

METABOLISM OF TOXIC SUBSTANCES

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The majority of chemicals normally regarded as foreign to the body are metabolized and transformed into other substances, irrespective of whether the foreign chemical is toxic or innocuous. In the case of toxic compounds, metabolism can play an important role in reducing or increasing the toxic effects, for a compound may produce signs of poisoning either because it is toxic per se or because it is converted in the body into a toxic substance. The extent to which a toxic substance can exert its deleterious effects may depend therefore on how effective the body is in metabolizing it. If a compound is toxic per se and is transformed in the body to a less toxic or non-toxic metabolite, then the more rapidly it is metabolized the less will be the toxic effect of a given dose. On the other hand, if it is metabolized to a more toxic agent, then the more rapidly it is metabolized the greater will be its toxic effect. There are, however, some compounds which do not undergo metabolic change in the body and obviously metabolism is not involved in their toxicity.

The metabolism of a toxic compound is an enzymic process. The metabolism of foreign compounds appears to occur in two phases. The reactions of the first phase include those which may be classified as oxidations, reductions and hydrolyses, whilst those of the second phase, often referred to as conjugations, are reactions classified as syntheses. During phase I reactions, the compound may acquire reactive groupings such as OH, NH₂, COOH and SH through which it can undergo the synthetic phase II reactions. If the foreign compound already possesses any of these reactive groupings, it could

Benzene is an example of a foreign compound which undergoes a two-phase metabolism. During the first phase of its metabolism, it is oxidized to phenol, and in the second phase the phenol so produced undergoes a synthetic reaction to form the conjugates, phenyl glucuronide and phenyl hydrogen sulphate. If phenol itself is administered, it can undergo the phase II reaction directly. Cyanide is a toxic ion which also can undergo conjugation directly to form the relatively non-toxic thiocyanate. Ethanol is an example of a compound which appears to be metabolized mainly by phase I reactions. Its main metabolite is carbon dioxide. Acetaldehyde and acetic acid are intermediates in the oxidation of ethanol and, although acetic acid contains a carboxyl group which is potentially capable of undergoing conjugation, this does not occur—presumably because acetic acid is rapidly metabolized to carbon dioxide through acetyl-coenzyme A.

The metabolism of toxic substances occurs principally in the liver and in those organs and tissues which facilitate entry to, or exit from, the body—namely the intestines, skin, kidneys and lungs. Most foreign compounds are normally ingested via the gastrointestinal tract, and after absorption are transported by the portal vein to the liver, where they tend to accumulate and undergo metabolism. It would thus appear that the enzymes which metabolize foreign compounds are strategically located at sites where they are best able to protect the body from the adverse effects of these compounds, and may have developed as a defence mechanism against the naturally occurring toxic chemicals of the diet and environment.

In the liver, many foreign compounds are metabolized by enzymes present in the endoplasmic reticulum of the hepatic cells. The major class of these enzymes is the microsomal mixed-function oxidases which metabolize foreign compounds, steroids and lipids by a process of hydroxylation or oxygenation, a phase I reaction (Parke, 1968). These enzymes require reduced nicotinamide-adenine dinucleotide phosphate (NADPH) and molecular oxygen, and contain a haemoprotein, cytochrome P-450, which is inhibited by carbon monoxide. The action of these enzymes is to introduce an atom of oxygen into the foreign molecule, which results in the hydroxylation of aromatic and alicyclic rings and alkyl side chains, the removal of alkyl groups from ethers and secondary and tertiary amines, the oxidative deamination of amines and the oxidation of sulphides to sulphoxides and sulphones. Many other enzymes concerned in the metabolism of foreign compounds are also located in the hepatic endoplasmic reticulum, as, for example, the glucuronyl transferases, enzymes that catalyse the synthesis of glucuronic acid conjugates, a phase II reaction.

Cytochrome P-450 and several other enzymes that metabolize toxic compounds are found also in the kidneys. Cytochrome P-450 is also present in the tissues of the gastrointestinal tract, where the enzyme, benzpyrene hydroxylase, which detoxifies benzpyrene and other orally ingested polycyclic hydrocarbons, is also found (Wattenberg, Leong & Strand, 1962). Blood plasma also contains a number of phase I enzymes, including the esterases which catalyse the hydrolysis of esters, such as

and amines and prodrugs. In the metabolism of naphthalene, some of the metabolites formed in the liver are further metabolized to 1,2-dihydroxynaphthalene and 1,2-naphthoquinone in tissues of the eye. This results in an increase in opacity and leads to retinal degeneration and lens opacity (van Heyningen & Pirie, 1957).

Aromatic amines, sulphonamides and hydrazides are metabolized by acetylation of the amino group, a phase II reaction which occurs in the reticulo-endothelial cells of the liver, spleen, lungs and gastrointestinal mucosa (Govier, 1965). Amino, both foreign and endogenous, may also undergo methylation, a conjugation reaction catalysed by a non-specific *N*-methyl transferase which is highly active in rabbit liver tissue (Axelrod, 1962).

Foreign compounds may also undergo metabolism in the gastrointestinal tract by the action of the gut microflora. Many of these reactions are reductive in nature, as in the hydroxylation of protocatechuic and hemoprotocatechuic acids (Dacre & Williams, 1968) and in the reductive scission of food dyes to toxic amine metabolites. Hydrolyses may also occur: for example, cycasin, the β -glucoside of methylglyoxymethanol and the toxic constituent of cycad nuts, is hydrolysed into its hepatocarcinogenic aglycone by glucosidases of the intestinal micro-organisms. The microflora of the gut also give rise to the formation of a number of toxic compounds, such as amines, indoles, and aromatic hydrocarbons (Scheline, 1968).

3. Excretion and the Nature of Metabolites

The metabolic transformation of a toxic compound in the body can result in the excretion in the urine of (i) the original compound; (ii) the products of oxidation, reduction or hydrolysis (phase I products); and (iii) conjugates (phase II products). The proportion in which these three types of products are excreted depends on several factors, including the chemical nature of the foreign compound, the dose, route of administration, species, strain, sex, diet and other environmental factors.

Metabolism, polarity and excretion in the urine. When a foreign compound is metabolized in the body, it is converted to more-polar compounds. Phase I products of metabolism are usually more polar than the parent compound, and phase II products are usually acidic compounds, such as glucuronic acid conjugates, ethereal sulphates, and glycine conjugates. These are water soluble and highly ionized at the pH of the blood and tend to be readily excreted by the kidney. The metabolic process thus converts lipid-soluble foreign compounds, which cannot be reabsorbed by the kidney tubules, into water-soluble products which are readily excreted; detoxication of such compounds may be a consequence of these processes. This may explain why many of the compounds that do not undergo metabolic transformations in the body are often strongly non-highly polar.

Excretion in the bile. Many toxic chemicals are excreted in bile, mostly as glucuronides and other conjugates, and after their decomposition products are finally voided in the faeces. Excretion by this route appears to be dependent on the nature of the compound excreted for, when the molecular weight is >400 , biliary excretion may be the major route of elimination of the compound from the body. Conjugates excreted in bile may be hydrolysed by the action of intestinal microflora to the original toxic substances or may be decomposed to new toxic substances. Polycyclic aromatic amines are

metabolized in the liver to *o*-hydroxyamines which are excreted as glucuronide conjugates in the bile. Hydrolysis of these glucuronides in the gut liberates the free *o*-hydroxyamines which are carcinogenic and could be the cause of intestinal tumours. The antibiotic chloramphenicol, which is gonitrogenic in rats, is excreted in the bile as chloramphenicol glucuronide and then undergoes hydrolysis and decomposition in the gut to form amines. These amines, which appear to be the gonitrogenic agents, are subsequently reabsorbed from the gut (Williams, Millburn & Smith, 1965).

Excretion by other routes. Toxic substances and their metabolites may also be excreted from the body by other routes. Many volatile compounds such as aromatic hydrocarbons are excreted unchanged in the expired air, as are volatile metabolites such as carbon dioxide. Nicotine and other basic compounds are secreted in the gastric juice into the stomach, and aflatoxin, pesticides and many drugs may be secreted into milk.

4. Two-Phase Metabolism and Toxicity

From the point of view of toxicity, phase I and phase II products may differ. Products of phase I metabolism may be less toxic, more toxic, or possess a different type of toxicity from their precursors. In contrast, the majority of phase II products are either non-toxic or considerably less toxic than the parent compound, or its phase I metabolites should they be formed. However, foreign compounds may also be concerned in certain synthetic reactions that produce toxic products or toxic consequences. These reactions are known as lethal syntheses and should be distinguished from the phase II or conjugation reactions. Lethal syntheses may come about as the result of a foreign compound or its phase I metabolite mimicking a natural intermediary metabolite, as, for example, fluoroacetate which mimics acetate, enters the tricarboxylic acid cycle and finally blocks this pathway of metabolism. Alternatively, certain important macromolecules may become slightly modified by the action of a foreign compound, for example, dimethylnitrosamine is converted by an oxidative process to an active methylating agent which reacts with nucleic acids. These nucleic acids may be important in the development of cancer (Craddock & Magee, 1966).

a. Phase I Reactions and Toxicity

As a result of phase I reactions, an initially toxic compound may be converted into less toxic substances or an initially non-toxic compound may be converted into more-toxic metabolites. A substance may also be converted into several metabolites, some of which may be more toxic and others less toxic than the original compound. Tetraethyltin is not itself biologically active but it shows the same toxic effects towards the central nervous system as does triethyltin, except that the symptoms are delayed in onset. This is apparently because of the hepatic de-ethylation of tetraethyltin to triethyltin which then exerts its toxic effects upon the animal (Cremer, 1958). Methanol causes blindness in man, and this is the result of its oxidation to formaldehyde which then disturbs oxidative phosphorylation in the retina (Kini & Cooper, 1961). The insecticide parathion is inactive until it has been converted in the tissues into paraoxon which is a powerful cholinesterase inhibitor (Gage, 1953). Again, the neurological effects of tri-*o*-cresyl phosphate (TOCP) are due in part to its oxidation and conversion to a toxic cyclic phosphorus compound (Eto, Casida & Eto, 1962).

though the glycoside amygdalin shows toxicity in some animals when it is given by mouth, but not when it is injected. In this case it appears that the glycoside is hydrolysed, probably by glycosidases of the gut flora, to mandelonitrile which then breaks down to give the highly toxic cyanide ion.

Examples of the reduction of toxicity and pharmacological activity of substances undergoing metabolism by phase I reactions are numerous. The carcinogen benzpyrene is considered to be a proximate carcinogen and undergoes metabolism by aromatic hydroxylation to give 3-hydroxybenzpyrene and 6-hydroxybenzpyrene, which are not carcinogenic. Many barbiturates are similarly detoxicated and inactivated by oxidation of their side chains, whether these be aliphatic, as in pentobarbital (5-ethyl-5-(1'-methylbutyl)barbituric acid), or aromatic, as in phenobarbital (5-ethyl-5-phenylbarbituric acid). Tyramine, a toxic hypertensive amine present in cheese, wine and certain other food-stuffs, is detoxicated by the enzyme monoamine oxidase and undergoes oxidative deamination to give *p*-hydroxyphenylacetic acid. The organophosphorus insecticide, malathion, an inhibitor of the enzyme cholinesterase, is metabolized in mammals by hydrolysis of the ethyl ester bonds to give the relatively non-toxic malathion diacid; and the chlorinated hydrocarbon insecticide dicophane (DDT; 1,1,1-trichloro-2,2-di-(4-chlorophenyl)ethane) is similarly detoxicated in mammals by metabolic dehydrochlorination to DDA (2,2-bis-(*p*-chlorophenyl)acetic acid).

b. Phase II Reactions and Toxicity

The conjugation of toxic compounds or their metabolites, the phase II reactions, almost invariably results in reduction of toxicity. Hydrazine, a chemical used as a rocket propellant, which is probably toxic by virtue of its reducing properties and its tendency to form hydrazides with physiologically essential carbonyl compounds such as pyridoxal, is detoxicated by acetylation to diacetylhydrazine. The corrosive poison, phenol, is detoxicated by conjugation with sulphuric or glucuronic acids; and the narcotic drug, morphine, is similarly inactivated by formation of the 3-glucuronide which is excreted in the bile. Salicylic acid, an antirheumatic drug that may uncouple oxidative phosphorylation, is deactivated by conjugation with glycine to form salicylic acid. The toxic solvent, chlorobenzene, is detoxicated by a similar conjugation with glutathione which, after further metabolism, is excreted as the acetylcysteine derivative, *p*-chlorophenylmercapturic acid. Finally, the conjugation of cyanide ion by the enzyme thiolphosphate sulphurtransferase gives the non-toxic thiocyanate.

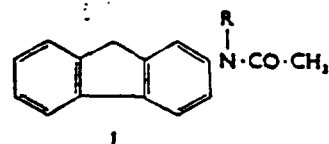
5. Factors Affecting the Metabolism of Foreign Compounds

The metabolism of a foreign compound is controlled by enzymes, and any factor which affects these enzymes also affects the metabolism of the compound and consequently its toxicity. Several factors are now known which affect this metabolism, these being species, strain (genetic factors), age, sex, disease, stress, diet, chronic administration, and the administration of other foreign compounds. The nature of the diet may also be important, since they may play a role in the metabolism of toxic compounds ingested orally or of compounds secreted in the bile. These organisms may vary from species to species, or even within a given species, dependant on the diet and environment.

a. Species

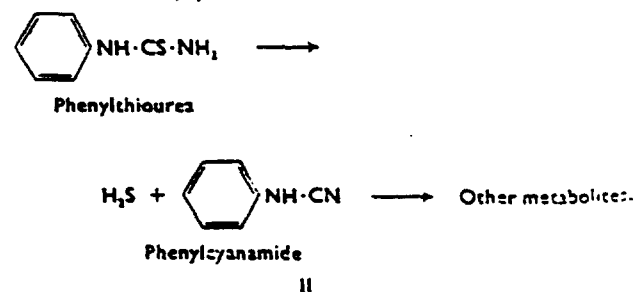
The safety of drugs and other chemicals used by man is assessed largely by tests on laboratory animals, but the activity and toxicity of these chemicals are not always the same in animals as they are in man. One reason for these differences is that the metabolism of a compound in laboratory animals often differs from that in man both qualitatively and quantitatively. Species differences in the metabolism of foreign compounds (see Williams, 1967a, 1967b; Smith, 1968) depend mainly upon variations in the enzymes which control phase I and phase II reactions. These variations in enzyme activity can be due to differences in the following: (i) the absolute amount or the specific activity of an enzyme; (ii) the amount or nature of natural inhibitors of an enzyme; (iii) the activity of an enzyme reversing the reaction; or (iv) the activities of enzymic reactions competing for the same substrate.

Examples of species differences in toxicity which appear to be related to differences in the metabolism of a compound are as follows. The carcinogenic activity of the 2-acetamidofluorene (I; R = H) is believed to be related to the conversion of the compound in vivo to *N*-hydroxy-2-acetamidofluorene (I; R = OH). The dog, cat, rabbit, rat, mouse and hamster are



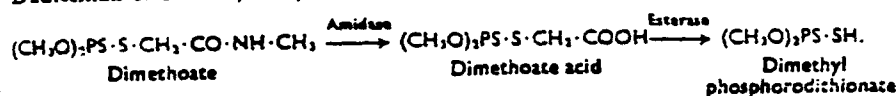
susceptible to this carcinogen and they all convert it in part to the *N*-hydroxy compound. The steppelcmming and guinea-pig are not susceptible and do not *N*-hydroxylate the carcinogen to any appreciable extent. Man and monkey also *N*-hydroxylate the compound, but it is not known whether they develop cancer from acetamidofluorene, although by inference from the evidence of other species they should (Weisburger, Grantham, Vanhorn, Steigbigel, Rall & Weisburger, 1964).

The development of certain rodenticides has been based on species differences in toxicity, a case in point being α -naphthylthiourea (ANTU) which is highly toxic to rats but not to domestic animals. A related compound, phenylthiourea, shows similar species differences in toxicity to ANTU (for rats the LD₅₀ of phenylthiourea is 5 mg./kg. body-wt.; for rabbits, 40 mg./kg.; for guinea-pigs, 250 mg./kg.; and for hens, about 1 g./kg.). There is evidence to suggest that in this case, also, toxicity is related to metabolism (B. Bringlee, R. L. Smith and R. T. Williams, unpublished data, 1964; see Williams, 1963). Phenylthiourea produces several urinary metabolites, including phenylcyanamide, and it has been postulated that its toxicity is due to the highly toxic hydrogen sulphide which is liberated in this reaction (II):



The toxicity of this compound is due to enzymic release of H_2S is supported by the fact that the less toxic 1-methyl-1-phenylthiourea (LD_{50} 200 mg/kg. body-wt. in rats) protects rats against a lethal dose of phenylthiourea, probably by competing for the enzyme releasing H_2S . Methylphenylthiourea would probably be metabolized similarly to give methanethiol, a product less toxic than H_2S . Phenylthiourea is desulphurized to about the same extent in the rat, rabbit and guinea-pig, 40-50% of the dose being excreted as sulphate in 24 hours, but the guinea-pig excretes more neutral sulphur compounds than the rat, and excretion of metabolites is more rapid. Moreover, guinea-pigs are less susceptible to H_2S than are rats, the approximate intraperitoneally administered LD_{50} being 2-3 mg/kg. body-wt. in rats and about 10 mg/kg. body-wt. in guinea-pigs. Species differences in the toxicity of phenylthiourea may therefore be related to species differences in the metabolism of the compound and in susceptibility to the toxic metabolite.

The insecticide dimethoate is 70 times more toxic to the cockroach and 325 times more toxic to the housefly than to the mouse. Dimethoate is detoxicated in the tissues by enzymic hydrolysis which can occur both at the amide and the thiol ester bonds. Studies with in-vitro preparations from various species have shown that the in-vitro rate of hydrolysis is inversely related to the toxicity of the compound (Uchida, Dauterman & O'Brien, 1964):



b. Strain: Genetic Factors

Differences in the individual susceptibility to toxic substances, and idiosyncrasies towards certain drugs and food-stuffs, are often the result of impaired detoxication of these compounds resulting from genetically determined variations in the enzymes concerned. Differences in phase I metabolism have been observed in the rate of metabolism in vitro of a number of foreign compounds in different strains of rats and dogs (Sieger, Alsleben, Liebenschütz & Remmer, 1964) and rabbits (Cram, Juchau & Fouts, 1965). From Table I it may be seen that variations by as much as a factor of 15 may occur in the rates of metabolism of toxic compounds in livers of different strains of rabbit. Furthermore, no correlation is apparent between the different metabolic reactions in the various strains. Cottontail rabbits in general appear to metabolize toxic compounds the least effectively, yet have the highest activity among the different strains for the metabolism of 3,4-benzpyrene. Similarly, Dutch rabbits have the highest activity for the metabolism for amphetamine and yet have the lowest activity for the metabolism of 3,4-benzpyrene.

Individual differences in the ability to metabolize drugs may have considerable bearing on therapeutic efficacy and toxicity. As a consequence of this, many investigations into genetic differences in the metabolism of toxic substances have been concerned with drugs. Hydrazine derivatives, such as isoniazid (isonicotinic acid hydrazide), hydralazine and phenelzine, and the sulphonamide, sulphadiazine, all of which are metabolically deactivated by acetylation, exhibit polymorphism of the enzyme involved, liver acetyltransferase, which divides human subjects into "slow" and "fast" deactivators (Evans, 1965). As would be expected, the "slow" deactivators experienced greater therapeutic efficacy and greater toxic side-effects than the "fast" deactivators. Similar genetic variants

have been observed in plasma "typing" (Shaw & Schur, 1958), an enzyme which hydrolyses many esters and deactivates drugs, such as the muscle relaxant succinylcholine (Harris, 1965). A deficiency of this enzyme in susceptible individuals can result in respiratory failure following the administration of normal doses of succinylcholine. Wide individual variability in the detoxication of phenazone (antipyrine), an antipyretic metabolized by hydroxylation, has been also observed in rats and man (Vesell & Page, 1968).

The toxicity of foreign compounds may also be affected by genetic variations in the activity of enzymes that are concerned only indirectly in the metabolism of these substances. For example, a number of drugs and other foreign compounds such as divicine and isouramil, pyrimidines that are present in the broad bean, produced haemolytic anaemia in certain susceptible individuals who have been found to have abnormally low levels of glucose-6-phosphate dehydrogenase activity in their red blood cells (Mager, Glaser, Razin, Izak, Bien & Noam, 1965).

c. Age

Newborn and foetal animals metabolize some toxic substances slowly (see Parke, 1968) because of the low activity of many of the respective enzymes which metabolize foreign compounds. The hepatic microsomal phase I enzymes, including cytochrome P-450, together with the phase II enzymes

responsible for the formation of glucuronic acid, glycine and glutathione conjugates are all at a low level or absent at birth. The enzymes which catalyse the formation of sulphate and acetyl conjugates are present, however, at normal levels in some species. In the newborn rat, glucuronyl transferase is already at adult level (Dutton, 1964). From birth onwards these enzymes develop rapidly, reaching adult levels in about 30 days in the rat and in about 8 weeks in man, and it is possible that their activity is partly stimulated by foreign chemicals present in the diet. In consequence of these enzyme deficiencies the newborn animal is generally more susceptible to the toxic

TABLE I. Differences in the rates of metabolism in vitro of toxic compounds in various strains of rabbit

(Cram, Juchau & Fouts, 1965)

Substrate	Major route of metabolism	µMoles of substrate metabolized/g. liver supernatant-N/100 ml. in the following strains of rabbit:			
		Dutch	English	New Zealand	Cottontails
Hexobarbital	Aliphatic hydroxylation	140	170	250	20
Aniline	Aromatic hydroxylation	35	130	100	70
3,4-Benzpyrene	Aromatic hydroxylation	25	35	30	50
Amidopyrine	Demethylation	70	220	80	15
Amphetamine	Oxidative deamination	150	90	80	10
p-Nitrobenzoic acid	Reduction	90	70	120	50

* The liver supernatant was prepared by homogenizing liver in 2 vol. 1.15% KCl solution, followed by centrifuging at 9,000 g for 30 min.

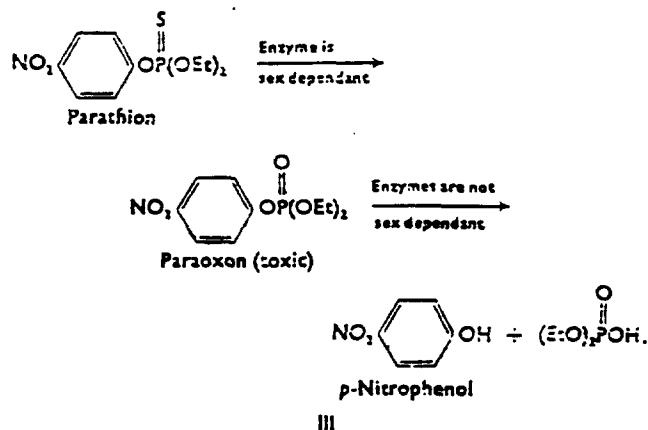
action of drugs and foreign compounds. From a study of the acute toxicity in newborn rats of a number of drugs, Yentry, Baskin & Finkelstein (1956) showed that most drugs are 2-10 times more toxic to 1-3-day-old rats than to adult rats.

The foetus with its lack of detoxicating enzymes would appear to be in a particularly vulnerable position, since many toxic compounds have been shown to cross the placental barrier and enter the foetus. Moreover, the protection of the maternal liver may also become impaired, since the level of hepatic cytochrome *P*-450 may be reduced in pregnancy and many of the detoxicating enzymes appear to be inhibited by the high plasma levels of progestogens that occur in pregnancy. Recent work has suggested that the human placenta, at least, has a slight ability to detoxicate foreign compounds, and this together with the somewhat diminished capacity of the maternal liver may afford reasonable protection to the foetus (M. Neale and D. V. Parke, unpublished data, 1969).

d. Sex

Certain foreign compounds show differential toxicity in male and female animals. Hurst (1953) gives a list of some 30 compounds that have been reported to be more toxic to one sex than to the other. In this list human beings were mentioned only twice, suggesting that women may be more susceptible than men to amidopyrine and benzene. When toxicity has been related to metabolism it does appear that rats show sex differences in toxicity more frequently than other species. Table II gives the LD₅₀ values of five relatively toxic compounds in male and female animals. Strychnine administered intravenously is equally toxic to male and female rats but, when given intraperitoneally or subcutaneously, it is more toxic to females. Kato, Chiesara & Vassanelli (1962a, 1962b) have shown that strychnine is more rapidly metabolized to less toxic metabolites by liver microsomal preparations from male rats than from females and is consequently less toxic to the males. Unlike strychnine, parathion is metabolized to a toxic metabolite, namely paraoxon and this occurs more rapidly in female rat-liver preparations than in male (Davison, 1955).

Parathion is thus less toxic to the male (DuBois, Doull, Salerno & Coon, 1949). In mice, cats and dogs, there is no sex difference in the toxicity of parathion and it would appear that the compound is metabolized equally well by both sexes. Paraoxon, the toxic metabolite of parathion, shows no sex difference in toxicity in the rat. It is metabolized by esterases to less toxic products and there is probably no sex difference in the activity of these enzymes (III):



Schradan, like parathion, is metabolized to a toxic substance but, unlike parathion, it is more toxic to male than to female rats, a consequence of its more rapid metabolism in male rat liver (Davison, 1955). Warfarin, the last compound in Table II, is less toxic to male than to female rats, and like strychnine is probably more rapidly metabolized in the male to less toxic metabolites. With these compounds, sex differences in toxicity are found only after puberty. It appears therefore that, as far as the rat is concerned, the metabolism of these compounds and hence their toxicity are under the influence of the sex hormones.

TABLE II. Sex differences in toxicity and metabolism

Compound	Species	Route	LD ₅₀ (mg/kg. body-wt.)		Susceptible sex	Toxicity of metabolites compared with parent compound	Rate of enzymic conversion	References
			Male (M)	Female (F)				
Strychnine	Rat	Intravenous	0.57	0.57	F	Less toxic	M > F	Kato, Chiesara & Vassanelli (1962a, 1962b)
	Rat	Intraperitoneal	2.82	1.62				
	Rat	Subcutaneous	4.01	1.81				
Parathion	Rat	Intravenous	7	4	F	More toxic	F > M F > M — —	DuBois, Doull, Salerno & Coon (1949); Davison (1955)
	Rat	Oral	15	6				
	Mouse	Intraperitoneal	10	9-10				
	Cat	Intraperitoneal	3-5	3-5				
Paraoxon	Rat	Intraperitoneal	1.2	1.2		Less toxic	—	DuBois et al. (1949)
	Rat	Oral	3.5	3.5				
Schradan	Rat	Intraperitoneal	7	27	M	More toxic	M > F	Davison (1955)
Warfarin	Rat	Oral	323	58	F	Less toxic	—	Hagan & Radomski (1953)

— means that no measurements have been made

e. Disease

It is well known that human patients and animals with liver disease are more than normally susceptible to the toxic effects of drugs and foreign chemicals, and since the liver is the major site of metabolic detoxication this is not surprising. Patients with liver diseases such as cirrhosis, obstructive jaundice and hepatitis showed impaired ability to form glucuronide and sulphate conjugates (Müting, 1963) and rabbits with experimental obstructive jaundice produced by ligation of the bile duct showed impairment of the hepatic phase I enzymes that oxidatively metabolize drugs (McLuen & Fouts, 1961). The amount of hepatic cytochrome *P*-450 and the activities of the microsomal enzymes of the liver are also diminished as the result of poisoning with the hepatotoxins carbon tetrachloride or dimethylnitrosamine (Smuckler, Arrhenius & Hultin, 1967). Liver damage may also change the pattern of metabolism, and the carcinogen 2-acetamidofluorene is metabolized to the carcinogenic *N*-hydroxy-2-acetamidofluorene to a greater extent in rats with liver damage than in normal animals (Margreth, Lotlikar, Miller & Miller, 1964).

f. Environment

Stress. Adverse environmental conditions such as cold and noise, which give rise to stress in animals, may result in an increase in the activity of the hepatic microsomal enzymes that is mediated through the pituitary-adrenal axis. The hydroxylation of acetanilide by rat-liver microsomes is almost doubled by previous exposure of the animals to cold stress, and the metabolism of 2-naphthylamine to 2-amino-1-naphthol in mice is increased more than 50% by exposure to cold and more than 100% by cold plus noise (Dewhurst, 1963). As 2-amino-1-naphthol is possibly the proximate carcinogen of 2-naphthylamine, the stress conditions may be considered, in this case, to result in an increase in toxicity.

Nutrition. For a discussion on nutritional aspects see the paper by McLean & McLean, p. 278 of this Bulletin.

Inhibition and stimulation of metabolism by foreign chemicals. The metabolism of a toxic compound by the hepatic microsomal enzymes may be initially inhibited by the presence of competing substrates, but subsequently stimulation of metabolism may result. Pre-treatment with drugs, steroids, food additives, toxic chemicals such as pesticides, polycyclic hydrocarbons, polycyclic amines, and normal constituents of food of no nutritional value such as β -ionone and saffrole (Parke & Rahman, 1969) can result in a twofold to tenfold increase in the activity of hepatic enzymes that metabolize drugs and toxic substances (Conney, 1967). This increase of enzyme activity differs according to the inducing chemical, but could be mediated through an increased rate of synthesis of enzyme protein, a decreased rate of turnover of enzyme, or an activation of enzyme possibly by changes of structure or conformation. The results of this phenomenon are also that chronic administration of a drug or toxic compound may enhance the activity of the enzymes that catalyse its metabolism. When metabolism results in a reduction in the toxicity of the compound, chronic administration will reduce the toxicity of a given dose. When metabolism first gives rise to a toxic

metabolite, which is subsequently detoxicated by further metabolism, the effects on over-all toxicity are consequently more complex.

Phenylhydrazide, an antineuronal blocking agent, inhibits the metabolism of coumarin anticoagulant drugs, such as dicoumarol, so that the usual therapeutic dose becomes excessive and spontaneous haemorrhage may occur (Solomon & Schrogie, 1966). On the other hand, pre-treatment with phenobarbital increases the metabolism of dicoumarol, thus reducing its anticoagulant activity. The development of tolerance to barbiturates and certain other drugs is similarly due to the stimulatory action of a drug on its own metabolism (Conney, 1967).

The induction of these enzymes by pre-treatment with foreign compounds does not necessarily increase the activity of all enzymes to the same extent. Certain metabolic pathways may be preferentially stimulated. The hepatocarcinogen, 2-acetamidofluorene, is metabolized by hydroxylation to give 5-hydroxy-, 7-hydroxy- and *N*-hydroxy-2-acetamidofluorene, the last compound being the proximate carcinogen. Continuous administration of 2-acetamidofluorene to rats results in a preferential induction of the *N*-hydroxylation. Pre-treatment with acetanilide, or 2-acetamidofluorene plus acetanilide, does not increase the extent of *N*-hydroxylation, and consequently prevents or delays carcinogenesis by 2-acetamidofluorene (Grantham, Mohan, Yamamoto, Weisburger & Weisburger, 1968; Yamamoto, Glass, Frankel, Weisburger & Weisburger, 1968). 7,12-Dimethylbenz[*a*]anthracene (DMBA), a carcinogen which also produces adrenal necrosis, is hydroxylated principally at the methyl groups. The 7-hydroxymethyl derivative is the active adrenocorticolytic agent, whereas the 12-hydroxymethyl derivative and ring-hydroxylated metabolites are probably true detoxication products. Pre-treatment with 3-methylcholanthrene and certain other polycyclic hydrocarbons protects against the DMBA-induced adrenal necrosis because of a preferential induction of the microsomal enzymes which shifts hydroxylation of DMBA from the methyl groups to the aromatic ring. Phenobarbital pre-treatment gives similar protection, but acts by a different induction mechanism involving the further hydroxylation of the 7-hydroxymethyl derivative to 7,12-di-(hydroxymethyl)benz[*a*]anthracene and other products (Boylan & Sims, 1967).

Cytochrome *P*-450, an essential component of the hepatic microsomal enzymes, is inhibited by carbon monoxide, and an atmosphere containing only 2% carbon monoxide inhibits the in-vitro hydroxylation of aniline and oxidative demethylation of amidopyrine in rats by about 50% (Kato, 1966). Inhibition of the hepatic microsomal enzymes is also produced by certain methylenedioxyphenyl and methylenedioxynaphthyl compounds such as piperonyl butoxide and sesamex (Metcalf, Fukuto, Wilkinson, Fahmy, El-Aziz & Metcalf, 1956). These compounds are used as insecticide synergists since, by inhibiting the insect enzymes which deactivate insecticides, they potentiate toxicity and insecticidal activity. It is pertinent to speculate on the possible effect of the chemicals which now contaminate the environment on the metabolism of toxic compounds in man.

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