Enzyme Systems that Metabolise Drugs and Other Xenobiotics.
Edited by Costas Ioannides

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1 Xenobiotic Metabolism: An Overview

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The meteoric advances in analytical technology that occurred during the last two decades have made possible the analysis of complex mixtures and the determination of chemicals present in minute quantities, thus allowing us to appreciate for the first time the number and diversity of chemicals to which we are exposed, from conception to death. To a surprisingly large number of people, the word 'chemicals' has become synonymous with manmade chemicals that have been synthesised by the chemist, and scant attention is usually paid to the myriad chemicals that occur in nature. Of the chemicals that humans ingest, 99.9% are natural, largely of plant origin (Ames and Gold 1998; Ames *et al.* 1990). These are chemicals that the plants generate to defend themselves and consequently are biologically active. Ames has estimated that North Americans consume some 5000–10 000 different chemicals and their breakdown products at a dose of 1.5 g per person per day. To this, one has to add the chemicals that are generated during cooking, 2 g of burnt material per person per day which also contains numerous chemicals, including many established chemical carcinogens such as heterocyclic amines and polycyclic aromatic hydrocarbons (Skog and Jägerstad 1998). Indeed, more than 1000 chemicals have been detected in coffee. Exposure to chemicals is, thus, continuous, inevitable and unavoidable.

Naturally occurring chemicals, similar to their anthropogenic counterparts, are biologically active, and thus capable of modulating physiological processes in humans. Undoubtedly the major source of human exposure to chemicals is diet. Many chemicals present in our everyday diet have been shown to display carcinogenicity in humans. Most of these derive from plants, such as hydrazines in the edible mushroom *agaricus bisporus* (Toth 1995), and safrole and estragole in spices (Mori *et al.* 1998). Furthermore, food may be contaminated by chemicals emanating from packages, such as the phthalate esters, or produced during storage, such as mycotoxins which are generated by contaminating fungi (Wang *et al.* 1998). Finally, potent chemical carcinogens, such as heterocyclic amines and polycyclic aromatic hydrocarbons, are

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generated during the normal cooking of food (Skog and Jägerstadt 1998). The biological activity of naturally occurring chemicals, however, is not always detrimental to the living organism, and it is increasingly being recognised that plant constituents in food, such as flavanols in tea and organosulphates in garlic, may possess anticancer activity (Mori and Nishikawa 1996; Ahmad *et al*. 1998), and this realisation has led to enormous current research effort, aimed at better defining these dietary anticancer chemicals and elucidating their mechanism of action (American Institute for Cancer Research 1997).

The body cannot exploit the chemicals to which it is exposed to generate energy, build new tissues, as chemical messengers or as cofactors, and consequently these chemicals are frequently referred to as xenobiotics (*Greek*: foreign to life). It recognises these as being foreign, and potentially detrimental, and its first line of defence is to eliminate them. Chemicals, however, that find their way into the body are lipophilic as they need to traverse lipoid membranes in order to reach the systemic circulation, through which they are distributed to the body. The capacity of the body to excrete lipophilic compounds is poor since, for example, lipophilic compounds that are excreted by glomerular filtration and active secretion in the kidneys are extensively reabsorbed because of their lipophilic character.

The body has, therefore, developed a number of efficient enzyme systems adept at metabolically converting lipophilic xenobiotics to hydrophilic metabolites, thus facilitating their elimination and minimising its exposure to them. The biological half-life of the chemical is shortened, thus reducing exposure and, moreover, the risk of accumulation on repeated or continuous exposure is decreased. In most cases, such metabolism also abolishes the biological activity of the xenobiotic, by, on the one hand, preventing its distribution to its biological receptor and, on the other, by markedly diminishing its affinity for the receptor, in comparison with the parent compound. Thus metabolism has a very profound effect on the biological activity of a chemical, regulating its nature, i.e. intensity and duration. It is unlikely that the human body could withstand the constant onslaught of chemicals and survive in such a hostile chemical environment if such effective defence mechanisms were not operative.

Metabolism of xenobiotics

The process of transforming lipophilic chemicals to polar entities occurs in two distinct metabolic phases. During Phase I, functionalisation, an atom of oxygen is incorporated into the chemical, and functional groups such as –OH, –COOH etc. are generated, as in the hydroxylation of diazepam (Figure 1.1); alternatively, such functional groups may be also formed following reduction, as in the case of the antitumour agent misonidazole (Figure 1.2), or unmasked, as in the deethylation of ethoxycoumarin (Figure 1.1). Such metabolites not only are more polar than the parent compound but, furthermore, are capable of undergoing Phase II metabolism, conjugation, where endogenous substrates, e.g. glucuronic acid and sulphate, are added to them to form highly hydrophilic molecules, ensuring in this way their elimination (Figure 1.1). Xenobiotics that already possess a functional group can bypass Phase I metabolism and directly participate in conjugation reactions; the mild analgesic

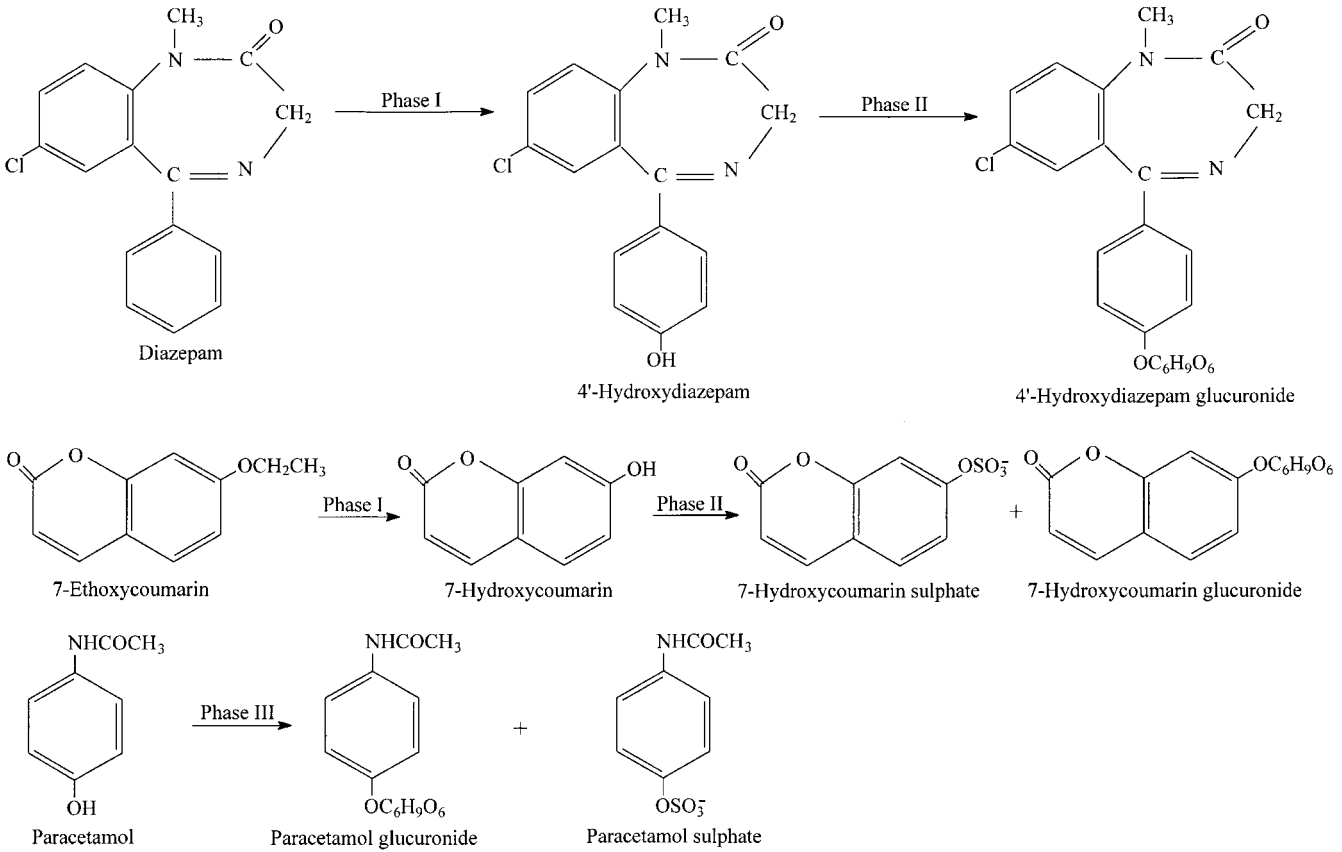


Figure 1.1 Phase I and Phase II metabolism of xenobiotics.

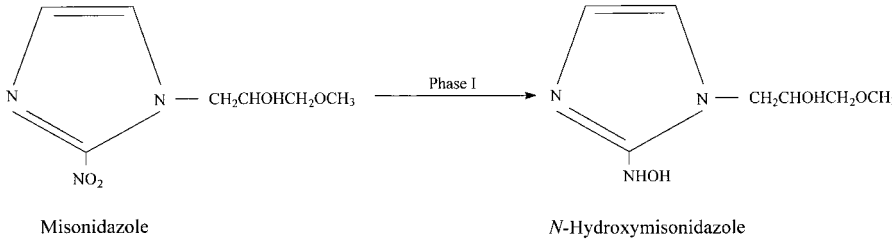


Figure 1.2 Reductive metabolism of xenobiotics.

paracetamol (acetaminophen) is metabolised primarily by sulphate and glucuronide conjugation (Figure 1.1).

It is also possible, but rare, that metabolism may lead to the formation of a more lipophilic metabolite. An important pathway in the metabolism of sulphohamides is *N*-acetylation, producing the less water-soluble acetylated metabolites (Figure 1.3). Such metabolites, as a result of their poor water solubility, may crystallise out in the kidney tubule, causing tissue necrosis and giving rise to a condition known as 'crystalluria'.

A number of enzyme systems contribute to xenobiotic metabolism, both Phase I and Phase II, the majority of which are localised in the endoplasmic reticulum and the cytosolic fraction of the cell. In mammals, they are encountered in every tissue, but particularly in the liver, which consequently functions as the principal site of xenobiotic metabolism. A Phase III metabolism is sometimes referred to and involves the further metabolism of products emanating from Phase II metabolism, the reactions being catalysed by enzymes that participate in the other two phases of metabolism. For example, conjugation with glutathione is an important route of metabolism, through which the cell detoxicates reactive metabolites and protects itself from their detrimental effects (see below). Glutathione conjugates may be further metabolised to mercapturates that are readily excreted in the urine and bile. This involves a sequential loss of glutamate and glycine to yield the cysteinyl derivative that is *N*-acetylated to generate the mercapturate. The cysteinyl derivative may be also metabolised by a pyridoxal phosphate-dependent β-lyase to form a thiol, releasing glutamate and ammonia (Figure 1.4). The thiol may give rise to toxicity or may undergo methylation (Anders and Dekant 1998).

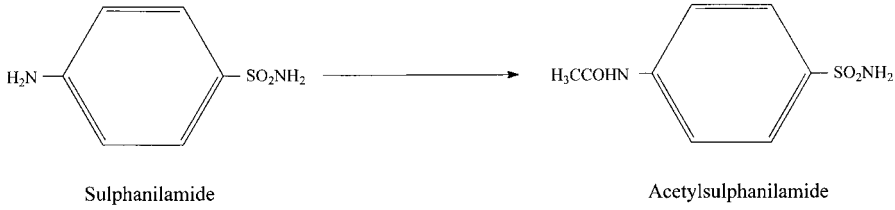


Figure 1.3 N-Acetylation of sulphanilamide.

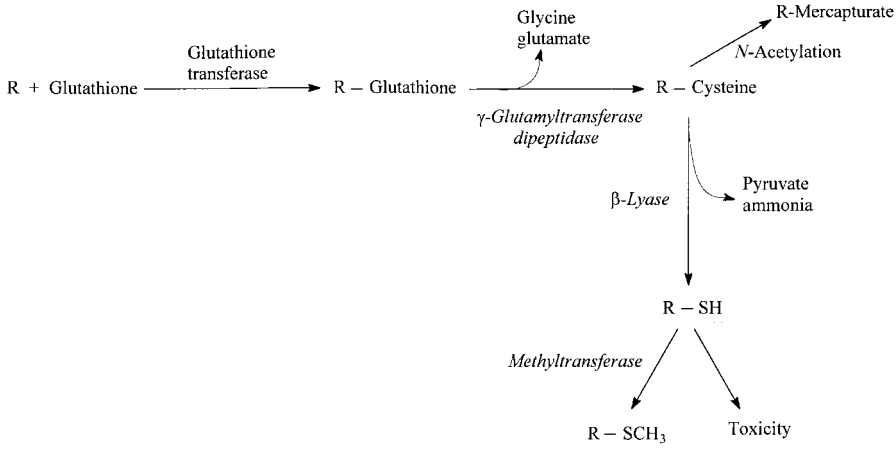


Figure 1.4 Phase III metabolism of glutathione conjugates.

Xenobiotic metabolism by gut microflora

Xenobiotics taken orally as well as those that are excreted into the bile may be subject to metabolism effected by gastrointestinal microorganisms (Rowland 1988). These microorganisms are particularly adept at carrying out reductive metabolism, e.g. nitro- and azo-reduction, and are also effective in catalysing hydrolytic reactions (see below). Metabolism by gut microflora of biliary excreted conjugates may result in cleavage of the conjugate releasing the less polar precursor which, as a result of the increased lipophilicity, may be reabsorbed through the intestine, and this cycling phenomenon is known as 'enterohepatic circulation'. The consequence is that the chemical burden of the living organism is increased since it is continuously re-exposed to the same chemical, until elimination is achieved by renal excretion and loss in the intestine.

Phase I metabolic pathways

AROMATIC HYDROXYLATION

This is one of the commonest pathways of metabolism leading to the generation of phenolic products which are then subject to metabolism by Phase II conjugation. Almost every compound containing a benzene ring undergoes aromatic hydroxylation. For example, the industrial chemical biphenyl is hydroxylated at two different sites, producing 2- and 4-hydroxybiphenyl (Figure 1.5). Aromatic hydroxylation may proceed via the formation of an epoxide which rearranges to a phenol (see below).

EPOXIDATION

This is a frequent pathway in the metabolism of many carcinogenic compounds such as aromatic hydrocarbons, mycotoxins, e.g. aflatoxin B_1 and vinyl chloride

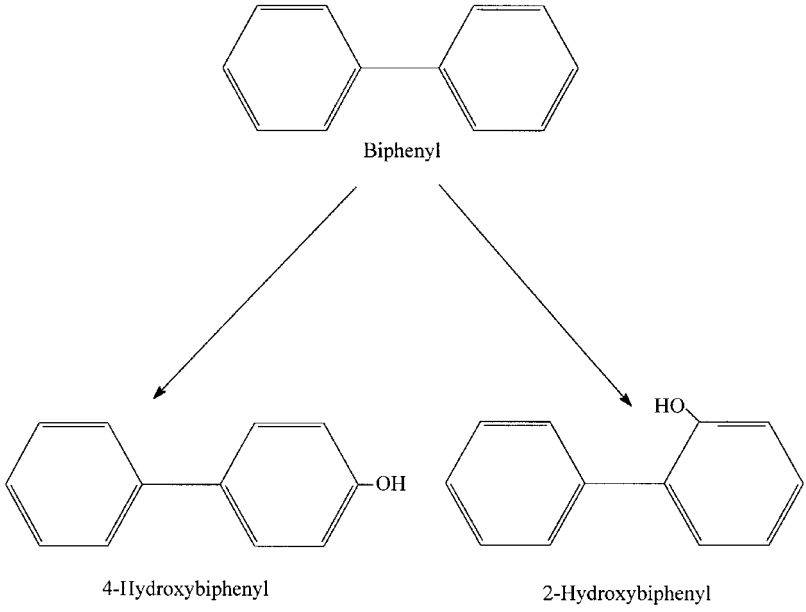


Figure 1.5 Biphenyl hydroxylation.

(Figure 1.6); an atom of oxygen is added across olefinic and acetylenic bonds. Such epoxides are frequently very reactive and have been implicated in the toxicity and carcinogenicity of many chemicals.

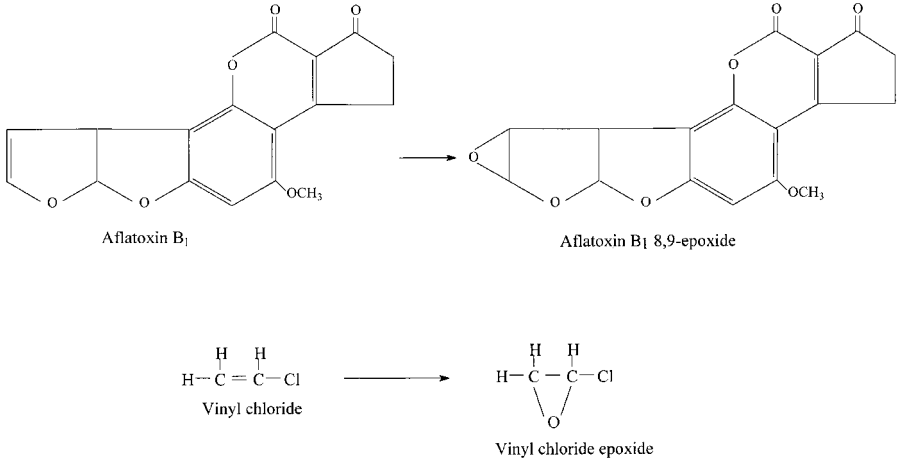


Figure 1.6 Epoxidation of aflatoxin B_1 and vinyl chloride.

ALIPHATIC HYDROXYLATION

Many medicinal drugs are metabolised and deactivated through aliphatic hydroxylation, including the barbiturate pentobarbitone (Figure 1.7). Further oxidation of the alcohol may occur, to yield the corresponding acid.

DEALKYLATION REACTIONS

This is a frequently observed reaction where alkyl groups attached to oxygen, nitrogen or sulphur may be dealkylated as in the case of ethoxycoumarin (Figure 1.1). These dealkylation reactions proceed through oxidation of the alkyl group and rearrangement resulting in loss of the alkyl group as the aldehyde (Figure 1.8).

NITROGEN AND SULPHUR OXIDATION

Nitrogen may be oxidised to form oxides and hydroxylamines, the latter being a pathway of metabolism of compounds having an exocyclic amino group, including many carcinogenic aromatic amines, and generally leads to the expression of toxicity and carcinogenicity (Figure 1.9).

OXIDATIVE DEAMINATION

This results in the release of ammonia and, as in the dealkylation reactions, it involves an initial carbon oxidation (see above) to generate an unstable intermediate (Figure 1.10).

OXIDATIVE DEHALOGENATION

Oxidative dehalogenation is an important pathway in the metabolism of halogenated hydrocarbons such as the anaesthetic halothane, which may also be metabolised by reductive dehalogenation (Figure 1.11).

NITROREDUCTION

Nitroreduction is catalysed not only by mammalian enzymes but also by microbial enzymes which thus contribute to the presystemic metabolism of xenobiotics. For example, the antibacterial drug chloramphenicol undergoes reduction, mediated largely by the gut flora (Figure 1.12).

AZOREDUCTION

Both microbial and mammalian enzymes can catalyse the azoreduction of chemicals. For example, the active form of the anti-inflammatory drug sulphasalazine is 5-aminosalicylic acid, which is released into the intestine following azoreduction catalysed by the gut flora (Figure 1.13).

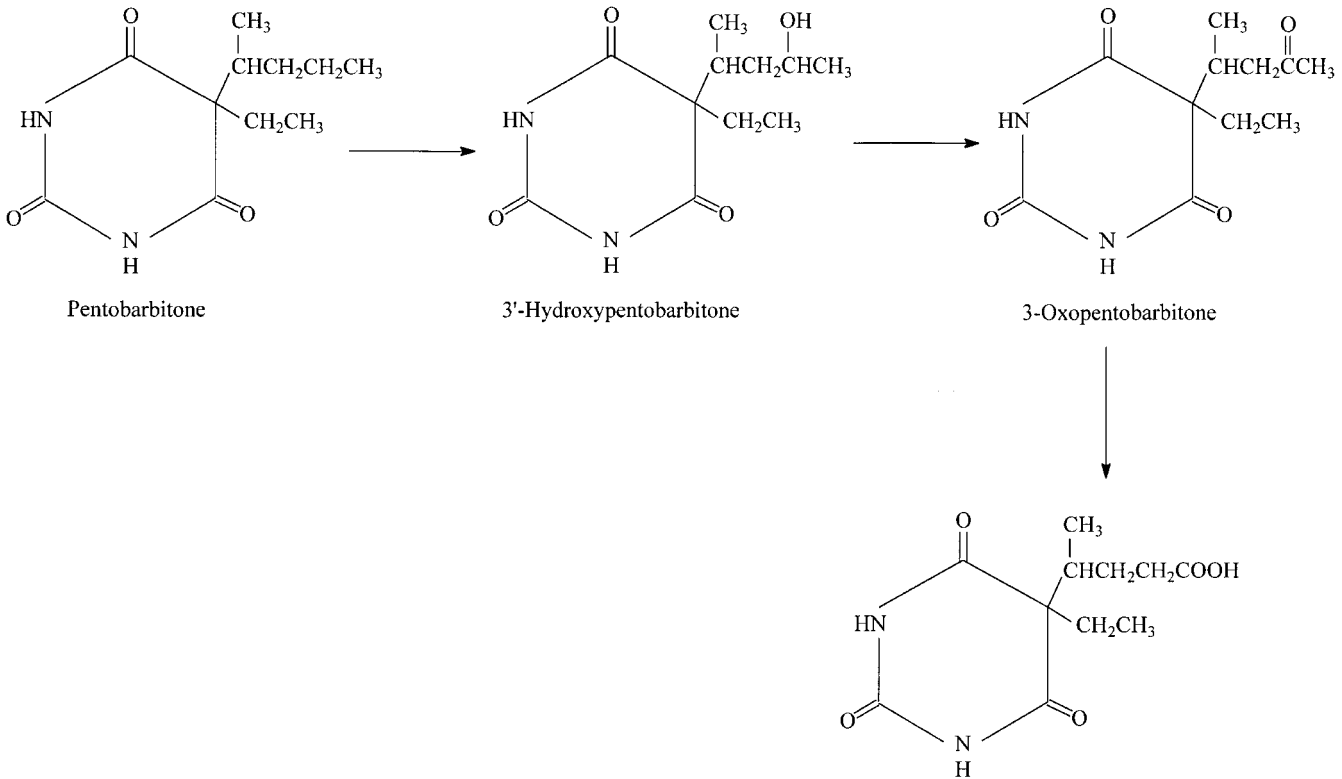


Figure 1.7 Metabolism of pentobarbitone by aliphatic hydroxylation.

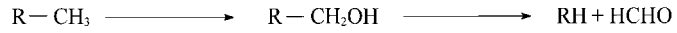


Figure 1.8 Mechanism of demethylation reactions.

REDUCTIVE DEHALOGENATION

This is an important pathway in the metabolism of halogenated hydrocarbons such as the carcinogen carbon tetrachloride and the anaesthetic halothane (Figure 1.11). Haloalkane-free radicals are initially formed as a result of the homolytic cleavage of the carbon–halogen bond.

HYDROLYSIS

A number of esters, amides and hydrazides are hydrolysed by esterases and amidases. The butyl ester of chlorambucil is hydrolysed to the free drug, the herbicide propanil is principally metabolised by amide hydrolysis, and the hydrazide isoniazid, an anti-tubercular drug, is hydrolysed to isonicotinic acid (Figure 1.14). These hydrolytic enzymes are present in many tissues, usually residing in the cytosol but also encountered in the endoplasmic reticulum. The plasma esterases also make a major contribution in the hydrolysis of many chemicals.

Phase II metabolic pathways

Conjugation reactions with endogenous substrates yield highly hydrophilic, and thus readily excretable, metabolites. However, functional groups may be also methylated or acetylated to produce less hydrophilic compounds.

GLUCURONIDE CONJUGATION

This is one of the most frequently utilised routes of conjugation where glucuronic acid, made available in the form of uridine diphosphate glucuronic acid (UDPGA), is added to the molecule thus conferring to it a high degree of hydrophilicity leading to its excretion in the bile and urine (Figure 1.1). The most readily conjugated functional groups are phenols and alcohols, yielding ester glucuronides, and carboxylic acids forming ether glucuronides. *N*-Glucuronidation is an important step in the metabolism of aromatic amines, many of which are carcinogenic, leading to their deactivation and excretion.

SULPHATE CONJUGATION

Conjugation with sulphate, facilitated by cytosolic sulphotransferases, is also a major route of Phase II metabolism, where inorganic sulphate, made available in the form of 3′-phosphoadenosine-5′-phosphosulphate (PAPS), is added to the molecule (Figure 1.1). This is the most important pathway in the metabolism of phenols and is a very

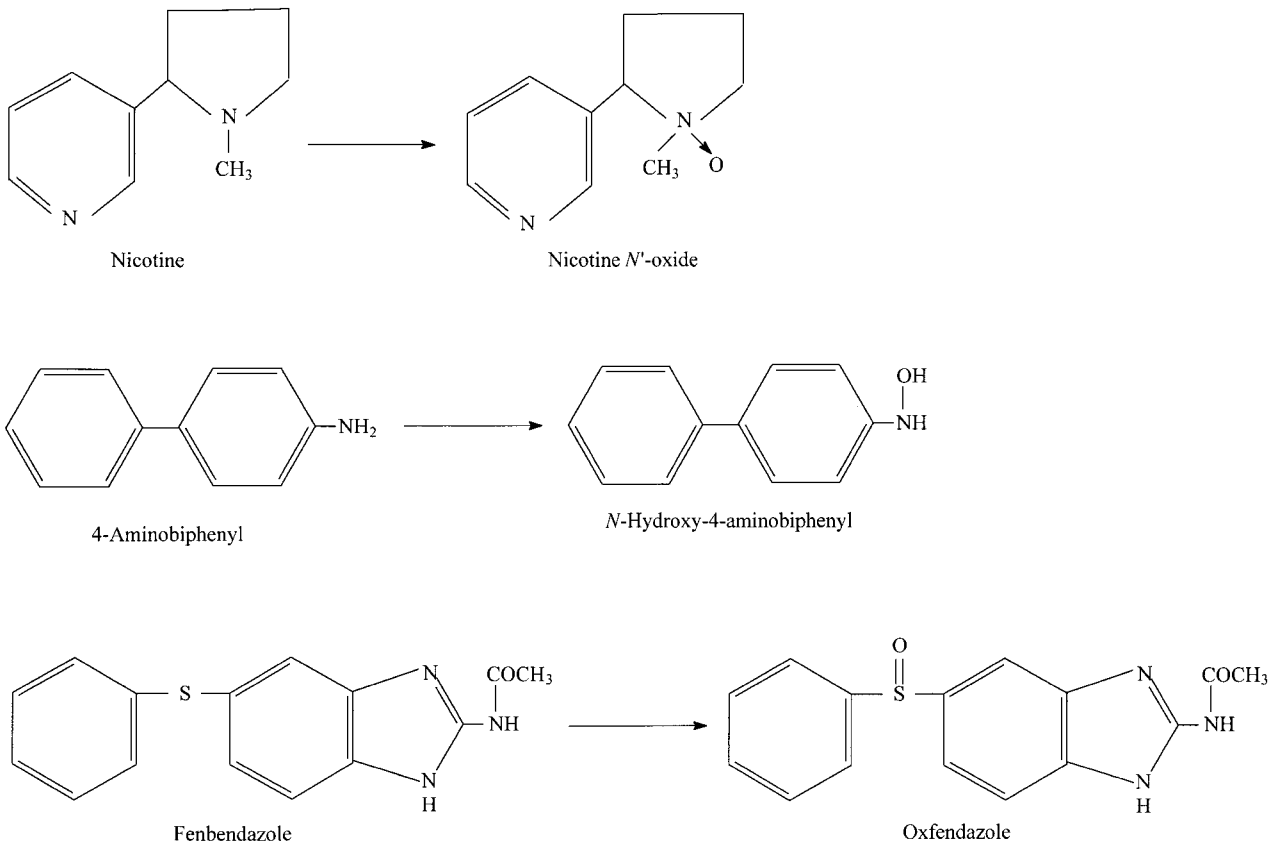


Figure 1.9 N- and S-oxidation of xenobiotics.

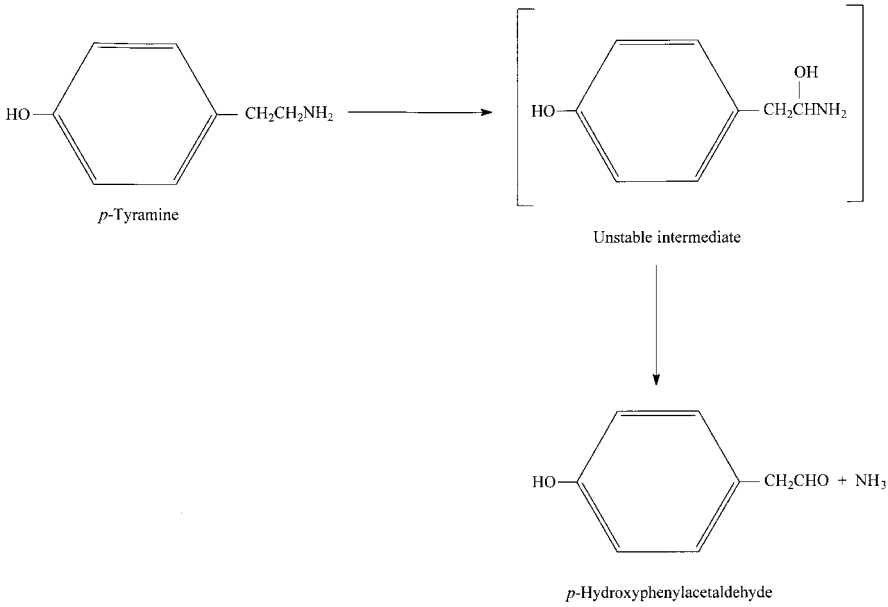


Figure 1.10 Oxidative deamination of *p*-tyramine.

efficient conjugating system as long as inorganic sulphate is available. Amino groups can also be sulphated to yield readily excretable sulphamates.

GLUTATHIONE CONJUGATION

This is one of the most important pathways of metabolism that allows the cell to defend itself from chemical insult. It utilises the nucleophilic tripeptide glutathione, possessing a nucleophilic sulphur atom, whose cellular concentrations are high (Vermeulen 1996), to detoxify reactive electrophiles. This reaction is catalysed by the glutathione *S*-transferases but may also occur chemically. These enzymes are localised primarily in the cytosol, and to a much lesser extent in the endoplasmic reticulum. The glutathione conjugate is excreted either unchanged or following further processing to produce the mercapturate (see above). Paracetamol, in addition to the deactivation reactions depicted in Figure 1.1, to a small extent undergoes Phase I oxidation to form an electrophilic quinoneimine, believed to be responsible for its hepatotoxicity during overdose. The cell neutralises this reactive intermediate by forming a glutathione conjugate, which is eventually excreted as the mercapturate, following Phase III metabolism. (Figure 1.15). The mercapturate is formed by sequential loss of the glutamyl and glycinyl moieties of glutathione. The resulting cysteinyl conjugate is *N*-acetylated to form the mercapturate (N-acetylcysteine derivative). The

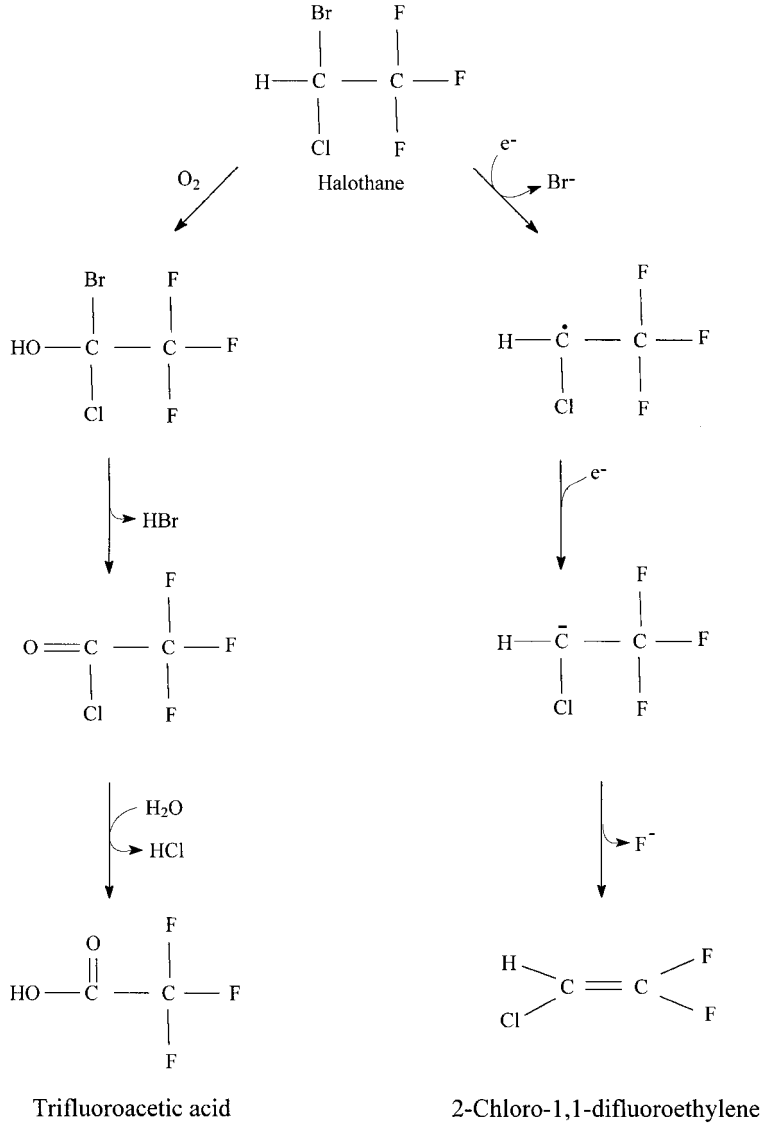


Figure 1.11 Oxidative and reductive dehalogenation of halothane.

glutathione conjugate is rarely excreted intact in the urine since its high molecular weight facilitates its excretion in the bile. The cysteine conjugate may be also further metabolised by the enzyme β-lyase (see above) to generate a thiol and release ammonia and pyruvate (Figure 1.15).

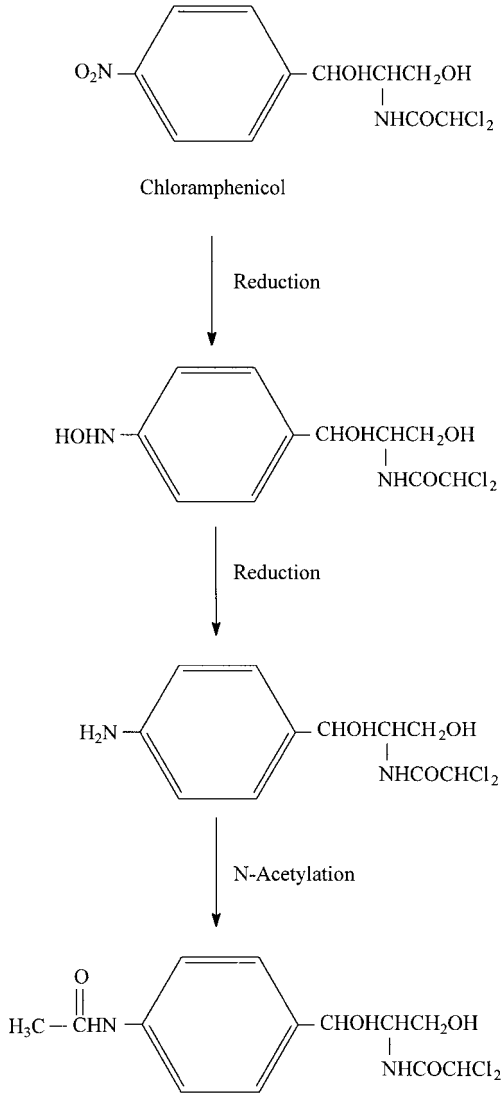


Figure 1.12 Nitroreduction of chloramphenicol.

AMINOACID CONJUGATION

The carboxylic group of organic acids may conjugate with aminoacids, glycine being the most common; other aminoacids found conjugated with xenobiotics are glutamine, taurine and ornithine. The carboxyl group of the xenobiotic forms a peptide

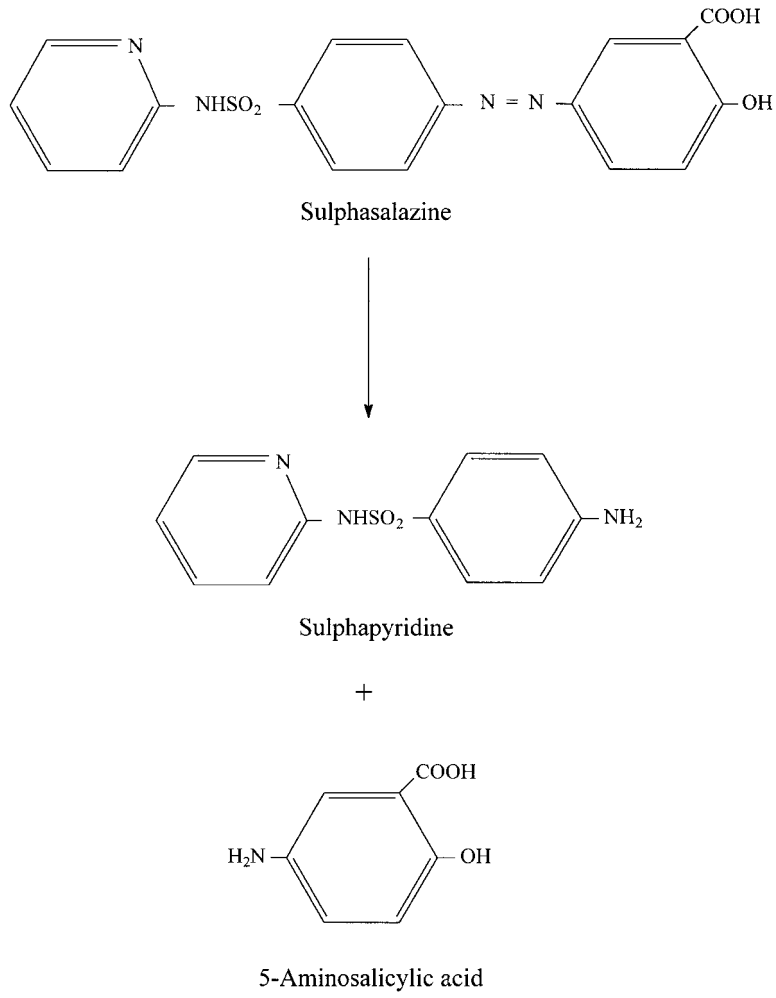


Figure 1.13 Azoreduction of sulphasalazine.

bond with the α-amino group of the aminoacid (Figure 1.16). Initially the carboxylic group reacts with CoA to form a derivative, which then interacts with the aminoacid.

HYDRATION

This involves addition of water to epoxides to form dihydrodiols. As epoxides are generally toxic entities, this is a very important route for their detoxification. Microsomal epoxide hydrolases catalyse the conversion of epoxides of polycyclic aromatic

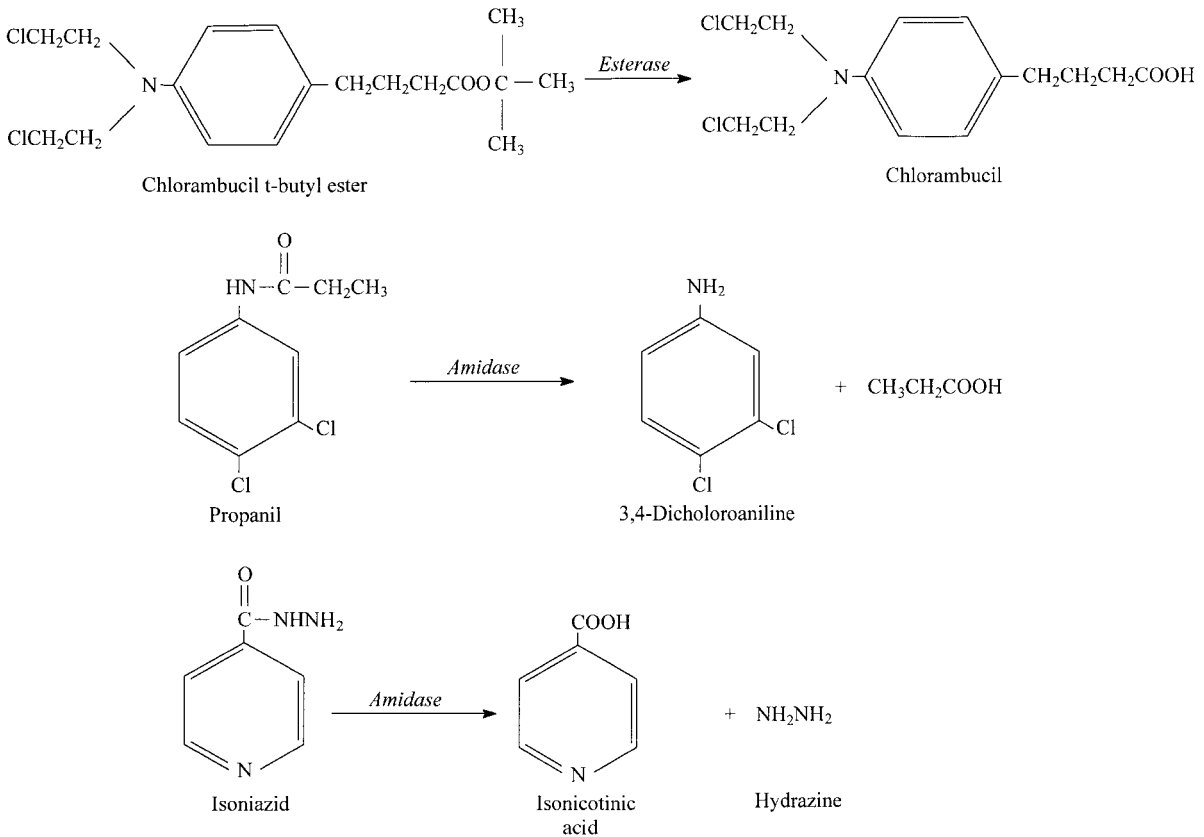


Figure 1.14 Metabolism of xenobiotics by hydrolysis.

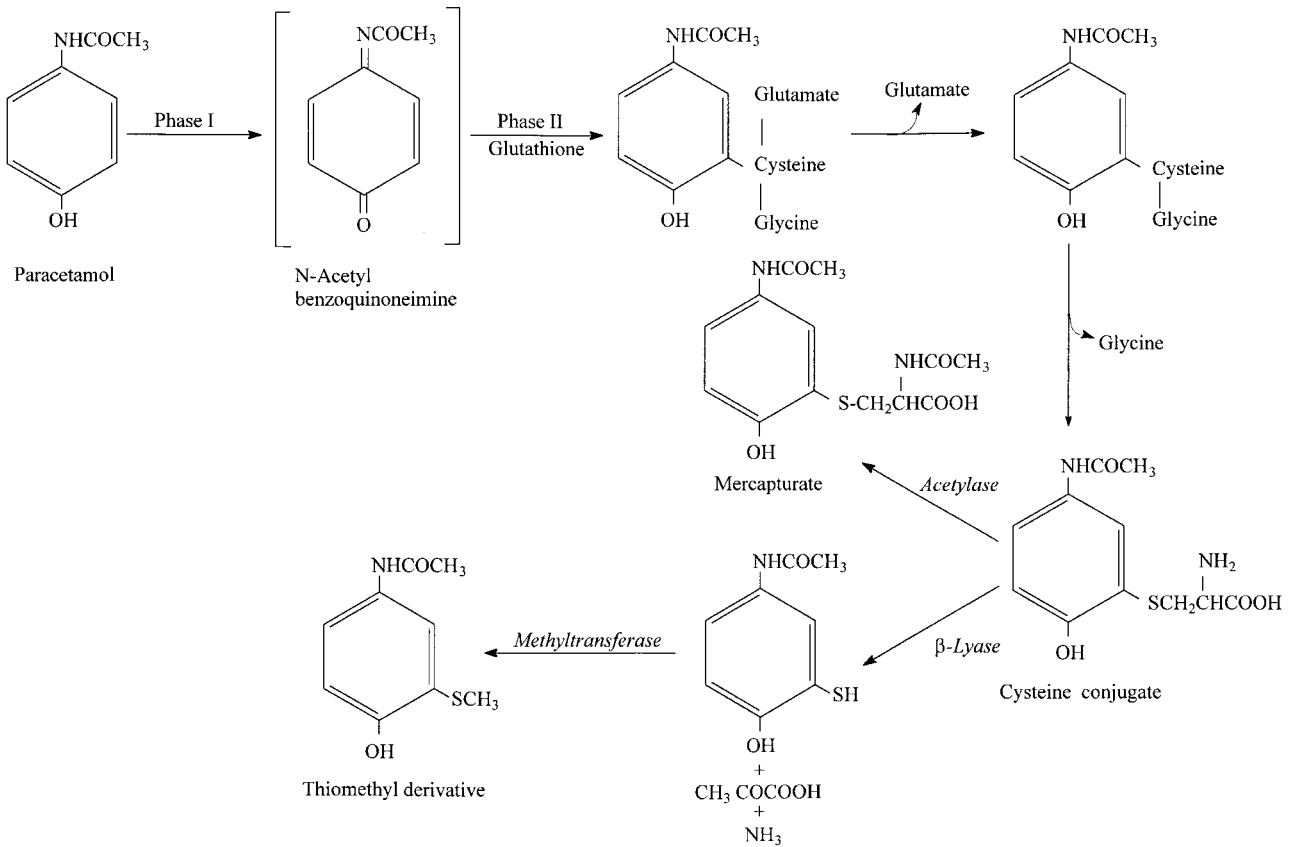


Figure 1.15 Metabolic activation of paracetamol.

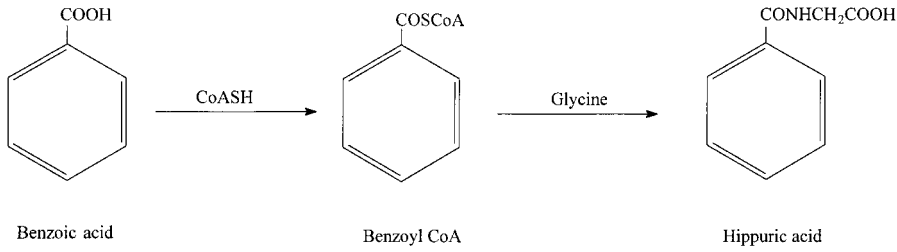


Figure 1.16 Conjugation of benzoic acid with glycine.

hydrocarbons to the corresponding dihydrodiols, as in the case of naphthalene (Figure 1.17).

METHYLATION

Hydroxyl as well as amino and thiol groups may be metabolised through methylation, the methyl donor being *S*-adenosyl methionine. The methyltransferases are primarily cytosolic enzymes, but are also present in the endoplasmic reticulum. Methylation is usually a minor metabolic route in xenobiotic metabolism, but plays a major role in the metabolism of endogenous substrates such as noradrenaline. Naturally-occurring polyphenolics, such as the tea flavonoid (-)-epicatechin, are metabolised through methylation (Figure 1.18). Thiols, emanating from the metabolism of glutathione conjugates, may be also subject to methylation (Figure 1.4).

ACETYLATION

This is an important metabolic route for aromatic and heterocyclic amines, hydrazines and sulphonamides. An amide bond is formed between the amino group of the chemical and acetate (Figure 1.3).

Bioactivation of xenobiotics

A well-documented paradox of xenobiotic metabolism is that, with certain chemicals, metabolism, both Phase I and Phase II, may generate highly reactive electrophilic

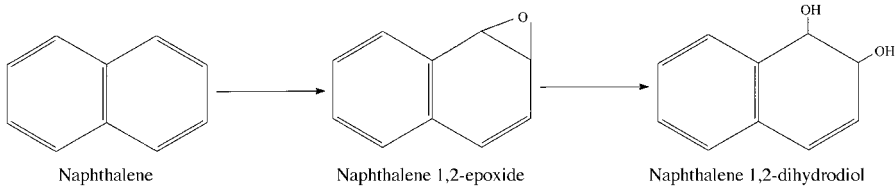


Figure 1.17 Metabolism of epoxides to dihydrodiols.

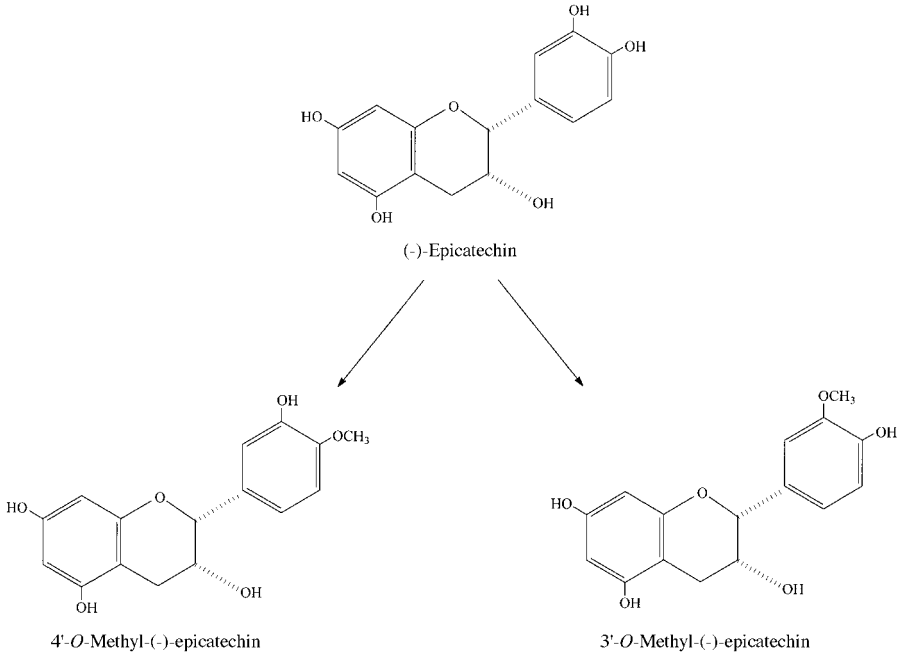


Figure 1.18 Methylation of flavonoids.

metabolites. Since these reactive species are generated intracellularly, they can readily and irreversibly interact with vital cellular macromolecules, such as DNA, RNA and proteins, to provoke various types of toxicity; thus, in this case, metabolism confers to the chemical adverse biological activity. The process through which inert chemicals are biotransformed to reactive intermediates capable of causing cellular damage is known as 'metabolic activation' or 'bioactivation' (Hinson *et al.* 1994). For these chemicals, toxicity/carcinogenicity is inextricably linked to their metabolism. Such metabolically derived reactive metabolites are epoxides, radicals, carbonium ions and nitrenium ions (Figure 1.19). The generated reactive intermediates may interact with DNA to form adducts which, if they escape the repair mechanisms of the cell, may be fixed and passed to the progeny, thus giving rise to a mutation (Figure 1.20). Reactive intermediates of chemicals may also induce DNA damage through an alternative mechanism that involves interaction with molecular oxygen to produce superoxide anions which, in the presence of traces of iron salts, can be transformed to the highly reactive hydroxyl radical ($OH^{\cdot}$), a powerful oxidant. It possesses an unpaired electron and so tends to form bonds with other species in order for the unpaired electrons to become paired. The hydroxyl radical, as well as other reactive oxygen species, can cause cellular damage similar to that resulting from the covalent interaction of the reactive species of chemicals with cellular components; they oxidise DNA to induce mutations, oxidise lipids to form lipid peroxides which appear to play an important

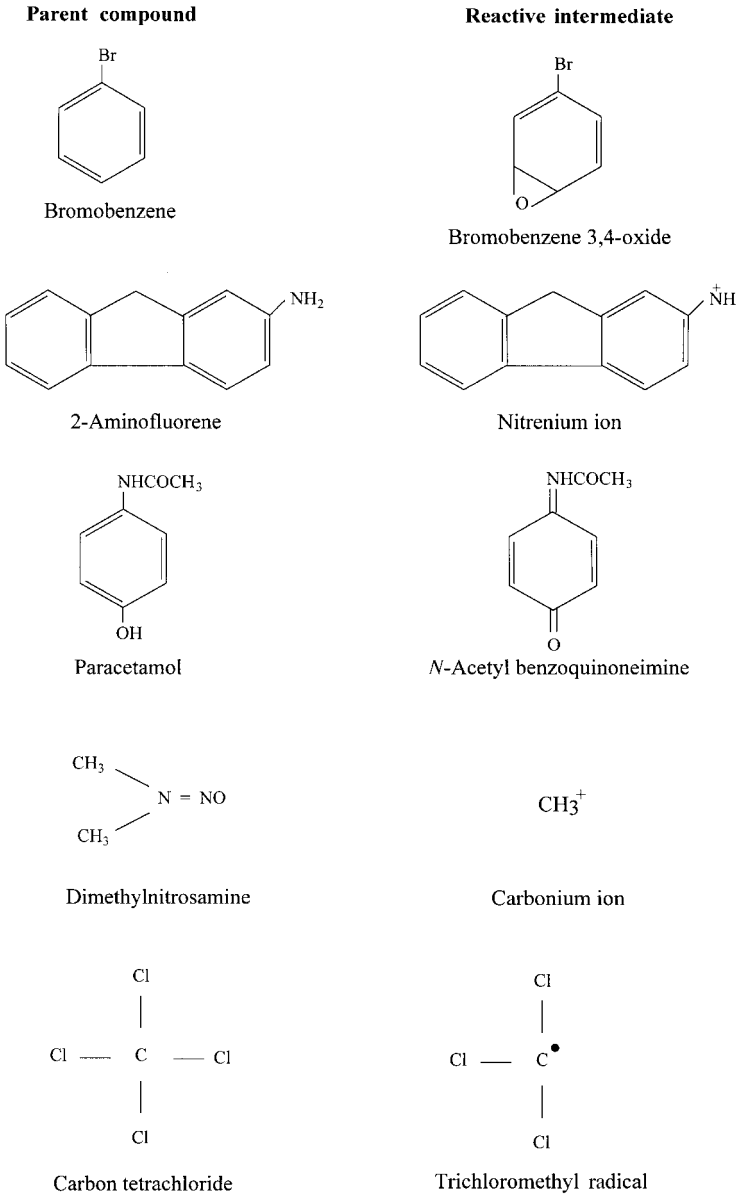


Figure 1.19 Reactive intermediates of toxic chemicals.

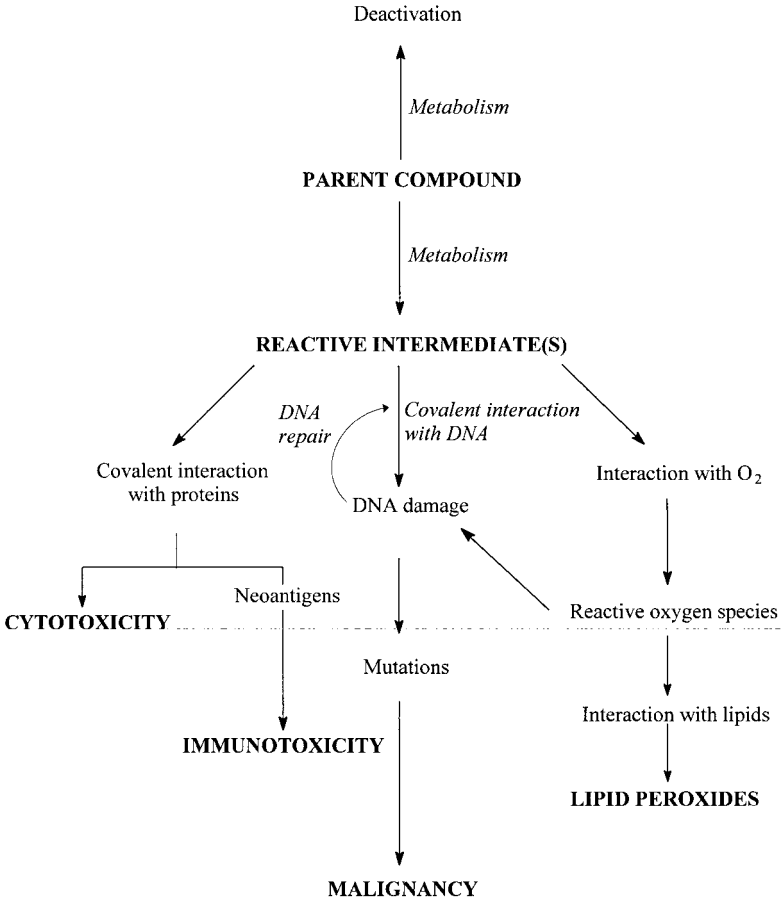


Figure 1.20 Bioactivation of chemicals.

role in the promotion and progression stages of chemical carcinogenesis, and also oxidise proteins (Wiseman and Halliwell 1996).

The reactive intermediates of chemicals may also interact covalently with proteins, disturbing physiological homeostasis, leading to cell death. In the last decade it has become apparent that reactive intermediates can also function as haptens, conferring on proteins antigenic potential and eliciting immunotoxicity (Pirmohamed and Park 1996; Dansette *et al.* 1998). Drugs such as tienilic acid, dihydralazine and halothane are metabolically converted to metabolites that bind covalently to proteins to generate neoantigens resulting in the production of autoantibodies. Subsequent exposure to these drugs may provoke an autoimmune response leading to hepatitis.

Not only oxidations, but also reductions can result in the bioactivation of chemicals,

as in the case of nitropolycyclic aromatic hydrocarbons, a ubiquitous class of carcinogenic environmental contaminants (Fu 1990). Reduction of the nitro group is the first step in their bioactivation, leading eventually to the production of the genotoxic nitrenium ion (Figure 1.21).

Gut microflora can also participate in the bioactivation of chemicals, the metabolic pathways including nitro- and azo-reduction (Chadwick *et al.* 1992). Intestinal metabolism is also responsible for the cleavage of glycosidic bonds releasing carcinogenic products, as in the case of cycasin (methylazoxymethanol-β-D-glucoside) which is hydrolysed to the aglycone, methylazoxymethanol (Figure 1.22).

The most effective protective mechanism against chemical reactive intermediates is their detoxication through conjugation with endogenous nucleophilic substrates such as the tripeptide glutathione; this process also renders it sufficiently polar for its facile excretion. In this way the cell hinders the interaction of the reactive intermediates with cellular components. Glutathione conjugates are excreted into the urine and bile usually following further processing to form mercapturates (Figure 1.15). It is, thus, not surprising that the cellular concentrations of glutathione are rather high (about 10 mM); the cytosolic enzyme glutathione reductase maintains glutathione in the reduced form. Unavailability of glutathione, as, for example, following depletion consequent to exposure to megadoses of chemicals or following inadequate nutrition, renders the cell vulnerable to the toxicity of xenobiotics. For example, bromobenzene forms an epoxide which appears to mediate its toxicity, following covalent binding to proteins. The epoxide may rearrange to form the 3- and 4-bromophenols or hydrated

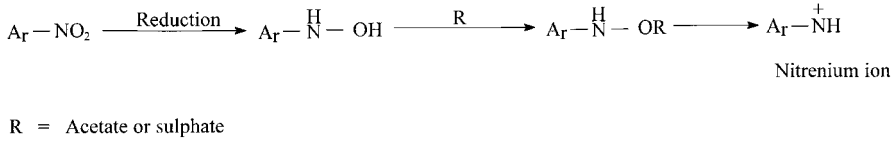


Figure 1.21 Bioactivation of chemicals through reductive metabolism.

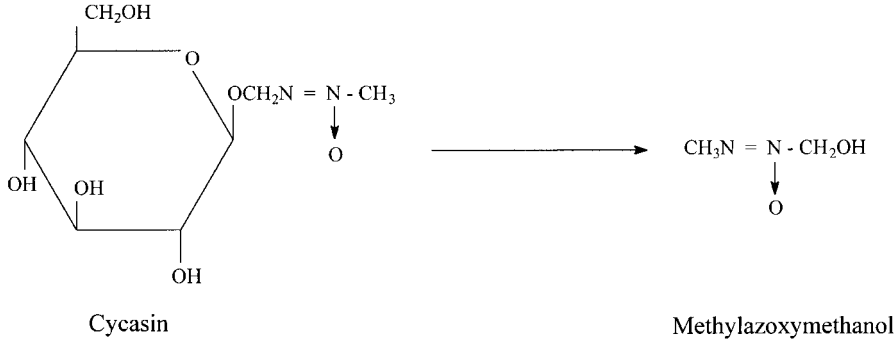


Figure 1.22 Bioactivation of chemicals catalysed by microbial enzymes.

by epoxide hydrolase to the 3,4-diol, all these being deactivation pathways. The epoxide is also effectively detoxicated through conjugation with glutathione (Figure 1.23). Bromobenzene is markedly more toxic to animals that have been starved, since this treatment leads to depletion of glutathione. Glutathione synthesis is impaired as a result of deficiency of its constituent aminoacids, particularly cysteine (Pessayre *et al.* 1979).

Balance of activation/deactivation

Clearly, a chemical is subject to a number of metabolic pathways, the majority of which will bring about its deactivation and facilitate its excretion. However, some routes of metabolism will transform the chemical to a metabolite capable of inducing toxicity and carcinogenicity. Obviously, the amount of reactive intermediate produced, and hence incidence and degree of toxicity, will be largely dependent on the

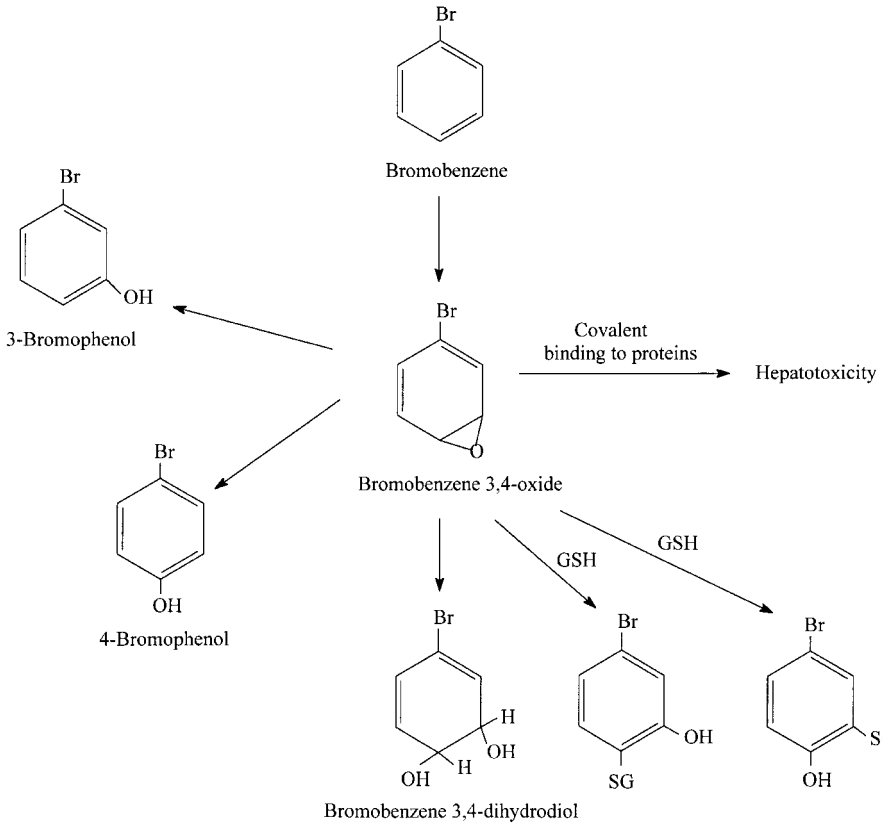


Figure 1.23 Metabolic activation and deactivation of bromobenzene.

competing pathways of activation and deactivation. The susceptibility of an animal species to the toxicity and carcinogenicity of a given chemical carcinogen is largely dependent on its complement of enzymes at the time of exposure, which determines whether activation or deactivation pathways are favoured. For example, the guinea pig is unable to carry out the N-hydroxylation of the carcinogen 2-acetylaminofluorene, the initial and rate limiting step in its bioactivation, and is consequently resistant to its carcinogenicity (Kawajiri *et al*. 1978). Similarly, the cynomolgous monkey lacks the enzyme—a cytochrome P450 protein, namely CYP1A2—that is responsible for the activation of the heterocyclic amine MeIQx (2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline), a food carcinogen, and is consequently refractive to its carcinogenicity (Ogawa *et al*. 1999), whereas rodents such as mice and rats, which catalyse the activation of this carcinogen, are also susceptible to its carcinogenicity (Kato *et al*. 1988; Snyderwine *et al*. 1997).

In most cases, under physiological conditions, the activation pathways represent minor routes of metabolism so that the generation of reactive intermediates is minimal, the low levels formed are effectively deactivated by the defensive mechanisms, and no toxicity is apparent. However, in certain situations, the activation pathways may assume a greater role, leading to enhanced production of reactive intermediates, overwhelming the deactivation pathways, resulting in their accumulation in the body, thus increasing the likelihood of an interaction with cellular components with ensuing toxicity. Such situations may arise when:

(a) Deactivation pathways are saturated or impaired. This can occur during exposure to large quantities of a chemical, rather than chronic exposure to low doses, resulting in depletion of the body of the conjugating substrates. As already discussed, paracetamol (acetaminophen) is effectively deactivated by being conjugated with sulphate and glucuronide, these being its principal pathways of metabolism (Figure 1.1). A small fraction of the dose is oxidised to form the *N*-acetylbenzoquinoneimine, a reactive intermediate that is readily deactivated through conjugation with glutathione (Figure 1.15). Intake of large doses, as in suicide cases, alters the quantitative metabolic profile of the drug. Sulphate and glucuronic acid conjugation pathways become saturated as a result of unavailability of the activated forms of sulphate (PAPS) and glucuronic acid (UDPGA) respectively; the rate of their utilisation exceeds the rate of supply. As a consequence, more of the metabolism is directed towards the oxidation pathway that now assumes greater importance. Initially conjugation with glutathione deactivates the quinoneimine, but eventually the extensive use of glutathione results in its depletion, thus allowing the covalent interaction of this reactive intermediate with the –SH groups of proteins, leading to its hepatotoxicity (Holtzman 1995; Cohen and Khairallah 1997). Indeed, the preferred treatment of paracetamol intoxication involves administration of *N*-acetylcysteine, which deacetylates to release cysteine, the rate-limiting aminoacid in the synthesis of glutathione, thus preventing its depletion. The toxicity of paracetamol is also markedly enhanced when glutathione levels are low, as, for example, when animals are starved (Pessayre *et al*. 1979). Similarly, genetic deficiencies in conjugating systems may lead to increased sensitivity to the toxicity of chemicals that rely heavily on these

enzymes for their deactivation. For example, in humans, glucuronyl transferase activity may be totally lacking in individuals with the Crigler–Najjar syndrome type I, a severe and fatal disease. These patients, being unable to eliminate bilirubin through glucuronidation, develop jaundice (de Wildt *et al*. 1999). Such patients may be sensitive to drugs whose principal pathway of metabolism is through glucuronidation. A milder condition is Gilbert's syndrome where the patient experiences only intermittent jaundice (de Wildt *et al*. 1999). These patients display low glucuronidation capacity, and when taking paracetamol they excrete less in the form of glucuronides, with more of the metabolism being directed towards oxidation forming the hepatotoxic *N*-acetylbenzoquinoneimine (Esteban and Perez-Mateo 1999). The anticancer drug iminotecan provokes severe toxicity in patients with Gilbert's syndrome as a result of suppressed glucuronidation (Wasserman *et al*. 1997). Moreover, intake of drugs metabolised by glucuronidation may induce jaundice, as the drug competes with bilirubin for glucuronidation (Burchell *et al*. 2000).

(b) The enzyme systems catalysing the activation pathways are selectively induced as a result of prior exposure to other chemicals (Okey 1990). One of the cytochrome P450 enzymes catalysing the bioactivation of paracetamol, namely CYP2E1, is stimulated by alcohol exposure and consequently chronic alcoholics are characterised by high levels of activity. As a result they are vulnerable to the hepatotoxicity of paracetamol (Seeff *et al* 1986; Zimmerman and Maddrey 1995). It is evident that any factor that disturbs the delicate balance of activation/deactivation will also influence the fate and toxicity of chemicals.

Although the outcome of Phase II conjugation reactions in the vast majority of cases is deactivation, these pathways can also generate reactive intermediates, and thus contribute in the bioactivation of chemicals (Kato and Yamazoe 1994; Glatt 1997; Glatt *et al*. 1998). For example, *O*-acetylation and *O*-sulphation of aromatic hydroxylamines generates unstable acetoxy and sulphatoxy esters which break down spontaneously to release a nitrenium ion, the species believed to bind to the DNA ultimate (Figure 1.21). Glutathione conjugates, as already discussed, can be converted to toxic thiols through the involvement of β-lyase (Koob and Dekant 1991) (Figure 1.4).

Fate of reactive intermediates

Liver is the principal site of the bioactivation of chemicals since most xenobiotic-metabolising enzyme systems are expressed in this tissue at high concentrations. Extrahepatic tissues contain a more restricted number of enzyme systems, generally being present at much lower concentrations compared to the liver. However, there are a few exceptions, for example prostaglandin synthetases, γ-glutamyltranspeptidase, the enzyme system involved in the metabolic conversion of glutathione conjugates to mercapturates, and some cytochrome P450 proteins are not appreciably expressed in the liver.

Since the liver is the centre of xenobiotic activation, it would be logical to expect that it would also be the major site for toxic and carcinogenic manifestations. In reality, however, the breast, lung and colon are far more frequent sites of tumorigen-

esis, despite their limited metabolic competence. It is becoming increasing apparent that the liver may function as the centre of production of reactive intermediates, but these may be exported systemically to other tissues where they can exert their deleterious effects.

Aromatic amines are potent urinary bladder carcinogens. In the liver they undergo *N*-hydroxylation, catalysed by cytochrome P450 enzymes and the flavin monooxygenase system, and the unstable hydroxylamines are trapped in the form of the more stable *N*-glucuronides. In the form of these glucuronides, the hydroxylamines are transported to the bladder where, under the acidic conditions prevailing in this tissue, the hydroxylamine is released and converted to the nitrenium ion, the ultimate carcinogen (Kadlubar *et al.* 1981). The nitrenium ion can also be formed *in situ* since the bladder has low levels of cytochrome P450 activity (Imaoka *et al.* 1997) and substantial levels of prostaglandin synthetase activity (Eling and Curtis 1992) that may contribute to the availability of the nitrenium ion (Figure 1.24). Similarly, the breast is a

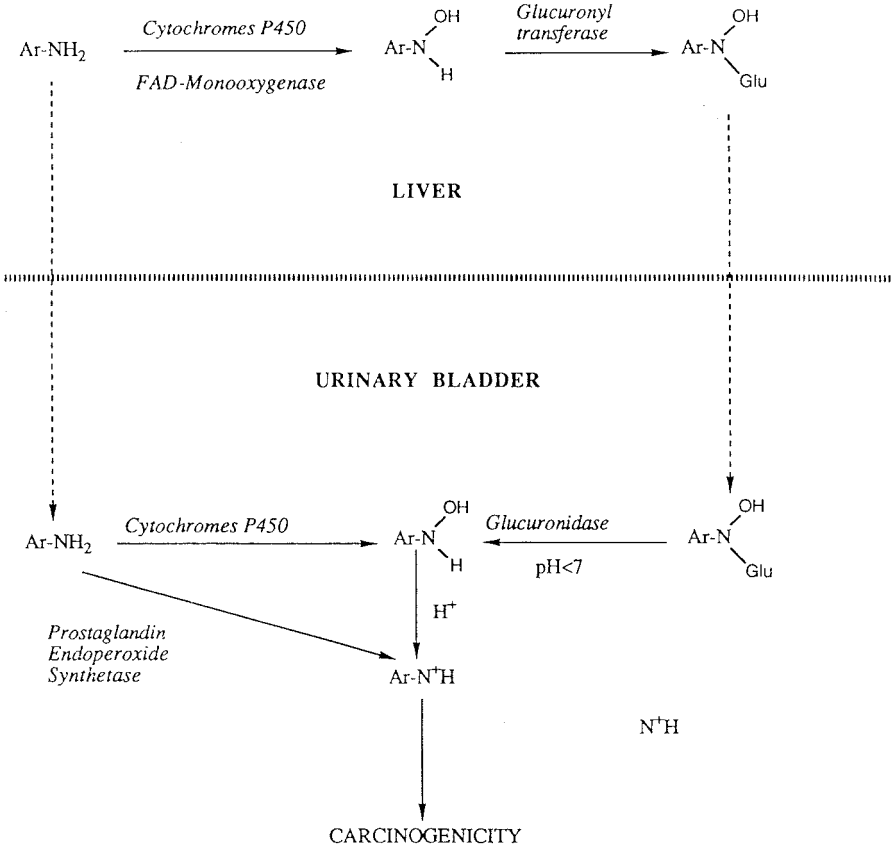


Figure 1.24 Transport of chemical reactive intermediates.

frequent site of tumorigenesis despite the fact that its capacity to metabolise and bioactivate chemicals through oxidation appears to be minimal (Davis *et al.* 1994). It has been proposed that heterocyclic carcinogenic amines, such as PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine), are N-oxidised in the liver and, in the form of either the hydroxylamine or following esterification to the acetoxyester, are then transported to extrahepatic tissues such as the colon to yield DNA adducts (Kaderlik *et al.* 1994). Extrahepatic tissues appear capable of catalysing the esterification of hydroxylamines but poor in catalysing the initial oxidation of the heterocyclic amine as a result of the very low levels of cytochrome P450 (Stone *et al.* 1998). In an elegant study, the liver from rats pretreated with benzo[a]pyrene was transplanted in untreated animals. The extent of DNA binding in the lung, liver and kidney was the same in both, those animals exposed directly to the carcinogen and those who received the liver transplants, indicating clearly that the liver was the source of the reactive intermediates that interacted with DNA, not only for the liver, but also for the lung and kidney (Wall *et al.* 1991).

Reactive oxygen species and chemical toxicity and carcinogenicity

Electrophilic intermediates can induce toxicity and carcinogenicity through an additional mechanism, involving interaction with molecular oxygen to yield short-lived highly reactive oxygen species capable of oxidising lipids and proteins and eliciting DNA damage. Indeed, reactive oxygen species are currently being implicated in the aetiology and progression of a number of major chronic diseases including atherosclerosis, cancer, cardiovascular disease, diabetes, rheumatoid arthritis, reperfusion injury and ischaemia (Parke *et al.* 1991; Martínez-Cayuela 1995; Oberley and Oberley 1995; Baynes 1995).

The most reactive oxygen species is the hydroxyl radical ($OH^{\cdot}$); other biologically relevant reactive oxygen species are the superoxy anion, another radical ($O_2^{-\cdot}$), and hydrogen peroxide (H_2O_2). In the presence of iron, the $OH^{\cdot}$ can be generated from either $O_2^{-\cdot}$ (Haber–Weiss reaction) or H_2O_2 (Fenton reaction).

Quinones, oxidation products of aromatic hydrocarbons, can undergo one-electron reductions to form the semiquinone radical (Figure 1.25), which may directly attack DNA. It causes DNA damage also indirectly, through reactive oxygen species produced as a consequence of their interaction with molecular oxygen (Aust *et al.* 1993).

However, the living organism is also endowed by defensive enzyme systems which effectively detoxify these reactive oxygen species, and prevent a state of 'oxidative stress', where the cell is unable to cope with the generation of reactive oxygen species. Such a state may ensue as a result of overproduction of reactive oxygen species and/or a decreased ability to deactivate them. Hydrogen peroxide is broken down enzymically by glutathione peroxidase, an enzyme present in the cytosol and mitochondria, and catalase, a peroxisomal enzyme (Figure 1.26). Superoxide dismutase, an enzyme localised in the mitochondria and cytosol, is an effective defence against the superoxy anion, and NAD(P)H-quinone reductase (DT-diaphorase), an enzyme found primarily in the cytosol, protects against quinone-derived oxygen radicals by converting the quinone to the hydroquinone through a two-electron reduction. An increasing number

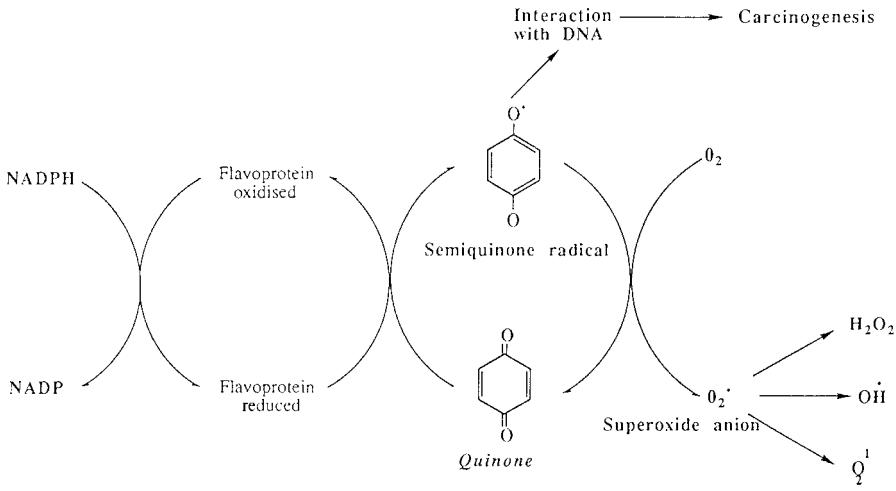
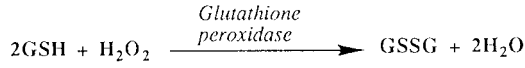
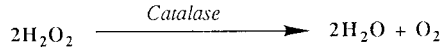
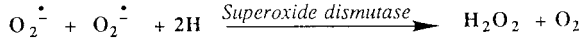


Figure 1.25 Mechanism of quinone toxicity.







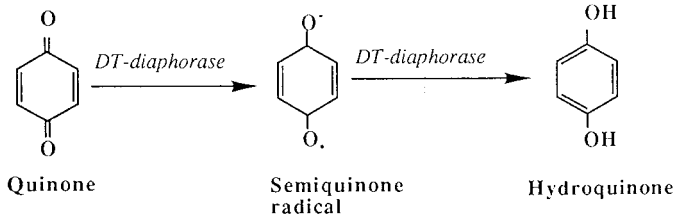


Figure 1.26 Enzyme systems protecting against reactive oxygen species.

of chemicals can function as antioxidants, scavenging oxygen radicals, and in this way afford protection against oxidative damage. Such antioxidants include endogenous chemicals, e.g. uric acid, vitamin E, melatonin etc., and numerous naturally occurring, plant-derived compounds that are ingested with food in substantial amounts, e.g. plant polyphenolics (Williamson 1998).

Conclusions

There can be no doubt that both the ever-increasing life expectancy and the high quality of life humans enjoy today could not have been achieved in the absence of the novel, not pre-existing in nature, chemicals that the chemist has synthesised. These possess biological activity that has been exploited to treat effectively, and in many instances cure, human disease and to provide a more hygienic and safe environment. It is not coincidental that the sharpest rise in life expectancy occurred at the same time as the birth of the pharmaceuticals industry. However, it must be also recognised that chemicals, both anthropogenic and naturally occurring, are also capable of inducing toxicity in humans. Manifestation of chemical toxicity is largely a consequence of the irreversible interaction of chemicals with cellular macromolecules such as DNA, RNA, lipids and proteins. However, most compounds lack the necessary chemical reactivity to participate in such interactions but can acquire it through metabolism. The reactive, toxic intermediates are generated intracellularly which allows them access to the vital components of the cell. Thus the view that metabolism is strictly a defensive mechanism against chemical injury is anachronistic. Clearly for a chemical to provoke toxicity at least two prerequisites must be fulfilled: (a) the chemical must be, or must have the propensity to be metabolically converted to a reactive intermediate(s), and (b) the living organism, at the time of exposure, must possess the necessary enzyme(s) required for the activation of the chemical. Both 2- and 4-aminobiphenyl can form genotoxic metabolites, the corresponding hydroxylamines, but in the case of the 2-isomer no enzyme appears to be capable of catalysing this *N*-oxidation, because it is situated in a conformationally hindered position (Ioannides *et al*. 1989). For this reason, the 4-aminobiphenyl is a potent human and animal carcinogen whereas the 2-isomer appears to be devoid of carcinogenicity.

It is apparent that toxicity is not simply a consequence of the intrinsic molecular structure of the chemical, but is also determined by the nature of the enzymes present at the time of exposure; these enzyme systems are in turn regulated genetically (Raunio and Pelkonen 1995; Cascorbi *et al*. 1995; van der Weide and Steijns 1999; Wormhoudt *et al*. 1999) but are also modulated by environmental factors such as diet (Ioannides 1999) and previous exposure to chemicals (Okey 1990; Tanaka 1998; Lin and Lu 1998) and the presence of disease (Ioannides *et al*. 1996; Iber *et al*. 1999). Toxicologists have the habit of referring to chemicals as being toxic or non-toxic, while in the strictest sense the vast majority of chemicals are innocuous, and it is the living organism that renders them toxic through metabolism. As we are becoming more competent in phenotyping humans for xenobiotic-metabolising activity, it may well become more appropriate to talk of 'toxicophilic' and 'toxicophobic' individuals rather than of toxic and non-toxic chemicals, depending on their propensity to bioactivate or detoxify chemicals. At present humans can be phenotyped for a number

of cytochrome P450 proteins, for N-acetylase, xanthine oxidase and flavin monooxygenase activities (Lucas *et al*. 1999; Chung *et al*. 2000), and these studies are bound to be extended to other enzyme systems, once appropriate chemical probes are identified. It is now possible, for example, to assess the effect of environmental factors such as diet on the expression of cytochrome P450 proteins in humans. For example, the cytochrome P450 enzyme, CYP1A2, was induced following the consumption of diets supplemented with Brassica vegetables but, in contrast, was suppressed when diets supplemented with apiaceous vegetables were consumed (Kall *et al*. 1996; Lampe *et al*. 2000). Such knowledge of a person's metabolic capacity will also enable us to identify individuals vulnerable to the toxicity of a certain chemical(s), and to develop drug dose regimens tailor-made to the needs of individuals, thus increasing efficacy and minimising the risk of adverse effects.

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