf drops of extracellular fluid and solutes are carried through in the resulting vacuoles of water.

Historically, passive diffusion has been viewed as by far the most important for foreign molecules. Filtration is important in the transfer of small molecules into interstitial fluid via the fenestrations in peripheral capillaries and it plays a major part in urinary excretion of drug molecules. Active transport is important for the absorption of a few drugs, but it is involved to a considerable extent in drug excretion processes. Transporters as mechanisms of reducing drug absorption and penetration of areas such as the brain are considered in Section 2.3.1.2. Facilitated diffusion is typified by the co-transport of sodium and glucose, and is the mechanism by which vitamin B_{12} is absorbed from the gastrointestinal tract. The transfer of small foreign compounds across membranes by pinocytosis is largely unknown, but it is believed to be the mechanism by which botulinum toxin is absorbed. Pinocytosis is important in nutrition and may be a mechanism by which small amounts of ionized molecules are absorbed.

Drug transfer will also be affected by the nature of the membrane. The basement membrane of peripheral capillaries has gaps or *fenestrations* through which small molecular mass drugs readily filter. Albumin ($M_r \sim 69{,}000$) is too large to be filtered and is excluded from interstitial fluid. At the glomerulus drugs up to about the size of albumin are freely filtered, while the fenestrations in the liver sinusoids are large enough for macromolecules such as lipoproteins to be filtered. Other membranes, notably those of the GI tract and the placenta do not have fenestrations. The observation from early studies that certain dyes did not enter the brain, led to the concept of the blood–brain barrier (BBB) – specialized capillaries with tightly packed endothelial cells. For these membranes drugs must traverse them either by passive diffusion or specialized carrier-mediated transport.

2.2.1 Passive diffusion

The rate of diffusion of a drug, dQ/dt, is a function of the concentration gradient across the membrane, ΔC, the surface area over which transfer occurs, A, the thickness of the membrane, Δx, and the diffusion coefficient, D, which is characteristic of the particular drug. The diffusion coefficient of a compound is a function of its solubility in the membrane, its relative molecular mass, and its steric configuration. Thus, the rate of diffusion is governed by Fick's law:

$$\frac{dQ}{dt} = RDA\frac{\Delta C}{\Delta x} \tag{2.1}$$

where R is the partition coefficient of the drug between the membrane and the aqueous phase. Because A, D, R and the distance over which diffusion is occurring are constants these can be combined into one constant, permeability, P, so that:

$$\text{rate of diffusion} = P\Delta C = P(C_1 - C_2) \tag{2.2}$$

2.2.1.1 pH-partition hypothesis

As far as foreign molecules are concerned, biological membranes behave as if they are simple lipid barriers. This is remarkable, in view of the obvious complexity of such membranes, but it should be remembered that they exist primarily to transfer nutrients, not to transfer drugs. Their function, as far as foreign molecules are concerned, is more likely to be one of exclusion than one of transfer, so that in a sense drugs are absorbed against the odds. The fact that drugs pass through biological membranes mostly by simple diffusion has been repeatedly verified in experimental work. In this transfer, drug molecules have to dissolve in the membrane and so lipophilic species diffuse freely, but polar, particularly ionized, molecules do not. Therefore whether a weak electrolyte is ionized or not, will be a major determinant of whether it will diffuse through a biological membrane. The ratio of ionized to non-ionized forms is a function of the pH of the aqueous environment and the acid dissociation constant, K_a, as explained in Section 1.6. This means that in any aqueous solution both ionized and non-ionized forms are present.

Calculation of the equilibrium distribution of aspirin between stomach contents and plasma water illustrates the importance of pH-partitioning (Figure 2.2). It will be readily appreciated that, at equilibrium,

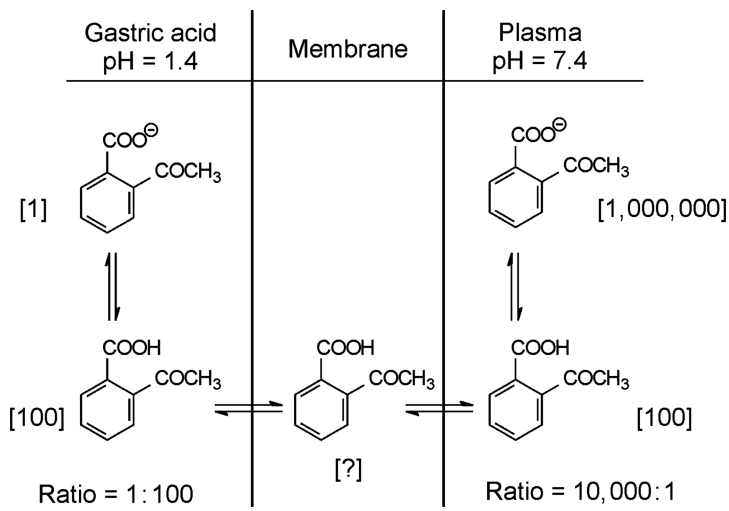

Figure 2.2 Equilibrium distribution of aspirin ($pK_a = 3.4$) between two solutions of pH 1.4 and pH 7.4 separated by a simple membrane. Relative concentrations are shown in [ ].

for the *non-ionized* species (which alone passes the lipid membrane) the concentration ratios will be the same:

$$\frac{\text{Concentration in membrane}}{\text{Concentration in gastric acid}} = \frac{\text{Concentration in membrane}}{\text{Concentration in plasma water}}$$

Thus for the non-ionized species the concentrations on each side of the membrane will be identical. For convenience we have designated this concentration [100]. However, from the Henderson–Hasselbalch equation (Section 1.6.1) the ratio of ionized to non-ionized aspirin at pH 1.4 is 1 : 100. In other words, for

every [100] non-ionized molecules of aspirin there is [1] ionized molecule. In the plasma (pH 7.4), aspirin will be highly ionized, the ratio being 10,000 : 1, i.e. for every 100 molecules of non-ionized aspirin there will be $10{,}000 \times 100 = 1{,}000{,}000$ molecules ionized. Therefore at equilibrium, the ratio of total material on the two sides will be:

$$\frac{\text{Total concentration in acid}}{\text{Total concentration in plasma}} = \frac{100+1}{1\,000\,000+100} = \frac{101}{1\,000\,100} \approx \frac{1}{9900}$$

or almost 10,000 to 1 in favour of plasma. If drug molecules are introduced into any part of the system, they will transfer between the various media, including the membrane, until this concentration ratio is achieved.

2.3 Drug administration

The route of drug administration will be determined by the nature of the drug and the indication for its use.

2.3.1 Oral administration

This route is popular as it is generally convenient, and requires no medical skill or sterile conditions. Thus it is appropriate for outpatient use and medicines bought over the counter (OTC). For those with difficulty swallowing tablets or capsules, the elderly or infants for example, the drug may be may be given as a solution or suspension in liquid.

However, the gastrointestinal (GI) tract is a harsh environment. Gastric pH is low and acid-labile drugs such as benzylpenicillin (penicillin G) and methicillin are inactivated. These drugs may be used in the very young or the elderly in whom gastric pH is higher, but generally they are given by injection. The presence of proteases makes the oral route unsuitable for proteins and peptides such as insulin and oxytocin.

Absorption occurs chiefly by passive diffusion of lipophilic molecules and active transport of drugs that are endogenous, levodopa, for example, or those that are structurally similar to endogenous compounds, such as the cytotoxic agent 5-fluorouracil. With passive diffusion the rate of absorption is proportional to concentration or amount of drug to be absorbed and the *fraction* absorbed in a given interval remains constant. With carrier-mediated mechanisms, active transport or facilitated diffusion, there is a limited capacity and the transporter can be saturated (Figure 2.3).

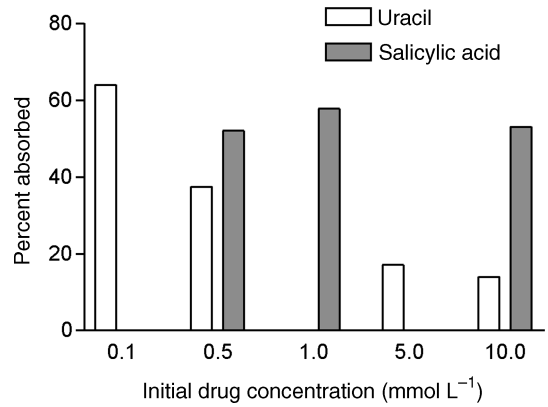

Figure 2.3 Absorption of uracil and salicylic acid from the rat small intestine as a function of initial drug concentration. Increasing concentrations of uracil saturate the active carrier so the proportion absorbed decreases, whereas for salicylic acid, which is absorbed by passive diffusion, the proportion absorbed is independent of the concentration. (Redrawn from the data of Brodie, in Binns, 1964.)

Being largely non-ionized in acid, aspirin can be absorbed from the stomach (Section 2.2.1.1), however most of the absorption occurs in the small intestine where the large surface area compensates for the less favourable degree of ionization. Weak bases cannot be absorbed until they have left the stomach, so delayed gastric emptying can delay the effect of such drugs. Quaternary ammonium compounds (Figure 2.4), which

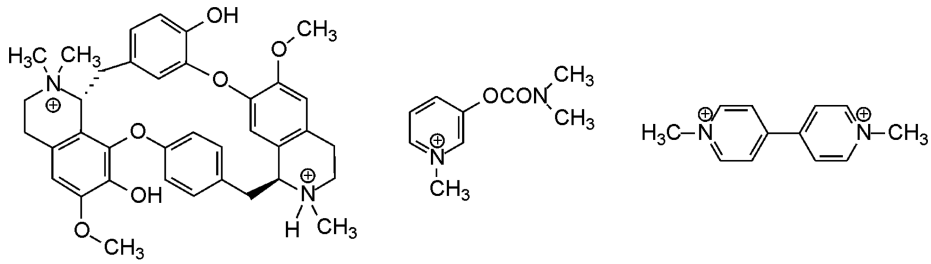

Figure 2.4 Some quaternary ammonium compounds.

are permanently ionized, would not be expected to be absorbed to an appreciable extent by passive diffusion and this appears to be the case. (+)-Tubocurarine, the purified alkaloid from the arrowhead poison, curare, is not absorbed and the oral absorption of pyridostigmine is very low and erratic, such that an oral dose is typically 30 to 60 times larger than an equivalent intravenous one. Of toxicological importance is the absorption of the diquaternary ammonium herbicide, paraquat. Its absorption is saturable which supports the hypothesis that a carrier (probably for choline) is involved.

2.3.1.1 Presystemic metabolism

Materials absorbed from the stomach and intestine are carried, via the mesenteric capillary network and the hepatic portal vein, to the liver. If the drug is largely metabolized as it passes through the liver, then little of it will reach the systemic circulation. The consequences of this *first-pass metabolism*, as it is sometimes known, will depend on whether or not the metabolites are pharmacologically active. In the case of glyceryl trinitrate (GTN, nitroglycerin), which is almost totally metabolized, the di- and mono-nitrate metabolites have very reduced activity and generally this drug is considered to be inactive when taken orally, although some clinical trials have confirmed at least some clinical value of high dose sustained-release oral preparations. GTN is generally given by more suitable, alternative routes. The extent to which a drug undergoes presystemic metabolism can be obtained by comparing the plasma concentration–time curves and areas under the curves (*AUC*), after an oral and an intravenous dose of the drug (Section 8.3, Equation 8.2). This is illustrated for chlorpromazine in Figure 2.5, where the systemic availability of chlorpromazine is markedly reduced after oral administration, as evidenced by the reduced $AUC_{\text{p.o.}}$ compared to the area under the intravenous curve, $AUC_{\text{i.v.}}$. The low oral bioavailability is known to be largely due to presystemic metabolism, because studies with radioactive drug and related phenothiazines show that radioactive metabolites appear in the systemic circulation.

First-pass metabolism may influence the relative proportions of metabolites produced, as with propranolol, for example. When given orally, pharmacologically active 4′-hydroxypropranolol is produced, however little of this metabolite is measurable when propranolol is given intravenously. It has been suggested that this

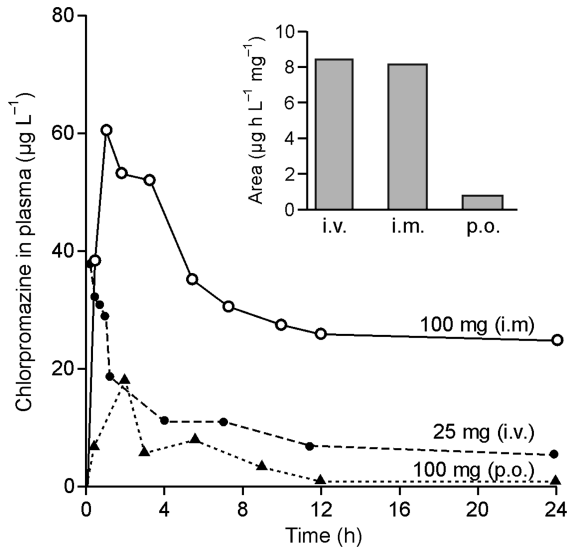

Figure 2.5 Plasma concentrations of chlorpromazine after three routes of administration and the areas under the curves after normalization for dosage (inset).

is because the high concentrations of propranolol reaching the liver after oral administration saturate the pathway that produces naphthoxylacetic acid, a major metabolite after intravenous (i.v.) injection. Comparison of the *AUC* values after oral and intravenous doses support this supposition (Figure 2.6). The

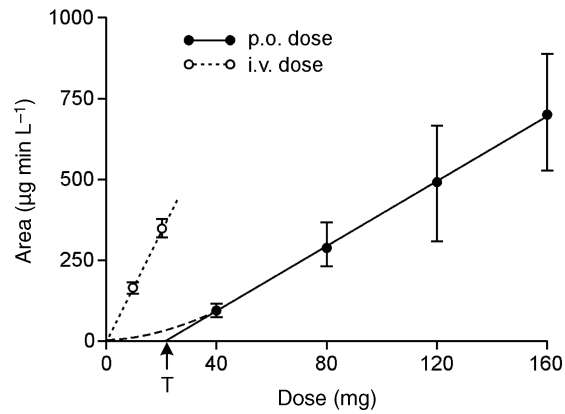

Figure 2.6 *AUC* values as a function of propranolol dose. The data provide evidence of saturable first-pass metabolism, with an apparent threshold dose (T) being required before any propranolol is measurable in plasma. (Redrawn from Shand & Rangno, 1972).

amount of drug reaching the systemic circulation is very reduced when given orally and, furthermore, there appears to be a threshold dose of approximately 20 mg below which little or no propranolol is measurable in the plasma, suggesting that at low doses all of the dose may be metabolized and that higher doses saturate some of the drug metabolizing pathways. When given to a patient with portocaval anastomosis the drug bypassed the liver and was completely available to the systemic circulation and no threshold was observed.

Saturation of first-pass metabolism may apply to other drugs that are normally extensively metabolized as they flow through the liver.

Drugs may be metabolized before they even reach the liver. GI tract mucosal cells contain several drug-metabolizing enzymes, probably the most important being cytochrome P450 3A4 (CYP3A4; Section 3.2.1.1). As described above, presystemic metabolism may render a drug inactive, or larger doses of a drug may have to be administered when given orally. On the other hand, prodrugs, that is drugs that are inactive until they have been metabolized (Section 3.2.1.1), will be activated by presystemic metabolism. Dose for dose such drugs may be more pharmacologically active when given orally than by other routes.

Inhibition of presystemic metabolism can have important consequences, including dangerous drug–drug or drug–food interactions (Section 17.6.2).

2.3.1.2 P-Glycoprotein

Not only are drugs metabolized by intestinal cells, drug that is not metabolized may be returned to the gut lumen by efflux. P-Glycoprotein (P-gp) transports substrates from the intracellular to the extracellular side of cell membranes. The gene expressing P-gp was first recognized in tumour cells and it was originally referred to as the multi-drug resistance (*MDR1*) gene. It is now known that the gene is highly expressed in the apical membrane of enterocytes lining the GI tract, renal proximal tubular cells, the canalicular membrane of hepatocytes, and other important blood–tissue barriers such as those of the brain, testes and placenta. Its location suggests that it has evolved to transport potentially toxic substances out of cells. P-gp is inducible and many of the observations that were once ascribed to enzyme induction or inhibition (Section 17.4) may in fact be due to changes in P-gp activity.

2.3.1.3 Gastrointestinal motility and splanchnic blood flow

Absorption is facilitated by thorough mixing of the drug within the gastrointestinal tract. Mixing increases the efficiency with which the drug is brought into contact with surfaces available for absorption. Therefore, in some instances, a reduction in gastrointestinal motility, for example by opiates or antimuscarinic drugs, may reduce absorption, including their own. On the other hand, excessive motility and peristalsis will reduce the transit time and this may be critical for drugs that are slowly absorbed or are only absorbed from particular regions of GI tract. The most favourable site for the majority of drugs is the small intestine with its large surface area due the presence of microvilli – sometimes referred to as the 'brush-border' because of its appearance. The transit through this part of the GI tract is usually about 3 to 4 hours. The surface area for absorption decreases from the duodenum to the rectum although the transit time in the large bowel is generally longer, 12–24 hours, possibly more. It is thought that some drugs are absorbed from particular regions, for example part of an oral dose of drug may be absorbed for 3 to 4 hours with little more appearing in the blood after that time. The remaining portion of the dose is expelled in the faeces. Increased intestinal motility may reduce the absorption of such a drug because the time it is in the optimal region for absorption is reduced. However, a drug that is rapidly and extensively absorbed from the duodenum will be less affected. Therefore it is sometimes difficult to predict how changes in GI motility will affect the oral availability of a drug.

Splanchnic blood flow will affect the rate of removal of the drug from the site of absorption, as blood flow and transport of drugs by plasma proteins is the major mechanism by which drugs are carried away from their sites of absorption and around the body. The blood flow to the GI tract, which represents approximately 30% of the cardiac output, is lower during the fasting state than after feeding. Weight for weight, the mucosa of the small intestine receives the largest proportion of the flow, followed by that of the colon and then the stomach.

Gastric emptying, or rather the lack of it, such as with the pyloric stenosis that often follows surgery, can have a major influence on oral availability. Tablets and capsules, particularly those designed not to release their contents until they reach the intestine will be trapped.

2.3.1.4 Food and drugs

Food may have a major, but not always predictable, effect on oral availability. Food generally delays gastric emptying but, as discussed above, increases splanchnic blood flow. It is often assumed that food delays absorption without necessarily reducing it. The absorption of griseofulvin, a very poorly soluble antifungal drug, is increased when taken with a 'fatty' meal. The constituents of a meal may interfere with the processes of absorption, such as the components of grapefruit juice (Section 17.6.2), or they may interact directly with the drug. Tetracylines chelate divalent metal ions to form unabsorbable complexes, and so their absorption is reduced by milk (high in calcium), magnesium containing antacids, and ferrous sulfate.

From the foregoing, it should be obvious that concomitant use of other drugs can affect oral absorption in a number of ways, including changing gastric pH or gut motility, or by forming unabsorbable complexes. Yet despite these problems (Figure 2.7), the oral route is the preferred one for outpatient and OTC medicines.

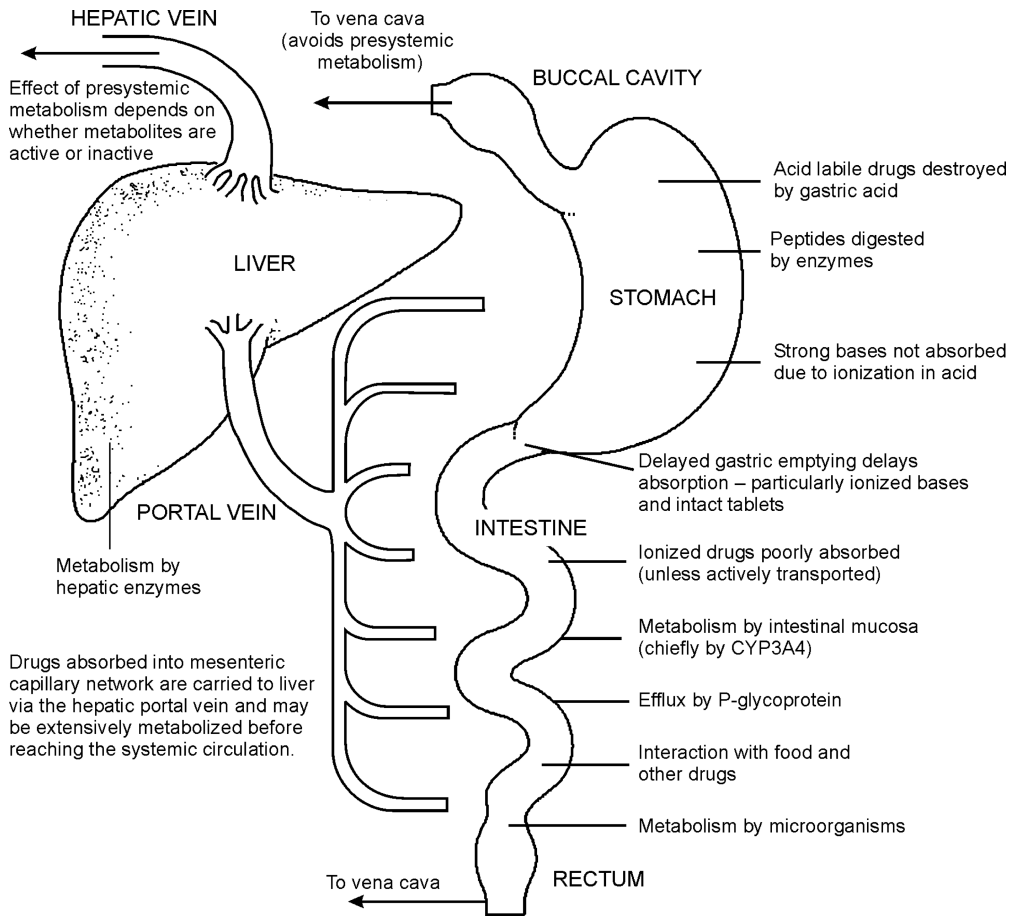

Figure 2.7 Some of the factors that may reduce oral availability. Other factors include GI motility, absorptive surface area and splanchnic blood flow.

2.3.2 Sublingual administration

Tablets are chewed or crushed and held under the tongue for the drug to be absorbed from the mouth. This occurs by diffusion and although the surface area for absorption is small, absorption occurs rapidly. Furthermore, materials absorbed across the buccal membrane avoid the hepatic portal system so this route is useful for drugs such as GTN with which a rapid effect is desired and which can be destroyed in the gastrointestinal tract before absorption or by presystemic metabolism (Section 2.3.1.1, Figure 2.7). Because GTN is volatile and may be lost from tablets, limiting its shelf-life, it is available as an aerosol for spraying into the mouth as a metered dose. Buprenorphine is available as a lozenge that can be held in the mouth to give sustained release of this potent opioid. Most orally administered drugs are deliberately formulated as tablets and capsules that are swallowed, thereby avoiding contact with the oral mucosa.

2.3.3 Rectal administration

Substances absorbed from the lower part of the rectum are carried to the vena cava and so avoid first-pass metabolism. However, this is not the chief reason for choosing this route of administration. The surface area for absorption is not especially large, but the blood supply is extremely efficient, and absorption can be quite rapid. This is considered sufficiently advantageous to warrant extensive use of this route of administration for a wide range of drugs in certain countries. Apart from situations in which a local effect is sought,in the United Kingdom suppositories tend to be used for more specialized purposes, such as the antiemetic, prochlorperazine (there is little point in taking it orally when one is vomiting). Diazepam suppositories are available for use in epileptic infants, when insertion of an intravenous cannula might be considered dangerous. Slow-release aminophylline is given rectally, often at night, to ease breathing in asthmatic children. Disadvantages of this route, apart from patient acceptability, include local irritation and inflammation and the possibility that the patient may need to defecate shortly after insertion of the suppository.

2.3.4 Intravenous and intra-arterial injections

Injecting a drug directly into the circulation avoids any problems of absorption and usually produces the most rapid onset of effects of any route. Peak blood concentrations occur immediately after a rapid 'bolus' injection, but if this presents a problem or a sustained effect is required, then the drug can be given as a slow intravenous infusion, over several minutes, hours or even days. For the longer periods, portable (ambulatory) pumps are available. These have been used to deliver insulin, opiates (usually in terminally ill patients), for treatment of iron poisoning and for the treatment of certain cancers, amongst other applications.

Intravenous injections are not used simply to overcome problems that may be encountered using other routes, but when the i.v. route is the most appropriate. Examples include the i.v. general anaesthetics (thiopental, propofol), muscle relaxants (tubocurarine, suxamethonium) and neostigmine (to reverse the effects of tubocurarine-like drugs); all these drug examples are used during surgery.

Intra-arterial injections are more specialized and are typically used in the treatment of certain tumours. By injecting a cytotoxic drug into an appropriate artery the drug is carried directly to the tumour.

Disadvantages of i.v. injections include the fact that sterile preparations and equipment are required. A high degree of skill is required, particularly to prevent extravasation (i.e. injection near to the vein or leakage from it), which can lead to serious tissue damage.

2.3.5 Intramuscular and subcutaneous injections

Some of the problems of low oral bioavailability can be avoided by intramuscular (i.m.) or subcutaneous (s.c.) injections. Unlike bolus i.v. injections there is no immediate peak plasma concentration as the drug has

to be absorbed from the injection site. Drugs, including ionized ones, enter the systemic circulation via the fenestrations in the capillary walls. Size does not appear to be a limiting factor up to approximately $M_r \sim 5000$, although the absorption is flow dependant and increasing local blood flow, for example by warming and massaging the injection site can increase the rate of absorption. The rate of absorption can be delayed by co-injection of a vasoconstrictor to reduce blood flow to the area; for example the use of adrenaline to prolong the effect of a local anaesthetic.

The rate of absorption may be faster or slower than that following oral administration. Absorption after i.m. injection of chlordiazepoxide or diazepam may be delayed because the drugs precipitate at the injection site. Some i.m. preparations may be formulated to provide sustained-release from the injection site; for example microcrystalline salts of penicillin G (i.m.) and various insulin preparations (s.c.).

Sterile preparations and equipment are required but as they require less skill, patients or their carers can be trained to perform i.m or s.c. injections.

2.3.6 Transdermal application

The epidermis behaves as a lipoprotein barrier while the dermis is porous and permeable to almost anything. Consequently, lipophilic molecules penetrate the skin easily and rapidly, whilst polar, ionized molecules penetrate poorly and slowly. For many years it has been known that chlorinated solvents and some organic nitro compounds were potentially toxic because they are rapidly absorbed through the skin. More recently, several pharmacological preparations, designed for absorption across the skin have been introduced. These may be in the form of ointments and creams to be rubbed on to the skin or patches to be stuck on. Some of the patches incorporate a rate-limiting membrane to ensure a steady, sustained-release of the drug. Generally the drugs have to be potent as well as lipophilic as large doses would be problematic. Examples of drugs applied to the skin for systemic effects include glyceryl trinitrate, hyoscine, buprenorphine, steroids (contraceptives and hormone replacement) and nicotine.

2.3.7 Insufflation

The nose with its rich blood supply, highly fenestrated capillaries and an epithelium with gaps around the goblet cells allows absorption of drugs that cannot be given orally. Peptide hormones, insulin, calcitonin and desmopressin can be given as nasal sprays. Furthermore, it has been suggested that some drugs can enter the central nervous system (CNS) directly via the nose and this route is being investigated for molecules which do not normally enter the brain.

2.3.8 Inhalation

Volatile and gaseous general anaesthetics are given via the lungs; their large surface area gives rapid absorption and onset of effect. Because most of these anaesthetics are also excreted via the lungs, the level of anaesthesia can be controlled by adjusting the partial pressure of the drug in the apparatus used to give it.

Other drugs frequently given by inhalation are those to relieve bronchiolar constriction in asthmatic patients. The β-adrenoceptor agonists, salbutamol and terbutaline, and the antimuscarinic drug, ipratropium, are examples. Applying these agents directly to the lungs gives rapid relief, reduces the dose of drug required and so lessens the severity of any systemic adverse effects. Disodium cromoglycate is very poorly absorbed when given orally but is absorbed into lung tissue after inhalation as a fine dry powder.

2.3.9 Other routes of administration

Drugs may be applied to various other sites, usually for a local effect. Intravaginal applications of antifungal creams are usually for local effects against *Candida albicans*. Prostaglandin pessaries may used to induce

labour. Lipophilic drugs such as physostigmine, pilocarpine and timolol are absorbed across the cornea and used to treat glaucoma. Tropicamide eye-drops may be used to dilate the pupil to aid ophthalmic examinations.

Drugs are generally well absorbed from the peritoneal cavity and although this route is rarely used in human beings, intraperitoneal injection (i.p.) is a convenient method for dosing laboratory animals.

2.4 Drug distribution

The majority of drugs have to be distributed to their site(s) of action. Only rare examples such as anticoagulants, heparin and the like, which have their effects in the bloodstream, do not. Drugs are carried by the circulation, often bound to plasma proteins from where they equilibrate with their sites of action or other storage sites (Figure 2.8).

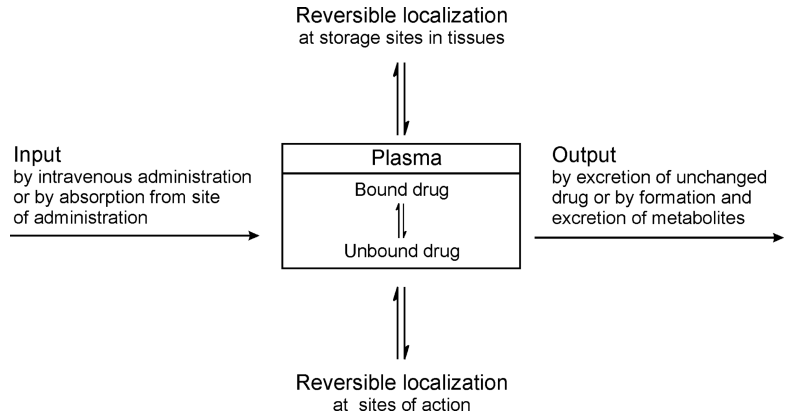

Figure 2.8 Scheme illustrating the key role of plasma in transporting drugs to sites of action, other storage sites and sites of elimination.

2.4.1 Extent of distribution

The extent and rate at which a drug is distributed is dependent on its physiochemical characteristics. Small lipophilic drugs that readily penetrate membranes are generally widely distributed, whereas polar, ionized ones and macromolecules are often contained in particular anatomical volumes. The body is made up of approximately 60% water, 18% protein, 15% fat and 7% minerals. Body water can be subdivided into that in the cells [intracellular water (ICF, 40% of total)] and the remaining extracellular water (ECF, 20%), which can be subdivided further into interstitial fluid (15%) and plasma (5%). The blood volume is 9% of total body water (TBW); the 4% of body water associated with the red cells is part of the intracellular volume.

2.4.1.1 Apparent volume of distribution

The extent of distribution of a drug is quantified by a parameter known as the apparent volume of distribution (V). This can be defined as 'the volume of fluid that is required to dissolve the amount of drug in the body (A) to give the same concentration as that in plasma at that time'. In other words:

$$V = \frac{A}{C} \tag{2.3}$$

where C is the plasma concentration. Immediately following a rapid intravenous injection of drug, the amount in the body is, for all intents and purposes, the dose, D, that has been injected. Therefore, if the plasma concentration was known at this time then the volume could be calculated. The practical situation is complicated because time has to be allowed for the drug to mix within the circulation and be distributed. Therefore, it is usual to measure the plasma concentrations over a period of time and to plot them, or ln C, against time (Figure 1.3) and to extrapolate to $t = 0$ to obtain a value of C_0, from which V can be calculated:

$$V = \frac{D}{C_0} \tag{2.4}$$

Although C_0 may be referred to as the concentration at time zero, it is the theoretical plasma concentration that would occur if it were possible for the drug to be distributed instantaneously, which of course it cannot.

Compounds such as Evans' blue, inulin and isotopically labelled water can be used to measure the volumes of plasma, ECF and TBW, respectively. Evans' blue binds so avidly to albumin that it does not leave the plasma. Inulin, a water-soluble polysaccharide does not enter cells and so can be used to estimate ECF. Of course, isotopically labelled water, whether ^2H_2O or ^3H_2O, distributes in TBW. Apparent volumes of distribution may give some indication of where a drug may be distributed (Table 2.1). Heparin is too large to

Table 2.1 Examples of apparent volumes of distribution

Compound	V (L kg^{-1})[a]	Notes
Evans' Blue[b]	0.05	Dye to measure plasma volume
Heparin	0.06	Macromolecule – cannot enter interstitial fluid
Inulin	0.21	Used to measure ECF
Penicillin G	0.2	Does not penetrate cells
Tubocurarine	0.2	Quaternary ammonium compound
Deuterium oxide (D_2O)	0.55–0.65	Isotopic labelled water – to measure TBW
Ethanol	0.65	Distributes in TBW
Antipyrine (phenazone)	0.6	Used to assess enzyme induction (Chapter 17)
Digoxin	5	Binds to Na^+/K^+ ATPase
Chlorpromazine	20	
Amiodarone	62	Little found in the CNS
Quinacrine	500	Intercalates in DNA

[a] Normalized to body weight.
[b] Compounds in italics used to measure anatomical volumes.

pass through the fenestrations in the peripheral capillaries and so is confined to plasma. Several drugs including the penicillins and tubocurarine do not readily enter cells but are small enough to filter into interstitial fluid. Drugs which can cross lipid cell membranes but are not concentrated (sequestered) in cells nor bound to plasma proteins have volumes approximately equal to those of TBW. Ethanol and the now obsolete antipyretic drug, antipyrine (phenazone), are examples of such compounds. Centrally acting drugs generally have to be lipophilic enough to cross the BBB and often have apparent volumes of distribution > 1 L kg^{-1}. However, the converse is not true, a drug such as digoxin has a large value of V because it binds to cardiac and skeletal muscle Na^+/K^+ ATPase.

2.4.2 Mechanisms of sequestration

Sequestration of drugs in various parts of the body arises because of differences in pH, binding to macromolecules, dissolution in lipids, transportation (usually against the concentration gradient), and what may be termed 'irreversible' binding.

2.4.2.1 pH differences

Local differences in pH may lead to high concentrations of weak electrolytes in one area relative to another because of differences in the degree of ionization. This is predictable from pH-partition considerations (Section 2.2.1.1.). For example the ionized to non-ionized ratio of salicylic acid ($pK_a = 3.0$) at pH 6.8 (intracellular pH) is 6,300 : 1 whereas at the pH of plasma (7.4) the ratio is 25,000 : 1. This represents a ratio of ~4 : 1 in favour of plasma water and is true for any weak acid. The converse is the case for bases, when the difference in intracellular and plasma pH means that bases will be distributed ~4 : 1 in favour of the more acidic fluid.

2.4.2.2 Binding to macromolecules

Drugs may be concentrated by binding to several types of macromolecules: plasma proteins, tissue proteins, including enzymes, and nucleic acids. Quinacrine and chloroquine are concentrated in tissues because of binding to DNA. Chlorthalidone binds to red cell carbonic anhydrase, whilst anticholinesterases such as neostigmine and pyridostigmine bind to red cell acetylcholinesterase. Binding to plasma proteins, which can lead to important differences in distribution and hence pharmacological activity is discussed in Section 2.5.3. The possible effects of protein binding on drug kinetics are considered in Section 7.4.1.

2.4.2.3 Dissolution in lipids

Lipophilic drugs are often concentrated in lipid cell membranes and fat deposits. This is thought to be simple partitioning, analogous to solvent:water partitioning. The distribution of thiopental in adipose tissue is an important determinant of its duration of action (Section 2.4.3.1).

2.4.2.4 Active transport

As mentioned earlier (Section 2.2) compounds that are structurally similar to endogenous molecules may be substrates for transport proteins. Guanethidine (and probably other adrenergic blocking drugs) is concentrated in cardiac tissue by active uptake. Amphetamine and other indirectly acting sympathomimetic drugs are actively transported into aminergic nerves. Paraquat is concentrated in the lungs because it is a substrate for a putrescine transport protein.

2.4.2.5 Special processes

This refers to binding processes which, for all intents and purposes, can be considered as irreversible. Examples include deposition of tetracyclines in bone and teeth and of drugs in hair. These areas have very poor blood supply so that penetration is slow and loss of drug by diffusion back to the blood is in effect zero. It is probable that drug residues are laid down as hair and teeth are formed, in areas of rich blood supply, and the deposits are carried away from these areas as the tissues grow.

Other examples of irreversible binding include covalent binding in tissues with a good blood supply. Such localization usually involves only a small proportion of the total amount of drug in the body but it is particularly important as a mechanism of drug toxicity (Chapter 18).

2.4.3 Kinetics of distribution

Drugs carried in the bloodstream will penetrate those tissues which they can, net transfer being down the concentration gradient, a process sometimes referred to as ‘random walk’. A drug placed at any point within

the system will diffuse backwards and forwards until characteristic equilibrium concentration ratios are reached (Figure 2.9). Within equilibrium of course, diffusion continues, but the relative concentrations at the various points do not change. Removal of drug from ECF, by metabolism and excretion, reduces the plasma concentration so that net movement is now from tissues to plasma.

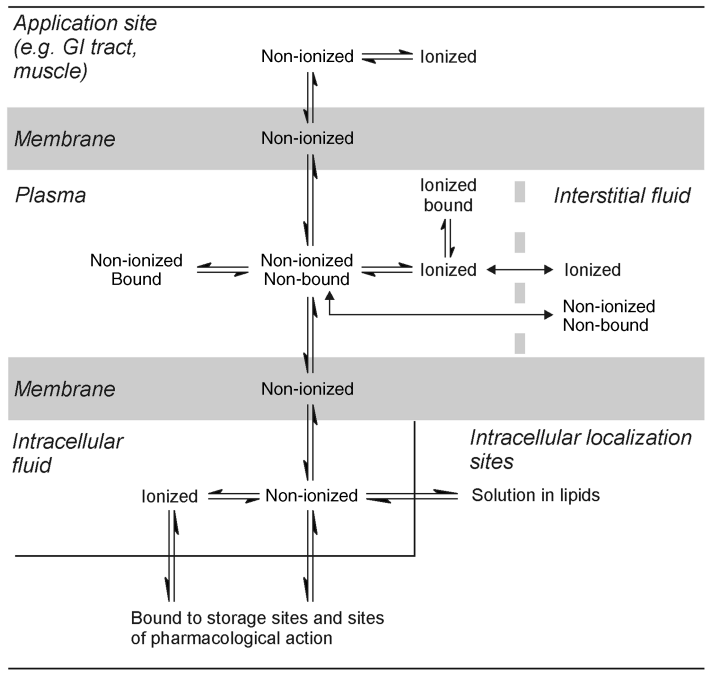

Figure 2.9 Simplified diagram for the equilibrium distribution of an ionizable drug of small relative molecular mass. The drug readily filters into interstitial fluid so the unbound concentrations are equal to those in plasma.

The result of tissue localization is a pattern throughout the body of concentrations in each tissue higher than concentrations in plasma by varying amounts. Highest concentrations are commonly found in liver, lung and spleen, but the significance of this, except perhaps in regard to drug metabolism and excretion by the liver, remains obscure. The speed with which a particular ratio is achieved is governed by the nature of the drug and the tissues in which it is distributed. A highly ionized drug like pyridostigmine rapidly enters interstitial fluid via capillary fenestrations and, because it does not enter cells, the equilibration time is very short. Because lipophilic drugs rapidly diffuse across cell walls, the rate-limiting step for equilibration is delivery of the drug to the tissue, that is, it is flow-limited. Thus the rate of equilibration will be a function of the vascularity of the particular tissue and is rapidly established with well-perfused tissues such as kidney, liver, lung and brain. Muscle is intermediate, in that a rising ratio can often be detected. Poorly perfused adipose tissue can require many hours for equilibrium to be achieved. Presumably drugs of intermediate lipophilicity will show a mixture of flow-limited and diffusion-limited equilibration. A number of drugs has been studied in detail with regard to tissue distribution.

2.4.3.1 Tissue distribution of thiopental

Thiopental is a lipophilic barbiturate which is used as a short-acting general anaesthetic. Brodie and his colleagues investigated the distribution of this drug in the 1950s as part of their studies to explain why repeated, or higher, doses gave disproportionate increases in duration of action (Brodie *et al*., 1952, 1956; Brodie and Hogben, 1957; Brodie, 1967; Mark *et al*., 1957). Their work made a major contribution to the understanding of the kinetics of drug distribution. After an intravenous injection in a dog, plasma and liver thiopental concentrations fell rapidly. The log(concentration)–time plots were parallel throughout the sampling period, showing that the liver:plasma concentrations had equilibrated by the time of the first sampling point [Figure 2.10(a)].

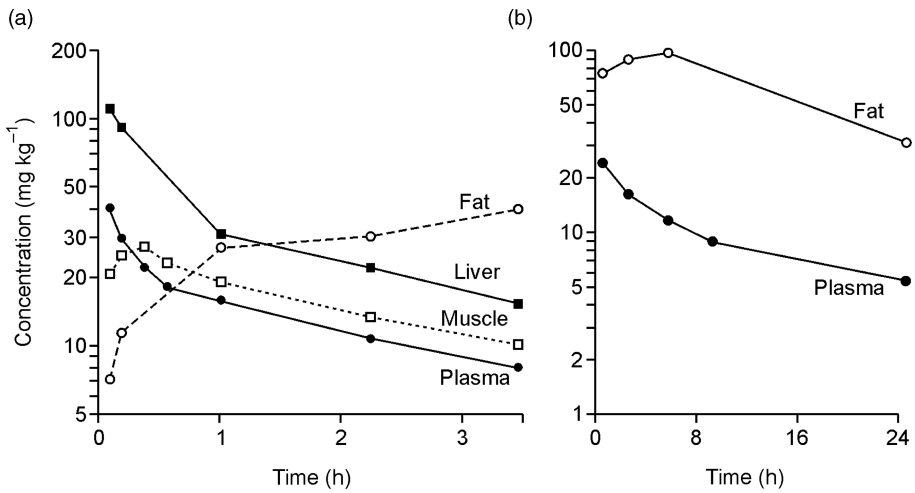

Figure 2.10 Concentrations of thiopental (logarithmic scale) in the plasma and various tissues of a dog after intravenous administration of 25 mg kg^{-1}. (a) 0–3.5 h (b) 0–24 h (Brodie *et al.*, 1952).

The concentration in skeletal muscle rose over the first 30 minutes and had equilibrated by about 1 hour after which the muscle to plasma ratios remained constant. Thiopental concentrations in fat continued to rise for several hours, equilibrated and then declined in parallel with the plasma concentration when plotted on a semi-logarithmic plot. [Figure 2.10(b)]. A slow rate of return from fat to plasma is responsible for the slow decline in plasma concentrations at later times. The peak concentrations in cerebrospinal fluid (CSF) were recorded at 10 minutes after which the concentrations were similar to those in plasma water [Figure 2.11(a)]. Clearly, the short duration of action of thiopental cannot be explained by it being rapidly removed from the body. However, in a separate study it was shown that brain concentrations rapidly equilibrated with those in the plasma [Figure 2.11(b)] and thus the steep decline in plasma concentrations is accompanied by a similar sharp fall in brain concentrations. Hence, the rapid onset and short duration of action of thiopental can be explained in terms of the kinetics of its distribution. After an i.v. injection, this lipophilic drug rapidly crosses the BBB to enter the brain giving an almost immediate loss of consciousness. Over the next few minutes, the plasma concentration declines, not due to elimination of the drug, but because of loss of the drug from the plasma to the less well-perfused tissues. Because of the rapid transfer between brain and plasma, brain concentrations quickly fall and the patient regains consciousness. In other words, the short duration of action of thiopental is due to redistribution of the drug from the brain, via the plasma, to less well-perfused tissues,

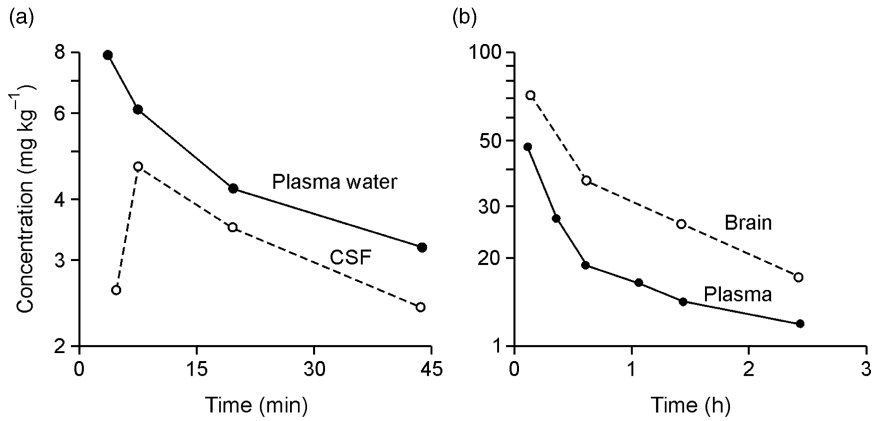

Figure 2.11 (a) Concentrations of thiopental in plasma water and cerebrospinal fluid (CSF) of a dog given 25 mg kg^{-1} intravenously (Brodie *et al.*, 1952). (b) Thiopental concentrations in the brain and plasma of a dog after intravenous administration of 40 mg kg^{-1} (redrawn from Brodie *et al.*, 1956).

probably muscle. This phenomenon probably occurs with many other lipophilic centrally acting drugs such as the related barbiturate, methohexital and the opioid, fentanyl. It has been shown to occur with propofol.

2.4.3.2 Tissue distribution of guanethidine

Guanethidine belongs to a class of antihypertensive drugs known as adrenergic neurone blockers that work by displacing noradrenaline (norepinephrine) from its storage vesicles. Guanethidine is different from the previous example, thiopental, because its distribution is dependent, in part, on active transport to maintain high tissue concentrations. This is reflected in the pattern of its distribution. Concentrations of guanethidine were measured in four tissues and plasma of rats over 24 hours (Figure 2.12). Equilibrium concentration

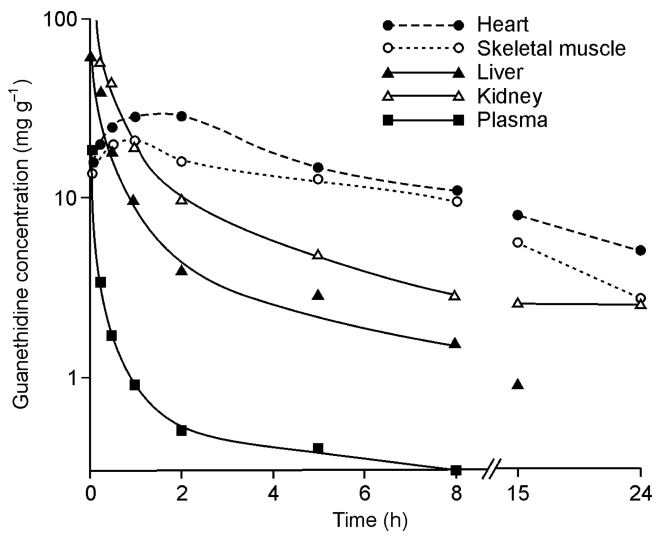

Figure 2.12 Mean concentrations of guanethidine in various rat tissues after 28 mg kg^{-1} (i.v.). Each point is the mean of data from four rats. (Redrawn from the data of Schanker & Morrison, 1965).

ratios were achieved almost instantaneously for liver, kidney and plasma. These ratios were maintained from the first sampling time (5 minutes) to the 8 hour collection time. It is difficult to conclude much for these tissues between 15 and 24 hours because of the paucity of data.

Skeletal muscle and heart showed initial rises in their concentrations, with peak concentrations between 1 and 2 hours. Extensive studies following up this observation have shown that the slow achievement of equilibrium in the case of muscle occurs as a result of a somewhat less efficient blood supply to this tissue. It is believed that localization in liver and kidney results from reversible process as discussed earlier.

Heart, however, is different. Guanethidine concentrations in heart are maintained at high concentrations by an active transport process. This accounts for the slow achievement of heart to plasma equilibrium ratios. The drug is thought to be taken up into noradrenergic nerve terminals by the high affinity neuronal transporter (Uptake 1) that is normally responsible for the reuptake of noradrenaline. The example of guanethidine illustrates how the tissue distribution of one drug may be more complex than another, particularly when active transport is involved and the simple model of passive diffusion does not apply.

2.4.4 Tissue distribution: more modern approaches

At one time tissue distribution studies of the type shown for thiopental and guanethidine were commonplace. The objective was first to determine which tissues showed selective uptake, in the hope of discovering sites of action, and second to aid the understanding of the time course of both the drug in the body and pharmacological effect. The thiopental studies described above provided a fundamental basis for the later concepts of pharmacokinetic compartment modelling, and also influenced dosing practices with intravenous anaesthetics. The guanethidine study, one of the last studies of this type to be published, showed that highly specific uptake at sites of action, using active transport mechanisms, could occur, and probably presaged modern effect-compartment PK/PD modelling (Chapter 14). Studies of this type were time-consuming, and they have largely been superseded by such techniques as microdialysis, and by imaging methods such as whole body autoradiography and positron emission tomography (PET).

2.4.4.1 Microdialysis

This technique involves insertion of very fine probes into the tissues of the living body, mostly into fluid spaces, such as the CSF, and other extracellular fluids where possible. The probes consist of at least two concentric tubes, and a semipermeable membrane separating them, positioned such that an artificial extracellular dialysis fluid can be slowly infused through the probe and past the membrane. Unbound drug molecules in the tissues surrounding the probe diffuse into the flowing dialysate, which is then collected for analysis.

Microdialysis was introduced for the measurement of extracellular concentrations of neurotransmitters in the brain. It has been used in both animals and humans, and is quite commonly used in intensive care units for measuring glucose and lactate. It can be used for both administration of drugs and for sampling fluids for drugs. Technical difficulties include determining the calculation corrections needed in quantification, because of the time over which samples are collected. The greatest challenge is determining the recovery of analyte. This is complicated by dilution of the sample and extracellular fluid by the dialysate, and the fact the analyte is not equilibrated between sample and the flowing dialysate. This is considered to be more important than the invasive nature of the technique. As examples of applications, this technique can be used to study transfer of drugs into the CSF through the choroid plexus, in the search for information on whether drugs then diffuse back into the brain tissue through the monocellular blood–CSF barrier, rather than diffusing out of the brain into the CSF, as a method of removal of drugs from the brain.

Cerebral concentrations of morphine were measured using microdialysis in a patient with a head injury (Figure 2.13). Samples were collected from two sites, an injured one and an uninjured one. Calculations of

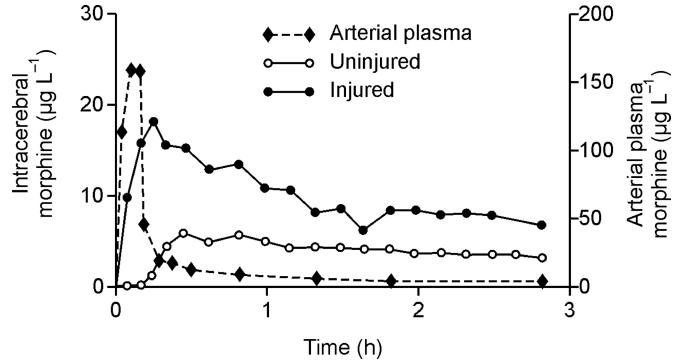

Figure 2.13 Morphine concentrations in a patient with a head injury. (Adapted from Ederoth *et al.*, 2004 and Upton, 2007).

the area under the curve of the unbound drug concentration in uninjured brain (open circles) was less than that in the arterial plasma (diamonds) or the injured part of the brain (closed circles). This was consistent with (i) active efflux of morphine from the brain, and (ii) a damaged blood-brain barrier in the injured area. Passive diffusion of morphine into the injured area was the major mechanism controlling brain exposure to the drug after the injury.

2.4.4.2 Autoradiography

In this approach, the radioactively labelled substance under investigation is administered to test animals, usually mice or rats, which are killed at suitable time intervals, and frozen sections of tissues or of the whole body prepared. An image of the radiation is obtained by placing the sections next to a photographic emulsion. Thus, if a whole body section is used, and the radioactivity is specifically localized in highly perfused organs such as the lungs and liver, the image will reveal this. Comparison with a normal photograph of the slice is used to identify which tissues contain the radioactivity. Densitometry can be used to quantify the relative amounts of radioactivity. This technique has been used to show highly-specific localization of endogenous materials, such as iodine in the thyroid gland, and cholecystokinin in the walls of the stomach and intestine. Less specific but no less valuable information is obtained with drugs.

Figure 2.14 shows three whole body autoradiographs of rat sections at different times after administration of hydralazine labelled with two different radioactive isotopes. Quite specific localization of this vasodilator drug can be seen in the arterial walls, the walls of the aorta, and the walls of the vena cava, amongst many other specific deposits, particularly those connected with the disposition of the drug, such as intestinal contents, the bladder, and the heart, kidney and liver. It can be presumed that the blood vessel labelling is connected with specificity for sites of action.

Autoradiography requires the synthesis of labelled drug, careful handling of materials, and long photographic exposures (up to 6 months). Also, there is no differentiation between parent drug and any metabolites, clearly a problem with drugs that are extensively metabolized. In the hydralazine study it can be presumed that the differences between the images obtained with ^{14}C and ^{3}H, which would have been at different locations within the hydralazine molecules, and therefore retained or lost in metabolites in different ways, reflect metabolic degradation and excretion. These problems have been overcome to some extent by using alternative analytical techniques, such as fluorescence imaging and radioluminography.

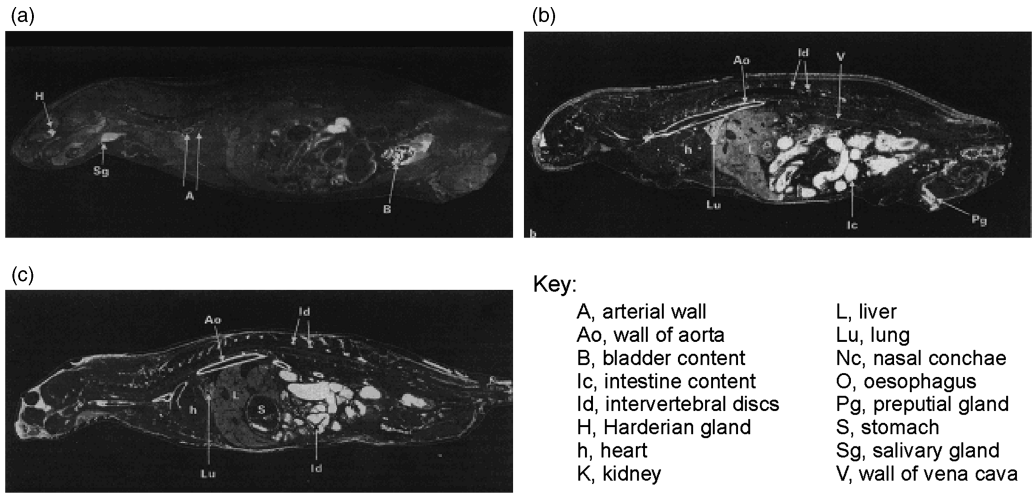

Figure 2.14 Whole-body autoradiographs of rats following intravenous administration of [^{3}H]-hydralazine (a and b) or [^{14}C]-hydralazine (c). (a) Five minutes (b and c) 6 h after injection. (From Baker *et al.,* 1985).

2.4.4.3 Positron emission tomography (PET)

Synthetic radioactive isotopes (e.g. ^{11}C, ^{13}N, ^{15}O and ^{18}F) with atomic masses less than the naturally occurring stable isotopes have half-life values of 2–110 minutes and emit positrons that interact with electrons to emit gamma radiation that can be detected outside the body. The isotopes are generated in a cyclotron and incorporated into the drug molecules immediately before the administration of the drug. PET scanning permits the production of images of live organisms including humans. The disposition of [^{11}C]-triamcinolone after intranasal administration is shown in Figure 2.15(a). The image is of a single

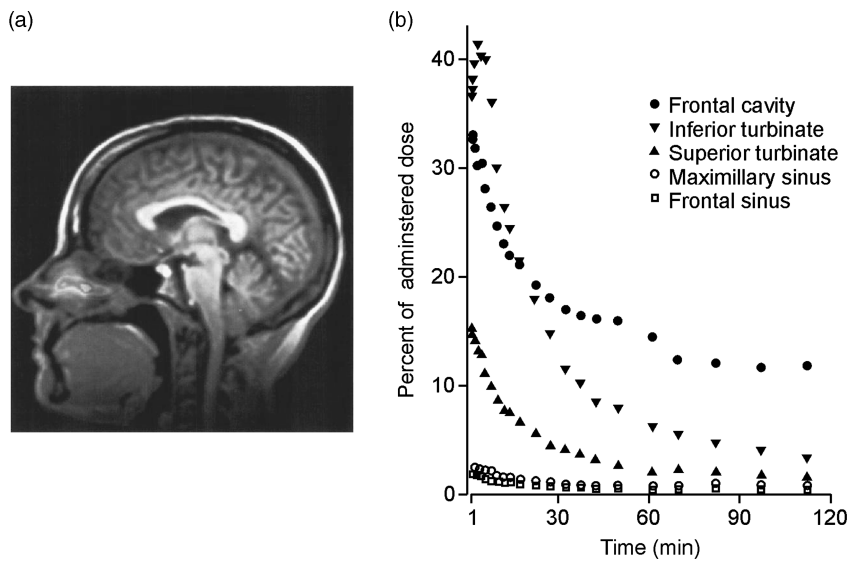

Figure 2.15 (a) PET scan of [^{11}C]- triamcinolone (see text for details).(b) Time course of [^{11}C]-triamcinolone in selected regions. Each point is the mean from three volunteers plotted at the midpoint of each PET scan. (Adapted from Berridge *et al.,* 1998).

sagittal 'slice' through a three-dimensional data set, 8 mm from the central plane of the head. The study showed that the drug was delivered rapidly into the turbinates and frontal regions of the nose and the sinuses. Thereafter, it was found to gradually diffuse away, some of it being swallowed, and also, some being absorbed into the systemic circulation. This technique is quantitative, and a graph of percentage of administered dose, against time up to 2 hours, in the frontal cavity, the turbinates, and the sinuses was generated [Figure 2.15(b)]. Estimates of the half-life of the drug in these locations were possible

Another application of this technique is exemplified by Figure 2.16. Dopamine receptors within the brain were labelled using the precursor, [^{18}F]-fluoroDOPA, to study the effect of drugs that modify dopamine

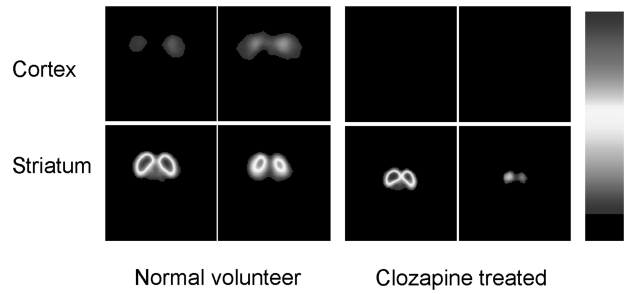

Figure 2.16 PET scans of the cortex and striatum after administration of [^{18}F]-fluoroDOPA, the precursor of [^{18}F]-fluorodopamine.

function in exerting their beneficial effect on psychiatric illness. The figure shows eight images, four generated within a healthy volunteer and four generated in a patient. Two areas of the brain were examined, and the disposition of [^{18}F]-fluorodopamine was studied at two different time points. The images clearly show clozapine reducing dopamine binding in the cortex of the brain.

2.5 Plasma protein binding

Binding of drugs to plasma proteins can have a major role in the overall distribution of a drug and may influence both its pharmacological activity and its kinetics. Most binding interactions are reversible, probably due to ionic and hydrophobic bonding – where lipophilic molecules associate with a hydrophobic part of the protein. Acids tend to bind to albumin and bases to α_1-acid glycoprotein and albumin. Covalent bonding, when it occurs, may result in antibody production and hypersensitivity reactions (Chapter 18). The extent of binding can be described by the fraction or percent of drug bound, β, or less commonly by the unbound fraction, α. The term 'free' is best avoided as the term is used by some to describe the non-ionized form or the non-conjugated form of a drug, such as morphine rather than morphine glucuronide, potentially resulting in untold confusion.

The interaction can be treated as any other reversible binding isotherm:

$$\text{Drug} + \text{Protein} \rightleftharpoons \text{Drug–protein complex}$$

If the molar concentration of bound drug is D_b and total concentration of protein is P_t, assuming one binding site per protein molecule then the concentration of protein without drug bound to it is $(P_t - D_b)$, so:

$$D_f + (P_t - D_b) \rightleftharpoons D_b$$

where D_f is the concentration of unbound drug. The equilibrium constant, K is:

$$K = \frac{k_{-1}}{k_1} = \frac{D_b}{D_f(P_t - D_b)} \tag{2.5}$$

Rearrangement gives:

$$\frac{D_b}{P_t} = \frac{KD_f}{(1 + KD_f)} = r \tag{2.6}$$

where r is the number of moles bound per total number of moles of protein. Equation 2.6 has been rearranged in a number of ways so that K and, usually, n can be estimated. Note that double reciprocal plot of Klotz [Figure 2.17(a)] and the Scatchard plot [Figure 2.17(b)] require the molar concentration of the protein to be

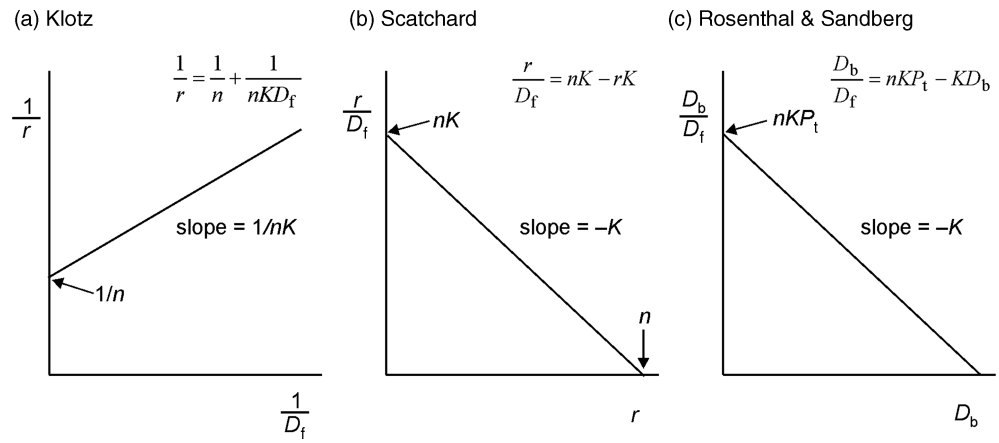

Figure 2.17 Diagram showing three methods for solving protein-binding data.

known, which of course means one has to know the characteristics of the protein to which the drug is binding. Plotting D_b/D_f against D_b overcomes this and is more useful when measuring binding in plasma. If P_t is known then n can be derived from the y-intercept [Figure 2.17(c)]. This plot is frequently used for receptor–ligand binding studies, and, although it is often referred to as a Scatchard plot, it was first proposed by Sandberg and Rosenthal (Rosenthal, 1967).

Curvature of binding plots is indicative of the existence of more that one class of site and the number of moles bound is the result of binding to all the sites:

$$r = \sum_{i=1}^{i} \frac{n_i K_i D_f}{1 + K_i D_f} \tag{2.7}$$

Frequently, protein binding has been evaluated in terms of the fraction bound, β, which for a single class of binding sites:

$$\beta = \left(1 + \frac{D_f}{nP_t} + \frac{1}{nKP_t}\right)^{-1} \tag{2.8}$$

Often a change in the fraction of drug bound in plasma is barely observable within the therapeutic range and when the concentration is increased over several orders of magnitude the change may be small (Figure 2.18). Experimentally, proteins appear to be able to 'mop-up' some drugs from aqueous solutions or suspensions, even when, in some cases the solubility in water as been exceeded. Scatchard plots are often curved when binding is studied over a large range of concentrations, suggesting that there is specific binding, with a small value of n and relatively large K, and non-specific binding which prevails at high concentrations where K is small but n is large.

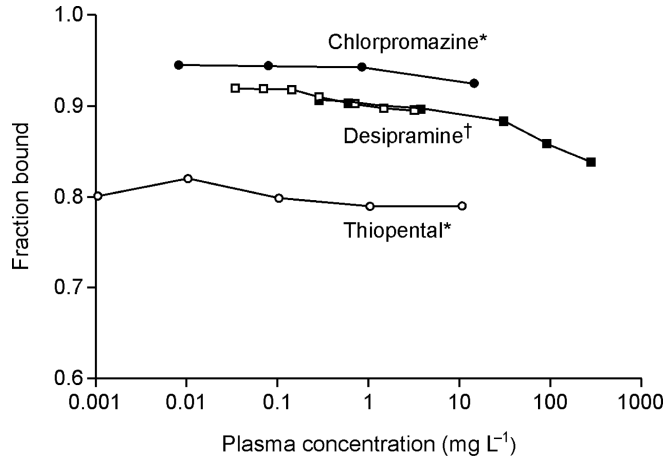

Figure 2.18 Fraction bound to human plasma protein for desipramine, chlorpromazine and thiopental (†two individuals, Borga *et al.*, 1969; *blood bank plasma, Curry, 1970).

Using a literature value of $K = 2.3 \times 10^4\,\mathrm{L\,mol^{-1}}$ and $n = 1$ for the binding of chlorpromazine to albumin ($M_r = 69{,}000$) and assuming the plasma concentration of albumin to be $40\,\mathrm{g\,L^{-1}}$, Equation 2.8 was used to calculate the fraction bound as a function of total concentration. The binding changed by <0.4% over a 100-fold range of drug concentration, so even specific binding can result in an almost constant degree of binding, as in this example, at therapeutic concentrations (Table 2.2).

Table 2.2 Calculated values of β for a range of chlorpromazine concentrations in man

Total concentration		Fraction bound (β)
$\mu g\,L^{-1}$	$mol\,L^{-1}$	
1.38	3.89×10^{-9}	0.9276
13.8	3.89×10^{-8}	0.9273
131.9	3.72×10^{-7}	0.9242
947.9	2.67×10^{-6}	0.9845
3093.1	8.71×10^{-6}	0.6767

Thus, for many drugs there appears to be excess binding capacity, however this may not be the case for less potent drugs that are used at higher doses. For example, therapeutic concentrations of phenylbutazone (approximately $50–100\,\mathrm{mg\,L^{-1}} = 0.16–0.32\,\mathrm{mmol\,L^{-1}}$) are not very far removed from the molar concentration of albumin in plasma ($0.58\,\mathrm{mmol\,L^{-1}}$). A phenylbutazone concentration of $\sim 180\,\mathrm{mg\,L^{-1}}$ represents a 1 : 1 ratio of drug: albumin and the fraction bound shows a marked increase in unbound concentration at concentrations higher than this (Figure 2.19).

2.5.1 Assessing protein binding

An obvious way of determining the extent of binding to plasma proteins is to separate and measure the concentration of the unbound drug. For highly protein-bound drugs, measuring the unbound drug concentration presents an analytical challenge and for drugs that are >99% bound it may not be possible to obtain accurate concentrations using standard laboratory methods, particularly if the analyte and protein

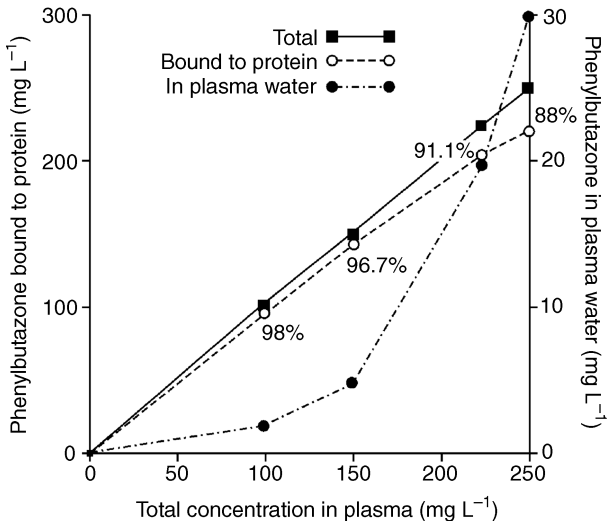

Figure 2.19 Binding of phenylbutazone to plasma protein as a function of phenylbutazone concentration. The percentages indicate the binding at 100, 150, 225 and 250 mg L^{-1} (Brodie & Hogben, 1957).

concentrations are those attained after therapeutic dosing. Under these conditions it may be necessary to use radiolabelled drug. A very small quantity of high specific activity labelled drug is added to plasma, incubated to ensure equilibration with non-labelled analyte, and the free and bound fractions are then separated for radioactive counting, usually by liquid scintillation spectrometry.

2.5.1.1 Equilibrium dialysis

This is the most unequivocal method for assessing the unbound fraction. The sample is placed on one side of a membrane that allows small molecular mass drugs to pass into the dialysate (usually pH 7.4 buffer) on the other side but not the protein [Figure 2.20(a)]. After equilibration, the concentrations on the protein (C_t) and buffer side (C_f) of the membrane are measured, the bound concentration, C_b being $C_t - C_f$. The technique is

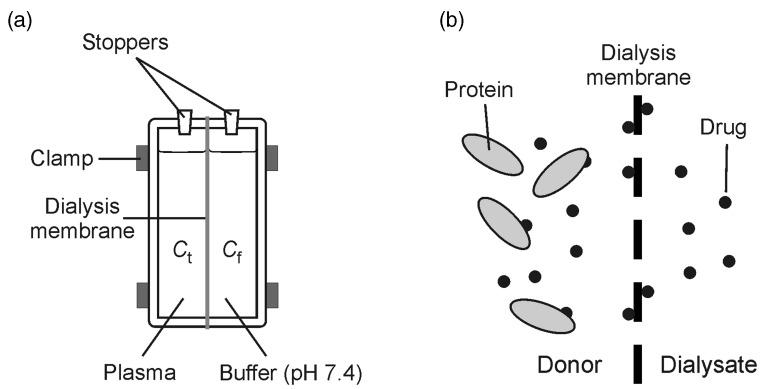

Figure 2.20 (a) Dialysis cell; the stoppers can be removed for sampling. (b) When equilibrated the unbound drug concentrations (C_f) on either side of the membrane are equal. Measuring the total drug concentration (C_t) on the protein side avoids errors due to binding of drug to the membrane.

not without its problems. The time for equilibration can be several hours, during which there may be microbial growth, particularly with plasma at 37 °C, possibly leading to changes in protein and analyte concentrations and analyte binding. If an antibiotic is added it cannot be assumed that it does not interfere with the binding. An advantage of dialysis is that the problem of adsorption of analyte to the membrane and apparatus is largely overcome by measuring the concentrations on either side of the membrane. Adsorption will reduce the concentrations in the donor and recipient (dialysate) solutions, but at equilibrium the unbound concentration in solution will be the same on either side of the membrane [Figure 2.20(b)]. Thus, it is possible to calculate the fraction bound or free and to relate this to the initial plasma concentration of analyte.

2.5.1.2 Ultrafiltration

Several ultrafiltration devices are available commercially. The filtration membranes used are made from a variety of materials and have relative molecular mass cut-offs in the range $M_r = 10{,}000$–$30{,}000$. Most devices are designed to be centrifuged to provide the filtration pressure. Ultrafiltration should not be confused with ultracentrifugation in which protein-bound and 'free' analyte can be separated as layers, often with the aid of a density gradient. This latter technique is particularly useful for investigating binding to lipoproteins which can be separated as layers floating on the surface of plasma, the density of which has been adjusted with potassium bromide. Ultrafiltration is more convenient and quicker than equilibrium dialysis, but the protein concentration in the retentate increases during filtration, potentially increasing the proportion of analyte bound. To minimize this problem as small a volume of ultrafiltrate should be collected as practicable, and the volume of retentate made up with an appropriate buffer solution periodically during centrifugation. Binding of the analyte to the filtration membrane (common with many lipophilic drugs) will reduce the concentration in the filtrate. Control experiments to ascertain the magnitude of this problem should be conducted. It is good practice to collect serial samples of filtrate for analysis to ensure that the sample is representative of the unbound concentration. Some filtration membranes need to be soaked in water or buffer before use and as a result the first ultrafiltrate collection(s) may be unrepresentatively dilute.

2.5.2 Molecular aspects of protein binding

Plasma protein binding is a physical interaction between a small molecule and a large molecule, and as such it is amenable to study using calorimetric and spectroscopic instrumentation, and analysis in thermodynamic terms. The purpose of such studies is to understand the sites and nature of the interaction on the protein surface and the effects, if any, of the binding drug on the protein, and to facilitate prediction of protein binding properties of new molecules, alone and in combination. Thus, among the spectroscopic techniques, changes in UV absorption or fluorescence spectra can be interpreted in terms of polarity of the drug. Also, nuclear magnetic resonance spectra can indicate which parts of the drug molecule are involved in the binding, and circular dichroism yields information on the three-dimensional structure of the binding site of the protein. Electron spin resonance spectroscopy permits study of the involvement of free radicals in the binding, when it occurs. A small sample from the literature relevant to this is presented below.

2.5.2.1 Microcalorimetry

Binding reactions are exothermic and can be studied by detecting the heat produced when separate solutions of the drug and protein are mixed under controlled conditions in flow-through cells. The technique has been applied mainly to drug binding to human serum albumin (HSA). The microcalorimeter is calibrated electrically, and the heat output measured in microvolts. Generally, a constant concentration of HSA and varied concentrations of drug are used, producing a diagram of the type in Figure 2.21. This shows the voltage produced with a range of concentrations of sulfathiazole, and seems to indicate a minor trend towards

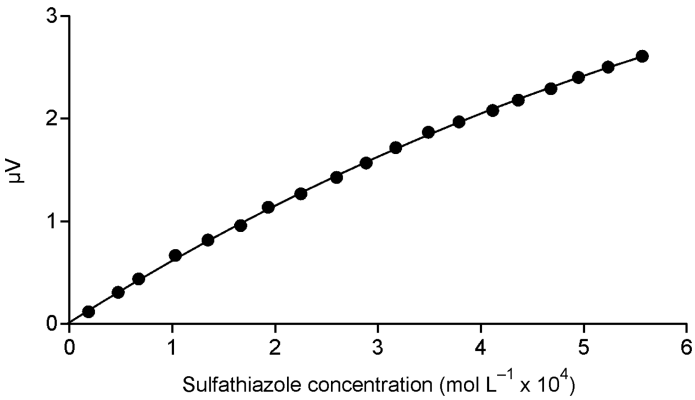

Figure 2.21 Heat produced (assessed as microvolts) as a function of sulfathiazole concentration at a fixed HSA concentration of 3.25×10^{-4} mol L^{-1} (Hardee *et al.*, 1984).

saturation. The voltage output associated with formation of one mole of complex is calculated, and the heat flux is calculated in microwatts – the numbers are quite small, in the range 1–40 μW.

This technique is particularly applicable to the study of drug interactions, as the heats of reaction for two separate drug–protein combinations should be additive. If they are less than additive it is proposed that a displacement of one drug by the other is occurring. In this case the interaction must be further studied using more traditional techniques such as dialysis and ultrafiltration. For example, it was possible to show both enhancement (greater than additive heat) and displacement (less than additive heat) with various combinations of non-steroidal anti-inflammatory drugs and coumarin anticoagulants (Hardee *et al.*, 1984).

2.5.2.2 Fluorescence spectroscopy

Binding of a fluorescent drug to protein may enhance the fluorescence and this can be used to study the interaction. For example, when warfarin binds to HSA, the fluorescent quantum yield of the drug increases eightfold and its emission maximum moves to shorter wavelengths. Rat and dog plasma albumins induce even greater 'blue shifts' in the emission [Figure 2.22(a)]. These changes can be interpreted in terms of the

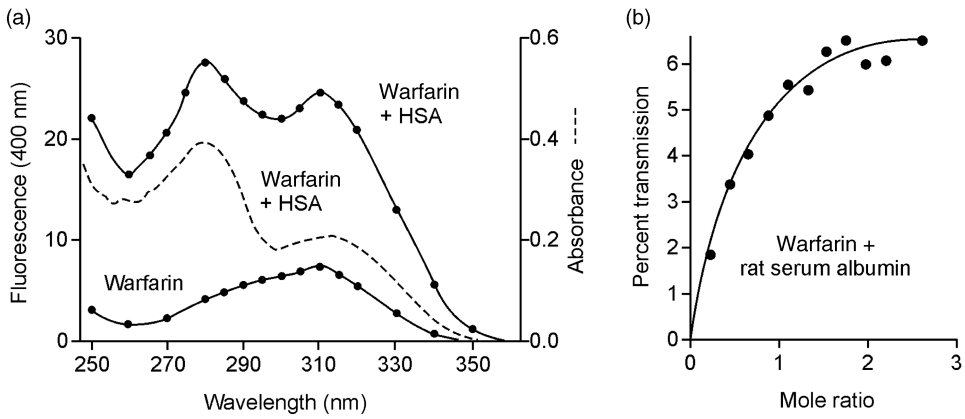

Figure 2.22 (a) Fluorescence emission spectrum of warfarin showing the increased quantum yield in the presence of human serum albumin (HSA). (b) Fluorescence titration of rat serum albumin (1×10^{-5} mol L^{-1}) with warfarin. Mole ratio = wafarin/rat serum albumin. (From Chignell, 1973).

polarity of the binding sites, and can be used to calculate apparent binding constants, using Scatchard plots, facilitating comparison with the comparable results of dialysis experiments. Thus, HSA has one specific binding site per molecule for warfarin, with an association constant of $8.2 \times 10^5\,\text{mol}\,\text{L}^{-1}$. A typical fluorimetric titration of rat plasma albumin and warfarin is shown in Figure 2.22(b). The titration appears to show saturation of the binding with increasing concentration of warfarin, while a Scatchard plot showed approximately one specific binding site per molecule of protein.

An alternative approach for drugs that do not show enhanced fluorescence on binding is to monitor the quenching of the native fluorescence of albumin that occurs when some drugs bind. It is important to correct for any effects due to light absorption by the drug which will affect the fluorescent yield. Spectroscopic techniques usually only detect specific binding which may be considered an advantage or a disadvantage, should the contribution from less specific binding be required.

2.5.2.3 Circular dichroism (CD)

This spectroscopic technique takes advantage of the differential absorption properties of left and right-handed circular polarized light. This is particularly applicable to the determination of the secondary structure of proteins; as stated earlier, it is possible to detect binding sites, and to observe changes in the α-helix of proteins, induced by binding of ions and organic molecules, including drugs. This technique is closely related to another approach, optical rotatory dispersion (ORD) (Chignell, 1973).

The power of CD is illustrated by studies conducted with dog serum albumin and copper and nickel (Mohanakrishnan & Chignell, 1984). Copper was described as showing a single positive 'extremum' at 664–667.5 nM; this was maximal at a Cu^{2+}/albumin molar ratio of three. Also, there was an absence of 'extrema' at 560–570 nM and 480–510 nM, said to indicate the absence of involvement of, for example, histidine residues – this aided the identification of the binding sites. In the case of nickel, it was shown that the binding was at the N-terminal tripeptide. Also, there was binding at sulfur-containing residues at relatively high Ni^{2+}/albumin ratios (Figure 2.23). The figure shows difference CD spectra of nickel binding at three different molar ratios. This technology has been recently reviewed by Ascoli *et al*. (2006).

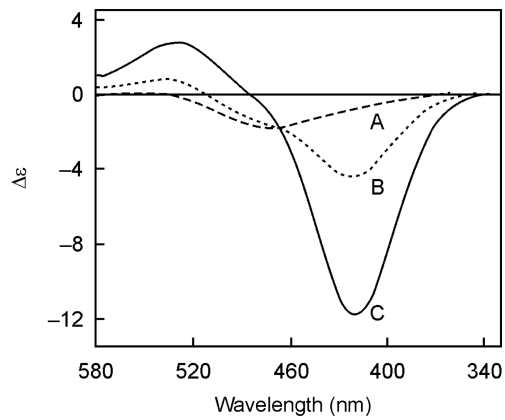

Figure 2.23 Difference circular dichroism spectra of nickel binding to 87.56 μM DSA (0.15 M NaCl pH 10.2). The Ni^{2+}/DSA ratios are 2 for spectrum A, 3 for spectrum B and 6 for spectrum C. (Mohanakrishnan & Chignell, 1984).

2.5.2.4 Electron paramagnetic resonance spectroscopy (EPR)

This is a spectroscopic technique for studying chemical species that have one or more unpaired electrons, such as organic and inorganic free radicals or inorganic complexes possessing a transition metal ion. This is similar to nuclear magnetic resonance, but it is the unpaired electrons that spin if excited instead of the atomic nuclei. Most chemical solvents do not give rise to EPR spectra. This technique has been applied to the search for free-radical mechanisms in covalent binding of drugs that oxidize to free radicals, such as phenothiazine drugs and halogenated solvents. Binding of this type would be expected to be associated with long-term persistence in the body, and possibly with an adverse clinical outcome. The technique can also employ 'spin labelling', in which a free radical is attached to a molecule of interest in order to track the disposition and fate of the compound so tagged. It has been proposed that this, and other spectroscopic techniques, would be especially applicable to studies of α_1-glycoprotein, which particularly binds basic drugs and remains relatively unstudied (Israili and Dayton, 2001). It is also of interest that albumin has a major role in the transport of nitric oxide, accounting for most of the antioxidant properties of human plasma (Fasano *et al*., 2005).

2.5.2.5 Commentary

Over the last 35 years or so, these, and other instrumental approaches to the study of plasma protein binding of drugs have undoubtedly enhanced our understanding of the chemistry of the phenomenon, and have certainly facilitated the partial prediction and prevention of drug binding interactions. They have made possible quantitative structure activity relationship (QSAR) studies, in the search for a measure of predictability, especially in relation to new chemical entities. However, an age-old question remains unanswered, whether *in vitro* experiments, even when conducted at 37 °C, reproduce faithfully the *in vivo* events, and studies of this type have not entered the mainstream of the new drug discovery process, or provided an explanation for many unexpected pharmacokinetic phenomena. It is still possible to treat protein binding with a sense of awe, as did Fasano *et al*. (2005), in their review entitled: 'The extraordinary ligand binding properties of human serum albumin'.

2.5.3 Pharmacological importance of binding to plasma proteins

Clearly plasma protein binding has a major influence on the distribution of drugs. Extensive binding to plasma proteins reduces the apparent volume of distribution because a larger proportion of the amount of drug in the body will be in the plasma. It is usual to measure the 'total' concentration of drug (i.e. bound + unbound) in plasma.

Binding to plasma proteins, provides an efficient way of transporting drugs in the circulation, sometimes at concentrations that exceed their solubility in plasma water. Binding has an important role in absorption, as it maintains a favourable concentration gradient for the unbound drug. It is generally assumed that plasma protein binding reduces the proportion of a dose of drug available to its receptors and so it can have a major influence on drug activity. Changes in the faction bound may occur because of:

- Displacement by a second drug. This may be clinically important with salicylate and valproic acid, displacing drugs that attain molar concentrations similar to that of the binding protein.
- Changes in protein concentration, often as result of disease (Chapter 12).
- Concentration dependent binding.

Many *in vitro* studies have demonstrated displacement of one drug by another, but *in vivo* the situation is more complex. The 'total' concentration of a displaced drug in plasma will be reduced as some of the liberated drug diffuses into tissues as new equilibria are established. The increased concentration of unbound drug may lead

to greater, possibly toxic, effects. Hence, measurement of the 'total' (bound + unbound) concentration of a drug in plasma may be misleading under certain circumstances. When phenytoin was displaced by salicylate, for example, the percentage unbound increased from 7.14 to 10.66%, and this was accompanied by a significant decrease in total serum phenytoin concentration from 13.5 to 10.3 mg L^{-1}. The salivary phenytoin concentration rose from 0.97 to 1.13 mg L^{-1} (Leonard *et al*., 1981).

The effect of protein binding on drug elimination is more complicated than it might at first appear. It is reasonable to assume that binding will reduce glomerular filtration, as the composition of the filtrate is in essence that of plasma water. However, other factors such as urine flow rate and reabsorption of drug from renal tubular fluid need to be considered (Section 3.3.1). The effects on drug metabolism are more complex. Briefly, binding reduces the rate of elimination of those drugs that are poorly extracted by the liver but not those that are extensively metabolized. In fact, for these drugs plasma protein binding can be considered as an efficient mechanism for delivering the drug to its site of metabolism. This is discussed in more detail in Section 7.4.1.

2.6 Summary

It is interesting to note that twentieth century man is fortunate that so many therapeutically useful compounds are successfully absorbed from the GI tract. The intestine is undoubtedly a very efficient organ for absorbing essential nutrients by active transport, but evolution appears to have designed it to exclude rather than transfer drugs, in that it is a lipid barrier, keeping out all but the most lipid-soluble foreign molecules. In addition, the intestine and the portal circulation are well endowed with enzymes which very effectively destroy many of the foreign molecules which penetrate the barrier. This design feature makes possible efficient transfer of food and virtual exclusion of non-food chemicals. Out of the minority of non-nutritive materials which do pass successfully from the gastrointestinal tract to the blood, a number have emerged as therapeutically useful drugs.

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