3

Drug Elimination

3.1 Introduction

The action of a drug may be terminated by redistribution away from its site of action as discussed in Section 2.4.3.1. However, most drugs have a limited duration of action because they are eliminated by metabolism and/or excretion. Some drugs, particularly polar, water-soluble ones, may be excreted in the urine unchanged but lipophilic drugs tend to be highly protein bound, which reduces glomerular filtration and any drug in kidney tubular fluid tends to be reabsorbed back into the body. Consequently, lipophilic drugs would have very long elimination half-lives if they were not metabolized into polar molecules that are more readily excreted.

3.2 Metabolism

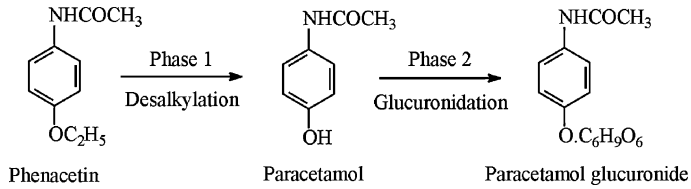
The liver is undoubtedly the major site of metabolism for the majority of drugs but most tissues have some metabolizing capacity. Other notable sites of metabolism include:

- Plasma and other body fluids – hydrolysis, mainly of esters
- Nerve terminals – metabolism of endogenous transmitters and drugs with similar structures
- Mucosal cells – responsible for presystemic metabolism of many drugs
- Kidney, lung and muscle – metabolize some drugs
- Brain – capable of glucuronidation of morphine
- Intestinal flora – metabolism of several drugs and hydrolysis of glucuronides.

Drug metabolism can be divided into two, sometimes three, distinct phases. Phase 1 reactions either insert, usually via oxidation, or reveal, by hydrolysis or desalkylation, reactive or functional groups that can undergo phase 2 reactions. For this reason, phase 1 reactions may be referred to as 'functionalization'. In phase 2 reactions, molecules with suitable functional groups are conjugated with endogenous compounds such as glucuronic acid, sulfate, acetate and amino acids, for example. Conjugation may be with products of phase 1 metabolism or if the drug has suitably reactive groups, conjugation may occur directly. Phenacetin is desalkylated to paracetamol which is then conjugated with either

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glucuronate or sulfate:



Clearly, paracetamol can be conjugated directly without introduction of a functional (reactive) group via a phase 1 reaction. The term phase 3 metabolism is sometimes used to describe the further metabolism of phase 2 conjugates, such as occurs with the glutathione conjugate of paracetamol.

3.2.1 Phase 1 metabolism

These reactions include reductions and hydrolyses, but the most common reactions are the oxidations catalysed by the mixed-function oxidase (MFO) system, so called because of the apparent lack of specificity for individual substrates. These enzymes are isolated by subjecting tissue homogenates to high centrifugal forces (100,000 ***g***) after the cell debris has been removed by centrifugation at 9,000 ***g*** (Figure 3.1). The pellet

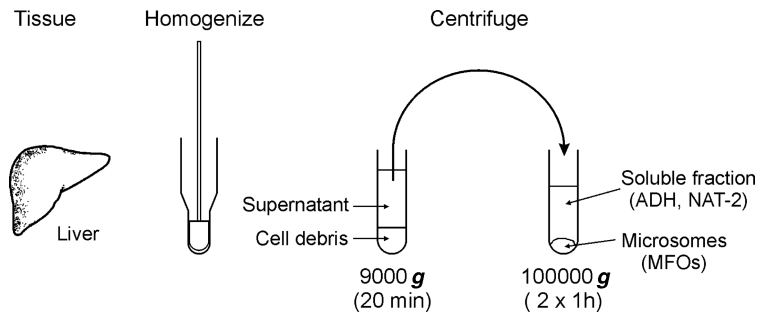


Figure 3.1 Isolation of mixed-function oxidases from liver.

comprises microsomes, small particles of smooth endoplasmic reticulum (SER) whilst the supernatant layer (soluble fraction) contains enzymes from the cytosol, such as alcohol dehydrogenase (ADH) and *N*-acetyltransferase type 2 (NAT2).

Microsomal oxidations involve a relatively complex chain of redox reactions and require (i) reduced nicotinamide adenine dinucleotide phosphate (NADPH), (ii) a flavoprotein (NADPH-cytochrome P450 reductase) or cytochrome b_5 (iii) molecular oxygen and (iv) a haem-containing protein, cytochrome P450, so called because in its reduced form it binds with carbon monoxide to produce a characteristic spectrum with a peak at 450 nm. Cytochromes, which comprise a superfamily (Section 3.2.1.1) with nearly 60 genes having been identified in man, catalyse the final step (Figure 3.2). Drug (DH) combines with the CYP, and the iron(III) in the complex is reduced to iron(II) by acquiring an electron from NADPH-P450 reductase. The reduced complex combines with oxygen [$(DH)Fe^{2+}O_2$)] and combination with a proton and a further electron (from NADPH-cytochrome P450 reductase or cytochrome b_5) produces a $DH–Fe^{2+}OOH$ complex. The addition of a proton liberates water and a Fe(III) oxene complex [$DH(FeO)^{3+}$], which extracts hydrogen from the drug with the formation of a pair of free radicals. Finally, the oxidized drug is

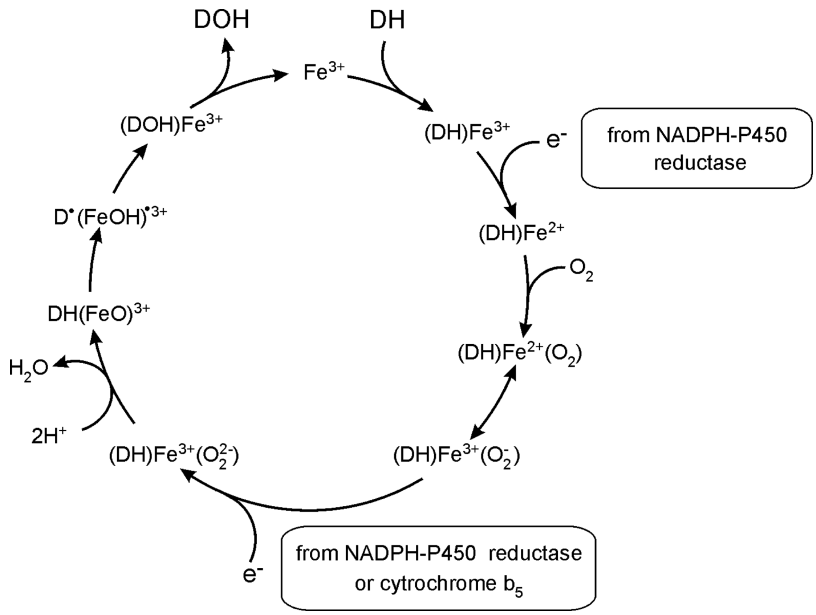


Figure 3.2 Diagrammatic representation of the final stage of incorporation of molecular oxygen into drug (DH) showing the various oxidation states of iron in the haem containing cytochrome.

released from the complex with the regeneration of the P450 enzyme. The last stage of the reaction involves free radicals and the same products can sometimes be produced by treating the drug with Fenton's reagent (a source of hydroxyl radicals). However, treating chlorpromazine, for example, with Fenton's reagent gives equal amounts of 3- and 7-hydroxychlorpromazine, whereas the major hydroxylated metabolite in humans is 7-hydroxychlorpromazine.

3.2.1.1 Cytochrome P450 superfamily

Although the Human Genome Project has identified 57 human genes that encode various forms of cytochrome P450, only three families, CYP1, CYP2, and CYP3, appear to be important in drug metabolism. The enzymes are classified on the basis of cDNA cloning according to similarities in amino acid sequence. A family contains genes that have at least a 40% sequence homology. Members of a subfamily (denoted by a letter) must have at least 55% identity. The individual gene products are identified by the final number.

The substrate specificity of CYP isoforms varies, as does that of their inhibitors and the agents that can induce their synthesis (Table 3.1). Note how several inhibitors and inducers appear several times. Ketoconazole and related drugs work by inhibiting fungal cytochromes whilst tranylcypromine binds covalently to monoamine oxidase and probably to other enzymes as well. Therefore, it might be predicted that these drugs would have the effects that they do. The H_2-receptor antagonist, cimetidine, inhibits several CYP isoforms and it is likely that a new drug will be required to show no or minimally significant interaction with cimetidine before approval from the licensing authorities is obtained, unless it can be shown categorically that the new drug will never be administered in a way in which an interaction could occur. Some drugs are both substrates and (competitive) inhibitors. Well-known enzyme inducing agents, phenobarbital, rifampicin (rifampin) and phenytoin feature large in Table 3.1. Note also that components of foods, particularly grapefruit, and herbal remedies, Saint John's wort, can inhibit or induce enzymes. The effects of enzyme inhibition and induction are discussed in Chapter 17.

Table 3.1 Some examples of drug-metabolizing cytochromes

Isoform	Substrates	Inhibitors	Inducers
CYP1A2	Caffeine, clozapine, fluvoxamine, olanzapine theophylline	Ciprofloxacin, cimetidine	Phenobarbital, phenytoin, rifampicin, tobacco smoke
CYP2A6	Cotinine, coumarin, nicotine	Ketoconazole, star fruit juice, grapefruit juice	Dexamethasone, rifampicin, phenobarbital
CYP2B6	Bupropion, cyclophosphamide[a], pethidine	Fluoxetine, orphenadrine	Carbamazepine, cyclophosphamide, phenobarbital
CYP2C9	Losartan[a], NSAIDs phenytoin, tolbutamide, *S*-warfarin	Amiodarone, ketoconazole, Tranylcypromine, valproic aicd	Phenobarbital, phenytoin, rifampicin, Saint John's wort
CYP2C19	Diazepam, omeprazole, phenytoin	Omeprazole, moclobemide, sulfaphenazole	Phenobarbital, phenytoin, rifampicin, Saint John's wort
CYP2D6	Codeine[a], debrisoquine, haloperidol, phenothiazines, SSRIs	Cimetidine, fluoxetine, quinidine, terbenafine	Glutethimide
CYP2E1	Enflurane, ethanol, halothane, paracetamol	Disulfiram, ethanol (acute), miconazole	Ethanol (chronic), isoniazid, rifampicin
CYP3A4	Clozapine, nifedipine, simvastatin[a]	Cimetidine, erythromycin, ketoconazole, SSRIs, grapefruit juice	Carbamazepine, phenobarbital, rifampicin, Saint John's wort
CYP3A5	Midazepam, caffeine		Dexamethasone

[a] Prodrug.

The distribution of the various CYPs also varies. CYP3A4, which metabolizes a large number of drugs, constitutes approximately 30% of the total CYP content of the liver and 70% of the total CYP in gastrointestinal epithelial cells. Many of its substrates are also transported by P-glycoprotein (P-gp), with which it appears to work in concert, so if a molecule escapes metabolism it is likely to be expelled into the gastrointestinal (GI) lumen by P-gp. CYP3A5 is the most abundant CYP3A form in the kidney. CYP3A7 is a foetal form of the enzyme that is believed to be important in the metabolism of endogenous steroids and *trans*-retinoic acid. It rapidly declines during the first week of life and is rarely expressed in adults. Although CYP2D6 represents only 2% of hepatic CYP, it metabolizes a large number of substrates, including many antipsychotic and antidepressant drugs, β-blockers, and the antihypertensive, debrisoquine. It is also responsible for activating the prodrug, tramadol, and for the *O*-desmethylation of codeine to morphine. It has been suggested that those with a deficit of CYP2D6 fail to obtain pain-relief when taking these drugs (Section 10.5.1).

3.2.1.2 Other oxidases

Not all oxidases are cytochromes. Monoamine oxidase (MAO) is a mitochondrial enzyme located in aminergic nerve terminals, the liver and intestinal mucosa. Not only does it deaminate endogenous neurotransmitters, noradrenaline (norepinephrine), dopamine, and serotonin, it is normally responsible for the first-pass metabolism of indirectly acting sympathomimetic amines (IASA) such as tyramine and ephedrine. When MAO is inhibited, for example by antidepressant drugs like tranylcypromine, the absorption of tyramine and subsequent displacement of elevated concentrations of noradrenaline can result in a dangerous hypertensive crisis (Table 17.3). Amphetamine is an IASA but it is a poor substrate for MAO because it has an α-methyl substituent; it tends to inhibit the enzyme.

Flavin-containing mono-oxygenases (FMOs) are microsomal enzymes that catalyse the NADPH-dependent oxidation of a large number of sulfur-, selenium-, and nitrogen-containing compounds, such as the *N*-oxidation of tertiary amines and stereospecific oxidation of sulfides. A genetic failure to express one isoform, FMO3, results in an inability to metabolize trimethylamine which leads to large quantities being excreted in the urine and sweat, a very distressing condition for the sufferers, who smell of putrefying fish.

Other phase 1 enzymes include the soluble enzyme, alcohol dehydrogenase (ADH), the mammalian form of which oxidizes several alcohols as well as ethanol, including methanol, ethylene glycol, and 2,2,2-trichloroethanol, the active metabolite of chloral hydrate. Xanthine oxidase metabolizes 6-mercaptopurine, a metabolite of azathioprine. The conversion of xanthine to uric acid may be inhibited by allopurinol, a drug sometimes used to treat gout.

3.2.1.3 Hydrolysis

Blood contains several esterases, including butyrylcholinesterase (BChE), a soluble enzyme (also known as pseudo- or plasma cholinesterase) and acetylcholinesterase (AChE) which is bound to the red cell membranes. Amides may be hydrolysed by plasma but the liver contains higher amidase activity as well as esterases. Esters are usually rapidly hydrolysed; examples include acetyl and carbamoyl esters (Figure 3.3).

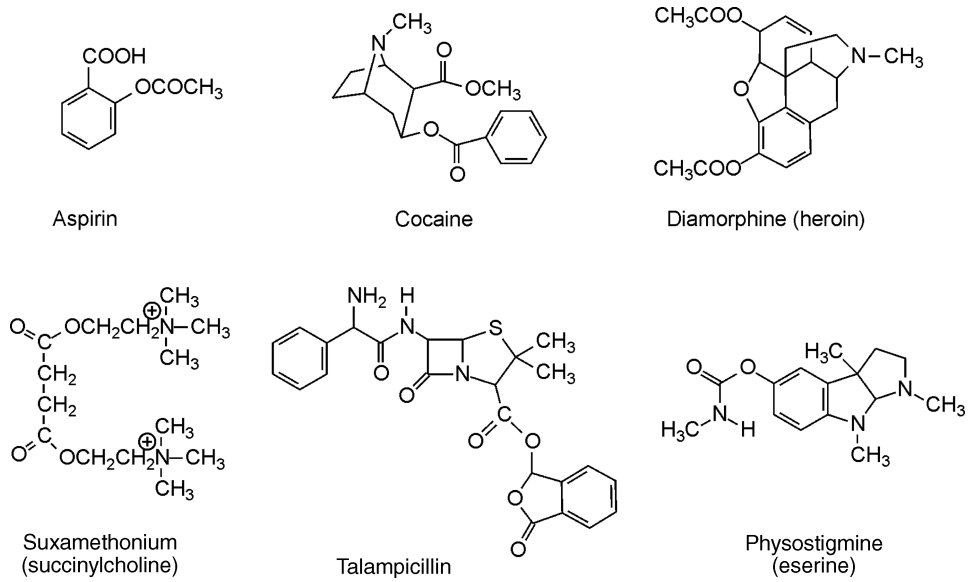


Figure 3.3 Examples of esters that are hydrolysed.

Several of the examples in Figure 3.3 are diesters. Hydrolysis of cocaine gives benzoylecgonine or methylecgonine which are further hydrolysed to ecgonine. Diamorphine is hydrolysed to 6-monoacetylmorphine (6-MAM) and then morphine. Suxamethonium is hydrolysed to succinylmonocholine and succinic acid. Carbamate esters such as physostigmine are hydrolysed by cholinesterases, but much more slowly than acetylcholine, and so are substrate inhibitors of the enzymes. Talampicillin is an example of a prodrug of ampicillin that was produced to increase the oral bioavailability of the parent drug.

Amides and hydrazides are hydrolysed (Figure 3.4). Procainamide is hydrolysed to *p*-aminobenzoic acid. Amide local anaesthetics, lidocaine (lignocaine) and prilocaine are hydrolysed more slowly than the esters such as procaine and benzocaine. The *o*-toluidine (2-methylaniline) produced from prilocaine is thought to be responsible for the methaemoglobinaemia seen with this anaesthetic. Hydrolysis of hydrazides may lead to reactive hydrazines.

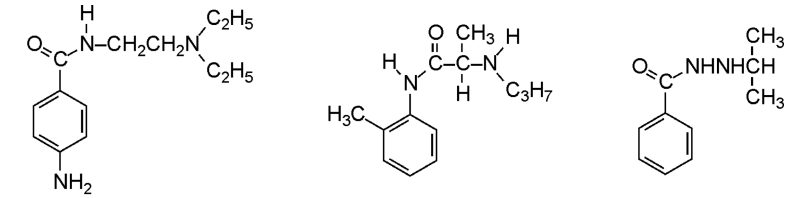


Figure 3.4 Examples of amides and hydrazides that are hydrolysed.

3.2.1.4 Reductions

Azo, nitro and dehalogenation reduction are catalysed by microsomal reductases. Several pathways are probably involved that are dependant on NADPH, cytochrome *c* reductase and flavoprotein enzymes. It is thought at azo- and nitro-reductases are different and the nitro-reductases can be divided into those that are oxygen-sensitive and those that are not. The nitro groups in nitrazepam, clonazepam and chloramphenicol are reduced to primary aromatic amines. The azo dye, prontosil, which is inactive *in vivo*, is reduced to sulfanilamide (Figure 3.5) from which the antimicrobial sulfonamides were developed.

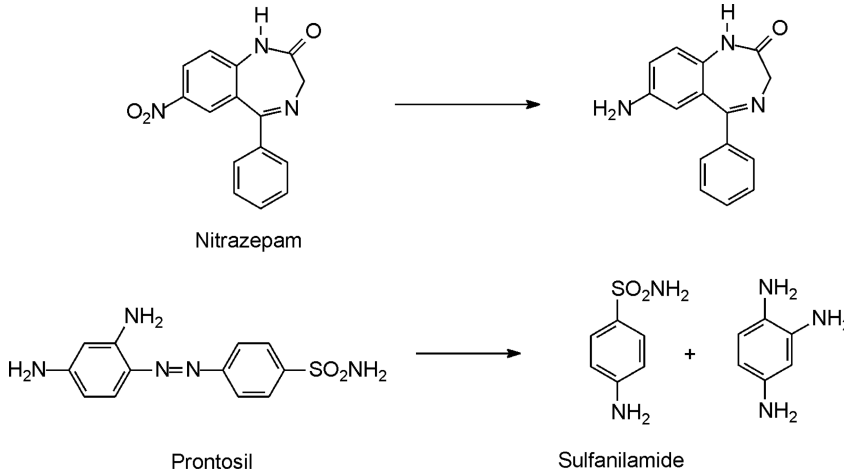


Figure 3.5 Examples of nitro and azo reduction.

Choral is reduced to trichloroethanol and the ketone group in warfarin is reduced to a secondary alcohol. These reactions are probably not microsomal and may be reversed oxidations.

3.2.2 Examples of phase 1 oxidation

3.2.2.1 Oxidation of alcohols

Alcohols may be oxidized via various routes including microsomal CYP2E1 and catalase but in mammals the major route is catalysed by cytosolistic ADH to the corresponding aldehyde or ketone. Ethanol and methanol are oxidized to acetaldehyde and formaldehyde, respectively. Further oxidation by aldehyde dehydrogenase (ALDH) gives either acetic acid or formic acid (Figure 3.6). Formaldehyde and formic acid are much more

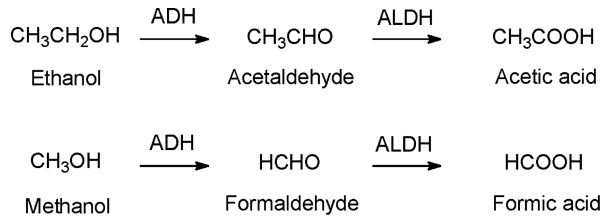


Figure 3.6 Oxidation of ethanol and methanol by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH).

toxic than acetaldehyde and acetic acid, so in methanol poisoning, ethanol may be given to compete with methanol for ADH and so reduce the rate of production of the toxic metabolites. Disulfiram inhibits ALDH so that after consumption of ethanol, acetaldehyde concentrations increase causing flushing, nausea and vomiting. The drug may be used in aversion therapy for alcoholics.

3.2.2.2 Aliphatic and aromatic hydroxylations

Microsomal hydroxylations are commonly catalysed by MFOs and occur with both aromatic and aliphatic moieties. Aromatic hydroxylations are exemplified by oxidation of phenobarbital to *p*-hydroxyphenobarbital, which is devoid of pharmacological activity, and by hydroxylation of phenothiazines and tricyclic antidepressants.

Examples of aliphatic hydroxylation include 4-hydroxlyation of debrisoquine (Section 10.5.1) and 3-hydroxylation of 1,4-benzodiazepines (Figure 3.7).

3.2.2.3 Oxidative desalkylation

Although cytochrome P450 catalysed oxidations insert an atom of oxygen into the drug, the initial product may be unstable and rearrange. This is the case with oxidative *N*- and *O*-desalkylation reactions. In the case of desmethylations, the methyl group is lost as formaldehyde, which contains the oxygen atom from the initial oxidation. Loss of larger alkyl groups results in the production of the corresponding aldehyde. With *in vitro* metabolism studies, the aldehyde may be trapped and measured as an indictor of the degree of desalkylation. *N*-Desalkylation is common with tertiary and secondary amines (Figure 3.7). Examples of drugs undergoing *O*-desalkylation include phenacetin (described earlier) and codeine (to give morphine).

3.2.2.4 N- and S-oxidation

Tertiary amines and sulfides are oxidized to *N*-oxides and sulfoxides, respectively. Sulfoxides may be further oxidized to sulfones. Thioridazine (Figure 3.8) is an interesting example of a drug that forms an *N*-oxide,

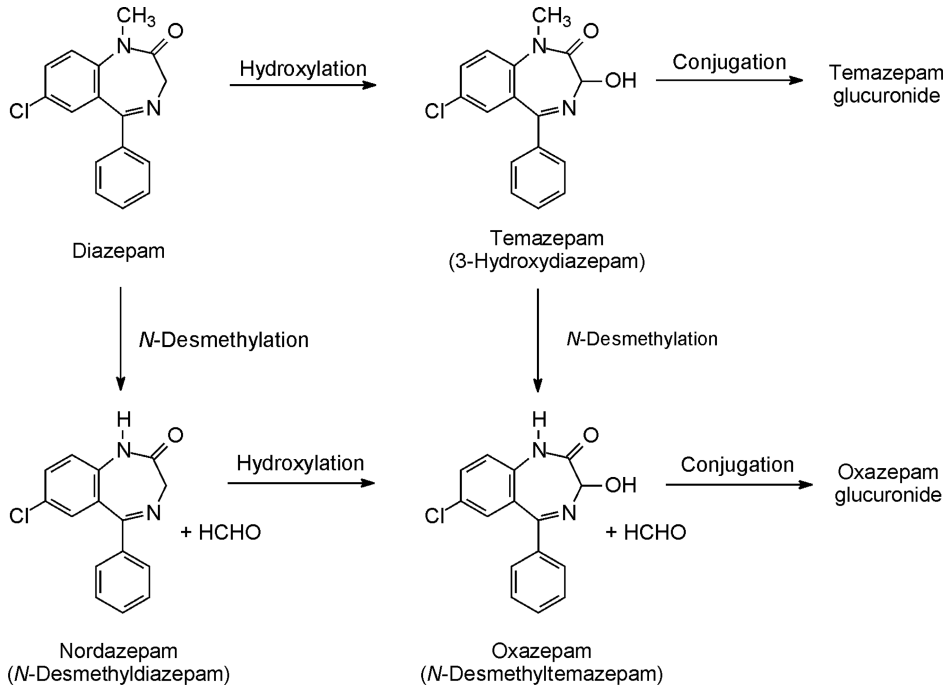


Figure 3.7 Metabolism of diazepam. Aliphatic hydroxylation gives temazepam, whilst *N*-desmethylation produces nordazepam and formaldehyde. Desmethylation of temazepam, or hydroxylation of nordazepam, gives oxazepam. The 3-hydroxy metabolites are conjugated with glucuronic acid and excreted in the urine.

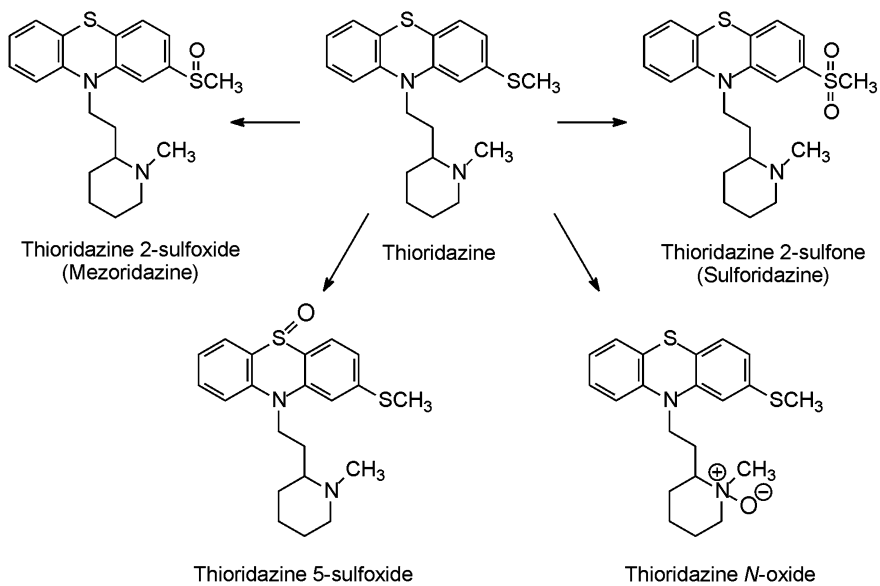


Figure 3.8 Some of the metabolic pathways of thioridazine.

a sulfone and two sulfoxides (more if the stereoisomers are taken into consideration). All phenothiazines form 5-sulfoxides, which are considered to be pharmacologically inactive. However, oxidation of the 2-thiomethyl group in thioridazine gives the 2-sulfoxide (mesoridazine) and 2-sulfone (sulforidazine) which are pharmacologically active and marketed as drugs in some countries.

N-Oxides are labile and are usually easily reduced to the parent amine or may spontaneously desalkylate to the secondary amine. A few drugs are marketed as *N*-oxides, chlordiazepoxide and minoxidil (see Figure 3.13), for example. Because of their labile nature, it is difficult to demonstrate whether the activity resides in the parent compound or one or more of its metabolites.

3.2.2.5 Oxidative deamination

Monoamine oxidase was mentioned earlier, and is responsible for deamination of endogenous amine transmitters, noradrenaline, dopamine and serotonin. Compounds with similar structures, such as ephedrine and tyramine, are deaminated to the corresponding aldehydes, which may be oxidized to carboxylic acids or reduced to alcohols. Other enzymes capable of deamination, include diamine oxidase and cytochromes; CYP2C3 for example, which has been shown to deaminate amphetamine to phenylacetone (Figure 3.9).

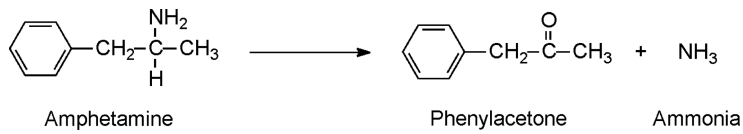


Figure 3.9 Deamination of amphetamine.

3.2.2.6 Desulfuration

Sulfur can be exchanged for oxygen. This exchange in the general anaesthetic, thiopental, produces the anxiolytic barbiturate, pentobarbital. In insects, malathion is converted to the much more toxic malaoxon (an example of lethal synthesis) whereas in mammals the major pathway is hydrolysis to the dicarboxylic acid (Figure 3.10).

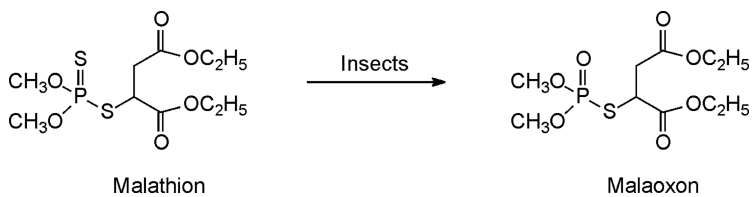


Figure 3.10 Insects convert malathion to malaoxon.

3.2.2.7 Combination of reactions

For those compounds with suitable groups, several of the reactions described above may occur. Figure 3.11 shows some of the metabolic pathways of chlorpromazine. Additionally, hydroxylation may occur at other

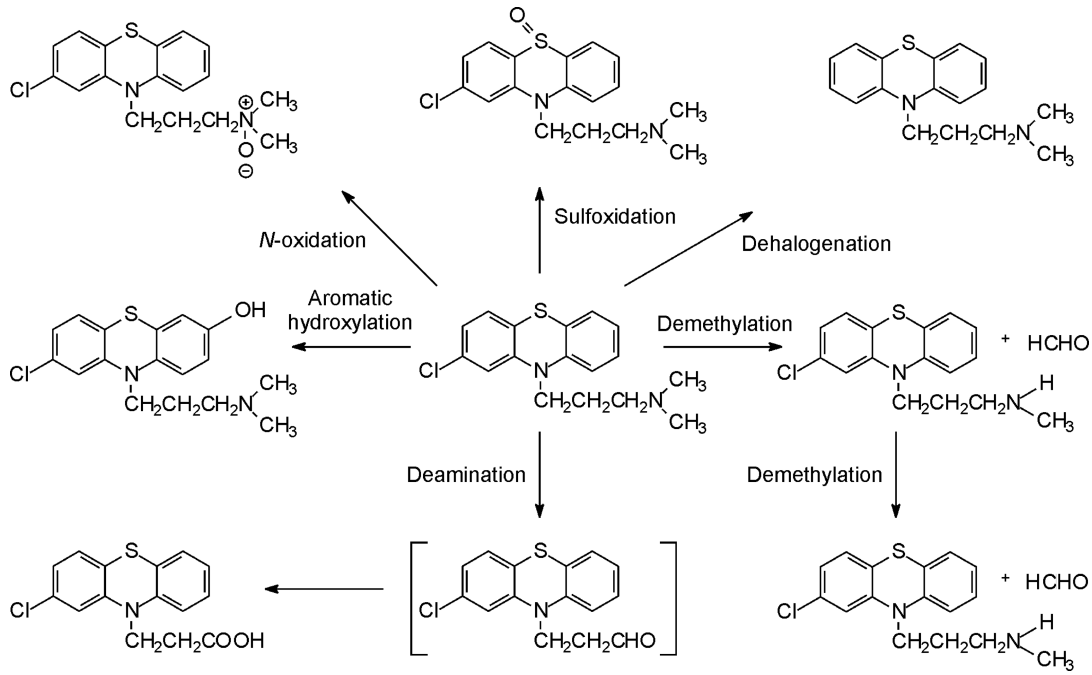


Figure 3.11 Examples of some of the pathways by which chlorpromazine is metabolized.

sites and that, plus conjugation reactions (Section 4.2.4), results in there being over 160 potential metabolites – and chlorpromazine is not the most chemically complex of the phenothiazine drugs.

3.2.3 Miscellaneous reactions

In this class are examples such as ring-opening, for example phenytoin to diphenylureidoacetic acid, and ring-formation, for example as occurs with proguanil. Transesterification can occur when ethanol is consumed. This has been shown to occur with methylphenidate (to ethylphenidate) and cocaine, when cocaethylene is formed. The ethyl homologue is active but has a longer half-life than cocaine and it has been suggested that this is why users take their cocaine with alcohol.

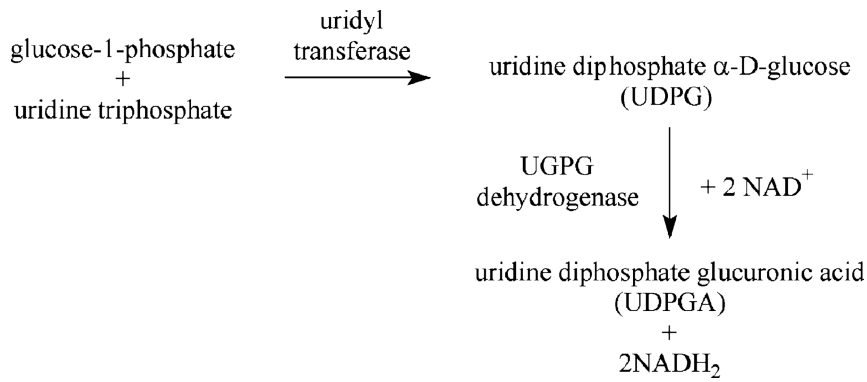
3.2.4 Phase 2 metabolism

Phase 2 reactions are those where molecules with suitably reactive groups, are conjugated with endogenous substances such as glucuronate, sulfate, acetate, amino acids and reduced glutathione (GSH). Usually the products are considerably less pharmacologically active, more water-soluble and more amenable to excretion, than the original substance. However there are some notable exceptions.

3.2.4.1 D-glucuronidation

This conjugation is a two stage process; the first stage being the synthesis of the donor molecule, uridine diphosphate glucuronic acid (UDPGA) in the cytosol. First glucose-1-phosphate is combined with uridine

triphosphate and then oxidized to the carboxylic acid:



The substrate–donor interaction is catalysed by UDP-glucuronyl transferases, which are membrane-bound enzymes. The reaction always produces the β-glucuronide (Figure 3.12).

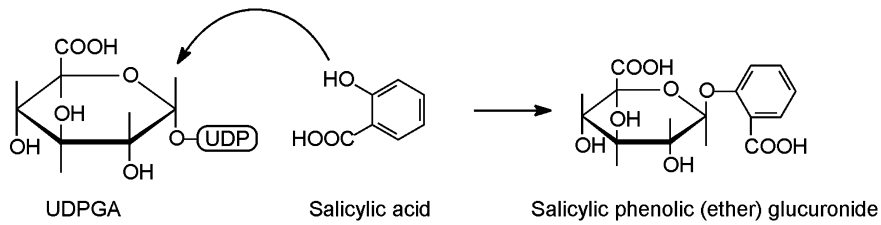


Figure 3.12 Glucuronidation of salicylic acid produces the β-glucuronide.

A large number of different glucuronides are possible, including *ether* glucuronides, formed with phenolic or alcoholic hydroxyl groups, *ester* glucuronides, formed with carboxylic acids, *N-glucuronides* formed with aromatic amines, alicyclic amines and sulfonamides and *S-glucuronides*, formed with various thiol compounds.

Generally, glucuronides are very water soluble, amenable to excretion via the kidney and bile (Sections 3.3.1 and 3.3.2), and pharmacologically inactive. However, morphine glucuronides are remarkable exceptions. There are three morphine glucuronides, 3-, 6-glucuronide and 3,6-diglucuronide. The 3-glucuronide accumulates in plasma to give higher concentrations than the parent compound and the 6-glucuronide is considered to be at least as active as morphine at μ-opioid receptors.

3.2.4.2 Sulfation

Ethereal sulfation is catalysed by several cytoplasmic sulfotransferases, depending on the substrate. The sulfate donor, 3′-phosphoadenosine-5′-phosphosulfate (PAPS), is formed from sulfate and ATP. Sulfate conjugates are usually very water soluble and readily excreted, and probably less pharmacologically active than the parent drug. The antihypertensive, minoxidil is interesting. First, it is an *N*-oxide and, second, sulfation occurs via this group (Figure 3.13). Minoxidil is also used to reduce or to prevent hair loss, and the sulfate conjugate has been shown to be 14 times more potent than minoxidil in stimulating cysteine incorporation in hair follicles.

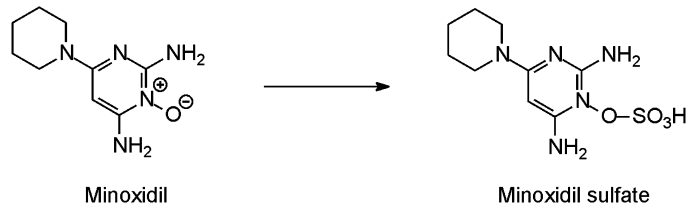


Figure 3.13 Sulfoxidation of minoxidil.

3.2.4.3 N-Acetylation

Aromatic primary amines and hydrazines are *N*-acetylated by *N*-acetyltransferase type 2 (NAT2) which is chiefly expressed in the liver, colon and intestinal epithelium. The donor molecule is acetylCo-A. NAT2 acetylates isoniazid, hydralazine, phenelzine, procainamide, dapsone and several antibacterial sulfonamides, including sulfadimidine (sulfamethazine) (Figure 3.14).

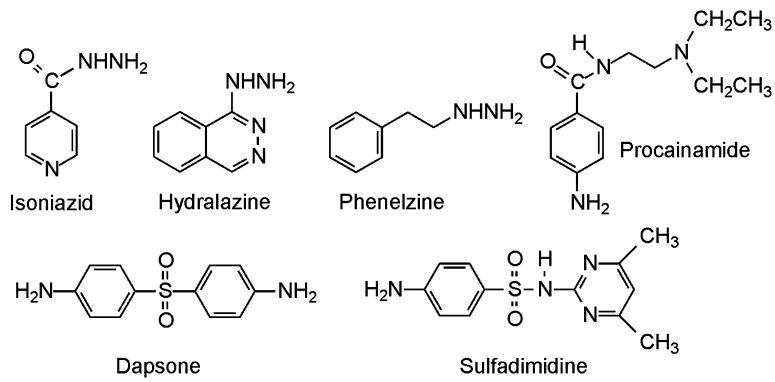


Figure 3.14 Examples of arylhydrazines and arylamines acetylated by human NAT2.

Most acetylated metabolites have reduced pharmacological activity and are considered inactive, this is particularly true of the sulfonamide antimicrobials. An exception is *N*-acetylprocainamide (acecainide) an antiarrhythmic agent that can be given either intravenously or orally.

It would be expected that acetylation would result in reduced aqueous solubility. This is only partially true; the acetyl metabolites of the older sulphonamides were less soluble in urine and caused crystalluria, but not the newer, more acidic, ones. Acetylation reduces the pK_a sufficiently to increase the degree of ionization at physiological pH values which more than compensates for the increase in lipophilicity.

3.2.4.4 Methylation

N-, *O*- and *S*-methylations are known and are common biochemical reactions, occurring with amines, phenols and thiols. The methyl donor is *S*-adenosylmethionine (SAM). Noradrenaline (norepinephrine) and related phenylethanolamines are *N*-methylated by phenylethanolamine-*N*-methyltransferase, which is found in the soluble fraction of adrenal homogenates. Histamine is *N*-methylated by imidazole *N*-methyltransferase.

Several enzymes are involved in *O*-methylation. Catecholol-*O*-methyltransferase (COMT) methylates both endogenous and foreign catecholamines, including isoprenaline (isoproteronol). Phenolic metabolites from phase 1 oxidations may be methylated, for example 7-hydroxychlorpromazine.

A number of exogenous thiols are methylated by *S*-methyltransferase. Endogenous substrates include the thiol containing amino acids, cysteine and homocysteine, and GSH. Inorganic sulfur, selenium and tellurium compounds are methylated to volatile derivatives.

3.2.4.5 Glycine conjugates

Aromatic amino acids, such as benzoic and salicylic acids, are conjugated with glycine to produce hippuric and salicyluric acids respectively. The acids react with coenzyme A, to form an acyl donor that reacts with glycine, and possibly other amino acids (Figure 3.15).

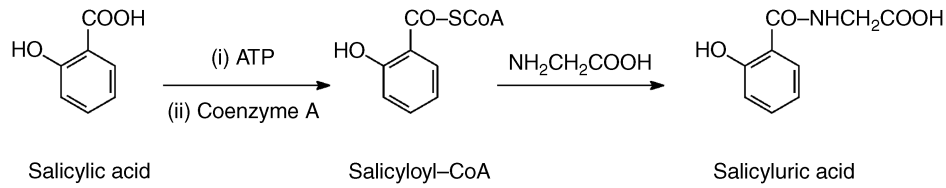


Figure 3.15 Formation of salicyluric acid.

3.2.4.6 Conjugation with glutathione

The reduced form of glutathione is an important reducing agent; one of its roles is to reduce methaemoglobin to haemoglobin. GSH usually acts as a nucleophile and can react chemically or enzymatically via a family of glutathione transferases. Substrates include aromatic nitro and halogen compounds and oxidized products of phase 1 metabolism. Probably the best known reaction of GSH is that with the reactive paracetamol metabolite, NAPQI (Figure 3.16). The GSH conjugate undergoes further (phase 3) metabolism to give the mercapurate derivative, which is excreted in urine.

3.2.5 Kinetics of metabolism

The simplest treatment of enzyme kinetics is that developed by Michaelis. The substrate combines with the enzyme and either dissociates or is converted to products:

$$\text{Enzyme} + \text{Substrate} \rightleftharpoons \text{Complex} \rightarrow \text{Products} + \text{Enzyme}$$

This is, in some ways, similar to the binding isotherms that have been derived for drug–receptor and drug–protein interactions but products are released. As the substrate concentration is increased, the enzyme becomes saturated with substrate so that the velocity, v, of the reaction cannot increase anymore [Figure 3.17(a)]. That is, for a given concentration of enzyme there is a maximum velocity, or rate, V_{max}. The rate of reaction v at any substrate concentration, C, is given by the Michaelis–Menten equation:

$$v = \frac{V_{max}.C}{K\text{m} + C} \tag{3.1}$$

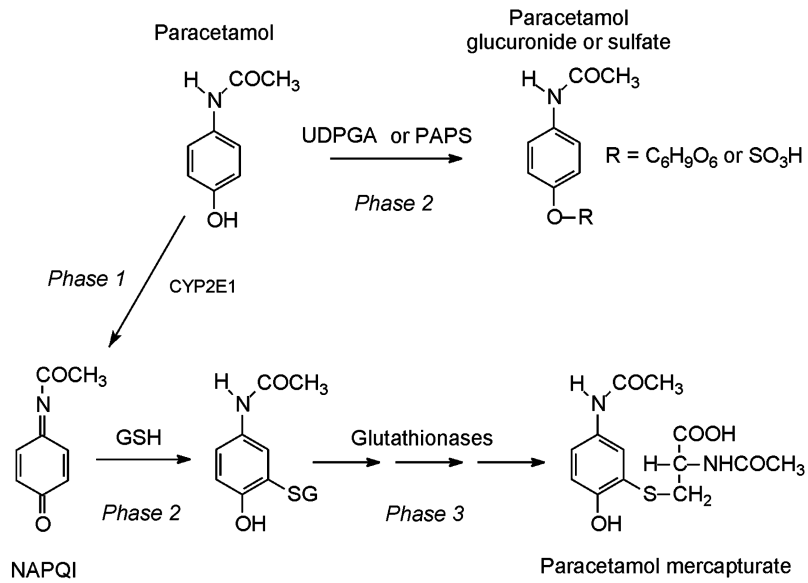


Figure 3.16 Metabolism of paracetamol.

where *K*m, which is numerically equal to the substrate concentration at one half V_{max}, is known as the Michaelis constant. This is easily demonstrated by substituting $V_{max}/2$ for v in Equation 3.1 and rearranging.

Values of *K*m and V_{max} can be derived by iteratively fitting v versus C values using relatively inexpensive computer software running on personal computers (Section 5.2.2). However, before such facilities were available Equation 3.1 was rearranged so that the constants could be obtained graphically. Lineweaver and Burk used a double reciprocal plot [Figure 17.1(a)]:

$$\frac{1}{v} = \frac{K\text{m}}{V_{max}C} + \frac{1}{V_{max}} \tag{3.2}$$

so that a plot of $1/v$ against $1/C$ is a straight line of slope $K\text{m}/V_{max}$ [Figure 3.17(b)]. Such plots can be used to investigate whether an enzyme inhibitor is competitive or non-competitive. An alternative 'linearization' of

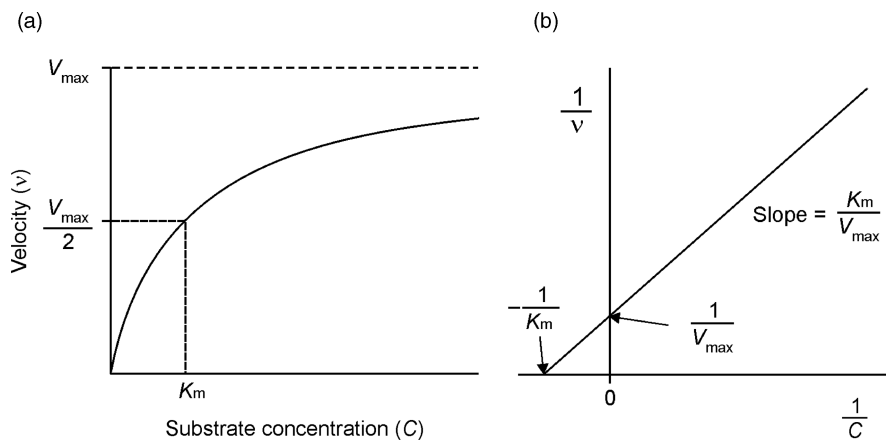


Figure 3.17 (a) Relationship between velocity and substrate concentration. (b) Lineweaver–Burk plot.

Equation 3.1 is the Eadie–Hofstee plot where v is plotted against v/C:

$$v = V_{\max} - \frac{v}{C}K\mathrm{m} \tag{3.3}$$

and Km is obtained from the slope and $V_{\max}$ from the y-intercept.

The velocities are usually obtained by conducting experiments at several initial substrate concentrations and measuring the formation of product or disappearance of substrate over a very short time period (e.g. up to 1 minute). The values can then be substituted into the Lineweaver–Burk or Eadie–Hofstee plot to obtain values of Km and $V_{\max}$. This approach avoids having to increase the substrate concentrations to the point where an accurate estimate of $V_{\max}$ can be obtained.

The majority of drugs exhibit first-order elimination kinetics (Section 1.5.3.1), even those that are extensively metabolized. The explanation is that the effective drug concentration is very low compared to that of the enzyme. Therefore at any time little enzyme is bound by drug and so that in effect the non-bound enzyme concentration is constant. At low substrate concentrations $K\mathrm{m} \gg C$, and so C makes a negligible contribution to the denominator of the Michaelis–Menten equation, i.e. $K\mathrm{m} + C \rightarrow K\mathrm{m}$ and Equation 3.1 becomes:

$$v \approx \frac{V_{\max}}{K\mathrm{m}}C \tag{3.4}$$

This is the equation of a first-order reaction because the rate is directly proportional to the substrate concentration. Applying a similar argument to the situation when $C \gg K\mathrm{m}$, the denominator approximates to C, as it is now Km that makes a negligible contribution to $K\mathrm{m} + C$ and:

$$v = \frac{V_{\max}C}{C} = V_{\max} \tag{3.5}$$

which is the situation described earlier when the enzyme is saturated. The reaction rate is constant and so the kinetics are zero-order. Ethanol kinetics approximate to zero-order at high concentrations and first-order at very low concentrations, but the kinetics can only be adequately described by the Michaelis–Menten equation at intermediate concentrations. Although most drugs exhibit first-order elimination kinetics at therapeutic concentrations, the anticonvulsant phenytoin is a notable exception and its time course can only be adequately described using Equation 3.1. Km and $V_{\max}$ apply when substrate is incubated with enzyme. However, *in vivo* the concentration is usually the plasma concentration and so the constants are not the same and should be referred to as 'apparent Km' and 'apparent $V_{\max}$'.

3.3 Excretion

The organs most involved in excretion are the kidney and liver. Although the liver is an important site of drug metabolism it is also an important site of excretion, drugs and their metabolites being excreted via the bile. Other sites might not be so important with regards to the amounts excreted but may be of toxicological significance. Excretion of drugs in milk may have consequences for a feeding infant. Expired air is used for medico-legal estimates of blood alcohol whilst measurement of drugs in saliva, sweat, hair and nails are often of forensic interest.

3.3.1 Urine

The functioning unit of the kidney is the nephron, there being over 1.2 million in a human kidney (Figure 3.18).

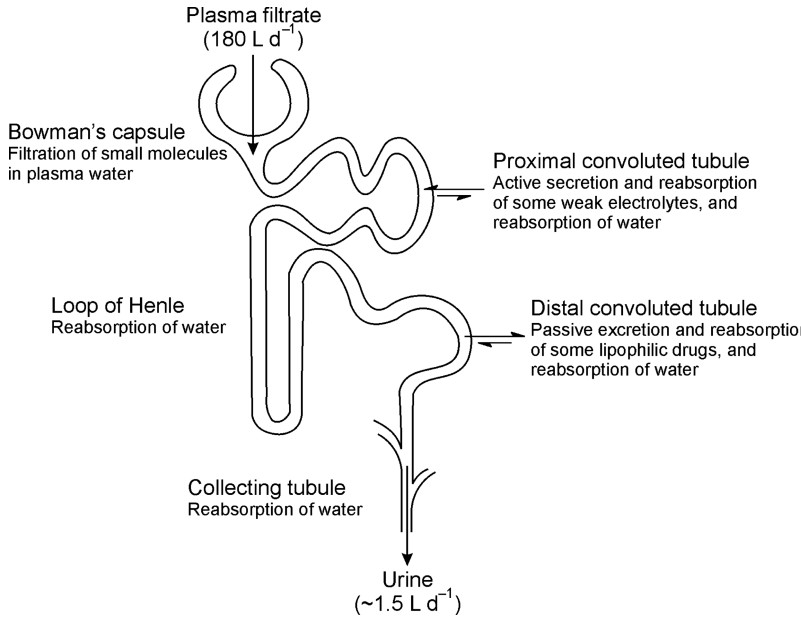


Figure 3.18 Diagrammatic representation of the principal processes involved in the renal excretion of drugs.

3.3.1.1 Glomerular filtration

The Bowman's capsule filters small molecules up to the size $M_r = \sim 66{,}000$. Peptides may be filtered. Appoximately 90% of the insulin ($M_r = \sim 6{,}000$) in plasma water appears in the ultrafiltrate whereas the figure for serum albumin ($M_r = \sim 69{,}000$) is normally $<0.1\%$. Approximately 180 L of plasma is filtered per day, equivalent to 13–15 times the volume of extracellular fluid. The plasma filtrate has the composition of plasma water, so small, unbound drugs are filtered, but bound drug is retained.

3.3.1.2 Active tubular secretion

The proximal convoluted tubular cells have at least three types of transport proteins. Organic anion transport proteins (OATP) and organic cation transporters (OCT) are thought to be in the basolateral membrane while P-gp is probably in the apical membrane. Many acidic drugs and their glucuronide and glycine conjugates are actively transported. Examples include penicillins, some sulfonamides and thiazide diuretics. Examples of basic drugs actively secreted include quinine, pethidine (meperidine), amiloride and triamterene.

Penicillins have short elimination half-lives (~0.5–1 h), which can be increased by co-administration of probenecid which competes for the OATP so that the renal excretion of the penicillin is reduced. Probenecid is no longer used for this purpose but it is sometimes used as a uricosuric drug, because it competes for the carrier that actively *reabsorbs* uric acid in the PCT, and so increases the excretion of uric acid.

3.3.1.3 Passive diffusion

To some extent the kidney tubule behaves like a typical lipid barrier. Lipophilic molecules may diffuse in either direction and, if it were a static system, the concentrations in tubular fluid and plasma water

would equilibrate. However, as a very large proportion of the filtered water is reabsorbed (Figure 3.18), the concentration of the tubular fluid markedly increases so the net movement of drug or metabolite is from tubular fluid to plasma, that is lipophilic molecules are reabsorbed. The reabsorption of weak electrolytes will be affected by the degree of ionization and the pH-partition hypothesis applies (Section 3.3.1.5).

Lipophilic drugs tend to be highly protein bound; they are hydrophobic and tend whenever possible to move from aqueous to non-aqueous environments. Protein binding reduces glomerular filtration and that, coupled with tubular reabsorption, means that little parent drug appears in the urine. This nicely illustrates the need for prior metabolism of such drugs as an aid to excretion – albeit as metabolites.

3.3.1.4 Renal clearance

The functioning of the kidney and how it handles substances has been investigated and described in terms of the physiological concept, renal clearance:

$$CL_R = \frac{U}{P} \times \text{urine flow rate} \tag{3.6}$$

where U is the concentration of substance in urine and P the concentration of substance in plasma. The ratio U/P is dimensionless so the units of clearance are those of urine flow – usually mL min^{-1}. Renal clearance can be defined as the volume of plasma flowing through the kidneys from which substance is removed per unit time, usually per minute. The two kidneys combined receive ~20–25% of the cardiac output ~1200 mL min^{-1} of blood, or ~650 mL min^{-1} plasma. If 10% of a substance was removed from the plasma as it passed through the kidneys, then the clearance would be 65 mL min^{-1}, or if it were 20% it would be 130 mL min^{-1}. The proportion removed is known as the extraction ratio, E, and so

$$CL_R = EQ \tag{3.7}$$

where Q is the plasma flow rate. It follows from Equation 3.7 that the maximum value of renal clearance cannot exceed the plasma flow, when $E = 1$.

The renal clearance of a substance that is freely filtered, not actively secreted by renal tubular cells and not reabsorbed is a measure of the glomerular filtration rate (GFR). Inulin is a highly water-soluble polysaccharide that is not bound to plasma proteins; it is not secreted, nor reabsorbed, and is used to assess GFR. Its value is maximal in males of about 20 years (125 mL min^{-1}, for both kidneys combined). It is less in women and declines with age. Creatinine clearance is sometimes used to assess GFR or serum creatinine is taken as an indicator of renal function, the premise being that reduced filtration is reflected in elevated serum creatinine. However, creatinine is a metabolite of creatine, chiefly found in skeletal muscle and can vary, so exogenously administered inulin is a more accurate method of measuring GFR. The interest in GFR is that it is an indicator of kidney function and doses of some drugs are adjusted on the basis of GFR.

p-Aminohippuric acid (PAH) is actively secreted and totally cleared as it passes through the kidney, that is, its extraction ratio is 1. This means that renal clearance of PAH equals the plasma flow through the kidneys, and PAH has been used to measure this. Kidney *blood* flow will be plasma flow divided by $(1 - H)$, where H is the haematocrit.

Because of tubular reabsorption, it is not always obvious from the renal clearance whether a drug undergoes active tubular secretion. Penicillin G has a renal clearance of approximately 350 mL min^{-1} and as this far exceeds GFR must be actively secreted into tubular fluid. However, the converse is not true. A drug

may be actively secreted but reabsorption may be such that $CL_R <$ GFR. Some indication may be obtained by comparing CL_R with the filtration rate of the unbound fraction. Competition between the protein carriers and plasma proteins probably means that plasma protein binding has little, if any, effect on active tubular secretion of drugs.

3.3.1.5 Effect of urine pH and flow rate

The pH of urine may vary, depending on diet and other factors, but pH 6.3–6.6 is a reasonable range under normal physiological conditions. Changing urine pH, say by administering sodium bicarbonate (sodium hydrogen carbonate, $NaHCO_3$) to make it alkaline, can have a major effect on the excretion of some weak acids and bases. The ionization of salicylic acid ($pK_a = 3.0$) at pH 6.3 is 99.95% whereas at pH 7.3 it is 99.995% ionized. This extremely small difference in the degree of ionization, represents a 10-fold difference in the proportion non-ionized (0.05–0.005%). The difference at pH 8.3, a value that can be easily obtained by giving sodium bicarbonate, is 100-fold.

Similar calculations can be performed for a base like amphetamine ($pK_a = 9.8$). At pH 7.8 the percentage non-ionized is 1% but at pH 4.8 the percentage falls to 0.001%, which makes a major change to the amount not reabsorbed and hence renal clearance. A similar situation exists for methamphetamine ($pK_a = 9.9$) and this is reflected in the amounts excreted at different pH values (Figure 3.19).

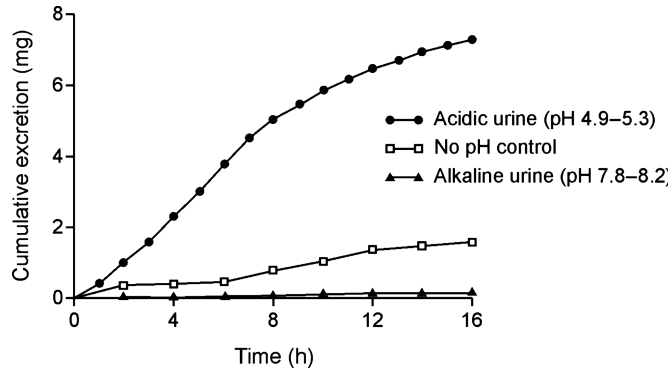


Figure 3.19 Effect of urine pH on amount of methamphetamine excreted in a single subject. (Redrawn from Beckett and Rowland, 1965).

3.3.2 Biliary excretion

Drugs excreted in the bile enter the duodenum via the hepatic duct. The rate of release of bile is not uniform but is increased in response to food. Those drugs and metabolites that remain in the GI tract will be removed from the body in the faeces; however there may be some reabsorption back into the body via the hepatic portal system (Section 2.3.1). When temoporfin, an anticancer drug, was injected intravenously, over 99.9% of the dose was recovered in the faeces, demonstrating how important biliary excretion can be. It also illustrates the important fact that finding an orally administered drug in faeces does not necessarily indicate that it has not been absorbed.

Bile canaliculi run between adjacent rows of hepatocytes to join the branching bile duct. Drugs may diffuse passively into the bile but this will result in relatively low concentrations similar to those of the unbound drug. Thus one would expect most drugs to be present in bile to some extent. Because the bile flow is

only ~0.5–0.8 mL min^{-1} the biliary clearance, CL_{Bile}, of such drugs will be low:

$$CL_{Bile} = \frac{\text{Concentration in bile}}{\text{Concentration in plasma}} \times \text{bile flow rate} \tag{3.8}$$

However, some drugs and, particularly, water-soluble metabolites such as glucuronides, are actively secreted into bile and can attain bile/plasma ratios in excess of 500 and the biliary clearance of these molecules may be several hundred mL min^{-1}. It is thought that there are at least three types of transport proteins, cationic and anionic ones and P-gp. There appears to be a molecular weight cut-off for active transport with substrates having to have M_r values > 325. This figure is for the rat and there are species variations. Above the minimum, the degree of biliary excretion increases with increasing M_r. Another feature is that polar, acidic molecules make good substrates. This is supported by the fact high concentrations of glucuronide metabolites are often found in bile. Glucuronides are polar and acidic and glucuronidation increases the molecular mass of the parent drug by 193 g mol^{-1}. It is likely that active transport has evolved to transport bile acids and compounds such as bilirubin glucuronide.

Suitable molecules may be actively transported without prior metabolism. The water-soluble cardiac glycoside, ouabain ($M_r = 728.8$) can have a bile:plasma ratio as high as 500. It is likely that temoporfin ($M_r = 680.7$) is actively transported into bile. Sulfobromophthalein ($M_r = 705.6$) is used to test liver function.

3.3.3 Expired air

The lungs are an important site for the excretion of volatile drugs and metabolites. The majority of gaseous and volatile general anaesthetics are excreted in expired air and because they are administered by the same route, the dose and hence the level of anaesthesia can be controlled by adjusting the partial pressures of these agents in the breathing apparatus. Deep alveolar air concentrations tend to equilibrate with those in blood, as would be predicted by Henry's law. This is the basis of breath alcohol measurements. At 34 °C the distribution between blood and alveolar air is 2100 : 1, that is 2100 L of air contain the same amount of ethanol as 1 litre of blood. Other volatile liquids are excreted via the lungs. For example, it has been shown that ~40% of an oral dose of benzene is excreted in expired air. Inorganic selenium salts are metabolized to dimethyl selenide (Section 3.2.1.2), which is exhaled, giving a garlic smell to the breath. Extensive metabolism of an organic compound will eventually produce carbon dioxide which may be excreted in expired air. Study protocols for metabolism studies with ^{14}C-labelled drug may include trapping any exhaled $^{14}CO_2$.

3.3.4 Saliva

Excretion of drugs in saliva appears to occur largely by passive diffusion. The pH of saliva can vary from 5.5, the lower limit for parotid saliva, to 8.4 in certain ruminants. The average pH of 'mixed' saliva is ~6.5, about 1 unit lower than plasma pH. Thus the unbound concentrations of basic drugs are usually higher in saliva than in plasma water whist the converse is true of weak acids. The saliva (S) to plasma (P) equilibrium ratio for an acid will be:

$$\frac{S}{P} = \frac{(1 + 10^{(pH(s) - pK_a)}) f_{u(p)}}{(1 + 10^{(pH(p) - pK_a)}) f_{u(s)}} \tag{3.9}$$

where pH(s) is the saliva pH, pH(p) is the plasma pH and $f_{u(p)}$ and $f_{u(s)}$ are the free fractions in plasma and saliva, respectively. There is less protein in saliva than in plasma – what there is, being chiefly antimicrobial and digestive enzymes secreted by the salivary glands. Consequently, the amount of drug-binding protein in

saliva is usually considered to be insignificant, so $f_{u(s)}$ is taken to be unity. For salicylic acid, $pK_a = 3.0$, which is 50–90% protein bound in plasma, depending on the dose, the S/P ratio would be expected to be 0.06–0.01 for a saliva pH of 6.5. The equivalent equation for bases is:

$$\frac{S}{P} = \frac{(1 + 10^{(pK_a - pH(s))}) f_{u(p)}}{(1 + 10^{(pK_a - pH(p))}) f_{u(s)}} \tag{3.10}$$

Pethidine, a weak base, $pK_a = 8.7$, is 40–50% bound to plasma proteins so, for a saliva pH of 6.5, the S/P ratio calculates to be 4.6–3.8. This is consistent with reports that pethidine concentrations in saliva are higher than those in plasma.

There is little evidence that active transport is important for secretion of drugs into saliva. Penicillin may be secreted as a study showed inhibition by probenecid, and metoprolol concentrations have been reported as being higher than predicted by Equation 3.10. Active transport of ions results in saliva being hypotonic, having less sodium but higher concentrations of potassium than plasma. S/P ratios for lithium are 2–3 and vary with plasma concentration, indicating that there is active secretion. The characteristics of this transport have been reported as being very similar to those for potassium.

The interest in salivary excretion has arisen (i) because it was seen as a non-invasive method of sampling for pharmacokinetic studies, for example, in calculating antipyrine half-lives, (ii) as a way of monitoring drugs, both therapeutic and drugs of abuse, and (iii) a method of estimating the plasma concentrations of the unbound drug. However, there are several caveats and considerations. Three pairs of glands are responsible for most of the saliva that comprises oral fluid. In the resting state the relative percentage flows are parotid (21), submandibular (70), sublingual (2) with minor glands making up the balance. On mechanical stimulation the percentage produced by the parotid increases (58) whilst that for the submandibular decreases (33). However, when flow is stimulated by citric acid, the parotid and submandibular flows are similar at approximately 45% of total. Salivary flow is under autonomic control. Stimulation of β-receptors increases the amount of protein and mucin and results in a more viscous fluid whereas α-adrenoceptor excitation increases protein. Muscarinic stimulation increases saliva flow which is accompanied by increases in sodium and bicarbonate concentrations leading to increased pH. Clearly drugs acting at these receptors will influence the composition of the oral fluid. Saliva flow shows diurnal variation and its composition may be affected by disease. Other issues include:

- Saliva concentrations may be low, particularly for highly protein bound drugs.
- Flow rate $\sim$1 mL min^{-1} – may result in insufficient sample, and/or long collection periods.
- Stimulating flow changes the composition – move to more constant pH 7.4. Drugs may be lost on materials chewed to stimulate flow.
- Effect of drugs on salivary glands.
- Some individuals, subjects and experimenters, find saliva unpleasant and foaming and frothing may be problematic. Special collecting aids overcome some of the problems.

Despite the problems, it is possible to use saliva for kinetic investigations when it is usual to validate against blood samples (Figure 3.20). However, it is drug monitoring that is driving the increasing interest in oral fluid analysis. For drugs of abuse, many of which are basic, sampling oral fluid is non-invasive and is done under supervision to reduce the risk of adulteration of the sample. Oral fluid is useful for situations where venepuncture may be difficult, such as the nervous patient or more importantly, for therapeutic drug monitoring in children, who are often less embarrassed than adults about spitting into a container. It has been suggested that phenytoin concentrations in saliva are a good approximation to the unbound concentration in serum and that this is a better indicator for dose adjustment than total serum concentrations.

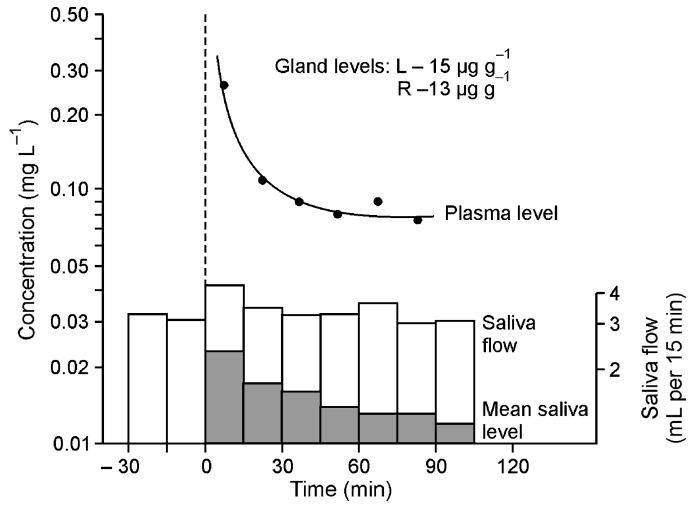


Figure 3.20 Concentrations of clonidine in plasma, submaxillary gland tissue (L = left, R = right) and submaxillary gland saliva, in a cat after 250 mg kg^{-1}, intravenously (Cho and Curry, 1969).

3.3.5 Stomach and intestine

A number of basic drugs, following i.v. doses are excreted into gastric juice in the stomach. This is predictable from the pH-partition hypothesis. Amongst the drugs studied this has been demonstrated for quinine and nicotine. Water-soluble drugs and ionized forms of weak electrolytes are likely to be excreted into the intestine in a way analogous the excretion to drugs into the stomach. With both the stomach and the intestine, diffusion from blood into the GI lumen is an essential part of the reversible diffusion reaction involved in the absorption of drugs. When the concentration gradient is favourable, for example after parenteral administration, the net movement will be into the GI lumen.

3.3.6 Breast milk

Excretion in milk is a minor pathway in terms of the amount of drug eliminated from the mother. It is of interest because of the potential effects of the drug on the suckling infant and the environmental exposure of populations to veterinary drugs in cows' milk.

A number of drugs appear in milk, usually in small concentrations suggesting that the mechanism of transfer is passive diffusion i.e. the breast behaves as a lipid membrane. Breast milk is slightly more acidic on average (pH 7.2) than plasma and contains some protein, chiefly whey proteins, lactoferrin and α-lactalbumin. Albumin is present at ~0.4 g L^{-1}, approximately 1/10th of the concentration in plasma. Assuming drugs enter milk by passive diffusion, a method for estimating the amount of a drug that will pass into milk has been proposed (Atkinson and Begg, 1990). Factors taken into consideration are the pK_a, the degree of protein binding in plasma and the lipophilicity, assessed as log $D_{7.2}$, (octanol partition coefficient at pH 7.2). The method predicted a milk/plasma ratio of 2.33 for venlafaxine, which was in good agreement with an average observed value of 2.5. Despite the high concentration of venlafaxine in milk (Figure 3.21), the proportion of the mothers' dose consumed by the infants ($n = 6$) was calculated to be <6% (mean = 3.2%).

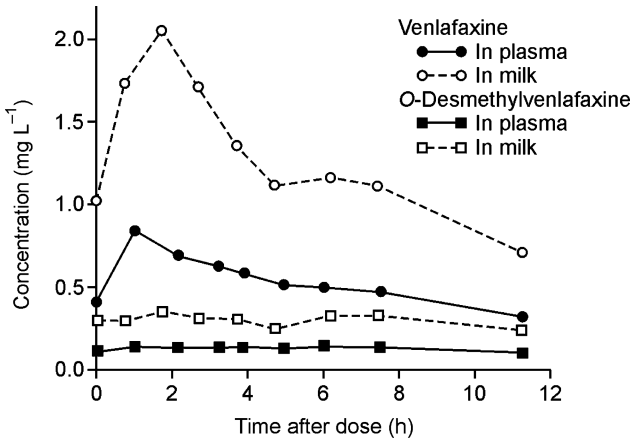


Figure 3.21 Steady-state venlafaxine and *O*-desmethylvenlafaxine concentrations in plasma and milk in a single subject. (Redrawn from the data of Ilett *et al.*, 2002.)

Interestingly, as the recommendations to breast feed increase, so the number of studies showing that the risk from maternal drug use is small, is increasing. However, it is wise to treat each case individually and to monitor the wellbeing of the infant. The death of a 13-day-old breast fed baby has been ascribed to the codeine his mother was taking for post-partum pain (Madadi *et al*., 2007). This was an unusual case because the mother was an ultrafast metabolizer of codeine to morphine (Section 10.5.1) and also rapidly converted morphine to morphine 6-glucuronide (Section 10.8.1).

3.3.7 Other routes of excretion

Amongst the remaining minor routes of excretion are sweat, keratinous tissues and genital secretions. The increasing interest in these routes of excretion is because the samples provide a way of demonstrating exposure to drugs, either by detecting the drug directly or one or more of its metabolites.

3.3.7.1 Sweat

There are two kinds of sweat gland, eccrine and apocrine. The latter, which form during puberty are associated with hair in the pubic region and under the armpits, and secrete a thick liquid into the hair follicle. Eccrine glands produce a watery sweat that is ~99% H_2O. It as long been known that drugs are excreted in sweat. This was demonstrated for quinine as early as 1844 and for morphine in 1942. There appear to have been few systematic studies as to the mechanisms involved, but a simple diffusion model probably applies as some basic drugs have sweat/plasma ratios >1 whilst weak acids have ratios <1 as would predicted from the pH-partition hypothesis for distribution drugs between plasma water and sweat, pH 5.8 (Johnson and Maibach, 1971).

3.3.7.2 Hair and nails

Drugs and their metabolites enter hair cells, probably by passive diffusion, as the hair is growing. Because the cells subsequently die, the drugs not only remain in the hair they are not metabolized and may be measured, months, years and even centuries later. This explains the forensic interest in measuring drugs in hair as a means of assessing previous exposure. For example, by examining segments of hair it is possible to determine periodic exposure to drugs and poisons.

3.3.7.3 Genital secretions

Obviously, excretion of drugs in vaginal secretions or seminal fluid is not going to play a major role in the removal of a drug from the body. Interest in these fluids arises because of the potential for drugs to interfere with sperm motility, have teratogenic effects or provide forensic evidence in a case of sexual assault. The excretion of drugs in semen has been reviewed by Pichini *et al*. (1994). In man approximately 30% of seminal fluid originates from the prostate and ~60% from seminal vessels. The mechanism by which drugs enter seminal fluid is probably by passive diffusion and the equilibrium plasma:seminal fluid ratio of weak electrolytes will be given by modified forms of the Henderson–Hasselbalch equations, analogous to Equations 3.9 and 3.10. However, the situation is complicated by the fact that prostatic fluid is acidic (pH ~ 6.6) while that of vesicular fluid is alkaline (pH ~ 7.8). Some antibiotics have been shown to have fluid/plasma ratios >1, for example ciprofloxin (5.8) and norfloxacin (5.4) but for many other drugs the ratio is less than one, for example, carbamazepine (0.4–0.7), phenytoin (0.17) and valproic acid (0.07), possibly reflecting the effect of plasma protein binding.

3.3.8 Cycling processes

Drugs that are excreted into saliva or the stomach are likely to be reabsorbed from the GI tract, particularly those that are readily absorbed when given orally. Therefore, excretion via these routes will make little contribution to the overall removal of such drugs from the body. Instead these drugs will 'trapped' in a cycling process: blood → saliva → intestine → blood, or blood → stomach → intestine → blood.

3.3.8.1 Enterohepatic cycling

This is a further, and possibly more significant, form of cycling. Drugs that are excreted in bile may be reabsorbed from the intestine, in much the same way as described above. However, as explained previously (Section 3.3.2), a number of drugs are excreted in bile as glucuronide metabolites. Water-soluble glucuronides are not normally absorbed from the GI tract. However, if the conjugate is hydrolysed back to the original drug by β-glucuronidases in intestinal micro-organisms then drug can be reabsorbed, conjugated and secreted again leading to cycling of drug and drug–conjugate (Figure 3.22). Obviously

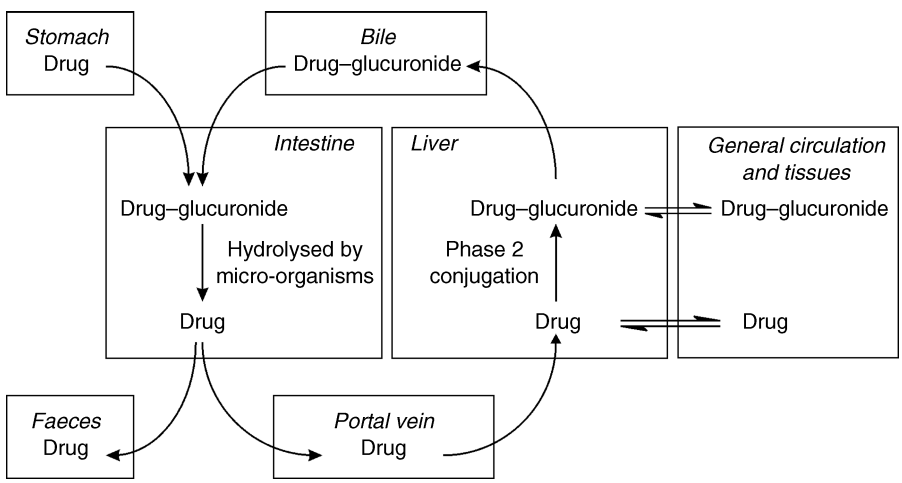


Figure 3.22 Enterohepatic recirculation. Orally administered drug enters the intestine from the stomach and is absorbed into the hepatic portal vein, carried to the liver and converted to the glucuronide which is secreted in the bile. Intestinal micro-organisms hydrolyse the glucuronide liberating parent drug, which can be reabsorbed.

only drugs that undergo phase 2 glucuronidation directly without a prior phase 1 reaction can do this. Examples of such drugs include stilboestrol, chloramphenicol, phenolphthalein and morphine.

3.3.8.2 Significance of cyclic processes

A drug trapped in a cycle persists in the body for longer than would otherwise occur and this reduces the rate of decline in plasma concentrations to some extent. Because the excretion of drugs via saliva and the stomach usually represent a steady release of small proportions of the dose it is most unlikely that the cycling will lead to any discernable fluctuations in plasma concentrations. On the other hand, the release of bile in man is pulsile and greater quantities are released in response to food, particularly fatty meals. Some studies have reported increases in plasma concentrations after meals, which have been ascribed to enterohepatic cycling. Rises in plasma concentrations of β-blocking drugs, benzodiazepines and phenothiazines have been observed at late times following intravenous injection.

Enteroheptic cycling will increase the half-life of a drug but the effect on the activity may be small if only a small proportion of the dose is being cycled. Furthermore, for the majority of drugs, drug molecules in the cycle are not available to the receptors, apart possibly from the laxative, phenolphthalein and the anti-diarrhoeal, morphine.

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4

Elementary Pharmacokinetics

4.1 Introduction

Pharmacokinetics is the science of using models (especially mathematical equations) to describe the time course of a drug and/or its metabolites in the body, usually in terms of the concentrations in plasma, although other fluids, such as whole blood or urine may be used. Further information may be gained from analysis of tissues or faeces. Probably the most important applications of the subject are in new drug development and in understanding and controlling optimum drug use. It is generally assumed that there is a relationship between the concentration of drug in plasma and the effects that are elicited. This is illustrated in Figure 4.1, which represents the time course of a drug in plasma following a single dose.

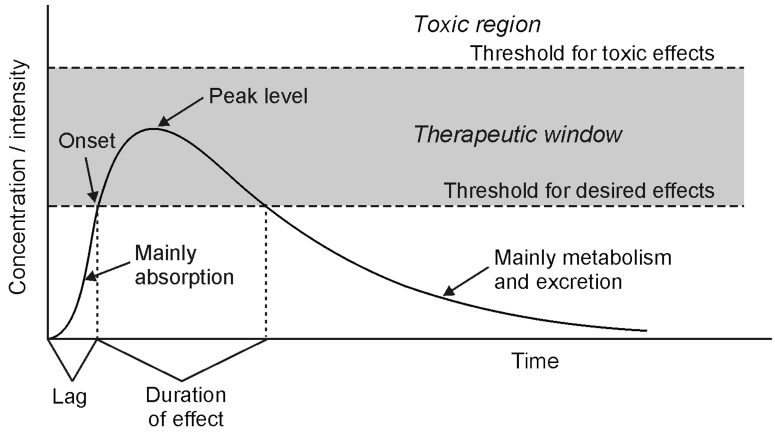


Figure 4.1 Model curve showing a theoretical perfect relationship between drug effect (intensity) and drug concentration following a single dose by any route other than intravenous injection.

If there is a minimum plasma concentration below which there is no therapeutic effect and a higher one, associated with the onset of unwanted, toxic, effects the intermediate concentrations must be those associated with wanted, therapeutic effects. This region is referred to as the 'therapeutic range' or 'therapeutic window'. If a suitably sized single oral dose, for example of aspirin for suppression of recurring inflammatory pain, is taken, there will be a lag-time while the tablet or capsule disintegrates and until sufficient drug has been absorbed for the plasma concentration to reach the threshold for effect to begin. The effect will cease when elimination has reduced the concentration to below the minimum concentration for the desired effect. The duration of effect will be the time between these two points. A larger dose may be taken to increase

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the intensity, or even the duration of effect, but it is clear from Figure 4.1 that there is a limit to the size of a single dose if toxic concentrations are to be avoided. Doubling the dose generally doubles the peak concentration, and may increase the intensity of the effect, but it will not necessarily double the duration of action. To obtain a longer duration of action, it is necessary to give smaller doses at regular intervals and if the dose and dosage intervals are chosen appropriately the concentrations can be maintained within the therapeutic window (Figure 4.2). The curves of this figure were generated using a simple pharmacokinetic model which will be explained later in this chapter.

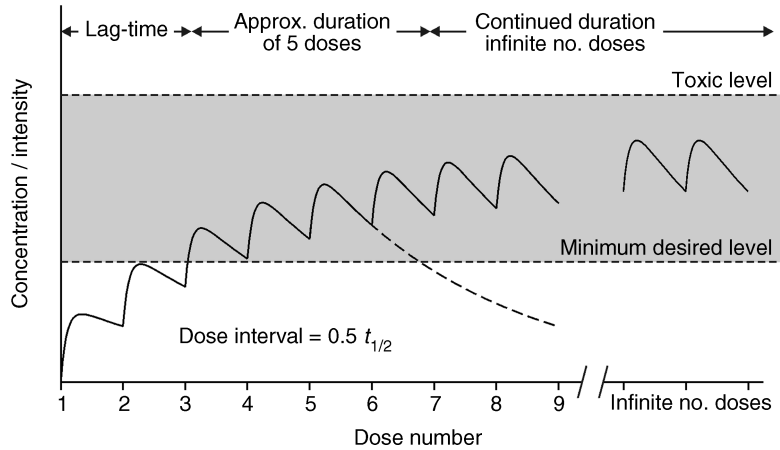


Figure 4.2 Model curves showing a theoretical perfect relationship between drug effect (intensity) and drug concentration during a series of eight doses, with a dose interval $0.5 \times t_{1/2}$ of the drug. The broken line following the fifth dose represents the decay if that had been the last dose.

The crucial point about deriving a model is that it can be used to predict what will happen when the parameters are changed. Pharmacokinetics can be used to:

- Assess the rate and extent of absorption
- Predict the effects of changing doses and routes of administration
- Model the effect of enzyme induction/inhibition and changes in excretion
- Model relationships between concentrations and effect (PK/PD).

Various types of model may be used, but probably the best known, and most widely used, are *compartmental* models. These assume that the drug in the body can be treated as if it were in (a) homogeneous solution(s) in one or more pools or 'compartments'. The concept of compartments is discussed in more detail in Section 5.1.5.

4.2 Single-compartment models

The simplest of compartment models is one where the drug is considered to be present as a well-stirred, homogeneous solution, rather like the solution of a dye in the flask depicted in Figure 4.3(a). The volume of the flask is analogous to the apparent volume of distribution of the drug, as discussed in Section 2.4.1.1. A drug is eliminated by metabolism and/or excretion as discussed earlier in Chapter 3 and, in the figure these processes are represented by the water flowing through the flask. In pharmacokinetics, the model is usually illustrated as a box or circle with an input and an output [Figure 4.3(b)].

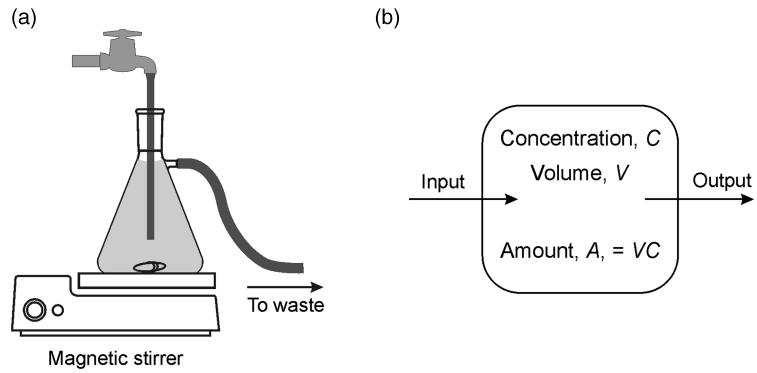


Figure 4.3 (a) Single compartment models are based on the concept of a well-stirred solution of drug. (b) Typical representation of a single compartment model.

Without any recourse to mathematics, it should already be apparent from Figure 4.3(a) that if dye (representing the drug) is rapidly introduced into the flask (representing a bolus i.v. injection) the flow of water will gradually wash out the dye and, furthermore:

- The faster the flow of water, the faster the dye will be removed, and
- The larger the flask, the longer it will take to remove the dye; although of course the initial concentration will be lower.

Intuitively, dye is being removed according to first-order elimination kinetics, observable in the flask as a gradual reduction in the colour of the solution, and this can be confirmed by writing a differential equation of the form of Equation 1.7 described in Section 1.5.3.1. The concentration, C is declining exponentially, and so is the amount, A. Because the volume is constant, $A = V \times C$, and the rate of elimination can be written:

$$-\frac{dA}{dt} = \lambda A = \lambda VC \tag{4.1}$$

where λ is a first-order elimination rate constant.

4.2.1 Systemic clearance

Systemic clearance, *CL*, also known as plasma clearance or whole body clearance, can be defined as the volume of plasma from which drug is removed per unit time, usually expressed as $\mathrm{mL\,min^{-1}}$ or $\mathrm{L\,h^{-1}}$. Clearance is the sum of all the *organ* clearances, so if a drug is eliminated by the kidney and the liver and no other routes:

$$CL = CL_R + CL_H \tag{4.2}$$

where CL_R and CL_H are the renal and hepatic clearances, respectively. Organ clearance is the volume of plasma flowing through the organ from which the drug is removed per unit time. The organ clearance cannot exceed the plasma flow rate through the organ (Equation 3.7, Section 3.4.1.1.). This is represented in Figure 4.4.

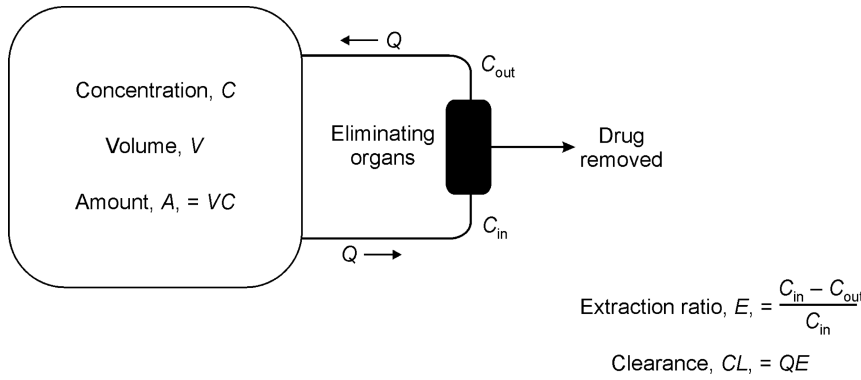


Figure 4.4 Schematic diagram to illustrate the concept of systemic clearance. The extraction ratio is the proportion of drug removed as it flows through the eliminating organs.

The clearance can be used to calculate the rate of elimination of a drug from its plasma concentration because:

$$\text{rate of elimination} = C \times CL \tag{4.3}$$

Provided *CL* is constant, this is a first-order equation because the concentration is declining exponentially and therefore Equations 4.1 and 4.3 are equal:

$$C \times CL = \lambda VC \tag{4.4}$$

and cancelling *C* from either side gives:

$$CL = \lambda V \tag{4.5}$$

Because the elimination kinetics are first-order, the half-life is given by Equation 1.10, which can be rearranged to:

$$\lambda = \frac{0.693}{t_{1/2}} \tag{4.6}$$

This, when substituted into Equation 4.5 and again rearranged, produces:

$$t_{1/2} = \frac{0.693\ V}{CL} \tag{4.7}$$

In Section 1.5.3 it was shown that Equation 4.6 arises from simple equations for first-order decay of concentrations in plasma incorporating the measured concentration at any time *t*, the theoretical concentration when $t = 0$, and using natural or common logarithms (Equation 1.10).

According to Equation 4.7, $t_{1/2}$, is a *dependent* variable that increases when the apparent volume of distribution increases and decreases when clearance increases. This is in agreement with the model of Figure 4.3(a), where the volume of the flask is analogous to *V* and the flow rate of water represents *CL*. Because no dye is returned to the flask, the extraction ratio, $E, = 1$ and so the clearance equals the flow, *Q*. A more general method for obtaining *CL* using the area under the plasma concentration–time curve (*AUC*) is described later (Equation 4.14, Section 4.2.3.2. and Section 5.1.1.4).

4.2.2 Why drugs have different elimination half-lives

The Büchner flask of Figure 4.3(a) may seem far removed from the complex anatomy and physiology of the body, but the single-compartment model, although it is not always applicable, is a useful introduction to PK

modelling. First, Equation 4.7 explains why drugs have different half-lives; it is because they have different volumes of distribution and/or systemic clearance. The apparent volume of distribution will depend upon the nature of the drug (Table 2.1) and the individual. Lipophilic drugs tend to have large apparent volumes of distribution and, if they enter adipose tissue, will be more widely distributed in someone who is obese. Also, changes in binding may result in changes in the distribution of drugs that are bound to plasma protein (Section 2.4.3.1). Clearance is a measure of how well the eliminating organs remove the drug. Hepatic clearance will increase when enzymes are induced and decrease in the presence of an enzyme inhibitor. Similarly, alkalinization of urine will increase the renal clearance of salicylate (Section 3.3.1.5). The clearance of chlorpromazine is higher than that of penicillin G, but being much more lipophilic, the volume of distribution of chlorpromazine is much higher and this results in a considerably longer half-life (Box 4.1).

Box 4.1 Effect of V and CL on elimination half-life

Penicillin G	*Chlorpromazine*
$V = 0.2\,\text{L kg}^{-1}$	$V = 20\,\text{L kg}^{-1}$
$CL_R = 320\,\text{mL min}^{-1}$	$CL_H = 600\,\text{mL min}^{-1}$
$t_{1/2} = \frac{0.693 \times 0.2}{320} \times \frac{1000}{60} \times 70^a$	$t_{1/2} = \frac{0.693 \times 20}{600} \times \frac{1000}{60} \times 70^a$
$\mathbf{t_{1/2} = 0.5\,h}$	$\mathbf{t_{1/2} = 27\,h}$

[a] 70 kg subject

4.2.3 Intravenous administration

When a drug is injected as a rapid intravenous dose (often referred to as a bolus dose), mixing within its volume of distribution is considered to be instantaneous – compartments are assumed to be well-stirred homogeneous solutions of drug. Clearly this must be an approximation because it takes a finite time to give the injection and a finite time for the drug to be distributed, even within blood. However, this may take less than a minute [Figure 4.5(a)] which is a negligible amount of time compared with the half-lives of the majority of drugs. There is no absorption involved and, after the initial mixing, the plasma concentrations show a monophasic exponential decline [Figure 4.5(b)]. If serial blood samples are taken and the plasma concentrations, C, of the drug are measured, then the rate constant, λ, can be obtained from the slope of the plot of ln C versus time. Back-extrapolation of the line to $t = 0$, gives a value for ln C_0 and hence C_0. This concentration is the theoretical plasma concentration of the drug, at $t = 0$, assuming instantaneous equilibration within its volume of distribution. At this time, *all* of the dose is in the body, as there has been no elimination, and the apparent volume of distribution, V, can be calculated as described in Section 2.4.1.1.

4.2.3.1 Half-life

The half-life, as defined earlier, is the time taken for the initial concentration to fall by one half. It would be better to refer to it as 'half-time', as there is no 'life' involved. However it is probably futile to rail against the use of half-life, as the term is used almost universally. There is, of course, a direct analogy with the half-life concept used with the decay of radioactive nuclei. Sometimes $t_{1/2}$ is qualified, for example, 'biological' or 'metabolic' half-life, adjectives that can be misleading, or worse, incorrect. Because it is measured in plasma, 'plasma half-life' has been suggested, but it is possible that $t_{1/2}$ refers to rising plasma concentrations, say after an oral dose. The important thing is to ensure that if a description is used, it is unambiguous.

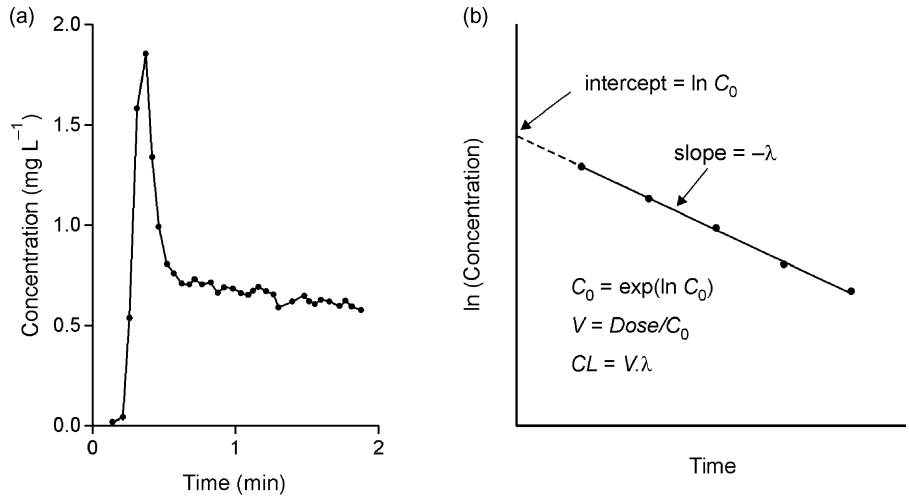


Figure 4.5 (a) Concentrations of phenylbutazone in one jugular vein of an anaesthetized sheep given a rapid intravenous dose in the other jugular vein (redrawn from McQueen and Wardell, 1971). (b) Back-extrapolation of experimental data, ln C versus t, to obtain a value of ln C_0 and hence C_0.

Half-life is a useful concept when applied to first-order reactions as it is constant. Thus, if 50% is lost in $1 \times t_{1/2}$ a further 50% of what is remaining will be lost in the next $t_{1/2}$, i.e. after $2 \times t_{1/2}$, 75% will have been lost and 25% of the original amount will remain. Note that the amount remaining, A, can be readily calculated from the number of half-lives that have elapsed, n,

$$A = \frac{A_0}{2^n} \tag{4.8}$$

where A_0 is the original amount. Note that n need not be an integer (Table 4.1). After five half-lives nearly 97% of the dose has been eliminated and after seven half-lives, >99% has been eliminated. Because the rate of elimination is higher when the concentrations are high shortly after the injection, almost 30% of the dose is lost in a time equivalent to $t_{1/2}$ divided by two.

Table 4.1 Proportion of dose eliminated or remaining as a function of the number of half-lives elapsed following an i.v. bolus dose of drug

Time elapsed (Number half-lives, n)	Amount eliminated (%)	Amount remaining (%)
0.5	29.3	70.7
1	50	50
1.5	64.6	35.4
2	75	25
3	87.5	12.5
5	96.87	3.13
7	99.22	0.78

Sometimes it is more convenient to obtain an estimate of the elimination half-life and to use Equation 4.6 to calculate the elimination rate constant, particularly when the concentration data are plotted on a logarithmic

scale (Figure 4.6). Note how the half-life is constant and can be obtained by choosing any convenient concentration that is easily divisible by 2. It is worth noting that the 'slope' of the line is not, as is frequently claimed equal to $-\lambda/2.303$. Any attempt to derive a slope between two time points will give the average slope of the C versus t curve, between those times.

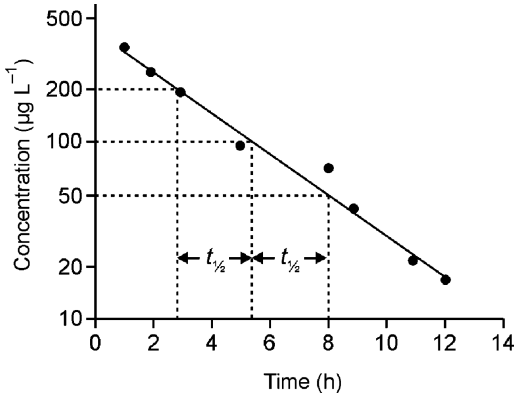


Figure 4.6 Prednisolone concentrations in a kidney transplant patient after an i.v. dose of 20 mg. (Redrawn from Gambertoglio *et al,* 1980).

4.2.3.2 Area under the curve

The area under the plasma concentration–time curve (*AUC*) can be computed from the integral of the equation defining the decay (see Appendix):

$$AUC = \int_0^\infty C\mathrm{d}t \tag{4.9}$$

C is given by Equation 1.8

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

so:

$$AUC = \int_0^\infty C_0 \exp(-\lambda t)\,\mathrm{d}t \tag{4.10}$$

$$AUC = \frac{C_0}{\lambda} \tag{4.11}$$

But from Equation 2.4, $C_0 = D/V$, so:

$$AUC = \frac{D}{\lambda V} \tag{4.12}$$

Therefore the *AUC* is directly proportional to the dose injected and inversely proportional to the volume of distribution and the elimination rate constant. This is to be expected as a larger volume will lead to lower plasma concentrations and the rate of elimination will be higher when λ is larger, again reducing the plasma

concentrations. Furthermore, $CL = \lambda V$ (Equation 4.5) so:

$$AUC = \frac{D}{CL} \tag{4.13}$$

and again, as might be expected, the *AUC* is reduced when *CL* is large. Equation 4.13 provides a way of calculating *CL* without use of Equation 1.8, i.e. without defining the model,

$$CL = \frac{D}{AUC} \tag{4.14}$$

because the *AUC* can be derived using the trapezoidal method (see Appendix).

4.2.4 Absorption

During drug absorption, the concentration gradient is very high and so the contribution of diffusion back from the pool of unabsorbed drug can be ignored and absorption can be considered as a non-reversible phenomenon. The model now requires an equation to describe the input (Figure 4.1). Absorption from the GI tract can be very variable for some drugs and the input function can be complex. The kinetics of absorption after i.m. injections are often first-order, and absorption after oral dosing may approximate to first-order kinetics. Thus, the rate of change of the amount of drug in the body is given by the rate at which it is being absorbed and the rate at which it is being eliminated:

$$\frac{dA}{dt} = k_a A_a - \lambda A \tag{4.15}$$

where k_a is the first-order rate constant of absorption and A_a is the amount of drug remaining to be absorbed. Provided all of the drug leaving the site of administration appears in the plasma, the rate of disappearance from the site of administration, which is basically inaccessible, must equal the rate of appearance in plasma. The rates of both processes can be accessed by k_a. Integration of Equation 4.15 produces:

$$A = \frac{A_0 k_a}{k_a - \lambda}[\exp(-\lambda t) - \exp(-k_a t)] \tag{4.16}$$

where A_0 is the dose, *D*. Dividing by *V* converts the amounts into plasma concentrations, and as all of the dose may not reach the systemic circulation, Equation 4.16 can be rewritten:

$$C = \frac{FD}{V}\frac{k_a}{k_a - \lambda}[\exp(-\lambda t) - \exp(-k_a t)] \tag{4.17}$$

where *F* is the fraction of the oral dose which reaches the systemic circulation (Section 8.3). Equation 4.17 can be written as:

$$C = C_0' \exp(-\lambda t) - C_0' \exp(-k_a t) \tag{4.18}$$

where $C_0' = \frac{FD}{V}\frac{k_a}{k_a - \lambda}$.

Thus it is clear, via Equation 4.18, that Equation 4.17 represents a concentration–time curve that is the *difference* of two exponential terms that have a common intercept on the *y*-axis [Figure 4.7(a)]. Both exponential terms asymptote to zero as *t* increases but the one with the largest rate constant decays the fastest, so that at later times Equation 4.18 approximates to a single exponential, as can be seen in Figure 4.7(b).

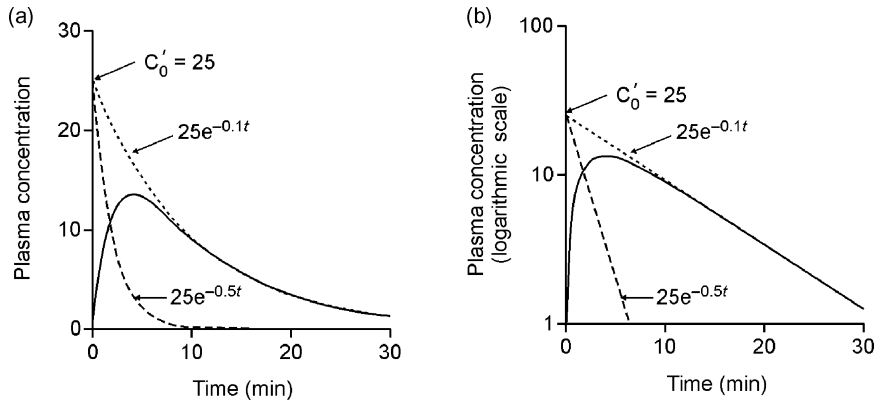


Figure 4.7 Simulated curves for first-order input into a single-compartment model, (a) linear scales, (b) semilogarithmic scales. $C_0' = 25$ arbitrary units, $k_a = 0.5\ \text{min}^{-1}$, $\lambda = 0.1\ \text{min}^{-1}$.

The maximum concentration, C_{max}, occurs when the rates of input and output are equal, and $dC/dt = 0$. The time of the peak, t_{max}, is given by differentiating Equation 4.17 with respect to t and rearranging:

$$t_{max} = \frac{1}{k_a - \lambda} \ln \frac{k_a}{\lambda} \tag{4.19}$$

Substitution into Equation 4.17 gives C_{max}, or it can be computed from:

$$C_{max} = \frac{D}{V} \left(\frac{\lambda}{k_a} \right)^{[\lambda/(k_a - \lambda)]} \tag{4.20}$$

For linear examples, t_{max} is independent of the dose, but C_{max} is directly proportional to the dose. This relationship does not hold for non-linear examples (Section 4.3). Also, the peak is not the time that absorption ceases and elimination starts. According to the model both start at $t = 0$ and continue to $t = \infty$. Absorption continues to make a significant contribution to the concentration–time curve at times beyond t_{max} as can be seen in Figure 4.7. The faction of the dose absorbed, f_{A_a} at t_{max} is a function of the relative sizes of the rate constants of absorption and elimination (Kaltenbach *et al.*, 1990):

$$f_{A_a} = 1 - \left(\frac{k_a}{\lambda} \right)^{-k_a/(k_a - \lambda)} \tag{4.21}$$

When $k_a = 2\lambda$, then 75% of the dose is absorbed by t_{max}, whereas for 90% of the dose to be absorbed when $t = t_{max}$, when k_a must equal 8λ. Therefore, it is important when performing pharmacokinetic experiments to ensure that data are collected for sufficient time for accurate assessment of the elimination rate constant, i.e. when absorption is making a negligible contribution to the concentration–time data. (Section 5.2.3).

4.2.4.1 Area under the curve

The *AUC* for the oral case is obtained in the same way as for the intravenous case, that is integration of the plasma concentration–time curve, Equation 4.17:

$$AUC = \int_0^\infty \frac{FD}{V} \frac{k_a}{k_a - \lambda} [\exp(-\lambda t) - \exp(-k_a t)] dt \tag{4.22}$$

$$AUC = \frac{FD}{\lambda V} \tag{4.23}$$

and, as $C_0 = D/V$:

$$AUC = F\frac{C_0}{\lambda} \tag{4.24}$$

Comparison of *AUC* after i.v. (Equation 4.11) and oral doses (Equation 4.24) gives:

$$F = \frac{AUC_{\text{p.o.}}}{AUC_{\text{i.v.}}} \tag{4.25}$$

Equation 4.25 is used to calculate the value of *F*, the respective areas usually being obtained by the trapezoidal method (see Appendix). The equation for clearance (see Equation 4.14) is:

$$CL = F\frac{D}{AUC} \tag{4.26}$$

This means that *CL* can *only be obtained after intravascular injection*, when *F* is known to be 1, otherwise the ratio of *D*/*AUC* gives *CL*/*F*. Similarly, because the amount of drug reaching the systemic circulation (*F*) can only be accessed from an i.v. injection it is not possible to determine the apparent volume of distribution from oral administration. There is, however, a concept known as 'apparent oral clearance', which uses these equations and literature values of *F*, obviously most of them less than unity, to calculate the clearance as if from an intravenous dose.

4.2.4.2 Special cases: $k_a < \lambda$ and $k_a = \lambda$

Drugs are usually formulated to be absorbed rapidly to give a rapid onset of effect. Consequently, one would expect k_a to be greater than λ. However, k_a can be less than λ. Indeed, that is the principle of sustained-release preparations. A growth and decay still occurs because when there is a large pool of drug to be absorbed, the *rate* of absorption still exceeds that of elimination (which initially is zero). In these special cases, because the absorption is rate-limiting, the half-life estimated from the decay phase is that for absorption and the rate constant of elimination is obtained from the steeper exponential curve. When the rate constants are substituted into Equation 4.17 the concentration curve is of the same form as that of Figure 4.7, but the rate constants appear to have been exchanged [Figure 4.8(a)]. This phenomenon has been referred to as 'flip-flop'. It is not possible from the concentration–time data to assign the rate constants to elimination or absorption. The only way to know which is which is from unambiguous data, such as with an independent i.v. dose. In the example of Figure 4.8(a), the rate constant of elimination was assigned by giving an intramuscular injection of a preparation that was not sustained-release.

If the absorption and elimination rate constants are equal then clearly Equation 4.17 is no longer applicable, as it reduces to zero at all times! By writing differential equations in which the rate constants are equal and *then* differentiating, an equation can be obtained for this situation (Gibaldi and Perrier, 1982):

$$C = \frac{FD}{V}kt\exp(-kt) \tag{4.27}$$

where $k = k_a = \lambda$. For this unusual situation:

$$t_{\max} = 1/k \tag{4.28}$$

and

$$C_{\max} = \frac{0.37FD}{V} \tag{4.29}$$

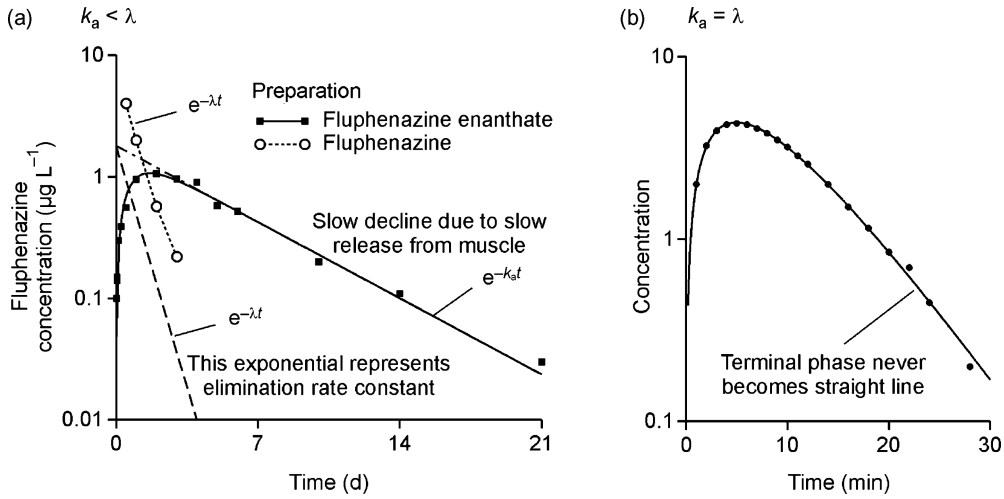


Figure 4.8 Examples of special cases. (a) Fluphenazine enanthate was developed so that the rate of release from muscle is rate limiting. Resolution of the data into its two exponentials makes λ appear to be the rate constant of absorption, but the decline after fluphenazine (open circles) confirms it is the elimination rate constant. (b) Methylene blue simulation of $k_a = \lambda$. The terminal decline never becomes straight and the parameters cannot be resolved using the method of residuals, however they can be resolved by iterative curve fitting to Equation 4.27 (solid line).

A crucial point about Equation 4.27 is that a plot of ln C versus time has no linear portion, so it is impossible to solve for k graphically. Although it is most unlikely that this situation would occur, it is easy to simulate using the dye model by connecting together two flasks of equal volume in series, so that the output of the first becomes the input of the second. This example illustrates the potential problem of solving for k_a and λ, when the values are similar.

4.2.4.3 Special case: lag time

The rise in plasma drug concentrations does not, in fact, commence immediately after an oral dose. Inevitably there is a lag phase because, even after administration of an oral solution, it takes a finite time for drug to reach its site of absorption. The lag time will be longer for tablets and capsules that have to disintegrate, and delayed gastric emptying may delay absorption even further. The lag time after consumption of enteric coated tablets may be several hours. The lag time, t_{lag}, moves the plasma concentration–time curve to the right so that Equation 4.17 becomes:

$$C = \frac{FD}{V}\frac{k_a}{k_a - \lambda}\left\{\exp\left[-\lambda(t - t_{lag})\right] - \exp\left[-k_a(t - t_{lag})\right]\right\} \tag{4.30}$$

An estimate of t_{lag} can be obtained by iterative curve-fitting of Equation 4.30 or, if the method of residuals is used the construction lines intersect at $t = t_{lag}$, rather than intersecting at the y-axis where $t = t_0$ (Section 5.2.1).

4.2.5 Infusions

Equations exist for the oral single dose situation in which absorption is at a constant rate, i.e. with zero order kinetics, and elimination occuring with first order kinetics. This can be important with controlled-release oral dosage forms. However, the principles are easily demonstrated by considering intravenous infusions.

For drugs that are given intravenously, an infusion over minutes or hours, rather than a bolus dose, may be appropriate, especially for safety reasons. Also, orally-administered drugs are often taken for extended periods of time, sometimes for the rest of a patient's life. Second and later doses of oral drugs given repeatedly are usually given before the previous dose has been completely eliminated, so that the drug accumulates in the body.

4.2.5.1 Zero-order input

Drugs may be infused using a motorized syringe pump and miniaturized versions are available for ambulatory patients. A constant rate infusion represents zero-order input, R_0, into the single-compartment model. At the start of the infusion the plasma concentration will rise as the infusion proceeds, however, for a drug that is eliminated according to first-order kinetics, it will not continue to rise indefinitely but 'level off' to what is referred to as the steady-state concentration, C^{ss}. This must be the case because as the plasma concentration increases the rate of elimination increases until the two rates become equal. The equation defining the plasma concentration during an infusion is:

$$C = C^{ss}[1 - \exp(-\lambda t)] \tag{4.31}$$

This is the equation of an exponential curve that asymptotes to C^{ss}. It is the same shape as the decay curve at the end of the infusion, but inverted. Because the curves are the same shape, it can be appreciated that during the infusion the concentration will be 50% of C^{ss} at $t = 1 \times t_{1/2}$, 75% of C^{ss} at $t = 2 \times t_{1/2}$, and so on. In fact, the values are the same as those for the percentage eliminated in Table 4.1, therefore after $7 \times t_{1/2}$, the concentration will be $>0.99C^{ss}$, even though according to Equation 4.31, C^{ss} is only achieved at infinite time [Figure 4.9(a)].

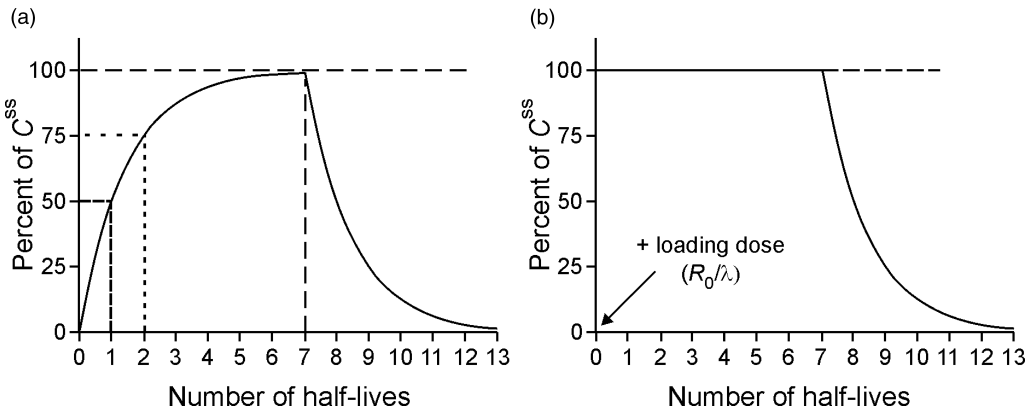


Figure 4.9 Comparison of attainment of steady-state concentrations as a function of elimination half-life (a) following a constant rate infusion for $7 \times t_{1/2}$ and (b) the same infusion with a loading dose injected at the start of the infusion.

Post-infusion the plasma concentration, $C_{postinf}$, decays according to an equation analogous to Equation 1.8, but rather than C_0, the concentration is concentration when the infusion was stopped, and the time is the time elapsed $(t - T)$ since the time at the end of the infusion period, T:

$$C_{postinf} = C^{ss}[1 - \exp(-\lambda T)]\exp[-\lambda(t - T)] \tag{4.32}$$

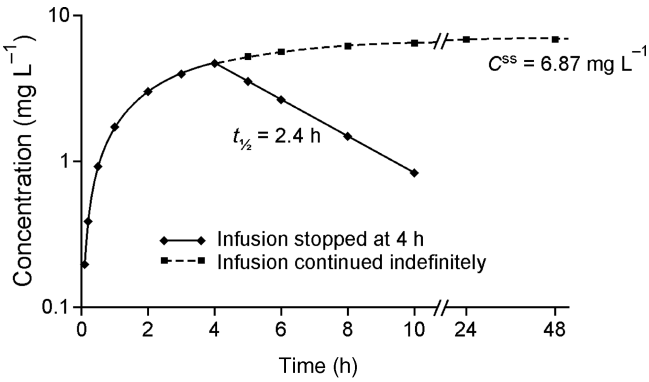


Figure 4.10 Amikacin infusion: simulated curves derived using Equations 4.5, 4.34, 4.31 and 4.32.

Figure 4.10 illustrates the principles of infusion using a simulation for the antibiotic, amikacin. In this type of simulation we ask 'what if' questions, in a sense the opposite of data analysis. The amikacin simulation shows concentrations in plasma to be expected if amikacin is infused at 0.625 mg min^{-1} (or 0.0089 mg min kg^{-1}) into a 70 kg subject in whom the clearance is 1.3 mL min^{-1} kg^{-1}. The apparent volume of distribution is 0.27 L kg^{-1}, the C^{ss} that results is approximately 7 mg L^{-1}. When the infusion is stopped, the drug concentration falls with a half-life of 2.4 hours. This simulation shows two 'what if' questions being asked, in that the effect of infusion to steady-state is compared with infusion for 4 hours after which the concentrations decline.

4.2.5.2 Loading dose

Because drugs with long elimination half-lives will approach steady-state slowly this may result in an unacceptable delay before therapeutic concentrations are achieved. This delay can be overcome by injecting a large bolus, known as a 'loading-dose', at the start of the infusion. The aim is to reach steady-state conditions immediately, and so the loading-dose must be such that the injection gives a plasma concentration of C^{ss}. This amount is, as always, the concentration multiplied by the apparent volume of distribution, so:

$$\text{loading dose} = VC^{ss} \tag{4.33}$$

The rate of elimination is the amount of drug multiplied by the elimination rate constant, and at steady-state this equals the rate of infusion, so:

$$R_0 = \lambda VC^{ss} \tag{4.34}$$

and so doubling the rate of infusion would double C^{ss}. Combining Equations 4.33 and 4.34 gives:

$$\text{loading dose} = R_0/\lambda \tag{4.35}$$

This equation was used to calculate a loading dose for the data of Figure 4.9(b).

Loading doses, both by injection and orally, are widely used with antibacterial drugs, such as sulfonamides, and in cancer therapy, such as with trastuzumab. They can also be used with non-steroidal anti-inflammatory drugs such as naproxen. However, there are situations in which pharmacokinetic considerations imply that a loading dose might make sense, but pharmacological considerations dictate otherwise. This occurs with tricyclic antidepressants, in that with these drugs the patient must initially develop a tolerance to

the acute cardiovascular effects, occurring at relatively low concentrations in plasma, so that the antidepressant effect, which occurs at relatively high concentrations, can be utilized. Thus the desired effect cannot be obtained after the first dose, only in part for pharmacokinetic reasons.

4.2.6 Multiple doses

When a sustained effect is required from oral dosing, drugs are usually prescribed as divided doses, taken over a period of time. Steady-state conditions will apply for drugs that are eliminated according to first-order kinetics, as they do for infusions, but now the plasma concentrations will fluctuate between doses. Because of this fluctuation this situation is sometimes referred to as 'pseudo-steady-state', or the 'pharmacokinetic steady-state'. The rate at which steady-state is achieved is the same as for the infusion, >99% after $7 \times t_{1/2}$. This is demonstrated schematically in Figure 4.11. An initial injection gives a concentration of 100 units, which falls to 50 units in $1 \times t_{1/2}$, when the dose is repeated. This gives 150 units, which falls to 75 in the next half-life, a further injection takes the concentration to 175 units, and so on, until at steady-state the peak and trough concentrations are 200 and 100 units, respectively.

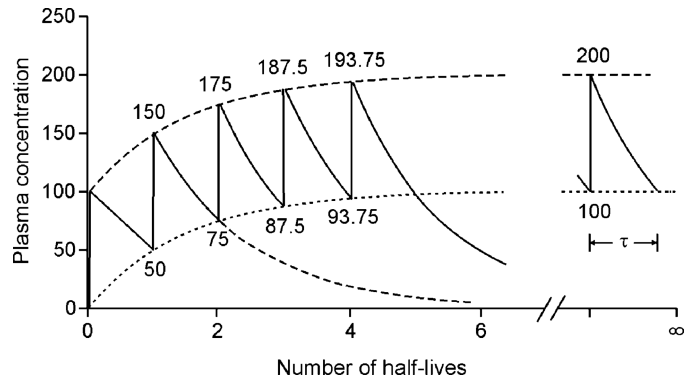


Figure 4.11 Modelled curve showing repeated i.v. injections at intervals equivalent to the half-life. Note the steeper decline in the curves as the concentration increases.

At steady-state, the maximum (peak) and the minimum (trough) concentrations after i.v. bolus doses can be calculated:

$$C_{\max}^{ss} = \frac{D}{V}\left(\frac{1}{1-\exp(-\lambda\tau)}\right) \tag{4.36}$$

$$C_{\min}^{ss} = \frac{D}{V}\left(\frac{1}{1-\exp(-\lambda\tau)}\right)\exp(-\lambda\tau) \tag{4.37}$$

where τ is the dosing interval. The 'average' steady-state is not the mean of the peak and trough concentrations but is based on the area under the curve as this is considered to give an estimate of the patient's exposure to the drug. It is the area under the curve divided by the dosage interval, τ, so from Equations 4.11 and 4.12:

$$C_{av}^{ss} = \frac{AUC}{\tau} = F\frac{C_0}{\lambda\tau} = \frac{FD}{V\lambda\tau} \tag{4.38}$$

The equation actually applies to both intravenous doses (when $F = 1$) and oral doses. With oral doses, because the average concentration is proportional to the dose and inversely proportional to τ then, for example, giving half the dose twice as often will give the same average concentration, but the peak to trough fluctuations will be less. It should be noted that the percent increase from the trough level in this situation is greater than the percent decrease from the peak. Thus, there is no validity in a statement such as: 'The concentration fluctuated by (X) percent.' Some authors favour the use of a 'fluctuation index, *FI*,' using a formula of the type:

$$FI = \frac{C_{max} - C_{min}}{C_{min}} \times 100 \tag{4.39}$$

where C_{max} and C_{min} are the peak and tough concentrations, respectively. There is, however, no unanimity on how such indices should be calculated, and one particularly interesting alternative uses the areas above and below the arithmetic or geometric mean steady-state concentration, so caution should be exercised in using the term fluctuation index.

Equations for multiple-dosing with first-order input (oral, i.m.) have been derived and can be found in standard texts such as Gibaldi and Perrier (1982). The situation is complex if the doses are given during the absorptive phase, however the equations that assume doses are given post-absorption are simpler and relatively easy to use. For example the concentration at any time t in the n^{th} dosage interval is given by:

$$C_n = \frac{FDk_a}{V(k_a - \lambda)}\left[\left(\frac{1-\exp(-n\lambda\tau)}{1-\exp(-\lambda\tau)}\right)\exp(-\lambda t) - \left(\frac{1-\exp(-nk_a\tau)}{1-\exp(-k_a\tau)}\right)\exp(-k_a t)\right] \tag{4.40}$$

where τ is the dosing interval. A loading dose can be calculated:

$$\text{loading dose} = D\left(\frac{1}{1-\exp(-\lambda\tau)}\right) \tag{4.41}$$

In this situation, D, the amount of drug given at the second and subsequent dose intervals, is often referred to as the 'maintenance' dose to distinguish it from the loading dose. The concentration–time curves of Figures 4.2 and 4.11 were simulated using Equations 4.40 and 4.41.

Note how in Figure 4.12 the drug with the shorter half-time approaches steady-state more quickly, as would be expected. However, the peak–trough fluctuations are greater because, having a shorter half-life, a

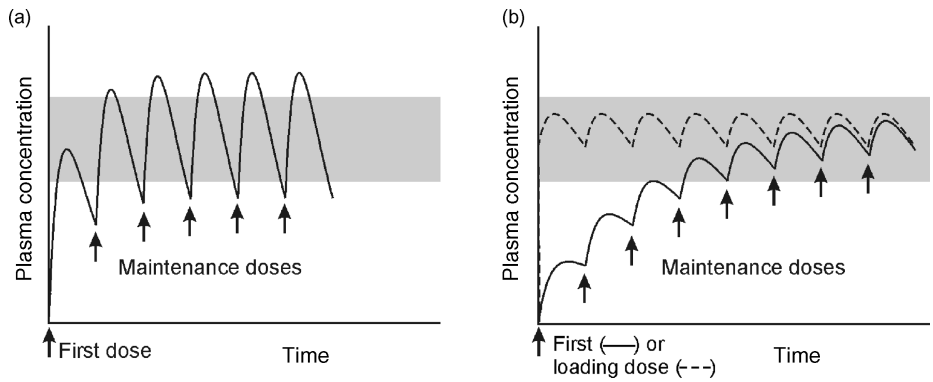


Figure 4.12 Examples of multiple dosing at regular intervals. (a) Drug with a short elimination half-life and (b) drug with a long elimination half-life, also showing the effect of a loading dose (broken line). The shaded area represents the therapeutic window.

greater amount of drug is eliminated between doses. As a consequence it may prove difficult to maintain the concentrations within a narrow therapeutic window. This situation can arise with morphine, when the patient may suffer pain at pre-dose, trough, concentrations and show signs of respiratory depression at peak concentrations. One way of dealing with such a situation is to give smaller doses more frequently as this reduces the difference between peak and trough levels whilst maintaining the same average concentration (Equation 4.38). An alternative is to use sustained-release preparations so that the 'effective' half-life is longer and the time course resembles that of Figure 4.12(b), when it takes longer to reach steady-state but the fluctuations are markedly reduced. A loading dose may be used to overcome any problems due to the delay in onset of effects.

Figure 4.13 shows the results of a simulation of lithium concentrations in a patient given 900 mg daily over a prolonged period of time. The half-life of lithium in this patient was 18 hours, the apparent volume of distribution was $0.66\,\mathrm{L\,kg^{-1}}$, and the clearance was $0.35\,\mathrm{mL\,min^{-1}\,kg^{-1}}$. The bioavailability ($F$) was unity. The figure shows the concentrations after the first dose, then the rise towards the pharmacokinetic steady-state – at this dosage in this patient the concentrations would have overshot the therapeutic range of 0.5–1.25 $\mathrm{mmol\,L^{-1}}$ and the dose would have needed to be reduced in order to reduce the risk of toxicity.

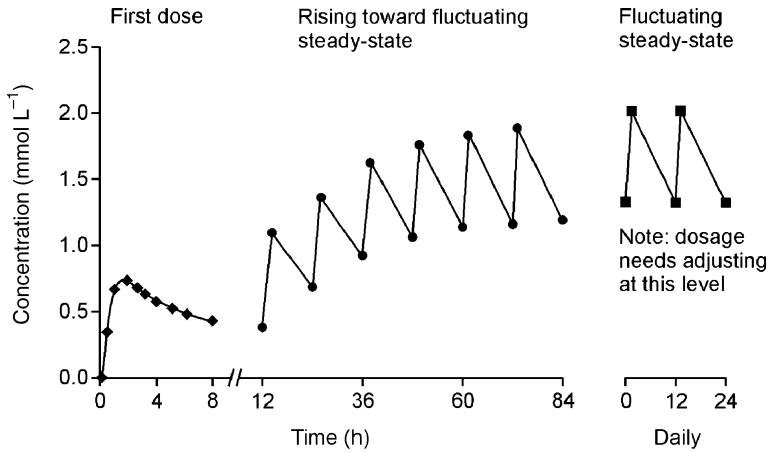


Figure 4.13 Calculation of lithium steady-state serum concentrations from the data of the first oral dose.

Whether or not fluctuation is desired often depends on the clinical objective. For example, a sleep aid drug taken night after night needs to have the maximum fluctuation achievable in order to prevent the occurrence of residual effects during the day. Hence, temazepam ($t_{1/2} \sim 12$ h) and with no active metabolites may be considered to be a more suitable hypnotic than diazepam ($t_{1/2} \sim 48$ h), which also has an active metabolite that accumulates in plasma (Section 6.2.4). On the other hand, an anticonvulsant is likely to be at its best if there is little or no fluctuation.

4.3 Non-linear kinetics

A feature of first-order kinetics is that C^{ss} is proportional to the dose administered; however there are situations when this is not the case and the kinetics are referred to as non-linear. The plasma concentrations

may increase disproportionately or may not increase as much as would be predicted from linear models (Figure 4.14). Antiepileptic drugs are examples of drugs exhibiting non-linear kinetics. Phenytoin metabolism tends to saturation at doses in the therapeutic range, whist gabapentin shows saturable absorption. Valproic acid concentrations may be less than predicted due to dose-dependent binding to plasma proteins, although the clinical effects may be greater (Section 2.5.3). Carbamazepine concentrations fall on repeated dosing because of auto-induction of enzymes. Phenobarbital shows linear kinetics at therapeutic doses but non-linear kinetics in overdose.

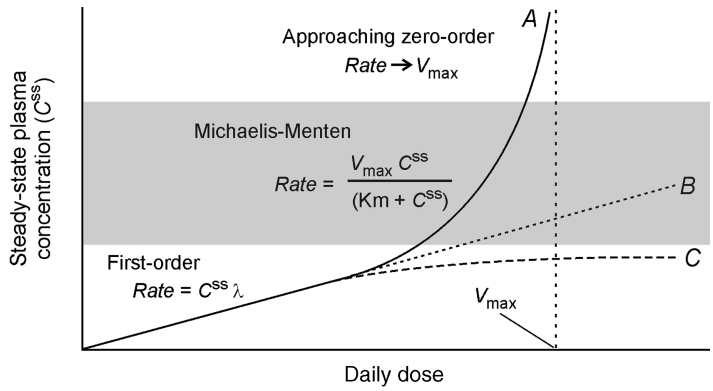


Figure 4.14 Comparison of non-linear kinetics. A: saturation of elimination, B: linear kinetics, C: saturation of absorption. Shaded area represents the therapeutic window.

Because half-life is dependent on apparent volume of distribution and clearance, non-linearity must be a result of non-linearity in one or both of these variables. The rate of elimination is $C \times CL$ (Equation 4.3), but for a drug eliminated according to Michaelis–Menten kinetics the rate is also given by Equation 3.1, so putting the equations equal to each other and dividing each side by C gives:

$$CL = \frac{V_{\max}}{K\mathrm{m} + C} \tag{4.42}$$

As concentration increases to the point where $C \gg K\mathrm{m}$ (zero-order case) then:

$$CL = \frac{V_{\max}}{C} \tag{4.43}$$

Thus, for non-linear kinetics where enzyme is becoming saturated with substrate, clearance will decrease with increasing drug concentrations. In the case of valproic acid, concentration dependent changes in protein binding result in concentration dependant changes in the apparent volume of distribution and hence nonlinear kinetics.

4.3.1 Phenytoin

Non-linear kinetics, such as those exhibited by phenytoin, can be modelled using the Michaelis–Menten equation. At low doses the kinetics approximate to first-order but the kinetics at higher doses can only be adequately described by Equation 3.1. As the dose is further increased the kinetics approach zero-order. This can result in C^{ss} increasing very rapidly for small increases in dose, making it difficult the maintain concentrations in the therapeutic window (Figure 4.14). Individual variations in apparent $K\mathrm{m}$ and $V_{\max}$ complicate things further [Figure 4.15(a)]. If it is necessary to individualize the dose a modification of

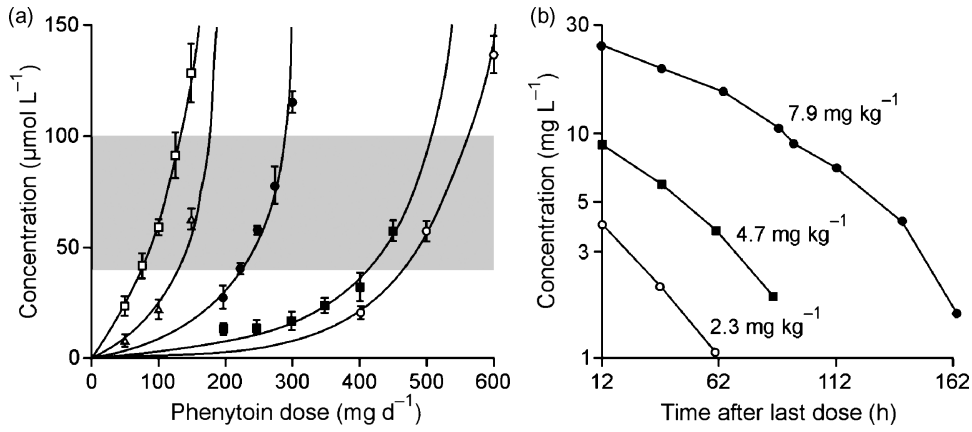


Figure 4.15 (a) Steady-state serum concentrations of phenytoin in 5 patients. The shaded area is the therapeutic window. (Redrawn from Richens and Dunlop, 1975) (b) Phenytoin plasma concentrations in a single subject given three dosage regimens, each lasting 3 days. (After Gerber and Wagner, 1972).

Equation 3.3 (Eadie–Hofstee) can be used. At steady-state, the daily dose, R, can be used as an estimate of the rate of elimination (mg day^{-1}) so:

$$R = V_{\max} - \frac{R}{C^{ss}} K\text{m} \tag{4.44}$$

Because there are two unknowns, C^{ss} values must be obtained for at least two dosage rates, when Equation 4.44 can be solved for $V_{\max}$ and Km. Alternatively a plot of R/C^{ss} versus R is a straight line of slope $-K$m and y-intercept, $V_{\max}$. The values can be used to calculate a daily dose that will give a concentration in the therapeutic range for that particular patient.

Non-linear kinetics are apparent from curvature of the ln C verses t plot [Figure 4.15(b)]. At high concentrations, the rate of elimination may be approaching $V_{\max}$ but, because the *proportionate* change is less than that at lower concentrations, the slope is less. As the concentration falls, the rate is less but the proportionate change tends to become constant as the kinetics approximate to first-order, when the slope of lines of a semilogarithmic plot will be the same, irrespective of the initial concentration.

4.3.2 Ethanol

Ethanol is metabolized primarily by liver alcohol dehydrogenase (ADH), although other routes of elimination exist, including metabolism by other enzymes, and excretion of unchanged drug in urine and expired air. Because the Km of human ADH is very low, and the 'dose' of ethanol, by pharmacological standards is high, the enzyme becomes saturated even with moderate drinking. In the 1930s Erik Widmark used a single-compartment model and zero-order kinetics to model blood ethanol concentrations. However, with advances in analytical methods it became clear that at low concentrations the kinetics were more akin to first-order decay. This led to the suggestion that at concentrations above 0.2 g L^{-1} the kinetics were zero-order whilst those below this concentration were first-order. Inspection of Equation 3.1 clearly shows that there cannot be an abrupt switch from first-order to zero-order kinetics and this can be demonstrated by substituting values into the equation (Table 4.2). If first-order kinetics applied, then the values calculated below 0.2 g L^{-1} would be directly proportional to the concentration. Clearly they are not. Similarly, if the kinetics were zero-order above 0.2 g L^{-1}, then the rate would be constant. Again this is not the case, although the rate does asymptote to $V_{\max}$ at very high concentrations.

Table 4.2 Calculated rates of ethanol metabolism as a function of concentration

C ($g\,L^{-1}$)	0.01	0.02	0.05	0.1	0.2	0.04	0.8	1.6	4.0	8.0
Rate ($g\,h^{-1}$)	0.73	1.33	2.67	4.0	5.33	6.4	7.11	7.54	7.8	7.9

$Km = 0.1\,g^{-1}$, $V_{mx} = 8\,g\,h^{-1}$

In a theoretical decay curve for ethanol [Figure 4.16(a)], the initial part of the curve is almost linear, reflecting approximately zero-order elimination, but the line becomes progressively more curved. Plotting the data with a logarithmic *y*-axis [Figure 4.16(b)] gives the typical shape expected for Michaelis–Menten elimination kinetics. At high concentrations the elimination rate approaches V_{max}, but the *proportionate* change is small, hence the shallow slope of the log-transformed curve. At later times (lower plasma concentrations) the rate of elimination is much lower, but the proportionate change is greater and will become constant when the elimination is first-order.

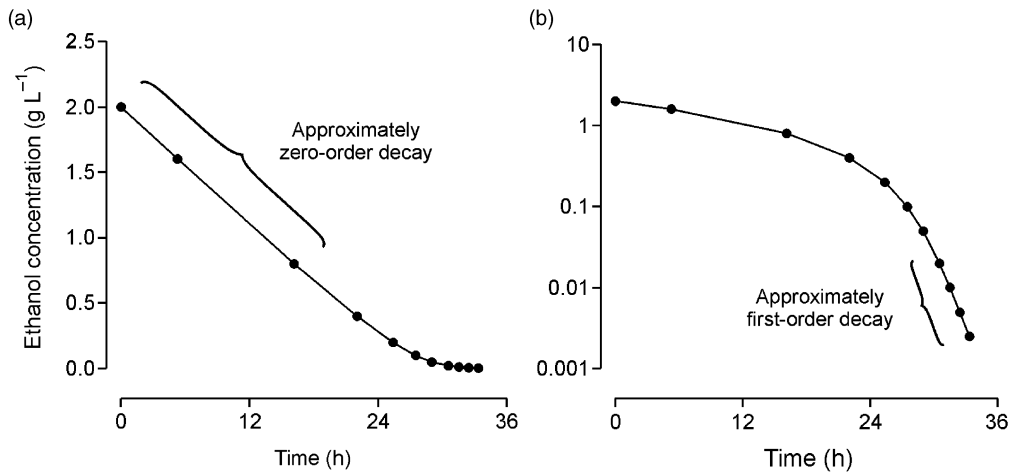


Figure 4.16 Simulated decay curve for ethanol ($C_0 = 2\,g\,L^{-1}$, $V_{max} = 8\,g\,h^{-1}$, $Km = 0.1\,g\,L^{-1}$).

4.4 Relationship between dose, onset and duration of effect

For a drug exhibiting single-compartment kinetics there are some simple theoretical relationships between the dose given and the duration and intensity of effect. Assuming that after an intravenous dose the effect ceases at a concentration C_{eff}, then the duration of effect increases by $1 \times t_{1/2}$ for every doubling of the dose [Figure 4.17(a)]. If the *y*-axis were dose and a horizontal line was drawn representing the duration of action then, the duration of action of the lowest dose would be less than that at higher doses, which would double with each doubling of the dose. Similarly, if at some concentration or time after the injection the effect wears off and the dose is repeated then the time for the second dose to wear off will be longer, because there is now more drug in the body. However if subsequent doses are given the time to reach the concentration at which the effect ceases is now constant between doses [Figure 4.17(b)]. If a horizontal line were drawn on Figure 4.17(a) then the duration of action of the lowest dose would be less than that at higher doses.

In the discussion above it has been assumed that the onset of effect was more or less instantaneous. The situation is more complex if the dose is not an i.v. bolus. As can be seen from Figure 4.1, increasing the size of

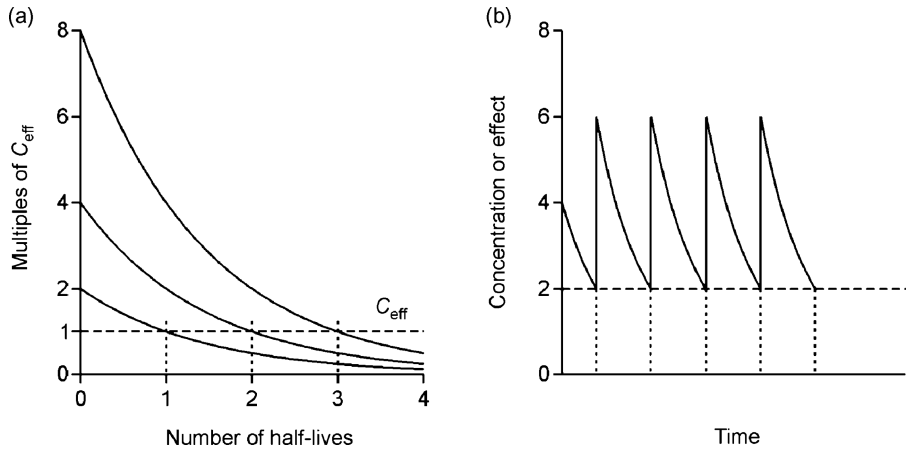


Figure 4.17 (a) Doubling the dose increases the time to reach the minimum effective concentration, C_{eff} by one elimination half-life. (b) Multiple doses given when the plasma concentration or effect has fallen to some arbitrary level. The duration of effect after the first dose is less than that after the second and subsequent doses. After the second dose the maximum concentration/effect is the same.

an oral dose decreases the delay before the onset of effects, but predicting the duration of effect is more difficult.

4.5 Limitations of single-compartment models

The astute reader will have noticed that most of the figures in this chapter use modelled data. This reflects the difficulty in finding convincing biological examples of data which fit a single-compartment model, although pharmacokinetic growth and decay curves are to be found throughout this book. For example, in Figure 4.6, which has been presented as representing decay from a single-compartment, the first sample was not collected until one hour after the injection. Thus, an earlier, faster, decay phase may have been missed. This is supported by the fact that the apparent volume of distribution, calculated using Equation 2.4, is ~50 L. This is larger than the volume of plasma or extracellular fluid, with which drug may be expected to equilibrate rapidly. In fact, it approximates to total body water, including water in tissues, into which a drug will penetrate relatively slowly, so in all probability analysis of earlier samples would have revealed another exponential decay representing distribution of the drug into tissues. Even ethanol concentrations, which are frequently treated as if they conform to single-compartment kinetics, show multi-exponential decay after intravenous infusion. Because it is now recognized that the timing of samples may influence conclusions about the most appropriate compartmental model, more recent studies are usually designed with sampling at sufficiently early collection times to define any distributional phase(s).

A related problem arises because of inhomogeneity of plasma. Thus absorption and metabolism/excretion do not take place in the same location within the single compartment. The drug must pass from one site to another through the very blood that is sampled, usually from the antecubital vein within that compartment, raising a question that is more than theoretical about the validity of the concept of homogeneity in this case. This is considered further in Chapter 7.

There are examples of what appear to be single-compartment models when some drugs are given orally or by i.m. injection. This is because the absorption phase masks more complex kinetics, as with fluphenazine enanthate described above [Figure 4.8(a)]. In such cases there is no alternative but to analyse the data as if from a single-compartment, even if other routes of administration (e.g. intravenous) show otherwise.

These situations may be referred to as apparent or pseudo single-compartment cases. Consequently, it is not unusual to find examples in the literature, for individual drugs, in which biexponential decay equations (two-compartment model) are used for intravenous doses, and biexponential growth and decay equations (one-compartment model) are used for oral doses.

4.6 Summary

The fundamental concepts of pharmacokinetics have been described including half-life, clearance, zero-order and first-order input into a single-compartment model, multiple dosing and attainment of steady-state. However, because single-compartment models are of limited use in describing the plasma concentration–time relationships for the majority of drugs, more complex models are required. These are the subject of the next chapter.

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