Chapter 4

Drugs as Toxic Substances

'There are no safe drugs, only safe ways of using them.'

'Doctors put drugs of which they know little, into our bodies of which they know less, to cure diseases of which they know nothing at all'.

Voltaire

Introduction

Most human beings and indeed many other animals are exposed to drugs sooner or later in their lives. However, drugs are substances designed to have biological activity and although the layman expects them to be perfectly safe it is not surprising that toxic effects do sometimes occur especially when drugs are wrongly used. However, drugs have made and will continue to make a major contribution to human health and we must accept a measure of risk attached to these benefits.

The tragedy which first made the public and probably also the medical profession fully aware of this unpleasant fact was that caused by the drug thalidomide. This event, perhaps more than any other, proved to be a major watershed for awareness of drug toxicity and the need for better legislation and testing of pharmaceuticals. Consequently, we will consider this as one of our examples which will also serve to illustrate the problem of teratogenesis.

There are several different types of drug toxicity: adverse effects or side effects occurring during proper therapeutic usage; acute toxicity due to overdosage; idiosyncratic reactions which occur during proper therapeutic usage but rarely; interactions with other drugs or other substances being taken concurrently which lead to toxic effects; and habitual abuse of drugs leading to chronic toxicity. Drug overdoses come within the bounds of clinical toxicology as does accidental ingestion of hazardous substances whereas abuse of drugs including their use for murder is the domain of the forensic toxicologist.

The basic mechanisms underlying these types of toxicity may also be summarized:

1. direct and predictable toxic effects due to altered or inhibited metabolism and occurring after overdoses;

2. toxic effects occurring after repeated therapeutic doses with a metabolic, pharmacological or maybe immunological basis;
3. direct but unpredictable toxic effects occurring after single therapeutic doses and due to idiosyncratic metabolism or a pharmacodynamic response;
4. toxic effects due to another drug or substance interfering with the disposition or pharmacological response of the drug in question.

Examples of some of these types of drug toxicity will be considered in this chapter.

Paracetamol

Overdosage with drugs is now one of the commonest means of committing suicide and one of the drugs most commonly involved in the UK is paracetamol with at least 200 deaths a year being due to overdoses of this drug. As well as intentional, suicidal overdoses, accidental poisoning has also occurred and recently been highlighted. This occurred as a result of patients and doctors being unaware that some proprietary preparations contain paracetamol. Thus, repeated self medication with paracetamol tablets possibly along with cold cures which may also contain the drug has lead to fatal over-dosage in at least one case (see *Pharmaceutical Journal,* Bibliography). Paracetamol is a minor analgesic which is very safe provided only the normal therapeutic dose of one or two tablets (500 mg) is taken. However, after over-doses, where fifteen or twenty tablets may be taken, fatal liver damage can result. Fortunately an understanding of the mechanism underlying paracetamol toxicity has led to a method of antidotal treatment which is now able to prevent the fatal outcome in many cases.

Paracetamol is metabolized mainly by conjugation with sulphate and glucuronic acid. Only a minor proportion is metabolized by oxidation which is catalyzed by the microsomal mono-oxygenases (Figure 4.1). This produces a metabolite which is toxic but is normally detoxified by reaction with glutathione (see Chapter 2). However, research in experimental animals has shown that after an overdose several changes take place in this metabolic scheme. The pathways of conjugation are saturated and cofactors, especially sulphate, are depleted. As a result more paracetamol is metabolized by the oxidative pathway giving rise to the toxic metabolite. Sufficient of this metabolite is produced in the liver to deplete all the glutathione available. Therefore, the toxic metabolite reacts with liver proteins instead of the glutathione and this causes direct tissue damage leading to hepatic necrosis.

Another factor of importance in relation to the susceptibility to toxicity is individual variation in metabolism, possibly as a result of the intake of other drugs. For example, excessive alcohol intake prior to paracetamol overdose may increase the liver damage as a result of induction of the particular isoenzyme of cytochrome P450 involved in the metabolic activation of paracetamol. The elucidation of this mechanism suggested a means of treatment

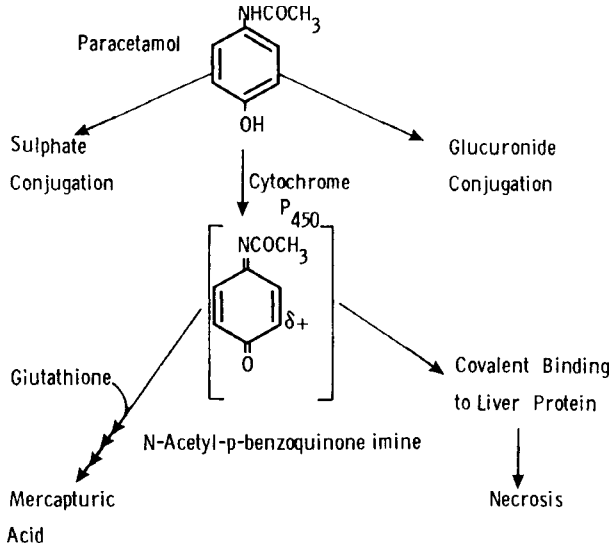

Figure 4.1. The metabolism of the analgesic drug paracetamol.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

with an antidote to either regenerate glutathione or replace it with an alternative. The currently accepted treatment uses N-acetylcysteine given either orally or intravenously. Provided this is given within 10–12 hours of the overdose fatal liver damage is usually avoided.

A number of other drugs are taken in overdose for purposes of suicide and these cause various toxic effects. Such drugs include aspirin, tranquillizers, barbiturates and opiates but antidotes are not available for most of these. Supportive measures, decreasing absorption, increasing elimination or altering the distribution of the drug are the major types of treatment.

Hydralazine

The second example is of a drug toxicity which follows normal therapeutic dosage leading to adverse effects in a significant number of patients. This example is of particular interest because it illustrates the importance of the combination of several factors in the development of and susceptibility to an adverse drug effect.

The drug in question is the antihypertensive drug hydralazine. This drug causes a syndrome known as lupus erythematosus which has some similarities with rheumatoid arthritis. When the drug was first introduced in the 1950s, relatively high doses were used and the incidence of the adverse effect was high, occurring in over 10 per cent of patients. The use of the drug declined. However, use of lower doses in combination therapy reduced the incidence of the adverse effect although a recent report estimates that the true incidence is still unacceptably high with an overall value of 6.7 per cent. Recent studies have

revealed that there are several factors which predispose patients to this particular adverse effect.

The factors so far defined are:

1. dose
2. acetylator phenotype
3. HLA type
4. sex
5. duration of therapy

We will examine each in turn.

Dose

This has already been mentioned. The incidence of the adverse effect seemed to be more common when doses of around 800 mg daily were used compared with doses of less than 200 mg daily, which are more commonly used now. One recent study showed more clearly that the incidence was dose related; as no cases were reported at doses of 50 mg daily, there was a 5.4 per cent incidence after 100 mg daily and a 10.4 per cent incidence with 200 mg daily.

Acetylator phenotype

Hydralazine is metabolized by the acetylation route which is a phase 2 metabolic transformation for foreign compounds which have an amine, sulphonamide or hydrazine group (see Chapter 2). This acetylation reaction is under genetic control in man and human populations can be divided into individuals of the rapid or slow acetylator phenotype. With hydralazine the adverse effect occurs almost exclusively in slow acetylators. As hydralazine undergoes acetylation it is probable that these differences in metabolism of the drug are responsible for the development of the syndrome. It may be that there is more of the parent drug available in slow acetylators which may initiate an immunological reaction. Alternatively, another pathway of metabolism may become more important in the slow acetylators (Figure 4.2). There is some evidence for this with the oxidative pathway, catalyzed by the mono-oxygenases being the most likely route. However there is now evidence that other enzymes, notably peroxidases such as those found in leucocytes are also able to activate hydralazine to yield the same metabolites (phthalazine and phthalazinone). However, which, if any, metabolite is responsible for the adverse effect is currently unknown.

HLA type

It was found that the patients who suffered the syndrome were more likely to have the HLA type (tissue type) DR4 than those not affected. That is, the incidence of HLA DR4 is 60 per cent in those patients with hydralazine induced lupus compared to an incidence of DR4 in the normal population of

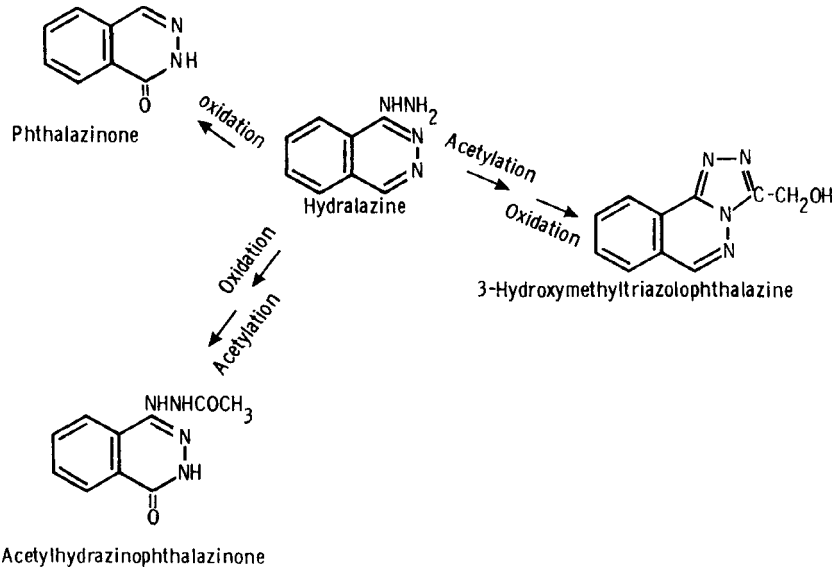

Figure 4.2. The metabolism of the antihypertensive drug hydralazine.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

27 per cent. The role, if any of the HLA type in the development of the syndrome is currently unknown; it may simply be a marker which has an association with a gene involved in the predisposition for the disease.

Sex

The adverse effect occurs more commonly in women than in men with a sex ratio of about 2:1 overall. However, one recent report quoted an incidence of 19.4 per cent in women taking 200 mg daily compared with an incidence in men of 4.9 per cent when measured three years after starting therapy. Currently there is no explanation for this sex difference as there is no evidence for any difference in acetylator phenotype or HLA type distribution between males and females nor for any difference in metabolism between males and females.

Duration of treatment

The final factor is the duration of treatment with the drug; it seems to require an average of 18 months' treatment for the development of the syndrome.

In view of these factors, the hydralazine-induced lupus syndrome is a particularly interesting example of an adverse drug reaction. The recognition of the predisposing factors allows an estimation of the likely incidence: the HLA type DR4 occurs in around 27 per cent of the population; females account for approximately 50 per cent; slow acetylators are approximately 50

per cent of the British population. Given a sufficiently high dose and duration of treatment these factors give an expected incidence of at least 7 per cent of the normal population. Although true incidence figures are hard to come by, the overall incidence in males and females as recently published is about 10 per cent. Alternatively, it can be regarded thus: a female, slow acetylator with the HLA type DR4 is very likely to suffer the adverse effect if a sufficient dose of the drug is given. This means that the adverse effect could be easily avoided if the prospective patients were screened for HLA type and acetylator phenotype.

The mechanism of hydralazine toxicity is currently unknown although it clearly has features characteristic of an allergic type of reaction. In fact, the adverse effect is usually manifested as a Type III immune reaction (see above, Figure 3.1).

Halothane

An example of an adverse drug effect which is a very rare, idiosyncratic, reaction is afforded by the widely used anaesthetic halothane. This may cause serious liver damage in between 1 in 10 000 and 1 in 100 000 patients. A mild liver dysfunction is more commonly seen but this probably involves a different mechanism.

The predisposing factors so far recognized in halothane hepatotoxicity are:

1. multiple exposures, which seem to sensitize the patient to future exposures;
2. sex, females being more commonly affected than males in the ratio 1.8:1;
3. obesity, 68 per cent of patients in one study were obese;
4. allergy, a previous history of allergy was found in one third of patients.

There is now good evidence that halothane causes hepatic damage via an immunological mechanism. The antibodies bind to the altered liver cell membrane and then killer lymphocytes attach to the antibodies. In response to this the killer lymphocytes lyze and destroy the liver cells of the patient, so causing hepatitis (Figure 4.3). The reactive metabolite involved in the immunological reaction is believed to be trifluoroacetylchloride which acylates proteins. This takes place in the vicinity of the endoplasmic reticulum and consequently enzymes such as cytochrome P450 are believed to be acylated and become anti-genie. Such antigens have been identified in liver.

The more common mild liver dysfunction is thought to be due to a direct toxic action of one of the halothane metabolites on the liver. The exact nature of the toxic metabolite is currently unclear although there is some evidence that the metabolite involved in the direct toxicity could either be a product of reductive or oxidative metabolism (Figure 2.21).

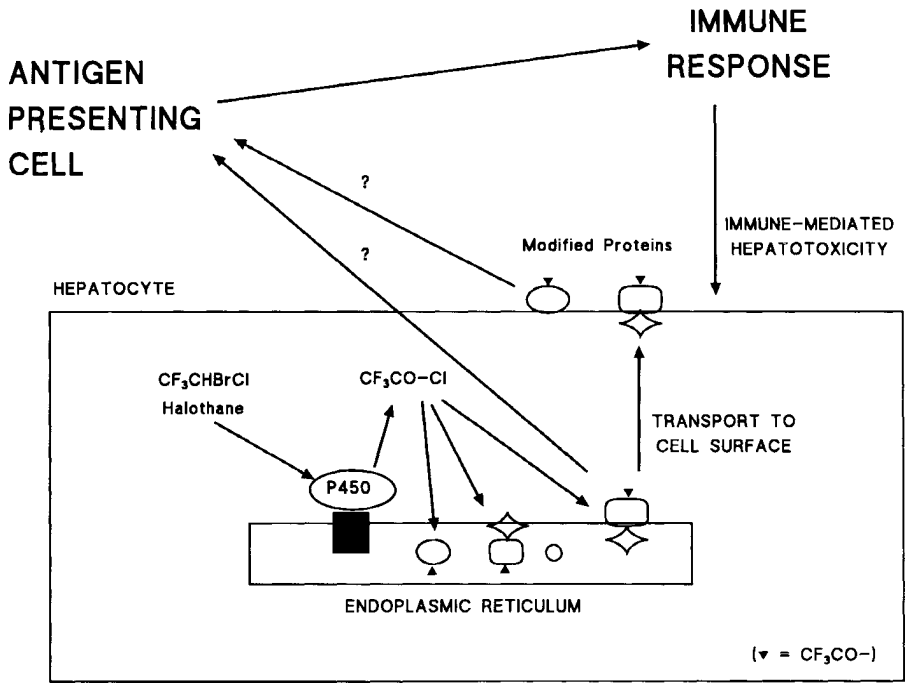

Figure 4.3. The hypothetical mechanism of hepatotoxicity of the anaesthetic drug halothane. Halothane is metabolized by cytochrome P450 (P450) to a reactive metabolite (CF_3COCI) the trifluoroacetyl part of which binds covalently to proteins in the endoplasmic reticulum (▼). The metabolite-protein conjugates are antigenic and elicit immune responses in susceptible patients. Adapted from Kenna, J.G. and Van Pelt, F.N.A.M. (1994) *Anaesth. Pharmacol. Rev.* **2**, 29.

As with hydralazine the knowledge of predisposing factors, in this case the extra risks after multiple exposures, should allow a reduction in the incidence of this adverse drug effect.

The examples used so far have involved a direct toxic effect on tissues mediated either directly or via an immunological mechanism and leading to pathological lesions. The next example illustrates a type of adverse drug reaction in which a pharmacological effect is involved again with a genetic factor.

Debrisoquine

Debrisoquine is a little used antihypertensive drug which was found to show marked inter-individual variation. After the normal recommended therapeutic dose is given this drug may cause an exaggerated pharmacological effect, namely an excessive fall in blood pressure in a few individuals who have a particular genetic predisposition. It has been discovered that about 5–10 per cent of the white population of Europe and North America have this genetic predisposition and are known as poor metabolizers of debrisoquine. This is due

to a defect in the mono-oxygenase system which catalyzes the hydroxylation of debrisoquine at the 4 position, the major metabolic reaction (Figure 4.4). Poor metabolizers have almost complete absence of the cytochrome P450 isozyme which catalyzes the hydroxylation of debrisoquine.

As this metabolic reaction is the major route for removal of the drug from the body, such patients have higher plasma levels of the unchanged drug after a normal therapeutic dose than normal subjects. As debrisoquine itself is responsible for the hypotensive effect the result is an excessive fall in blood pressure (Figure 4.5). This is another example of unexpected toxicity occurring in a small proportion of the patients exposed. In this case, however, the metabolic mechanism seems fairly clear.

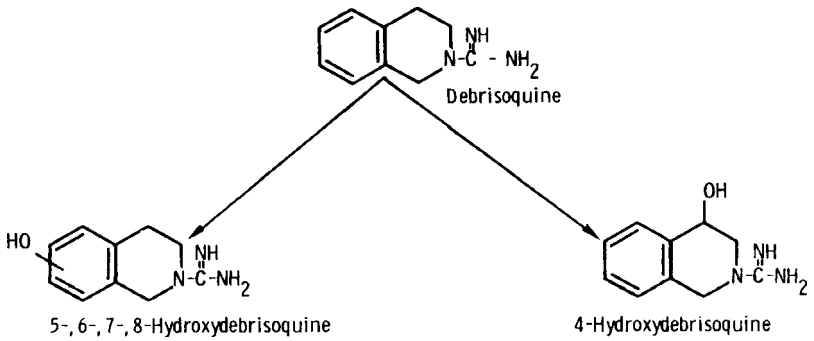

Figure 4.4. The metabolism of the antihypertensive drug debrisoquine.
From Timbrell, J.A., *Principles of Biochemical Toxicology*, Taylor & Francis, London, 1991.

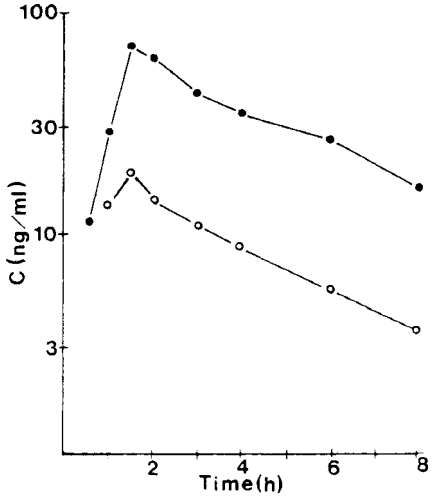

Figure 4.5. The plasma concentration (C) of debrisoquine after a single oral dose (10 mg) in human subjects of the extensive (○) and poor (●) metabolizer phenotypes.
Data from Sloan *et al.* (1983) *British Journal of Clinical Pharmacology,* **15**, 443.

A similar example is the genetically determined toxicity of succinylcholine. This again results from reduced metabolism in certain individuals due to an enzyme variant. Succinylcholine is a muscle relaxant which normally is rapidly removed by metabolic hydrolysis and its duration of action is correspondingly short. In individuals with a defect in the cholinesterase enzyme responsible for the hydrolysis, however, metabolism is slow and consequently relaxation of muscle is excessive and prolonged.

Thalidomide

Thalidomide became notorious as the drug which caused limb deformities in children born to women who had used the drug during pregnancy. The drug is now a well established human teratogen. The thalidomide disaster is particularly important as it was the watershed for drug safety evaluation because it was perhaps the first major example of drug-induced toxicity. Thalidomide is a sedative drug, which was sometimes used for the treatment of morning sickness, and which seemed to be relatively non-toxic. However, it eventually became apparent that its use by pregnant women was associated with a very rare and characteristic limb deformity known as phocomelia in which the arms and legs of the infant were foreshortened. It became clear that these deformities were associated with the use of thalidomide on days 24–29 of pregnancy. The malformations were initially not reproducible in rats or rabbits and had not been detected in the limited toxicity studies carried out by the company manufacturing the drug. The mechanism underlying the effect is still not understood. Thalidomide is an unstable molecule and gives rise to a number of polar metabolites which are derivatives of glutamine and glutamic acid but the ultimate toxic metabolite has not yet been identified. Interestingly one of the isomers of thalidomide (the S-enantiomer) is more embryotoxic than the other. This is an illustration of the importance of chirality as a chemical factor affecting toxicity which has only relatively recently been recognized.

Thalidomide is an exceptionally potent teratogen, but because it has very low maternal toxicity in humans and low toxicity to experimental animals it was allowed to be marketed and used as a drug by pregnant women. It was detected as the cause of the deformities from epidemiological data, when an astute physician associated the exceedingly unusual effects with use of the drug.

There are many other examples of adverse drug reactions which can be found in the literature and the interested reader should consult the references given in the bibliography at the end of this chapter.

Drug Interactions

The problem of interactions between drugs is a major one, particularly with the growth in polypharmacy and multiple drug prescribing. Although the physician and the pharmacist should be aware of such interactions new and unexpected

interactions can and do appear. Interactions may be due to any one of a number of mechanisms, such as interference in the metabolism of one drug by another, interference in the disposition of one drug by another or alteration of the pharmacological response to one drug by another.

Many drugs may interfere with the metabolism of another drug either by inducing or inhibiting the enzymes involved (see Chapter 2). The best known example is that of barbiturates, such as phenobarbital, which induce the mono-oxygenase enzymes and so, by altering the rate or route of metabolism of other drugs, may alter their toxicity. Paracetamol overdoses are more severe if such inducing drugs have been taken, because metabolism via the toxic pathway catalyzed by the microsomal mono-oxygenases is enhanced (see above). Enzyme induction may also decrease the pharmacological or toxicological effects of a compound. For example, use of the antitubercular drug rifampicin, which is also a microsomal enzyme inducer, increases the metabolism of contraceptive steroids and so reduces their efficacy, sometimes resulting in unwanted pregnancies. Whether the toxicity of a particular drug will be increased or decreased will depend on the particular drugs involved and the mechanism of the toxic effect.

Interference in the disposition of one drug by another is a common interaction, particularly involving displacement of a drug from a binding site, typically from binding to plasma proteins. A well-known example of this is the displacement of the anticoagulant warfarin from plasma protein binding sites by phenylbutazone, an anti-inflammatory drug. This results in an elevated plasma level of warfarin leading to excessive anticoagulant activity and haemorrhage.

Altered Responsiveness: Glucose-6-phosphate Dehydrogenase Deficiency

Occasionally drug toxicity may occur in some individuals due to an unusual sensitivity, i.e. idiosyncrasy. Perhaps the best known example of this is the acute, drug-induced haemolytic anaemia due to a deficiency in the enzyme glucose-6-phosphate dehydrogenase. This enzyme, which has a major role in intermediary metabolism in the pentose phosphate pathway, is important in maintaining the NADPH concentration in the red blood cell. NADPH is necessary for maintaining the level of reduced glutathione in the red cell, which in turn protects the red cell from oxidizing substances such as the metabolites of certain drugs:

$$\text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{NADP}^+$$

$$\text{Glucose-6-phosphate} + \text{NADP}^+ \xrightarrow{\text{G6PD}} \text{6-phosphogluconate} + \text{NADPH}$$

Patients who have this particular genetic defect suffer acute haemolytic anaemia when they take drugs such as the antimalarial primaquine or are exposed to certain types of foreign compounds such as aniline derivatives. Fava beans

contain a substance which will precipitate haemolytic anaemia in susceptible individuals, hence the term favism.

The deficiency in glucose-6-phosphate dehydrogenase activity is the result of variants in the enzyme rather than complete absence. The enzyme variants are intrinsic to the red blood cell and so red blood cells from victims will be responsive *in vitro*. On challenge with a suitable drug these red blood cells will lyze and it can be shown that the level of glutathione is lower than in non-sufferers and in fact the glutathione level shows a bimodal distribution. It is a genetic defect carried on the X chromosome, so it is sex-linked but the inheritance is not simple. Overall 5–10 per cent of Negro males suffer the deficiency and will suffer acute haemolytic anaemia if challenged with drugs such as primaquine. The highest incidence (53 per cent) is found in male Sephardic Jews from Kurdistan. There are many compounds which will cause haemolytic anaemia in susceptible individuals, some of which require metabolism to reactive metabolites, others not.

Abuse of drugs, alcohol and certain volatile solvents is becoming increasingly common in modern society and consequently so also is toxicity due to this abuse. It is widely known that repeated use of certain drugs leads to habituation and with some drugs to addiction. In some cases the social and related effects of this addiction may be sufficient to indirectly lead to morbidity and death. In other cases actual pathological damage may result as is the case with cocaine which causes liver damage and may also destroy the nasal passages when inhaled. The toxic effects of chronic alcohol abuse on the liver and brain are widely known as are the many hazards of smoking tobacco. Both alcohol and tobacco are addictive drugs and cause far more widespread damage to public health than the more notorious hard drugs such as heroin and cocaine. Drug abuse also causes indirect effects on human health, such as injury following driving under the influence of drugs, child abuse occurring as a result of drug use, and the AIDS virus spreading via intravenous drug users.

Questions

1. Discuss the role of genetic factors in drug toxicity. Use examples to illustrate your answer.
2. 'An understanding of the mechanism underlying the toxicity of paracetamol led to a means of treatment for overdoses.' Explain this statement.

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