9

Factors Affecting Plasma Concentrations

9.1 Introduction

The factors that affect plasma concentrations of drugs include:

- Those associated with the disposition and fate such as route of administration (Chapter 2) and rate of elimination (Chapter 3).
- Pharmaceutical factors such as tablet and capsule properties (Chapter 8).
- General physiological factors such as time of administration of dose and food, diet and nutritional state, weight, sex, hormone balance including pregnancy, circadian rhythms, genetics (Chapter 10) and age (Chapter 11).
- Pathological state, especially diseases of the organs involved in disposition and fate (Chapter 12) and.
- Drug interactions (Chapter 17).

This chapter is concerned with those general physiological factors that are not the subjects of individual chapters.

In considering factors that affect plasma concentrations of drugs following single doses it is essential to refer to the standard rising and falling patterns discussed at the beginning of Chapter 4 and shown in model form in Figures 4.1 and 4.2. For single doses (Figure 4.1), we need to recall that with regard to plasma concentrations:

- A change in the *extent* of absorption will change the concentrations at all times, leading to a larger or a smaller area under the concentration–time curve (*AUC*).
- A change in the *rate* of absorption, with no changes in the extent, will lead to changes in one direction at early time points, with changes in the opposite direction at later time points, and no change in *AUC*.
- A change in the rates of metabolism and/or excretion will lead to changes in the concentrations in the same direction at all points, and to changes in *AUC*.
- Changes in tissue localization will lead to changes in concentrations in the opposite direction, e.g. increased tissue binding leading to reduced plasma concentrations.
- Any one factor can change one or more of the pharmacokinetic properties of the drug.

In regard to multiple-dosing, it is essential to refer to the standard pattern shown in Figure 4.2. With i.v. infusions, we note the constant pharmacokinetic steady-state concentration, and the time to reach that steady-state. With oral dosing, in addition to the average concentration within each dosage period and the time to reach that average, and the pharmacokinetic pseudo steady-state that it represents, it is necessary to consider

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the fluctuation between peaks and troughs. Thus factors affecting the rate and extent of absorption of a drug in different ways, and also factors affecting elimination, will cause complex changes in the time to reach a plateau and in the height of that plateau.

By applying the concepts discussed above, it should be possible to deduce the influence of any factor when it is described in terms of rate and extent of absorption, metabolism, excretion, or tissue localization. The factors affecting plasma concentrations are considered in this light in the following sections of this chapter.

9.2 Time of administration of dose

9.2.1 Time of day, and association of dosing with meal times

The dosing of drugs 'three times a day with meals' was at one time deeply ingrained in the practice of medicine. This was at a time when there was little pharmacokinetic knowledge, when most drugs were given according to this regimen, either because they were antacids or antispasmodics designed to reduce the unwanted discomfort experienced after the wanted enjoyment of a fine meal, or because the frequency of dosing was unimportant in comparison with the need to provide a reminder that it was time for a dose, and so increase compliance. At one time, new drugs were introduced with this regimen for no reason other than that this was standard practice.

Combining drug dosing with eating is not always good science, and the influence of food on drug absorption is considered in the next section. Also, a lunchtime dose is notoriously subject to being forgotten, partly because people are erratic in their lunch habits, and partly because people are at work at lunchtime and do not wish to be seen taking tablets and capsules by their workmates, or are just too busy to remember. The dose in the middle of the day is particularly difficult in the case of schoolchildren who need medication, so there is a powerful drive to design drugs with pharmacokinetic properties such that doses can be given twice a day or, better still, once a day. Additionally, there are variations in the receptivity of the body to doses given in the morning rather than the evening. This is considered in the circadian rhythms section. This is quite separate from the obvious fact that we want sleep-inducing drugs to exert their effects only at night, or when we want to sleep.

Generally speaking, daily doses are scheduled to be taken in the morning. However, pharmacological needs in this context can be quite subtle. For example, cholesterol synthesis is greater at night. The 'statin' drugs, which inhibit cholesterol synthesis, vary in first-pass effects, which can in turn be affected by diurnal rhythms. They also vary in that some of them are prodrugs, and also in drug interactions. In some cases, these drugs are recommended to be taken at bedtime, as a way of achieving greater efficacy. This would emerge as more cholesterol reduction per unit of dose during therapy. This timing of dose recommendation is not always followed – it might be most achievable by using once a day controlled-release technology. Controlled-release products have the potential to be less affected by daily rhythms.

An example of a dosing schedule based on a combination of pharmacological and pharmacokinetic knowledge leads to the recommendation that sedative antihistamines used in the treatment of allergic disorders be taken at bedtime, so that the sedative effect is experienced at night, leaving the residual antihistamine effect, sometimes exerted by metabolites with relatively long half-life values, to prevail the following day. Another example based on pharmacology is that some antihypertensive drugs are commonly recommended not to be taken at bedtime, as, if patients under the influence of these drugs arise during the night they are at considerable risk of orthostatic hypotension causing them to fall over at a time when their defensive reflexes are slow. A further example is the complex situation that can arise in patients taking several drugs, with the potential for absorption interactions. In this case it can be necessary to space out the various doses during the day, to avoid the effects of the various drugs on each other. Drug interactions are the subject of Chapter 17.

9.3 Food, diet and nutrition

9.3.1 Physical interaction with food

The presence of food in the gastrointestinal tract, particularly the stomach:

- Provides *adsorbing* surfaces to which drugs can adhere, in competition with sites of *absorption*.
- Prevents free access of drug molecules to the absorbing surface by reducing the efficiency of mixing.
- May provide lipid layers in which drug molecules will dissolve, mostly reducing their availability for absorption.
- Stimulates gastric acid secretion, which affects the ionization of weak electrolytes, and can cause chemical decomposition of some drugs.
- Modifies gastric emptying time, and hence changes the rate of movement of drugs from the stomach to the intestine.

It is thus not surprising that food can markedly reduce the rate, and sometimes also the extent, of absorption. This can be turned to good effect if a relatively low, prolonged effect is wanted, or if, as with some of the non-steroidal anti-inflammatory drugs reduction in gastrointestinal irritation and bleeding is required. It can be a nuisance if the highest possible peak concentration, or the earliest possible effect is required, or if bioavailability (Chapter 8) is reduced, so prescribing instructions sometimes include advice on timing of doses in relation to food, depending on the clinical need.

Food in the stomach both stimulates and delays gastric emptying. In the fasting state, the stomach experiences periodic waves of peristalsis which cause any accumulated fluid to pass through the pyloric sphincter. When solid or semisolid food enters the empty stomach, the pyloric sphincter closes or remains closed. A slurry (called 'chyme') is then created by the combined effect of stomach acid and the grinding effect of stomach muscle, so that virtually no solids enter the intestine. Once the slurry is formed, the pyloric sphincter opens to allow the slurry through. Fatty food, especially, slows gastric emptying. Drug particles become caught up in this process when taken with food. There are drugs, both therapeutic and non-therapeutic, that shorten or lengthen the gastric emptying time. Alcohol is one of the more important non-therapeutic drugs that slows this process, and when alcoholic beverages are combined with fatty meals late at night, and followed quickly by sleep, quite prolonged gastric emptying can occur, preserving the experience of a full stomach into the next morning.

Griseofulvin is an interesting example of a drug where absorption is faster and more complete when the dose is accompanied by dietary lipid – this drug is absorbed along with the fat molecules in the course of normal lipid absorption. Another interesting case arises with the anti-obesity drug orlistat, which exerts its effect by inhibiting gastric and pancreatic lipases, slowing the conversion of dietary fat into absorbable products, and hence reducing the absorption of those products. At the same time it can reduce the absorption of dietary fat-soluble vitamins, such as vitamins A and E, and β-carotene, and of fat-soluble drugs, such as warfarin and ciclosporin.

9.3.2 Macronutrients

The role of nutrition in the drug-metabolizing enzyme system in animals was reviewed by Campbell and Hayes in 1974. No specific role for carbohydrates had been shown, although sugar intake (glucose, sucrose and fructose) had been shown to decrease enzyme activity and to prolong hexobarbital sleeping times in mice (a pharmacological method of evaluating microsomal oxidation). A fat-free diet had been shown to depress activity, with decreased V_{max} for various substrates. Protein lack had been shown to reduce activity, as had vitamin deficiency, particularly with vitamins B, C and E and, to a lesser extent, vitamin A. Calcium, magnesium and iron deficiencies had been shown to reduce activity, as did starvation.

Early human studies supported these observations, with a highly significant partial correlation between protein intake and dosage requirements for digoxin. A lesser correlation was shown with fat intake, and this was ascribed to influences on renal clearance. Antipyrine and theophylline oxidation were early study topics, with half-life reductions being shown when subjects changed to a low carbohydrate/high protein diet (antipyrine from 16.2 to 9.5 h; theophylline from 8.1 to 5.2 h). A change to a high carbohydrate/low protein diet reversed these effects. These changes were ascribed to metabolic differences. Early studies on fasting in obese subjects showed no effect on antipyrine half-life, or on that of tolbutamide, but did cause changes in apparent volumes of distribution, in part because of greater fluid volumes, as well as fat masses, in obese people. Reduced dietary protein intake reduces synthesis of plasma proteins such as albumin.

More recent data support the above conclusions. Long-term consumption of a high-protein diet has been shown to increase the clearance of propranolol and antipyrine, a high-carbohydrate diet has been shown to reduce the clearance of theophylline, but a high-fat diet has been shown to increase the clearance of ciclosporin after i.v. injection and the fraction absorbed after oral dosing. Restriction of calorific intake has been shown to reduce the clearance of aminopyrine, as has intravenous nutrition with glucose. In regard to glucuronidation, a lack of sensitivity to diet has been shown with paracetamol and oxazepam. The changes that occur with alterations in dietary protein intake are supported by studies in patients on total parenteral nutrition (TPN). They can be detected within a few days of dietary changes.

At one time it was believed that dietary protein could change the activity of the P-450 system in an acute way, as it was shown that the extent of systemic availability of propranolol could be increased by as much as 70% within 5 minutes of consumption of a high-protein meal. The effect lasted for about 30 minutes. This occurred with both i.v. and oral (immediate-release) but not controlled-release propranolol. This could only be explained by a meal-related reduction in presystemic drug elimination, as the apparent volume of distribution, protein binding, plasma half-life and oral clearance of the enantiomers of propranolol were unaffected in these circumstances, as was the metabolic pattern. The explanation was that the meal caused a dramatic increase in hepatic blood flow, and as such, similar effects might be expected with all meals of any composition. However, it was shown that protein caused a particularly large increase in hepatic blood flow, raising the portal vein concentrations of propranolol after both i.v. and oral doses to levels that partially saturate the enzymes responsible for presystemic elimination. These levels were not reached with controlled-release propranolol. Similar observations have been made with several other high extraction ratio drugs, notably oral metoprolol, labetalol and intravenous lidocaine, and also with hydralazine, which is metabolized by acetylation. Similarly, although it was discovered in a long-term study, the observation with ciclosporin has been at least in part ascribed to an absorption influence in the belief that the ciclosporin complexed with fat passes membranes more rapidly.

9.3.3 Micronutrients

Pyridoxine supplementation can decrease the systemic availability of phenytoin and phenobarbital in epileptic patients. Levodopa can be affected similarly. This appears to be an effect on intrinsic clearance. Folic acid supplementation has a similar effect on phenytoin metabolism. Ascorbic acid can compete with ethinyloestradiol for sulfate conjugation, and dietary supplements can cause enhanced bioavailability of the steroid. Similar results have been observed with oral contraceptives, with an enhanced effect on blood clotting being observed. Also, high dose supplementation with ascorbic acid can impair antipyrine metabolism. Enriched vitamin K in certain plant foods can cause resistance to warfarin. However, the same plant foods can stimulate drug metabolism, and this result may have been caused by an increase rate of warfarin metabolism.

A variety of effects related to intake of particular dietary constituents, food additives, and herbal supplements have received intense study. These include cruciferous vegetables (such as cabbage, broccoli, cauliflower and spinach), which contain indoles that have the ability to increase the activity of the P-450

system. For example, the now-obsolete analgesic phenacetin (acetophenetidin) was shown to have 50% reduced bioavailability under such influences, and paracetamol metabolism is also similarly enhanced. The enzyme-inducing influence of polycyclic hydrocarbons in barbequed (charcoal-broiled) and smoked food has received much attention. In contrast, food additives, particularly butylated hydroxyanisole and butylated hydroxytoluene (BHT), which are approved preservatives in processed food and beverages, have been shown not to affect the pharmacokinetic properties of antipyrine and paracetamol. St. John's wort and grapefruit juice, a medicinal herb and a popular beverage, respectively, interact with a variety of drugs to a considerable extent, and are considered in Chapter 17.

9.4 Smoking

The properties of approximately one third of the one hundred most prescribed drugs are known to be affected by, or have the potential to be affected by, cigarette smoking. The most common influence is through enzyme induction, exemplified in clearance studies, but there are also influences on absorption. The main enzyme inducing agents are polycyclic aromatic hydrocarbon (PAH) compounds, among the thousands of constituents of cigarette smoke. These compounds induce the activity of CYP1A1, CYP1A2 and possibly CYP2E1. One model PAH is 3-methylcholanthrene, which was one of the first inducers of drug metabolism to be discovered. This compound is still used as a tool in drug metabolism and carcinogenicity research. Nicotine is primarily metabolized by CYP2A6, and it is also an inducer, increasing the activity of the same enzymes as those affected by the PAH compounds, and also inducing CYP2B1 and CYP2B2, but this effect is probably not clinically important. Examples of drugs affected by smoking include theophylline, propranolol, diazepam and chlordiazepoxide. The induction of propranolol metabolism is associated with a decreased effect on blood pressure and heart rate reduction. More recent drug examples include caffeine, imipramine, haloperidol, pentazocine, flecainide and oestradiol. Effects on oestrogen kinetics can negate the efficacy of oral contraception. When smokers give up smoking, this induction dissipates at a rate related to the turnover of microsomal protein. This includes past-smokers who use nicotine replacement therapy to help them achieve their 'cure'. Cigarette smoking also results in relatively fast clearance of heparin, possibly related to enhanced heparin binding to antithrombin III.

Insulin absorption from subcutaneous sites of injection can be relatively slow in smokers, because of the vasoconstriction caused by nicotine. However, and in contrast, absorption of inhaled insulin can be enhanced, leading to earlier and higher maximum insulin levels. Inhaled corticosteroids can also be affected leading to reduced effects in asthmatics who smoke. There is also the potential for a pharmacodynamic interaction involving sedative effects of central depressant drugs, such as the benzodiazepines and opioid analgesics, and the stimulant effect of nicotine, with the one effect competing with the other. This is analogous to the interaction between amphetamine and amylobarbital, given as a single regimen, in which each is thought to negate the effect of the other. This combination was once widely prescribed as an antidepressant despite the lack of evidence as to its efficacy.

9.5 Circadian rhythms

Circadian rhythm refers to a cycle in biochemical, physiological and behavioural processes of approximately 24 hours. The term, from the Latin *circa* ('around') and *diem* or *dies* ('day'), is attributed to Franz Hallberg. The formal study of such temporal rhythms is chronobiology and more recently the term 'chronopharmacokinetics' has been coined. Other related terms, such as 'chronokinetics' and 'chronopharmacodynamics' are to be found in the literature, together with 'diurnal variation,' and 'chronopharmacology'. Circadian rhythms are internally generated, but they can be reset by external cues, such as daylight, and even by conscious decisions, such as eating schedule. Functionally, they facilitate adaptation by the body to environmental changes and maintenance of homeostasis within a particular 'daily round'.

Central control is through the master 'biological clock' – the suprachiasmatic nucleus (SCN), or nuclei, a pair of cell groups in the hypothalamus. The SCN receives input from the retina and relays it to the pineal gland, which secretes melatonin, with production peaking at night. In the absence of input on the light/dark cycle, the body adjusts to a day varying from about 23.5 to 24.65 hours. The SCN processes information on, and also influences, hunger/satiety cycles, body temperature, and many other functions. In fact, food is a very important synchronizer (or 'zeitgeber') for this mechanism of homeostasis. There are believed to be other, independent peripheral 'oscillators' in the body with similar functions in relation to specific organs, including the liver. These clocks can take from 1–7 days to reset when influences are imposed voluntarily, such as with travel across time zones, and changes in the timing of meals, leading to the familiar total body disruption associated with long-distance air travel.

Diurnal variations occur in all of the physiological processes of importance in pharmacokinetics, such as gastric emptying time, gastric and urinary pH, and blood flow to the liver and kidneys. Consequently, diurnal variations are observable in at least drug absorption, metabolism and excretion. However, it should be noted that such variations occur in parallel with, for example, physical activity, and it can be difficult to determine the component contributions to pharmacokinetic variation in any particular case. Cardiovascular and non-steroidal antiinflammatory drugs have received special consideration in this context.

9.5.1 Absorption

Several studies have shown nifedipine and propranolol to have increased C_{max} and earlier t_{max} values after morning compared with evening administration. Because these differences occurred only with immediate-release dosage forms, and not with sustained-release dosage forms, and as these drugs are lipophilic, these differences have been attributed to the relatively short gastric emptying time and high gastrointestinal perfusion in the morning. This is presumed to result in faster absorption, and it is probably a general phenomenon. It is also thought that this would not be the case with less lipophilic drugs, such as atenolol, which has been shown to be less susceptible to this effect. However, it is important to note that drugs in this class are used for the treatment of hypertension. Blood pressure itself shows a circadian rhythm, thus introducing a pharmacodynamic sensitivity component. Time-dependent drug absorption is almost always a consequence of time-dependent gastric emptying, which is affected most obviously by physical activity, rather than the biological clock. However, physical activity can reset the biological clock. Thus the desire to go to the gymnasium may be triggered by the biological clock, or cause the biological clock to reset. Similar considerations apply to the effects of food, which are considered elsewhere in this chapter.

Studies with non-steroidal antiinflammatory drugs have revealed a number of observations, including, for example with diclofenac, highest C_{max} and highest AUC, but no differences in t_{max} and $t_{1/2}$, with morning doses. With indomethacin (Figure 9.1) and ketoprofen, similar absorption differences were noted, but additionally the terminal half-lives were higher in the evening. Additionally, with ketoprofen given i.v., there was an indication of slower elimination in the evening. In the case of salicylic acid, urinary elimination is apparently relatively slow in the morning.

Circadian rhythms in protein binding have been reported, dependent on temporal changes in plasma protein concentrations. This has been noted as significant with such highly protein bound drugs as carbamazepine, diazepam, phenytoin, valproic acid and cisplatin. This would be pharmacologically important only with highly protein-bound drugs that have low apparent volumes of distribution.

9.5.2 The liver

Animal investigations have shown circadian rhythms in hepatic blood flow and liver enzyme activity including expression of the cytochrome P-450 enzymes. Cytochrome P-450 enzymes metabolize melatonin

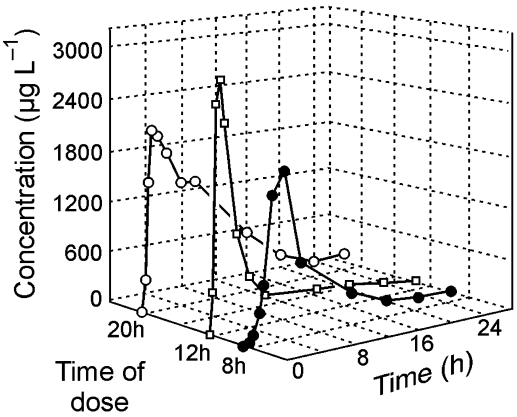

Figure 9.1 Indomethacin plasma concentrations according to time of dose, after oral doses of a sustained-release preparation, 75 mg h^{-1} (adapted from Bruguerolle, 1998).

in humans. Several examples of model compounds have been studied in rats, including, in particular, the *O*-dealkylase enzymes that detoxify various coumarin compounds. Higher levels of activity were shown during the dark period of the 24-hour cycle, but only in males. Similarly, erythromycin *N*-demethylase activity is relatively high in rats during the dark period, in this case in the second third of that period. While emphasis has been placed on the biological clock influencing P-450 activity, it should be noted that there is significant feedback with activity of CYP proteins affecting circadian rhythms – the pregnane X receptor (PXR) has a role in this – and, of course, the rat is a nocturnal animal.

In humans, plasma concentrations of 5-fluorouracil (5-FU) are subject to circadian variation in dihydropyridine dehydrogenase, which has relatively low activity at night. During constant rate i.v. infusion equilibrium concentrations were highest at 1 am, indicating relatively slow metabolism at that time. However, several studies in humans have failed to show a significant correlation between diurnal changes in enzyme activity and pharmacokinetics, including studies with tacrolimus, a substrate for CYP3A4, in spite of changes being noted in the hydrocortisol/cortisol ratio, an established biomarker for CYP3A4 activity. Using the constant rate infusion technique with theophylline, no diurnal variations were observed, although with ranitidine, relatively high concentrations were seen at 10 pm. With diclofenac there was no diurnal variation in half-life, while indomethacin and ketoprofen showed relatively high half-life values at 7 and 8 pm, respectively, but the highest salicylic acid half-life was at 6 am. The half-life of paracetamol was 15% longer at 6 am compared with 2 pm. Thus while the general picture prevails of relatively slow metabolism of drugs in humans at night, the opposite of the situation in rats, no general rule can be applied. It could be that, with all of the other influences at work in any particular case, the diurnal variation in enzyme activity is a minor factor. Hepatic blood flow may be more influential, for example, one study estimated hepatic blood flow in supine healthy individuals was greatest at 8 am. As a result, it might not be clear whether a difference seen with a drug with hepatic blood flow dependent properties was an effect of posture or a biological clock.

9.5.3 The kidney

Glomerular filtration, renal blood flow, urinary pH and tubular reabsorption have all been shown to have higher values during the daytime in humans. pH-dependent effects have been shown with salicylic acid and sulfasymazine, with faster excretion in the evening when the pH is relatively high. This is probably a food effect, with food choices made in the evening causing slight alkalinization of the urine.

9.5.4 Intravenous and other injected doses

Several drugs have been studied using intravenous doses, in attempts to eliminate the drug absorption component. Ketoprofen, 5-FU, theophylline and ranitidine have already been mentioned (see for example, Section 9.5.1). Other examples have included bupivacaine, for which peridural infusion over 36 hours revealed higher clearance in the early morning, and terbutaline, for which there were highest concentrations at 11 pm, and etoposide, with which no time-dependent variations were seen.

9.5.5 Pharmacodynamics

Theophylline is of especial interest because, as a drug with a narrow therapeutic index, the relationship between plasma concentration and clinical efficacy, as assessed by peak expiratory flow rate is important. Notwithstanding observations reported after i.v. administration, regarding the rate of metabolism being relatively slow in the morning, there is a contrasting report of therapeutically significant concentrations in patients being found after evening, but not morning, administration.

9.6 Weight and obesity

9.6.1 General principles

To standardize exposure between experimental animal species, drugs may be administered on a weight-corrected basis (usually mg kg^{-1}). Laboratory animals are obviously considerably smaller than humans and this is considered in detail in Chapter 15. Clinically, intravenous dosing apart, doses are mostly given in multiples of unit doses, as represented by a number of tablets or capsules, and while higher doses may be given to heavier people, exact proportionality in dosing is rarely attempted. Within a group of individuals, changes in dose on a mg kg^{-1} basis mostly lead to proportionate changes in concentrations in plasma and tissues, and it might be reasonable to suppose that changes in weights of individuals will lead to proportionate changes in concentrations. However, it must be remembered that there can be different reasons for differences in body weight. Clearly, sumo wrestlers, ballet dancers, long distance cyclists and competitive swimmers, for example have differences in both weight and in the proportions of the various constituents of their weight. It is necessary to consider the *proportion* of bone, fat, muscle, and fluid as there may be different pharmacokinetic consequences of changes in the relative amounts of these components, when weight increases or decreases. This in turn leads us to take note of the lipophilicity or otherwise of any compound under investigation for influences of weight changes and differences, and especially consider localization of drugs in lipid deposits as well as their binding to muscle. Because tissue localization reflects equilibrium distribution between plasma water and binding or other sites in tissues, binding to plasma protein is also a part of this equation (Figure 2.12). It is relatively easy to make seemingly logical predictions concerning the likely effect of lipophilicity differences among a group of anaesthetics, based on data considered in Chapter 2, and to make similar predictions on the likely response rate of a lean person compared with a fat person in the case of thiopental anaesthesia. However, such predictions rarely match experimental data.

Human pharmacokinetic studies are, generally speaking, conducted in subjects and patients in the 'normal' range with regard to their weight and age. Rarely does a pharmacokinetic protocol involve tight control of the weight of the subjects, even less frequently are objective measures of weight used as selection criteria for inclusion in a study. There are in fact many objective measures of weight. For example, body mass index (BMI), historically known as Quetelet's number (or index), provides a convenient and useful indicator of body fat. BMI is the body weight in kilograms divided by the square of the height in metres. Individuals with values of 25–30 are considered to be overweight, and those with values >30 are designated as obese. Obesity is further classified as moderate (BMI 30–35), severe (35–40), and morbid (>40). Obviously, it is

possible to have a very muscular body without excess fat, and thus have a relatively high BMI, as this index does not differentiate adipose tissue and muscle mass. Muscle has a higher density than fat, so the small framed but muscular ballet dancer mentioned earlier will have a higher specific gravity than the sumo wrestler. Nevertheless, generally speaking, a high BMI indicates an excessive body content of fat.

Ideal body weight (IBW) is a concept derived from data collected by the Metropolitan Life Insurance Company of New York. It relates weight to mortality data. It is an estimate of desirable weight corrected for sex, height and frame size. IBW is considered relevant only to life expectancy, and not to pharmacokinetics. However, there is a concept of lean body weight (LBW):

$$\text{Males}: \ \text{LBW} = 1.1 \times \text{TBW} - 0.0128 \times \text{BMI} \times \text{TBW}$$
$$\text{Females}: \ \text{LBW} = 1.07 \times \text{TBW} - 0.0148 \times \text{BMI} \times \text{TBW}$$

where in this instance TBW is total body weight (not to be confused with total body water). Some investigators use lean body *mass* (LBM), which has been derived by substituting the equation for BMI into the equations above:

$$\text{Males}: \ \text{LBM in kg} = 1.1 \times \text{TBW in kg} - 128(\text{TBW}/\text{height in cm})^2$$
$$\text{Females}: \ \text{LBM in kg} = 1.07 \times \text{TBW in kg} - 148(\text{TBW}/\text{height in cm})^2$$

It has been suggested that dosing of different drugs should be based on TBW, depending on their lipophilicity. Lipophilic drugs would be adjusted based on TBW, and hydrophilic drugs on LBW.

9.6.2 Obesity

Differences in distribution of drugs associated with obesity, for reasons connected with body size and composition might be expected, and because obese people have reduced cardiac output and slower tissue perfusion. A broad selection of drugs has been studied in the search for differences in their pharmacokinetic properties that can be related to obesity. Generally speaking, no consistent or important influences of obesity on drug absorption have been identified. However, there is no shortage of differences in apparent volume of distribution, both when expressed using volume units and also when weight corrected volume units are used. There is also no shortage of examples of obesity affecting clearance and half-life, but there is no consistent pattern of any obesity-induced changes in plasma protein binding of drugs or in renal excretion. There is a close association with cardiac output and obesity, with the potential to explain the changes seen in clearance when it is affected by, for example, hepatic blood flow. Table 9.1 shows a representative selection of examples of pharmacokinetic observations in obesity (Cheymol, 2000).

Table 9.1 Selection of pharmacokinetic observations in obesity. Each pair of numbers separated by / is the mean from control/obese patients

Drug	Therapeutic group	V (L)[a]	V (L kg^{-1})	CL (L h^{-1})	$t_{1/2}$ (h)
Ciprofloxacin	Anti-infective	219/269[b]	3.08/2.46[b]	44.6/53.8[b]	4.0/4.2
Ifosfamide	Anticancer	33.7/42.8[b]	0.53/0.55	4.33/4.56	4.9/6.4
Carbamazepine	Anticonvulsant	69.7/98.4[b]	0.96/0.87[b]	1.38/1.19	31.0/59.4[b]
Propofol	Sedative/anaesthetic	13.0/17.9	2.09/1.8	1.70/1.46	4.1/4.05
Dexfenfluramine	Appetite suppressant	668.7/969.7[b]	11.3/10.2	37.3/43.9	13.5/17.8
Propranolol	β-Blocker	180.0/226.8	3.1/2.4	41.6/46.2	3.4/3.9

[a] V_{ss} for propranolol.
[b] Significantly different.

The only consistent theme evident from these data is a totally expected increase in apparent volume of distribution in obesity. However, quite commonly, this is accompanied by a decrease in the value corrected for body weight, indicating that the drugs tend not to penetrate the excess weight proportionately. It would be expected that lipophilic drugs would show relatively high apparent volumes of distribution when the excess weight is primarily fat, and that hydrophilic drugs would show relatively high apparent volumes of distribution when the excess weight is primarily water, but this proposed pattern is not always confirmed by data. It is also possible that excess fat in particular, given the relatively low perfusion rate of fat, could require longer times for equilibration of drug concentrations and that apparent volumes of distribution in fatty obese people have been underestimated. Livers of obese people show fatty infiltration, and clearance can be lower, higher or no different in obesity, although studies with antipyrine have suggested that there are no specific effects on hepatic intrinsic clearance. However, the erythromycin breath test (Section 10.2.2) has shown a strong negative correlation with the percentage of IBW. Other examples, not included in Table 9.1, support this conclusion, and the examples in the table are typical of their groups. However, among the anti-infectives, vancomycin differs from ciprofloxacin by showing a shorter half-life in obesity. The anaesthesia group are of particular interest in not showing dramatic effects of retention in lipid in obese patients, in spite of their lipophilicity. The renal clearance of lithium, which, as an inorganic cation that is excreted unchanged, was found to be relatively fast in obese people in spite of there being no difference in the creatinine clearance in the patient groups studied. Finally, some oncologists dose anticancer drugs on the basis of IBW rather than TBW, as these drugs tend to be lipophobic.

9.7 Sex

There has been for many years a general belief that females are more sensitive to drugs than males. This has been based on observations with alcohol, on extrapolations from knowledge of glomerular filtration rates in males and females, on early studies that showed that male rats tend to metabolize drugs relatively rapidly, and on clinical observations, to some extent made by anaesthetists. However, differences in human beings have never been as prominent as those observed in laboratory rats.

9.7.1 Absorption and bioavailability

Gastric emptying time is relatively long in females, and this is thought to reflect effects of oestrogen. This can be expected to cause delays in absorption of drugs, with the same *AUC*, but longer lag times and lower rate constants of absorption, and hence lower C_{max} and later t_{max} values. There is no particular abundance of data supporting this proposed general rule. In fact, relatively fast absorption of salicylate has been shown in females, and a population study with mizolastine, an orally administered antihistamine drug, also demonstrated relatively slow absorption in males. However, absorption of ferrous sulfate has been shown to be relatively fast in prepubertal girls, apparently attributed to a hormonal effect, which would have to be on the carrier mechanism for iron.

Gastric alcohol dehydrogenase levels are relatively low in females. This leads to lesser presystemic metabolic losses, and so to relatively high blood alcohol concnetrations. This accounts, at least in part, for the observations of sensitivity differences with this drug. In contrast, intestinal concentrations of CYP3A4 do not show a similar, or indeed, any consistent pattern. One positive observation of a difference was made with oral verapamil, which is cleared relatively quickly in men, a difference not observed after i.v. doses.

9.7.2 Distribution

On average, body weight and BMI are relatively low in females, who also have a higher percentage of fat, and a relatively large plasma volume – and organ blood flow is also relatively high in women. On the basis of

general principles, theories can be generated that these differences would be expected to cause tissue distribution differences, depending on the lipophilicity of the drug. A limited number of examples either do or do not support such theories. For example, vecuronium, a skeletal muscle relaxant, shows relatively fast onset of effect and relatively long duration of action in females, not immediately explained by assumptions about lipohilicity, tissue distribution and duration of effect. However, diazepam shows a relatively high apparent volume of distribution in females, and metronidazole, which has low lipophilicity shows a relatively low apparent volume of distribution in females. Also, metronidazole shows higher clearance in females supported by the lower *AUC*. Protein binding may make a contribution in this context, but observations made to date are inconsistent.

9.7.3 Metabolism

Although there are more data on metabolism, the pattern is no more consistent than in regard to absorption and distribution. There does seem to be a general trend of relatively fast rates of metabolism of CYP3A4 substrates in females, and males seem to have relatively high P-gp levels. In relevant physiology, cardiac output and hepatic blood flow are relatively low in females.

In vitro studies have mostly shown relatively high CYP3A4 concentrations in female tissue samples, and erythromycin and isofsamide have been shown to be metabolized more rapidly. However, it should be noted that *in vitro* experiments do not necessarily reproduce the hormonal differences that occur *in vivo*, and this could lead to inconsistent observations. However, *in vivo*, i.v. studies have shown relatively fast metabolism of erythromycin in females. With midazolam, the overall weight of evidence is that there are basically no differences between the sexes with either i.v. or oral doses. However, oral, but not i.v., verapamil is cleared more rapidly in males. This is reflected in blood pressure changes. The midazolam and verapamil data together seem to provide insight into the relative significance of the enzyme exposure and P-gp transport. To reach the enzymes, the substrate needs to penetrate the hepatocytes, and to do this it must by-pass the effect of P-gp. Midazolam is a substrate for CYP3A4 but not for P-gp-mediated efflux, whereas verapamil is a substrate for both. Thus verapamil shows sex differences because of differences in P-gp affecting the exposure of oral doses to CYP3A4, with relatively low intracellular concentrations of verapamil in males and faster presystemic metabolism. Generally-speaking, drugs that are substrates for both CYP3A4 and P-gp show sex-related differences, but those that are only substrates for CYP3A4 do not. Most of the work in this context has been with CYP3A4. In regard to CYP2D6 and CYP1A2 there is evidence of metabolism by males being relatively fast. In the case of CYP2C19 there seems to be no difference.

Hormonal changes *per se* seem to have little effect. Studies during the menstrual cycle with midazolam after oral and i.v. doses, of eletriptan, a migraine treatment, and with dextromethorphan have shown no effects. Premenopausal patients apparently metabolize midazolam faster than do postmenopausal patients, but this is not reversed by hormone replacement treatment. Analogous studies with erythromycin have shown no differences.

9.7.4 Excretion

The differences between males and females in glomerular filtration rate are relatively small and in proportion to body weight, so there are sex differences that are really weight differences. There is a small amount of evidence of sex differences in active tubular secretion of drugs, mainly obtained from studies with frusemide (furosemide), which is a substrate for a renal tubular transporter – the clearance in females is lower than that in males. Similarly, amantidine, which is a substrate for the organic cation transporter, shows faster clearance in males. The renal clearance of acetylsulfadimidine, the metabolite of sulfadimidine (sulfamethazine), is reduced in females (Table 9.2).

Table 9.2 Mean urinary clearance values for sulfadimidine and *N*-acetylsulfadimidine in 54 subjects (Curry 1980)

Compound	Renal clearance (mL min^{-1})	
	Male ($n = 35$)	Female ($n = 19$)
Sulfadimidine	3.82	3.93
N-Acetylsulfadimidine	21.16*	22.20[a]

[a]Sex difference significant $p < 0.5$ (*t*-test).

9.7.5 Effects

Links between sex differences in pharmacokinetics and effect have been sought with prednisolone, for which there are pharmacokinetic differences but no pharmacodynamic differences. In contrast, vecuronium shows a relatively high effect in females because of differences in the apparent volume of distribution. Verapamil sex differences, with which there is a correlation between effect and pharmacokinetic properties, have already been mentioned. In regard to centrally-acting drugs, a limited number of studies has confirmed that there are cases of relatively high pharmacological sensitivity in females. Undoubtedly, part of the sex difference in alcohol response is the result of pharmacokinetic influences, but there seems to be a pharmacodynamic contribution to this. With morphine, there is a relatively narrow therapeutic index in females, with a 60% higher incidence of nausea and vomiting associated with a comparable analgesic effect than that in males. With diazepam and some antidepressants, there is evidence of relatively high pharmacodynamic sensitivity in females. With propofol, there is about 30–40% higher effect in females. This is not attributable to differences in pharmacokinetics.

9.8 Pregnancy

Pregnancy leads to a wide variety of anatomical, physiological and biochemical changes, and all of them have the capacity to modify the pharmacokinetic properties of drugs. While there has always been a tendency to discourage the use of medication during pregnancy, because of the risk of teratogenic effects, many patients have to continue with chronic medication, such as with antiepileptics, antiasthmatics and antidepressants, during pregnancy. There is also an ongoing need for acute treatments, such as with anti-infective agents during pregnancy, and it has been estimated that pregnant women receive an average of 1.3 prescriptions per clinic visit.

9.8.1 Physiological and biochemical changes

The cardiovascular system shows profound changes in pregnancy. Cardiac output, heart rate and stroke volume increase, and peripheral resistance and blood pressure (except in abnormal situations) decrease. Plasma volume can also increase. Total hepatic blood flow can increase by over 50% above non-pregnant rates, especially in the third trimester. Renal blood flow and glomerular filtration rate also increase by as much as 50%, as the result of renal vasodilatation. Thus changes in drug absorption, tissue distribution, metabolism and excretion can all be proposed as likely. However, the pharmacokinetics of the majority of drugs remain to be studied in this condition (Hodge and Tracy, 2007). Renal excretion has been investigated the most. For example, in one study the renal clearance of atenolol, a drug commonly studied for its renal elimination because of its near dependence on the kidney for its removal from the body,

was 12% above the postpartum level during the third trimester. Similarly, the renal clearance of digoxin, which is 80% excreted unchanged, increased by 21% and the clearance of lithium doubled during pregnancy.

The isoforms of the P-450 system show variable changes during pregnancy. For example, CYP1A2 and CYP2C19 show decreases in activity, and the half-life of theophylline, metabolized by CYP1A2, has been shown to be increased sufficiently to lead to a need for dosage reduction. Proguanil, which is converted to an active metabolite, cycloguanil, by CYP2C19, shows sufficiently decreased conversion to require an increase in dosage in pregnancy. Other isoforms show increased activity, so that, for example, fluoxetine (CYP2D6) shows relatively fast metabolism and relatively low plasma concentrations during pregnancy, and dosage increases may be needed. Phenytoin (CYP2C9) also shows increased clearance, and dosage adjustment based on maintenance of the optimum plasma concentration may be needed. There is also increased clearance of nicotine (CYP2A6) and methadone (CYP3A4). Conjugating enzymes also show a variety of effects. For example, the clearances of lorazepam (UGT2B7) and paracetamol (UGT1A1) are increased while that of caffeine (NAT2) and lamotrigine (UGT1A4) are decreased. The anticipated changes in half-life and *AUC* occur.

9.8.2 Hormonal effects

The significance of hormonal changes *per se* is not clear. Studies in pregnancy *per se* are in short supply, but much can be learned from studies during oral contraceptive use. There is considerable evidence of a component of hormonal control over the activity of CYP1A2, the activity of which is reduced among women taking oral contraceptives, although studies have shown no correlation between either oestrogen or progesterone levels and the activity of this isoform. An analogous situation exists with CYP2A6, the activity of which is increased during oral contraceptive use. The metabolism of omeprazole (CYP2C19) is decreased during oral contraceptive use, while that of dextromethorphan (CYP2D6) is apparently unchanged. Nifedipine and midazolam (CYP3A4) show decreased metabolism in users of oral contraceptives, and it is theorized that this may be due to inactivation of the CYP3A4 enzyme by oestrogen. However, this apparently does not occur in pregnancy, as neither oestrogen nor progesterone has been shown to inhibit, nor, for that matter, induce the enzyme *in vitro* or *in vivo*, although medroxyprogesterone has been shown to have an inhibitory effect on the human enzyme *in vitro*. Similar incomplete information is to be found for the conjugating enzymes.

9.8.3 Transporters

Studies of transporters, especially P-gp, multi-drug resistance associated protein, and breast cancer-resistance protein have been mostly restricted to the role of transporters in the placenta. There is some evidence that hormonal changes may induce or inhibit the expression of transporter proteins affecting intestinal uptake and efflux, and renal excretion and reabsorption. For example, *in vitro* accumulation studies with digoxin appear to indicate potential for changes in the disposition of this drug in the intestine and kidney during pregnancy, although details remain unclear. In the placenta, although approximately twenty transporter proteins have been identified, few have been linked to xenobiotic transport. For example, P-gp is expressed on the maternal side of the placenta, and the use of knock-out mice has shown that the experimental teratogen, avermectin is at least in part prevented from reaching the foetus by this efflux protein. Also, measurement of foetal levels of digoxin, paclitaxel and saquinovar, all P-gp substrates, has been used to demonstrate a similar exclusion. Measurement of P-gp levels in the human placenta have been considered to provide evidence of similar exclusions in humans. P-gp expression is relatively high in the earlier stages of pregnancy, the time when the foetus is most susceptible to teratogenic damage. Analogous,

but less complete information, has been obtained for multi-drug resistance associated protein and breast cancer-resistance protein.

9.8.4 The foetus

Some of the enzymes involved in drug metabolism are present as early as the sixth week of gestation, although appreciable levels are mostly not reached until after birth. CYP3A7 is the predominant enzyme in the foetus, actually declining in activity after the end of the first week from birth. Data from *in vitro* studies support the belief that this enzyme has a role in detoxifying certain endogenous compounds, notably dehydroepiandrosterone-3-sulfate, and also the potentially toxic metabolites of retinoic acid. Enzymes detected as present also include CYP2C9, CYP2C19 and CYP2D6, and it can be presumed that these enzymes are present to metabolize exogenous molecules that fail to be excluded by transporters such as P-gp. There is relatively little expression of UGT activity in the foetus, although *in vitro* studies have shown measurable glucuronidation of morphine in cells from foetal livers.

9.9 Ambulation, posture and exercise

In the course of any day, humans stand up and walk, sit, lie down for rest and sleep, and deliberately exercise their bodies. Exercise can be described as 'acute' (relatively short-lived as in a 'work-out') or 'chronic' (a programme of training continued over a relatively long period of time). These activities obviously involve changes in posture among other body features. The distinction between acute and chronic exercise is important, as, for example, during acute exercise blood is shunted to the working organs, primarily the muscles, at the expense of other organs, some of them crucial in drug disposition and pharmacokinetics, such as the gastrointestinal (GI) tract and the liver. In acute exercise, heart rate, cardiac output, systolic blood pressure, and pulmonary ventilation all increase, while hepatic blood flow is decreased. In chronic exercise, cardiac output is relatively high, although resting heart rate is lowered, so perfusion of all tissues, including the GI tract and the liver, the brain, the kidneys, and tissues in which drug molecules are stored such as voluntary muscle and fat deposits is increased. This is assessed as increased regional blood flow. Also, blood volume and activity of oxidative enzymes is increased, while fat mass is reduced. Thus there is potential in both acute and chronic exercise for all of the drug disposition sites to operate differently. Maximal oxygen uptake ($VO_{2_{max}}$) expressed as mL min^{-1} or mL min^{-1} kg^{-1}, is commonly used as the best measure of aerobic (cardiorespiratory) fitness, higher values being associated with higher fitness levels. Maximal oxygen uptake is considered to be reached when oxygen consumption shows no further increase with increased exercise workload, and represents the maximal capacity of the system to extract, deliver and utilize oxygen.

9.9.1 The gastrointestinal tract

Drug absorption is affected in several different ways by posture and body movement. For example, alcohol absorption is relatively slow when the body is in the supine position, for example during alcohol-induced sleep, as opposed to when standing and/or walking, and this has been attributed to relatively slow gastric emptying consequent on the adoption of the supine position, and also to a specific pharmacological effect of alcohol slowing gastric emptying. Exercise has little effect on gastric emptying until the intensity exceeds 70% of maximum oxygen uptake. Both acute and chronic exercise then have the ability to both shorten and lengthen gastric emptying time depending on the type of stomach contents involved. Intestinal transit time shows only minor changes with exercise, and then only with chronic exercise, when

it is shortened. Intestinal blood flow is reduced in acute exercise, as the blood is shunted to the working organs, principally voluntary muscle, but it is increased by chronic exercise. The absorption of quinidine, salicylate and sulfadimidine (sulfamethazine) is unaffected by the level of acute exercise. In contrast, sulfamethazole, tetracycline and doxycycline showed increases in C_{max} on acute exercise, but the comparison was with bed rest (as opposed to upright but not exercising), which is a different posture. The *rate* of absorption of digoxin has been shown to be higher during acute exercise, again in contrast with the body in the supine position. One solitary chronic exercise absorption study with asozemide in rats, showed no effect.

9.9.2 Transdermal absorption

Exercise increases skin temperature, and can cause vasodilation, or vasoconstriction if compensatory mechanisms of the vasomotor centre prevail. In this context, glyceryl trinitrate (GTN, nitroglycerin) plasma concentrations obtained from skin patches have been shown to increase three-fold in exercise, with a weak correlation with skin temperature. However, GTN concentrations in plasma are very sensitive to posture, and to hepatic blood flow, and observations attributed to exercise may well be related to changes in the metabolism of the drug in blood vessel walls, caused by changes in cardiac output (Figure 9.2).

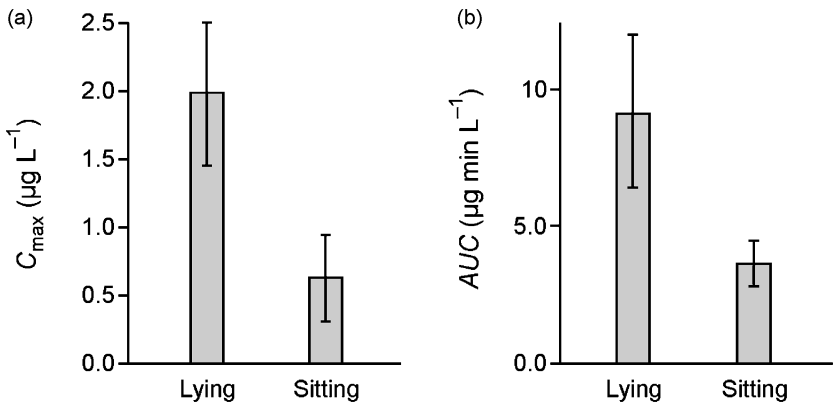

Figure 9.2 (a) Maximum concentration and (b) area under the curve for glyceryl nitrate in two matched groups of healthy volunteers, one group lying down (face up) and the other sitting. Data are mean ± SEM. (Adapted from Curry and Kwon, 1985.)

9.9.3 Tissue distribution

Because blood is shunted to the active tissues in acute exercise, changes in apparent volume of distribution are to be expected, and animal studies with atropine, theophylline and antipyrine, in which reductions in apparent volume of distribution were observed, support this.

There were no changes in plasma protein binding of verapamil and propranolol during acute exercise. However, digoxin concentrations in skeletal muscle have been shown to be increased during exercise, with corresponding decreases in concentrations in erythrocytes. Thus a shunting from red cells to active tissues occurs, but most likely with no impact on myocardial concentrations.

Chronic exercise is likely to be associated with reduction in fat mass and an increase in lean mass, with potential for effects on tissue distribution related to muscle binding and fat uptake. This has been simulated

for thiopental, using the kind of information discussed in Section 2.4.4.1. In this work, it was shown that the apparent volume of distribution would increase by as much as 76% in overweight individuals (physically inactive) when compared with lean individuals (exercising regularly). Chronic exercise has been shown to increase the apparent volume of distribution of antipyrine in mares, but only by 8%, although studies in humans with antipyrine and procainamide have not led to comparable conclusions.

9.9.4 Subcutaneous and intramuscular injections

Subcutaneous and i.m injections are greatly affected by blood flow to the injection site (Section 2.3.5). Thus injections into the legs are released into the blood more rapidly during ambulation and/or acute exercise. It is thought that this is more likely to be the result of a 'massage' effect of the muscles rubbing against each other and against the skin, rather than the blood flow effect *per se*, although it would be counter-intuitive to think that there would be no 'mixing' effect resulting from increased delivery of blood to the absorption site. This massage effect is analogous to the hand rubbing of the injection site widely practised as an effective means of helping absorption of injections, although this practice may have more to do with psychological soothing than drug absorption. Data in this context has come almost entirely from insulin (subcutaneous injections) and atropine (intramuscular injections). It is important for diabetic patients self-injecting with insulin to know and understand the significance, to their own particular needs, of the site of injection in relation to their level of physical activity.

9.9.5 The liver

Hepatic blood flow is reduced during acute exercise. This has been demonstrated with indocyanine green clearance studies and has obvious implications for drugs with blood flow-dependent clearance, such as lidocaine, the plasma concentrations of which are higher during exercise. However, verapamil and propranolol showed no change in clearance, and there is some evidence that the lidocaine observation is more related to posture than to hepatic blood flow controlled by blood flow shunting. Theophylline clearance is reduced in exercise, but there is evidence from antipyrine, diazepam, and sulfadimidine acetylation plasma studies that there are basically no effects on enzyme activity. Chronic exercise apparently does not cause a long-term increase in liver blood flow, and, generally speaking, few positive effects have been shown on drug metabolism. In one study, a relatively short half-life was observed with aminopyrine in trained athletes, and an analogous observation has been made with antipyrine. Prednisolone has also been shown to have a faster elimination rate in trained athletes.

9.9.6 The kidneys

Renal blood flow is reduced as the result of exercise, as is the glomerular filtration rate, shown by creatinine clearance. Plasma protein concentrations can change either way with exercise, because of fluid shunting, and urinary pH being reduced. As a consequence, atenolol renal clearance is reduced, as is that of digoxin, along with active tubular secretion of procainamide.

9.9.7 Body temperature

Acute exercise increases body temperature by 1–2 °C. It has been suggested that this could increase the kinetic energy of drug molecules, increasing the rate of diffusion across membranes in the gastrointestinal tract and kidney, and increasing the activity of the drug metabolizing enzymes. However, available data do not seem to support this.

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10

Pharmacogenetics and Pharmacogenomics

10.1 Introduction

Genetic differences in drug response may be due to differences in pharmacodynamics (different receptor populations) or in drug disposition (differences in drug metabolizing enzymes and transporters). When genetic differences are due to a single gene mutation and the incidence is $>1\%$ then such differences may be detectable in population studies as a bi- or trimodal distribution. There is interest in such polymorphisms because metabolism inactivates or activates not only drugs, but also carcinogens and procarcinogens, and much of the recent literature is devoted to assessing the role of genetics as a risk factor in cancer. The term pharmacogenetics is usually applied the study of drug interactions with a relatively restricted number of genes, whereas pharmacogenomics aims to study the effect of the entire complement of genes (i.e. the genome) on drug action. As this is a rapidly developing field, anything written one year is likely to be superseded within a few years. Thus, this chapter will use selected examples to illustrate the principles involved.

10.1.1 Terminology

In mammalian cells, *chromosomes* are thread-like structures in the nucleus, comprised of DNA and associated proteins. Typically there are two sets of chromosomes, arranged in pairs (*diploid*), one set being inherited from each parent. *Genes* are sequences of nucleic acids located on regions (*loci*) of chromosomes that define the characteristics or traits of the organism. They can be considered as the basic units of heredity. Different forms of a gene are known as *alleles* and it is possible to inherit the same alleles, in which case the individual is referred to as a *homozygote* and said to be *homozygous*, or when the alleles are different, a *heterozygote*. The genetic make up of an organism is known as the *genotype*. *Phenotype* refers to the physical characteristics exhibited and these can be influenced by inherited and environmental factors. The phenotype in heterozygotes will be largely determined by the interaction of the different alleles which can often be referred to as *dominant* or *recessive*. In the simplest case, a dominant allele will produce the same phenotype as that when both dominant alleles are present. Dominant alleles may be denoted R, and recessive ones r, so that heterozygotes are Rr whilst homozygotes are either RR or rr. The different phenotypes are referred to as being *polymorphic* (having different forms). Clinically it is the phenotype that is important but knowledge of the genotype may help to explain the phenomenon.

By convention genes (e.g. *CYP2C19*) and alleles (e.g. *CYP2C19*1*) are written in italics, and in capitals when referring to human genes, whilst the gene products (enzymes, transporters, etc.) are written in the standard font (e.g. CYP2C19*1 or CYP2C19.1). The term *wild-type* may be encountered. It was introduced to describe the form of allele found in nature, that is it was considered to be the 'standard' or 'normal' allele,

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others being mutant alleles. However, most genes exist in a variety of forms, the frequency of which varies depending upon the geographic range of the species and, in the case of humans, the extent to which populations have migrated and interbred.

Often alleles occur because of a *single-nucleotide polymorphism* (SNP), which can give rise to a protein in which one amino acid is substituted for another. In the case of enzymes this may result in reduced activity or no activity. The site(s) of the SNPs may be identified, for example in CYP1B1*3 cytosine is replaced by guanine, C4326G; this produces an enzyme where leucine at 432 is replaced by valine, Leu432Val. Occasionally SNPs lead to enzymes with increased activity. A *null allele* is one that either produces no protein or the protein lacks any function.

10.2 Methods for the study of pharmacogenetics

10.2.1 Studies in twins

An obvious way to investigate whether a phenomenon is genetically related is to investigate it in twins. The major influence of genetic control on drug disposition has been demonstrated by studying the half-lives of several drugs including antipyrine, dicoumarol, phenylbutazone and nortriptyline in identical (monovular) and fraternal (biovular) twins. The similarity in values for identical twins can be striking as with the study by Vesell and Page (1968) who investigated the elimination half-life of phenylbutazone after oral doses (Table 10.1).

Table 10.1 Paired plasma half-life values (days) for the decline in phenylbutazone in seven pairs of identical and non-identical twins (Vesell and Page, 1968)

	1	2	3	4	5	6	7
Identical twins	2.8	2.6	2.8	4.0	3.9	1.9	3.2
	2.8	2.6	2.9	4.0	4.1	2.1	2.9
Non-identical twins	7.3	2.9	2.6	1.9	2.1	2.3	2.8
	3.6	3.0	2.3	2.1	1.2	3.3	3.5

10.2.2 Phenotyping and genotyping

Early observations of the influence of genetics on drug disposition arose because subjects could be classified according to their phenotype, for example they were either 'slow' or 'fast' acetylators of isoniazid (see below). Today phenotyping may be carried out systematically, using drugs as 'probes' to determine a subject's metabolizer status. Mixtures of drugs have been developed and some bear the name of institutions in which they were developed, for example, the Pittsburgh, Indiana and Karolinska Cocktails (Table 10.2) These drugs may also be used when investigating the effects of age, sex and drug interactions on enzyme activity (see Section 17.10.2). The tests may simply determine the concentration of the test drug in plasma or urine at a defined time after it has been administered, or specific metabolites may be measured. Alternatively, serial samples may be collected so that *AUC* and oral clearance values can be calculated. The erythromycin breath test involves administering [^{14}C-methyl]-erythromycin and collecting breath (in a balloon) for measurement of $^{14}CO_2$, which reflects the degree of desmethylation. Intravenous and oral administration of midazolam has been used to differentiate between hepatic and gut wall (+ hepatic) metabolism by CYP3A4. Additionally, inhibitors may be given in an attempt to confirm the identity of the enzyme involved. A limitation to this approach is the lack of specificity of some substrates and inhibitors for some enzymes and transporters. This is a particular problem with CYP3A4, CYP3A5 and P-glycoprotein (P-gp), which have similar substrate specificities.

Table 10.2 Examples of substances used to phenotype individuals for drug metabolizing activity

Enzyme	Probe	Measurement	Sample/time
CYP1A2	Caffeine[a]	Caffeine/1,7-dimethylxanthine (paraxanthine)	Plasma – 8 h
	Caffeine[b]	Paraxanthine/Caffeine	Serum – 6 h
	Caffeine[c]		Plasma – 4 h
CYP2B6	Bupropion[d]	Hydroxylation	
CYP2C8	Amodiaquine[d]	Desethylation	
CYP2C9	Losartan[c]	5-Carboxylic acid metabolite (E-3174)	Urine – 8 h
	Tolbutamide[b]		Serum – serial samples
CYP2C19	Mephenytoin[a]	4-Hydroxymephenytoin	Urine – 8 h
	Omeprazole[c]	5-Hydroxyomeprazole	Plasma – 3.5 h
CYP2D6	Debrisoquine[a]	4′-Hydroxydebrisoquine/ (4′-Hydroxydebrisoquine + debrisoquine)	Urine – 8 h
	Dextromethorpran[b]	Dextrorphan	Urine – serial samples
	Debrisoquine[c]	4′-Hydroxydebrisoquine/ (4′-Hydroxydebrisoquine + debrisoquine)	Urine – 8 h
CYP2E1	Chlorzoxazone[a]	6-Hydroxychlorzoxazone/ chlorzoxazone	Plasma – 4 h
CYP3A4	Dapsone[a]	Dapsone hydroxylamine/ (Hydroxylamine + dapsone)	Urine – 8 h
(Hepatic)	Midazolam (i.v.) [b]		Serum – serial samples
(Hepatic + intestinal wall)	Midazolam (p.o) [b]	1′-Hydroxymidazolam	
	Cortisol	6-β-Hydroxycortisol/cortisol	Urine
	[^{14}C]-Erythromycin	[^{14}C]-CO_2	Breath – 20 min
CYP3A4/5	Quinine[c]	3-Hydroxyquinine	Plasma – 16 h
	Dextromethorphan	3-Methoxymorphinan	
CYP3A5	Midazolam[d]	1′-Hydroxymidazolam	
NAT2	Dapsone[a]	Monacetyldapsone	Plasma – 8 h
	Sulfadimidine	Acetylsulfadimidine	Plasma – 8 h

[a]Pittsburg cocktail (Frye *et al*., 1997); [b]Indiana cocktail (Wang *et al*., 2001); [c]Karolinska cocktail (Christensen *et al*., 2003); [d]O'Donnell *et al*., 2007.

Polymerase chain reactions (PCR), originally with restriction fragment length polymorphism (RFLP), are used to sequence genes and so identify particular genotypes and variant alleles. Furthermore, transfection of cDNA into organisms (e.g. *Escherichia coli*) or cell lines (e.g. COS) allows production of recombinant enzymes which can be sequenced to identify changes in amino acid composition and enzyme activity, expressed as *K*m and V_{max}, values for the substrates of interest. This can lead to confusion as a 'mutant' enzyme may be more active per weight of protein than the wild-type when tested *in vitro*, but if the variant allele results in much less enzyme being expressed, then the *in vivo* activity may be reduced.

10.3 *N*-acetyltransferase

There are two major forms of arylamine *N*-acetyltransferase. Substrates of type 1 include *p*-aminobenzoic acid, *p*-aminosalicylic acid and endogenous *p*-aminobenzylglutamate, whereas a number of primary

aromatic amine and hydrazine drugs are acetylated by *N*-acetyltransferase type 2 (NAT2), including the examples of Figure 3.14. Over 30 variants of the *NAT2* gene have been identified. *NAT2*4* is considered the 'wild-type' allele.

10.3.1 Isoniazid

The bimodal nature of metabolism of the anti-tuberculosis drug, isoniazid, was known in the early 1950s, but it was the classic experiments of Evans *et al.* (1960) that demonstrated the genetic nature of the polymorphism. Plasma isoniazid concentrations were shown to be bimodally distributed when 483 subjects were given identical doses. A subset of results from 267 members of 53 families confirmed the hereditary nature of the phenomenon. Subjects with plasma concentrations $<2\,\text{mg}\,\text{L}^{-1}$ were referred to as 'rapid inactivators' whilst those with lower concentrations were classed as 'slow-inactivators' (Figure 10.1).

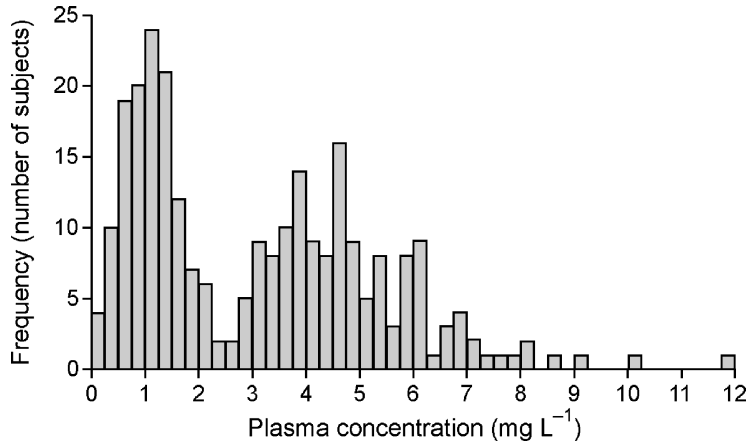

Figure 10.1 Frequency distribution for plasma isoniazid concentrations 6 hours after oral doses of $9.7\,\text{mg}\,\text{kg}^{-1}$ in 267 members of 53 families. (Redrawn from Evans *et al.*, 1960.)

The rapid allele (R) is dominant, and so only homozygotes (rr) are slow acetylators. The distribution of fast to slow acetylators is approximately 50 : 50 in Caucasian and African Americans, so the gene frequency of the slow gene(s) must be ~75%. Inuits and Japanese are primarily fast acetylators (95%) but some Mediterranean Jews are mainly slow (20% fast). Fast acetylators may require higher doses. Slow acetylators may develop a peripheral neuropathy due to the imine (Schiff's base) formed between isoniazid and pyridoxal, which depletes the vitamin, whereas rapid acetylators are prone to hepatotoxicity which is probably caused by *N*-acetylhydrazine that is released from the acetyl metabolite (Section 18.6.1).

It is sometimes possible to identify heterozygotes, but not from histograms of the type shown in Figure 10.1. For example, systemic clearance, rather than plasma concentration, was shown to correlate with the number of *NAT2*4* alleles (Kinzig-Schippers *et al.*, 2005).

10.3.2 Sulfonamides

The acetylation of the primary amine groups of several sulfonamides, including sulfadimidine (sulfamethazine) shows the same polymorphism as that of isoniazid. Because sulfadimidine concentrations could be measured using a relatively simple colorimetric assay, this drug has been used to test for acetylator status.

Following a test dose of 0.5 g, the proportion of N^4-acetylsulfadimidine in plasma was bimodally distributed, with an antinode at ~40% (Figure 10.2). Thus, those with >40% acetyl metabolite in plasma are fast acetylators. With test doses of 2 g the antinode was ~25%, presumably showing that at the higher dose the metabolism is becoming saturated. As with isoniazid, it has been shown that sulfadimidine elimination half-lives can be assigned to three distinct groups, reflecting the three phenotypes.

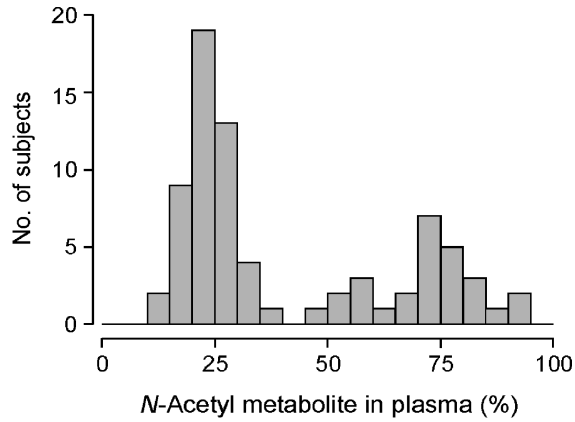

Figure 10.2 Distribution for percent *N*-acetyl metabolite in plasma 6 hours after a test dose sulfadimidine (0.5 g, p.o.). (Redrawn from Whelpton *et al.*, 1981.)

10.3.3 Other drugs

Other substrates of NAT2 include hydralazine, phenelzine and dapsone. The acetyl metabolites of these compounds are considered inactive in comparison to the parent drug. Generally, fast acetylators may not respond adequately to treatment whilst slow acetylators are more prone to adverse effects. Procainamide is unusual in that the acetyl metabolite has similar pharmacological properties to the parent drug and is marketed as acecainide.

10.4 Plasma cholinesterase

Several genotypes for plasma cholinesterase (pseudocholinesterase) have been discovered. Approximately 94% of the population are homozygous for the 'normal' allele and are designated EuEu. Of the atypical alleles, the one coding for a dibucaine-resistant form of the enzyme (Ea) is probably the most important clinically. EaEa homozygotes show prolonged paralysis when given the muscle relaxants suxamethonium (succinylcholine) and miracurium and may be sensitive to other drugs including, procaine, cocaine, pilocarpine, huperazine A and donepezil. Fluoride resistant (Ef) and silent (Es) alleles have been identified.

10.4.1 Suxamethonium

In normal subjects the duration of action of suxamethonium is approximately 5–10 minutes. Most of the injected dose is hydrolysed so that only some 5–10% reaches the acetylcholine receptors of the motor endplate. Drug that diffuses from the receptors is hydrolysed by the normally functioning enzyme. Approximately 1 in 3,000 people remain paralysed for an unusually long period following the drug (Figure 10.3). Should this occur during an operation then mechanical ventilation must be continued until the patient can breathe normally.

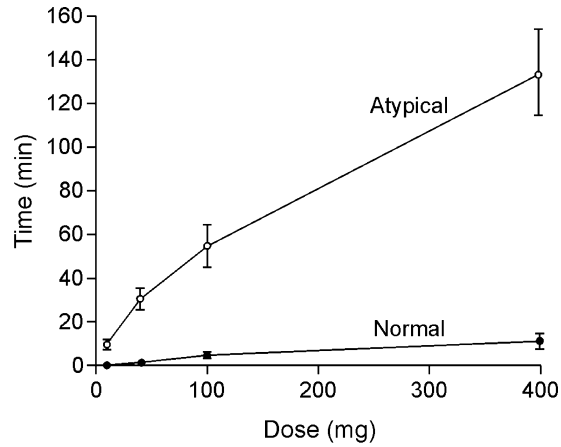

Figure 10.3 Duration of apnoea in adult male patients with normal and atypical cholinesterase (Kalow & Gunn, 1957).

As well as taking a family history, patients can be tested using standard cholinesterase assays in the presence of a standard concentration of the local anaesthetic dibucaine, which inhibits the normal enzyme activity by 80%. The enzyme from EaEa homozygotes is only inhibited by 20%. These values are known as the dibucaine number. Heterozygotes (EuEa) have dibucaine numbers of ~60, and although these individuals may have a longer duration of apnoea, it rarely lasts for more than 1 hour, and is not considered clinically important. A more serious situation arises in homozgyotes carrying the Es gene who have no pseudocholinesterase activity and the duration of apnoea may be over 8 hours. The frequency of this polymorphism is 1 : 100,000.

Demonstrating polymorphic hydrolysis of other drugs is complicated by the fact that they may also be substrates for the many other esterases that exist in plasma.

10.5 Cytochrome P450 polymorphisms

It is probable that polymorphisms exist for all the drug metabolizing cytochromes and several important clinical differences have been demonstrated for CYP2D6, CYP2C9 and CYP2C19. Individuals with two functioning genes are referred to as extensive metabolizers (EMs) and those with no, or only one, functioning gene, are classed as poor (PMs) and intermediate (IMs) metabolizers, respectively. People with more than two functioning genes are ultrarapid metabolizers (UMs).

10.5.1 Cytochrome 2D6

One of the first observations of a polymorphism in microsomal drug metabolizing enzymes was the exaggerated response to the obsolete antihypertensive, debrisoquine. The metabolism to the 4-hydroxy metabolite is catalysed by CYP2D6 and has been used to phenotype poor metabolizers (Table 10.2). Urine is collected for 8 hours following a test dose of debrisoquine (10 mg, p.o.) and the metabolic ratio (*MR*) calculated from:

$$MR = \frac{\%\text{ of dose as debrisoquine}}{\%\text{ of dose as 4-hydroxydebrisoquine}} \qquad (10.1)$$

Poor metabolizers are defined as those having a log(*MR*) value > 1.1 (i.e. a ratio > 12.5). At the same time as polymorphisms in debrisoquine metabolism were being investigated similar patterns were demonstrated for sparteine and nortriptyline. Subsequently, it was shown that the differences were due to different *CYP2D6* alleles. A large number of drugs are substrates for CYP2D6, including several β-blockers, neuroleptics and SSRIs (Table 3.1). It has been claimed that PMs obtain no pain relief from codeine as they are unable to metabolize it to morphine, while UMs show an exaggerated response to codeine. Tamoxifen is another drug that relies on CYP2D6 for its activation and PMs do not respond as well as EMs do to this drug.

The distribution of PMs varies amongst different ethnic groups. In Europeans it is ~7%, which means that some 20–30 million Europeans have no CYP2D6 enzymes. However it has also been estimated that 15–20 million Europeans have multiple copies of the gene. Approximately 2% of Swedish and 7% of Spanish people are UMs. The figure may be as high as 29% for Ethiopians. Many of these patients fail to respond to standard doses of CYP2D6 substrates and may be classed as non-responders. Figure 10.4 shows plasma nortriptyline concentrations in subjects carrying different numbers of functional genes (0–13) after a single dose. The differences in concentrations will be even more marked after multiple dosing to steady-state concentrations.

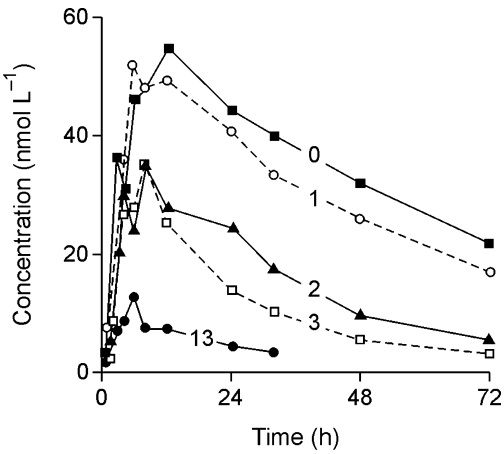

Figure 10.4 Mean plasma concentration of nortriptyline after a single oral dose in subjects with varying numbers (0–13) of *CYP2D6* genes as indicated on the lines. There were five subjects in each group apart from $n = 1$ for the subject with 13 genes (redrawn from Dalén *et al,* 1998).

10.5.2 Cytochrome 2C9

It has been estimated that CYP2C9 catalyses approximately 10% of P-450-mediated drug metabolism. *CYP2C9*2* and *CYP2C9*3* alleles arise from SNPs and the enzymes have been estimated to confer 70% and 10% of the intrinsic clearance of the wild-type enzyme (CYP2C9*1), respectively. Approximately, 35% of Caucasians have at least one *2 or *3 allele. Although rare, *CYP2C9*6* is a null allele, conferring no enzyme activity.

Substrates of this enzyme include phenytoin, tolbutamide, valproate, and warfarin (Table 10.3). In the past there have been reports of unusually long half-lives of some of these drugs in a small number of patients. Adverse drug reactions to phenytoin have be ascribed to patients having defective *CYP2C9* alleles, particularly in *3/*3 diplotypes, who comprise ~0.4% of Caucasians.

Table 10.3 Examples of CYP2C9 substrates

Analgesics	**Anticonvulsants**	**Oral hypoglycaemics**	**NSAIDs**
Paracetamol	Phenytoin	Tolbutamide	*S*-Naproxen
Phenacetin	Phenobarbital	Glibenclamide	Diclofenac
Aminopyrine	Valproate	Glipizide	Celecoxib
Oral anticoagulants	**Angiotensin II antagonists**	**SSRIs**	**Others**
Warfarin	Losartan	Sertraline	Fluvastatin
Dicoumerol	Candesartan	Venlafaxine	Sildenafil

NSAIDs, non-steroidal anti-inflammatory drugs.

10.5.3 Cytochrome 2C19

Polymorphic 4′-hydroxylation of *S*-mephenytoin was reported in the 1980s and it has since been shown that the enzyme responsible is CYP2C19. This enzyme catalyses the metabolism of several frequently prescribed drugs. It catalyses hydroxylation of the proton pump inhibitor, omeprazole and the desmethylation of diazepam, imipramine and citalopram. The incidence of poor metabolizers is higher in East Asian populations (13–23%) compared with Caucasians (2–5%). *CYP2C19*1* is the wild-type allele whilst CYP2C19*2 or CYP2C19*3 are considered defective mutants that result in reduced enzyme activity. Thus, following a single dose of omeprazole the mean *AUC* value for *2/*2 homozygotes was nearly 10 times that measured for *1/*1. The *AUC* for heterozygotes was only twice that of the EM subjects.

The degree to which the pH of gastric contents was increased and the success of ulcer treatment with omeprazole was highly dependant on phenotype, with 100% success in PMs, but only 25% in EMs. It has been suggested that CYP2C19 genotyping is cost effective in predicting response to omeprazole and amoxicillin in the treatment of *Helicobacter pylori* infection and peptic ulcer.

10.5.4 Cytochromes 3A4/5

The *CYP3* alleles are clustered on chromosome 7 along with the *MDR1* gene that encodes P-gp. As CYP3A4/5 have been estimated to metabolize some 50% of commonly-used drugs and have important roles in first-pass metabolism, polymorphisms could have major affects on bioavailability, and hence pharmacological/toxicological activity. However, because of the similar substrate/inhibiter specificities of CYP3A4/5 and P-gp it is not always possible to ascertain whether individual differences in bioavailability are due to polymorphisms in the enzymes or the transporter. The situation is further complicated by the fact that high levels of these enzymes are expressed in enterocytes and hepatocytes. The enzymes can accommodate both small drugs, such as midazolam, and larger substrates, including ciclosporin. *CYP3A4* alleles have been identified but their frequencies are low.

Higher levels of CYP3A5 are expressed in Africans and saquinavir *AUC*s were 34% lower in 'CYP3A5 producers'. Two alleles arising from SNPs, *CYP3A5*3* and *CYP3A5*6* have relatively high frequencies and some subjects may not have any functioning CYP3A5. Dosage adjustments of immunosupressants, ciclosporin and tacrolimus may be required for some individuals, but the effect of polymorphism in *MDR1* may be a contributory factor (Section 10.6).

10.5.5 Other cytochrome P450 polymorphisms

Polymorphisms in CYP2B6 have been identified of which the variant *CYP2B6*6* appears to be the most important. The frequency of homozygotes for this allele is 3% in whites and 20% in blacks, and these individuals have higher plasma concentrations and increased adverse reactions with the anti-HIV

drug, efavirenz. Cyclophosphamide is a prodrug with complicated activation and inactivation pathways, some of which are non-enzymatic. The first step, oxidation to 4-hydroxycyclophosphamide, is catalysed by CYP2B6 and individuals with the variant *CYP2B6*6* produce a protein that metabolizes the drug at a faster rate. However, the amount of enzyme expressed by *6/*6 homozygotes was considerably less than those with the wild-type allele. Polymorphism in nicotine oxidation has been attributed to an inactive variant allele of *CYP2A6*. An alternative explanation is that in some Asian subjects a *CYP2A6* gene may be deleted and may be responsible for reduced nicotine metabolism. Some individuals may have multiple copies of *CYP2A6*.

10.6 Alcohol dehydrogenase and acetaldehyde dehydrogenase

Alcohol dehydrogenase (ADH) is a dimeric enzyme made up of six separate subunits, encoded by three genes, ADH_1, ADH_2 and ADH_3. Many combinations of isoenzymes exist, leading to different rates of metabolism amongst white, black African, and Asian populations.

Aldehyde dehydrogenase (ALDH) is a mitochondrial enzyme. Some Asians have ALDH different from that of Caucasians and about 50% of Asians (principally Chinese) have inactive ALDH, leading to flushing and other unpleasant effects when these individuals consume ethanol. These effects are similar to those seen with disulfiram.

10.7 Thiopurine methyltransferase

Phenotyping or genotyping of thiopurine methyltransferase (TPMT) is used to guide treatment with azathioprine to avoid life-threatening acute toxicity. The incidence of very low TPMT activities is relatively high (1 : 300), whilst 11% of subjects have intermediate activity. 6-Mercaptopurine, derived from azathioprine is normally metabolized via one of three pathways: (i) methylation by TPMT, (ii) oxidation by xanthine oxidase or (iii) by hypoxanthine phosphoribosyltransferase to active thiopurine metabolites including 6- thioguanine nucleotides. Patients with low TPMT activity have unusually high levels of 6-thioguanidine incorporated into DNA, which is, in part, responsible for azathioprine toxicity.

10.8 Phase 2 enzymes

Because of the historical importance of *NAT*2 polymorphism and the fact that it provides a simple, but clear, example of the issues involved, this phase 2 enzyme was discussed earlier. Other phase 2 enzymes, the UDP-glucuronosyltransferases (UGT), sulfotransferases (SULT) and glutathione transferases (GST) are superfamilies, much as the cytochrome P450s are a superfamily.

10.8.1 UDP-glucuronosyltransferases

The two major classes of *UGT* genes are *UGT*1 and *UGT*2 which produce at least 18 enzymes. UGT1A1 catalyses the glucuronidation of bilirubin. The wild-type allele is *UGT1A1*1*, but a common mutant, *UGT1A1*28* leads to a mild form of hyperbilirubinaemia, known as Gilbert's syndrome, which occurs in 5–10% of the population. As a consequence sufferers may be prone to the adverse effects of drugs or metabolites metabolized by UGT1A1. This has been shown to be the case with the anticancer drug irinotecan, which is metabolized to the active, and potentially toxic, metabolite known as SN-38. Glucuronidation and inactivation of this compound is catalysed by UGT1A1 and those with Gilbert's syndrome are more prone to neuropenia and diarrhoea. In 94% of people with Gilbert's syndrome two other UGTs are affected, one of which is thought to catalyse the glucuronidation of paracetamol.

3-Glucuronidation of morphine is catalysed by several UGTs, but only UGT2B7 has been shown to catalyse both 3- and 6-glucuronidation. Some studies of the allelic variant, *UGT2B7*2* have shown that the rate of glucuronidation is greater in carriers of this allele, however other studies have failed to substantiate this claim, and whether this genotype has any clinical significance is equivocal. Of course the situation is complicated by the fact that the 3-glucuronide is inactive whereas the 6-glucuronide is analgesic.

10.8.2 Sulfotransferases

The most widely studied sulfotransferase is SULT1A1, also known as 'thermostable' or phenol SULT, as it catalyses sulfation of a large number of endogenous and exogenous phenols including paracetamol and the 4-hydroxy active metabolite of tamoxifen. SULT1A1 and 1A3 are highly expressed in platelets thereby facilitating study. A common variant is SULT1A1.2, in which G638A substitution results in Arg213His in the allozyme. This enzyme is less thermally stable, has reduced activity compared to SULT1A1.1 and a shorter half-life. SULT1A1.3 arises from a A667G SNP leading to a Met223Val substitution in the enzyme.

Ethnic variations have been described. The frequency of the *1A1*1* allele is 0.914 in Chinese but only 0.656 and 0.477 in Caucasians and African Americans, respectively. The frequency of the *1A1*2* allele is 0.332 and 0.294 in the latter groups. The incidence of the *1A1*3* allele is 0.229 in African Americans. Much of the research into the functional effects these alleles has concerned sulfation of flavanoids, which may protect against cancer, 17-β-oestradiol, which has been implicated in breast cancer, and 4-hydroxytamoxifen. It has been suggested that women carrying the *1A1*2* or *1A1*3* alleles sulfate the active metabolite of tamoxifen to a lesser extent and so have higher exposure to the drug. However they also have reduced sulfation of oestradiol. Despite the role of sulfation in the metabolism of paracetamol, there appears to be no definitive observation on the impact of SULT variants.

10.8.3 Glutathione S-transferases

Human GST families are designated by uppercase Greek letters, such as alpha (A), mu (M) pi (P) and theta (T). Polymorphisms are known in the *GSTP1* gene, and deletions occur frequently with *GSTM1* so that 50% of the population are homozygous for the null allele. The frequency of deletions with *GSTT1* is ethnically determined, ∼20% of Europeans and ∼60% of Orientals and Africans being *GSTT1*0/*0*. High levels of GSTT1 are found in erythrocytes. Thus, it would appear that there is potential for phenotypic polymorphism in GSH conjugation, however most studies have concentrated on the role of these alleles in the development of cancers.

With regard to the effects on drug disposition, the clearance of busulfan, an alkylating agent used for treating chronic myelogenous leukaemia, was significantly lower in *GSTA1*A/*B* heterozygotes than in those with homogenous *GSTA1*A/*A*. The plasma concentrations were correspondingly higher in the heterogeneous group. Ekhart *et al*. (2009) investigated the effects of pharmacogenetics on the oxidation and conjugation of the alkylating anticancer drug thiotepa, which is metabolized to tepa. The conclusion was that patients who were homozygous for a *GSTP1* variant allele had greater exposure to the two compounds.

10.9 Transporters

The widespread distribution of multifunctional transport proteins results in these being of prime importance in pharmacogenetics. Interest is primarily in those genes that encode P-gp, multi-drug resistance associated protein, organic anion transport polypeptides, and organic cationic transporters. Some of these transporters

are located in the basolateral membranes whilst others are in the luminal membrane and may work in concert to eliminate drugs. The complexity of these combinations can be appreciated from the simplified diagram of Figure 10.5.

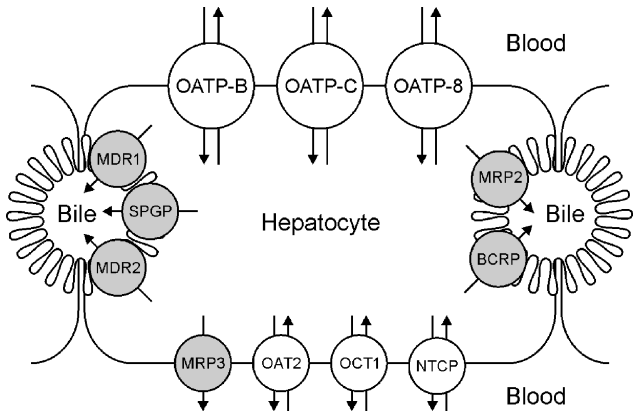

Figure 10.5 Simplified diagram of hepatic transporters. Organic anion transport proteins (OATP-B, OATP-C and OATP-8), organic anion transporter 2 (OAT2), organic cation transporter 1 (OCT1) and sodium-dependent taurocholate transporting polypeptide (NTCP) facilitate the uptake of solutes into hepatocytes. Multidrug resistance-associated protein 3 (MRP3) is an efflux pump. The hepatic canalicular efflux proteins include multidrug resistance proteins (MDR1 and MDR2), MRP2, sister of P-glycoprotein (SPGP) and breast cancer resistance protein (BCRP). The efflux proteins utilize ATP (not shown). Adapted from Tirona & Kim, 2002.

The *MDR1* gene which encodes for P-gp is highly polymorphic. One variant, C3435T, has been shown to result in half the normal P-gp expression in the duodenum and this was associated with higher digoxin concentrations in these subjects. Some 24% of the subjects were homozygous for this SNP. Similarly, the frequency in children being treated for HIV, was C/C (44%) C/T (46%) and T/T (10%). The children that were heterozygous, had higher plasma nelfinavir concentrations and lower oral clearance (although it is not clear whether the clearance values were corrected for differences in bioavailability). Children in this C/T group responded more quickly to treatment than the others. On the other hand, Kim *et al*. (2001) found conflicting results with fexofenadine but pointed out that additional SNPs were possible – C1236T and G677T, and Chowbay *et al*. (2003) showed that haplotypes where all three positions were substituted with thiamine (T-T-T) had increased *AUC* and C_{max} values for ciclosporin.

Allelic variations have been described in the various OATP families. An example of a functional effect of mutations is the clearance of pravastatin, which is transported into hepatocytes by OATP-C and into rena tubular cells by OAT3 (Nishizato *et al.*, 2003). In subjects with the *OATP-C* 15* variant the total and non-renal clearances were lower than those subjects without this allele. The one subject who was homozygous **15/*15,* had the lowest *CL* value and the largest *AUC*.

An investigation of the role of mutations in organic anion transporter (OCT) genes, in the disposition of the loop diuretic, torasemide, revealed that mutations in OCT4 (located in the luminal membrane of proximal tubular cells) rather than OCT1 or OCT3 variants (found in the basolateral membrane) reduced the renal clearance of this drug.

OCT2 is the major organic cation transporter in the basolateral membrane of renal proximal tubular cells and metformin is a suitable probe as 98% of a dose is eliminated via the kidneys, and the renal clearance (CL_R) ranges from ~400–600 mL min^{-1}, indicating tubular secretion. There is little plasma protein binding so creatinine clearance, CL_{cr} has been used to estimate filtration, enabling the contribution from secretion, CL_{sec} to be estimated:

$$CL_{sec} = CL_R - CL_{cr} \tag{10.2}$$

At least 28 variants of OCT2 have been reported. A study in Chinese subjects to investigate the functional effects of a common variant (G808T), showed the clearance of metformin was lowest in T/T homozygotes, while T/G heterzygotes had intermediate clearance values. Cimetidine, a specific inhibitor, reduced the metformin *AUC* in G/G homozygotes but had little effect on the *AUC* measured in T/T subjects. In a study in Caucasians, low activity OCT1 was associated with reduced metformin clearance.

10.10 Pharmacodynamic differences

Naturally this chapter concentrates on the effects of pharmacogenetics on drug disposition but it is worth remembering that variations in drug response may be due to, wholly or in part, differences in drug targets. Genetic variants in β-adrenoceptors have been long known. Differences in sensitivity to warfarin also arise because of mutations in the *VKORC1* gene which encodes for vitamin K epoxide reductase. African Americans are relatively resistant to warfarin. Recent interest has been into polymorphism in the gene that codes for angiotensin-converting enzyme (ACE).

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