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Metabolism of Sulfur-Containing Xenobiotics

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METABOLISM OF SULFUR-CONTAINING XENOBIOTICS

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SUMMARY

Organisms dwell within environments which are superabundant in chemical entities. Some are essential for the growth, repair and general maintenance of living systems, but many are apparently unnecessary, 'foreign' to intermediary metabolism and potentially dangerous. Nevertheless, they still gain entry and have to be dealt with.

Up to one-third of these compounds may contain a reactive sulfur centre which can be exploited by a living system via chemical modification in the desire to hasten removal and minimize any deleterious effects which may arise during the passage of these 'foreign' molecules through the organism. Numerous enzyme-catalysed chemical pathways have evolved and are able to undertake this task. It is these metabolic options which are available to the sulfur centre within sulfur-containing xenobiotics that are discussed in this review.

INTRODUCTION

Sulfur is a major nutrient element for which a perfect biogeochemical cycling has evolved with intermediate exchange between the atmospheric, aquatic and terrestrial phases of the environment. The primary sources of sulfur are the sulfides which are to be found throughout the earth's crust. These are converted to inorganic sulfate by climatic processes and microorganisms which also incorporate the sulfur into organic molecules. Both plants and microbes are able to reduce sulfate to the thiol level, its lowest oxidation state, such as that found in the amino acids, cysteine and methionine. These pathways are lacking in animals; generally divalent organic sulfur is transformed into compounds possessing higher oxidation states and eventually to sulfate (Fig. 1).

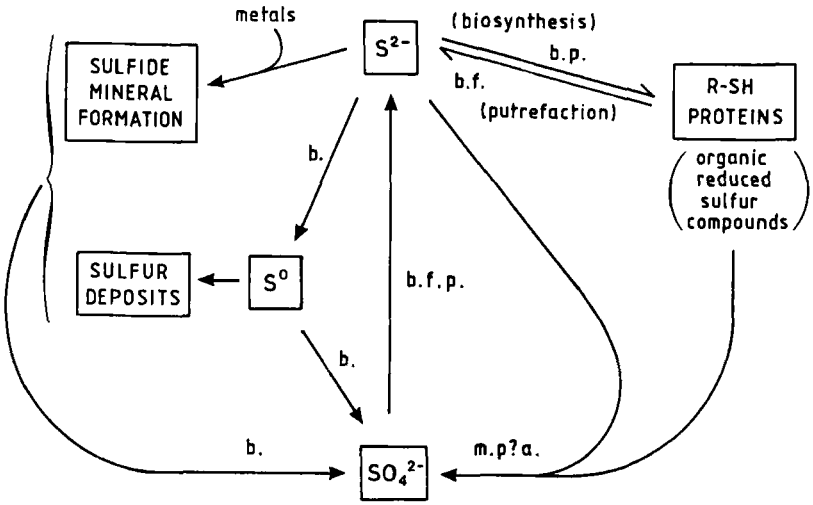

FIGURE 1. The biogeochemical sulfur cycle. (Footnote for Fig. 1.) a = animals; b = bacteria; f = fungi; m = microorganisms; p = plants

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Biological sulfate reduction, a primitive form of respiration, may have been occurring as early as 3–5 billion years ago, which is soon after the probable time of the emergence of life on earth.[1] Other evidence suggests that photolithotropic sulfur oxidizers, primordial photosynthesizers, may have even preceded the sulfate reducers, both developing on an anaerobic planet prior to the evolution of the water-splitting reaction of green plant photosynthesis and the appearance of an oxygen-rich atmosphere.[2-4] Ancestor tracing based on RNA sequence analysis has concluded that all living organisms originated in a sulfur-metabolising thermophilic organism.[5,6]

Reduced sulfur appears in organic compounds essential to all organisms as constituents of proteins, coenzymes and major cellular metabolites which dictate structure, binding site characteristics and transport system function. In addition, owing to their relative ease of oxidation they protect against oxygen toxicity and radiation damage. Oxidized sulfur, in the form of sulfinates and sulfates, is also prevalent in a number of organisms and serves important structural functions as well as providing charged anionic binding sites for movement, maintenance and regulation of appropriate charge separation.

Electronic expansion into the d-orbitals allows sulfur to assume several valencies at a number of oxidation states ranging from -2 (e.g. thiols, sulfides) to $+6$ (e.g. sulfates). This permits sulfur to form a series of oxyanions, and compounds at theses intermediate oxidation states are chemically and biologically active. Such compounds can undergo redox reactions with energy being liberated as the oxidation state of sulfur increases. Oxidation of sulfur from the -2 oxidation state to that of the most stable state, $+6$, gives a free energy release (ΔG) of around 700–800 KJ, mol^{-1}. This is quite considerable in biological terms; the free energy of hydrolysis of ATP at pH 7 is 31.2 KJ, mol^{-1}.[7] For organisms using sulfate as a sole source of sulfur considerable energy must be invested in reducing it whereas species who receive sulfur in the reduced state can derive appreciable amounts of energy oxidising it. However, within intermediary metabolism this energy may be largely wasted as the dioxygenases mainly involved in oxidising sulfur from the -2 to $+4$ state appear not to be coupled to ATP synthesis.[7] Sulfite oxidase, the enzyme catalysing the conversion of sulfite ($+4$) to sulfate ($+6$) links this oxidation to the reduction of cytochrome c and in intact mitochondria causes the phosphorylation of ADP.[8]

The wide number of chemical reactions occurring within living systems are made possible by the assistance of catalytic enzymes which present idealised local electromolecular environments in which the reactions can take place. Those reactions which are fundamental for the continuance of life constitute a central core of intermediary metabolism. These are surrounded by a less essential, but nevertheless important, series of enzyme-mediated reactions. It is amongst these latter reactions that those of xenobiotic metabolism can be placed. The enzymes involved in quintessential metabolism generally utilize only one, or at most only a few, substrates and there is rarely competition amongst different enzymes for substrates. However those enzymes involved in xenobiotic metabolism usually possess a broad substrate specificity linked to lower catalytic rates and a given

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TABLE 1. Major metabolic options available to the sulfur moiety within sulfur-containing xenobiotics

Disulfide formation and cleavage
(thiol oxidation; disulfide reduction)
Addition and removal of oxygen
(sulfoxide, sulfone and sulfate formation; sulfoxide reduction)
Conjugation reactions
(glucuronidation; glucosidation; methylation, sulfation; thioamide formation)
Miscellaneous reactions
(C—S and N—S bond cleavage; substitution with oxygen)

chemical may serve as a substrate for more than one enzyme. The interaction of the sulfur moiety within sulfur-containing compounds with this array of enzymes can result in a variety of chemical modifications of the molecule; it is these xenobiotic metabolic pathways which are outlined in this review (Table 1).

Interestingly, it has been demonstrated that the energy released by the chemical oxidation (via bromine in pyridine) of the sulfur atom in xenobiotic sulfides (thioethers, thiolactones, thiazolidones) can be trapped in a chemical system resulting in the formation of ADP and ATP in excellent yields from AMP and P_i.[9-12] Furthermore, it has been suggested that in living systems this may be involved in the mechanism of oxidative phosphorylation.[9] It would be intriguing if the presumed incidental metabolism of certain chemicals regarded as xenobiotics was not only beneficial in terms of their own detoxication but also played a role in supplementing energy provision. Such metabolism may also provide protective mechanisms. The sequential oxidation of a surplus xenobiotic sulfide to sulfate, with the addition of four oxygen atoms to every one of sulfur and the production of a water soluble ion may be an efficient and economical way of removing excess and potentially damaging oxidising capacity.[13]

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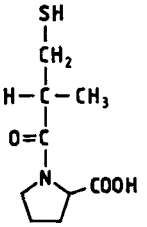
DISULFIDE FORMATION AND CLEAVAGE

Thiols (R—SH), also known as thioalcohols or mercaptans, are the sulfur analogues of alcohols (R—OH) and their chemistry has been extensively reviewed elsewhere.[14] Many have distinctive and objectionable odours. Ethanethiol is present in human breath, being implicated in the mephitic 'foetor hepaticus' associated with certain liver diseases[15] whilst skunks employ butanethiol and isopentanethiol, secreted from their anal glands, to effectively deter unwelcome guests.[16] Thiols are stronger acids than alcohols since the S—H bond is much weaker than the corresponding O—H bond. Consequently, the thiol group is facile, liberating a thiolate ion ($R—S^-$) on ionisation, a reactive species which acts as a powerful nucleophile participating in substitution reactions at saturated carbon atoms to give thioethers. This reaction is exploited in the preparation of many substituted thiols.

Oxidation at the sulfur atom within the thiol group may lead to the sequential

production of sulfenic, sulfinic and sulfonic acids. These reactions are discussed in the next section. Under aerobic conditions the relatively high reactivity of thiols favours their oxidation to disulfides, thereby limiting the amount of free thiols entering an organism from its surroundings. Under reducing conditions within the cell the free thiols may be regenerated from the ingested disulfide.

Captopril **1**, a compound employed in the treatment of hypertension, and penicillamine **2**, an antirheumatic agent, are both thiol-containing drugs which yield



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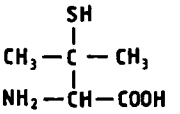

2

(A) captopril **1**, penicillamine **2**

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disulfides as major metabolites. In addition to forming disulfides with other drug molecules these compounds are capable of forming mixed disulfides where the other thiol group belongs to an endogenous compound such as cysteine or glutathione or those within the structure of proteins. It is also quite probable that these drug disulfides may readily interchange with endogenous disulfide moieties, leading to further mixed disulfide formation and drug-protein complexes. The formation of such drug conjugates with plasma proteins may be linked to the unwelcome toxicity of the above compounds.[17,18] The formation of disulfides from thiols can proceed as a purely chemical reaction where the oxidising conditions are favourable, but enzymes, generally known as thiol oxidases, can also catalyse such conversions. Two different enzyme systems have now been partially characterised. The first type was detected in bovine milk and was shown to be a non-flavoprotein containing iron which possessed a broad substrate specificity.[19] The second type, present in the cytosolic fraction of the male reproductive tract, was purified from rat seminal vesicle and was found to be a monomeric flavoprotein also possessing a wide catchment area with low molecular weight thiols such as 2-mercaptoethanol and dithiothreitol serving as substrates.[20]

Dithioic acids are the sulfur analogues of carboxylic acids and this class of compounds was amongst the earliest discovered in organosulfur chemistry.[21] Since this time a large number of dithiocarbamates and related compounds have been prepared and many have found applications in agriculture and medicine.[22] Dithioic acids are rarely encountered in nature and the only one studied in any great detail is diethyldithiocarbamic acid **3**, the pharmacologically active metabolite of tetraethylthiuram disulfide **4**. Oxidative pathways are thought to contribute to the metabolism of diethyldithiocarbamic acid[23] although these are generally minor routes of metabolism for dithioic acids, the majority undergo

extensive conjugation at the −SH group (Fig. 2). Diethyldithiocarbamic acid is itself marketed as a chelating agent employed as an antidote for heavy metal poisoning.

Disulfides (R—S—S—R′) are sulfur analogues of peroxides (R—O—O—R′) and are present in numerous xenobiotics and many components of the diet (e.g. dimethyl disulfide in onions). As previously mentioned, the major routes of metabolism for disulfides are reduction to the thiol and by oxidation, eventually yielding sulfate. In the 1930's it was shown that bread cultures of the mould *Scopulariopsis brevicaulis* caused fission of the S—S link of disulfides evolving pungent smelling thiols and alkylmethyl sulfides.[16,24] It is now known that disulfides such as diethyl disulfide and tetraethylthiuram disulfide **4** are readily reduced to ethanethiol and diethyldithiocarbamic acid **3** respectively. Tetraethylthiuram disulfide **4** is a compound employed in the treatment of chronic alcoholism and consists of two thiocarbamoyl groups attached via a disulfide bond.[22] It was developed as a result of observations made within the rubber industry, where workers exposed to the vulcanising agent, tetramethylthiuram

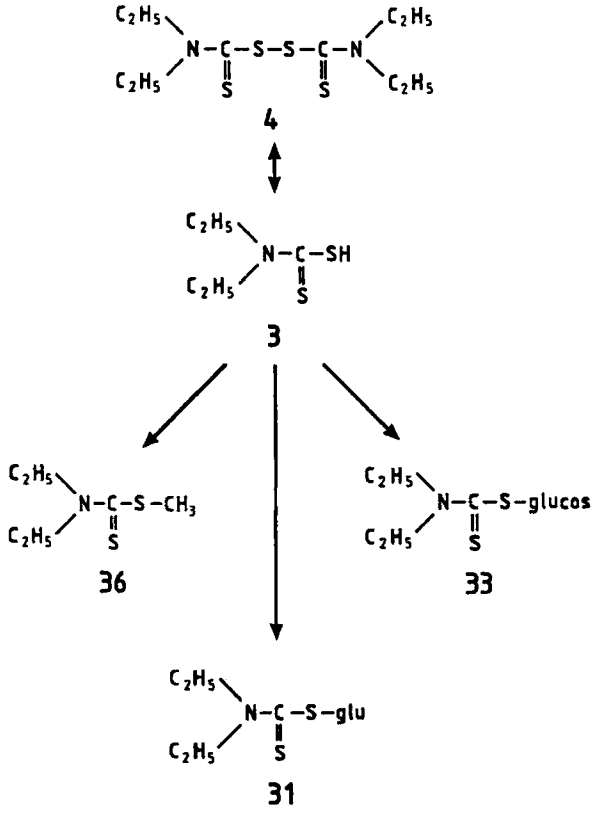

FIGURE 2. Metabolism of diethyldithiocarbamic acid.

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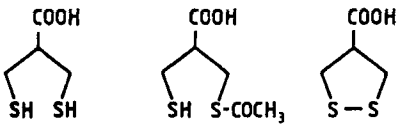
disulfide, became sensitised to alcohol. This was later shown to be due to the compound's potent inhibition of an enzyme involved in the degradation of alcohol, aldehyde dehydrogenase, thereby permitting toxic acetaldehyde to accumulate in the body. The enzymes characterizing the reduction of disulfides have not been thoroughly investigated but are thought to be microsomal thioltransferases. They are broadly specific for aliphatic monothiols and dithiols and appear to play a major role in maintaining mercaptans in the reduced state.[25,26]

Amongst the myriad of chemicals contained within the diet the sulfur-containing compounds are renowned for their organoleptic properties. Vegetables such as onions, leeks, chives and asparagus are well known for the volatile products obtained during heating or cooking which impart the characteristic smell and taste. Over a century ago investigations were conducted into the nature and origins of the strange sulfur odour emanating from the urine of certain individuals only after consuming asparagus.[27] During the following years the novel compound 3,3'-dimercaptoisobutyric acid **5** was isolated from asparagus juice[28] and its *S*-acetyl derivative **6** together with the cyclic oxidised form 1,2-dithiolane-4-carboxylic acid (asparagusic acid) **7** were later identified in asparagus shoots.[29]



5 6 7

(B) 3,3'-dimercaptoisobutyric acid **5**, *S*-acetyl derivative of **5(6)**, 1,2-dithiolane-4-carboxylic acid (asparagusic acid) **7**

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Recent investigation into the asparagus-induced urinary odour have shown that it is, in part, due to the excretion of low molecular weight sulfur compounds (methanethiol, dimethyl sulfide, dimethyl disulfide, bis(methylthio)methane, dimethyl sulfoxide, dimethyl sulfone) suggesting that in some individuals extensive degradation of asparagusic acid presumably takes place.[30] Such compounds and other related molecules are interesting examples of cyclic disulfides whose metabolism has yet to be investigated in any detail.

ADDITION AND REMOVAL OF OXYGEN

Electronic Aspects

The oxidation of sulfur as a metabolic option vis-a-vis sulfur-containing xenobiotics is now widely accepted within the field of foreign compound metabolism despite being relatively recent in its recognition.[13] The sulfide moiety is frequently encountered in xenobiotics and, together with the sulfoxide and sulfone

groups, exists within a wide range of pharmaceutical, agricultural and industrial chemicals to which man and other living creatures are exposed.

Compared to the corresponding sulfides, considerable electron density has been drained off the sulfur within sulfoxides by the addition of an electronegative oxygen atom and by ensuing orbital rehybridisation of the sulfur atom. The sulfoxide function is highly polarised and the oxygen of the S—O group can participate in hydrogen bond formation thereby permitting loose associations with other molecules.[31-34] At areas of high local concentrations, perhaps at excretory sites, the sulfoxides may associate with themselves into aggregates or clusters.[35] When a sulfide is converted to a sulfoxide the high polarity of these metabolites would be expected to promote their renal elimination and limit their access to membrane-bound enzymes which may mediate sulfone production. Conversely, the charge separation around the sulfoxide bond may lead to a stronger interaction of these molecules with the protein and lipid environments through which they travel. This may slow their overall passage through the body when compared to the movement of their corresponding sulfides.[36] The addition of an oxygen atom to a sulfide moiety clearly modifies the physiochemical properties of a molecule leading to alterations in its biological activity and potential changes in toxicity or therapeutic effectiveness.[36,37]

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With the formation of a sulfone, the weakly basic pair of sulfur electrons present in the sulfoxide become bonded to the additional oxygen. If this were viewed as coordination of the sulfur lone pair by oxygen, the net effect would be to drain electron density off the sulfur and from the existing sulfur-oxygen bond. This would cause an appreciable decrease in electron density on the sulfur in the sulfone (an increase in positive charge on the sulfur atom) with concomitant d-orbital contraction[38,39] and a decrease in the electron density on the two sulfone oxygens relative to the original sulfoxide oxygen.[40] As a result the sulfone is a poorer electron donor (weaker base) than the sulfoxide and participates less in hydrogen bond formation.[31,40] With the increased symmetry afforded to the sulfone grouping and stability contributions from resonance hybrids the sulfone function itself is relatively unreactive, which is in marked contrast to the chemical reactivity of the sulfoxides.[41] Within living systems sulfone metabolites generally assume less quantitative importance than their corresponding sulfoxides.

Just as oxygen can be added to a sulfide or sulfoxide, so can it be removed from a sulfoxide or sulfone. The sulfide, sulfoxide and sulfone groups exist as members of a series of interconvertible redox states. In some cases, as with the antitubercular compound ethionamide[42] or the non-steroidal antiinflammatory agent sulindac,[43] a redox equilibrium is set up within the body between the sulfide and sulfoxide, irrelevant of which was initially administered. Redox interconversions between the sulfoxide and sulfone are much less frequent. The electrochemical redox potential for sulfones is apparently similar to that of the corresponding sulfoxides[44] which suggests that the relative lack of reduction of sulfones may be related to the substrate specificity of reductase enzymes rather than insurmountable redox potentials.[37]

Addition of Oxygen

Within xenobiotics the thioether function may exist as part of a hydrocarbon chain (dialkyl sulfide; e.g. dimethyl sulfide), as a bridge between an aromatic ring and an alkyl group (alkyl aryl sulfide; e.g. *p*-methylthioaniline) or between two aromatic rings (diaryl sulfide; e.g. 4,4′-diaminodiphenyl sulfide), or as part of a heterocyclic structure.[45] The sulfur moieties within thiols, thiocarbamides, thiocarbamates, dithiocarbamates (dithioic acids), thioamides and disulfides existing within xenobiotic molecules may also be susceptible to oxidation (Table 2).

The industrial solvent dimethyl sulfide **8** is metabolised to dimethyl sulfone **9** in both man and other animals.[46,47] Administration of the sulfoxide **10** itself leads to the urinary excretion of the unchanged compound and smaller amounts of the sulfone.[48–50] Little degradation of these molecules takes place; demethylation is not an important pathway[51,52] and the sulfur moiety is not exhaustively oxidised to sulfate[53,54] (Fig. 3). A similar observation has been made for diethyl sulfide in dogs.[55] The ethylthiomethyl side-chain of the phosphodithioate insecticide phorate has been shown to form a sulfoxide metabolite when incubated with centrifuged extracts from soybean and bean root and also in the intact plant treated with this compound.[56] Similarly, the ethylthioethyl moiety of the anticholinesterase agent systox (an isomeric mixture of dementon-O and dementon-S) has been shown to form both a sulfoxide and a sulfone derivative in the cockroach and in the mouse.[57]

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TABLE 2. Xenobiotic structures capable of undergoing metabolic sulfoxidation.

xenobiotic moiety	chemical example	
heterocycle		
4-membered	alitame	artificial sweetener
5-membered	dibenzothiophen	industrial chemical
6-membered	phenothiazine	anthelmintic
7-membered	clothiapine	antipsychotic
sulfide	dimethyl sulfide	industrial solvent
thiol	2-pyridinethiol *N*-oxide	antiseptic
thiocarbamide	propylthiouracil	thyroid inhibitor
thiocarbamate	drepamon	herbicide
thioamide	thioacetamide	industrial chemical
(dithioic acid/dithiocarbamate)		
(disulfide)		

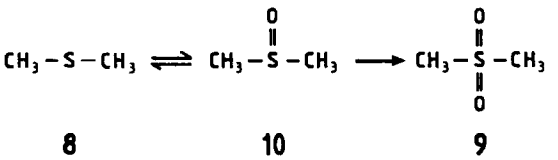


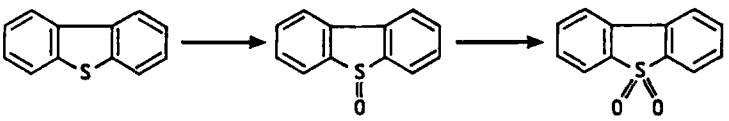
FIGURE 3. Interconversion of dimethyl sulfide, sulfoxide and sulfone.

The sulfone metabolite of the alkylaryl sulfide, *p*-methylthioaniline was shown to be formed in mice, rats and rabbits after the administration of the sulfide and was isolated from mouse urine as the *N*-acetyl sulfone derivative.[58] It was assumed, although not investigated, that the sulfoxide had been formed as an intermediate. Such an assumption would be now generally accepted. This brief article in 1948 may have been the first definite report of xenobiotic sulfone formation. Previous references to the sulfone of methylene blue (1905) must be regarded with caution as the metabolite identified may have been an *N*-demethylation product.[59] Conversion of 2,2′-dichlorodiethyl sulfide to its sulfoxide and sulfone was postulated as a requirement for the toxic action of mustard gas (1921) and investigations in rabbits with the sulfoxide derivative suggested that this was metabolised to the sulfone (1946), but neither of these claims were proven.[60,61]

The dissimilar nature of the substituents on the sulfide moiety produces a centre of asymmetry with the electron pairs on the sulfur atom in alkylaryl sulfides being prochiral (enantiotropic). The resulting sulfoxide metabolites may then exist as two optical isomers or enantiomers.[62] The asymmetric complex contained within optically active sulfoxides is destroyed by reduction or oxidation, the sulfides or sulfones so produced being optically inactive.[63]

Dibenzothiophen **11**, a molecule where the sulfur atom is contained within a five-membered ring, is converted to its sulfoxide **12** and sulfone **13** metabolites both *in vivo* and *in vitro* in the rat.[64,65] The first of these reactions has also been shown to occur in marine ecosystems[66] (Fig. 4). The relatively stable metabolic products result from the fusion of the thiophen ring to adjacent aromatic systems. The sulfoxide and sulfone of thiophen itself are highly reactive and unstable and the metabolism of this cyclic system contained within drugs is usually via oxidation at surrounding carbon atoms. Tetrahydrothiophen, which contains a reduced thiophen ring, has the properties of an aliphatic sulfide and forms stable *S*-oxidized products.

In phenothiazine **14** the sulfide linkage is contained within a six-membered heterocyclic ring and, together with an imino bridge, forms a link between two aromatic systems. Phenothiazine, an anthelminitic, is the parent molecule of a multitude of drugs which have for many years found varied and extensive use in clinical practice. During studies on the metabolism of this compound the sulfoxide metabolite **15** was identified in the blood, aqueous humour and lacrimal fluid of calves, in the plasma of young pigs and in sheep who had received large doses of the vermifuge[67] (Fig. 5). This was the first authenticated report of xeno-



11 12 13

FIGURE 4. Metabolism of dibenzothiophen.

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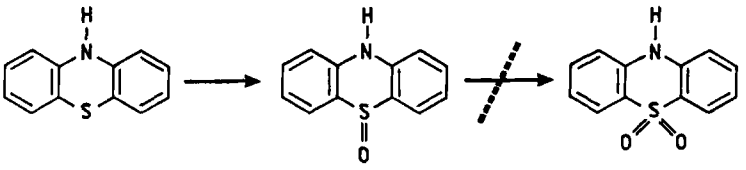

14 15

FIGURE 5. Metabolism of phenothiazine.

biotic sulfoxidation (1947) and the sulfoxide has since been observed as a urinary metabolite in many species including man (Fig. 5).[68] Ring sulfoxidation is now regarded as an important route of metabolism for this type of compound.[69] The general lack of further oxidation of phenothiazine ring sulfoxides is in agreement with other observations that strained cyclic sulfides are only oxidized as far as their sulfoxides, which are their major sulfur oxidation products. Further oxidation to their sulfones is seldom encountered. For certain more flexible acyclic sulfides (e.g. dimethyl sulfide **8**, diethyl disulfide) oxidation to the sulfone is a major pathway.[69]

The enzyme-mediated oxygenation of thiols provides unstable sulfenic acids and then sulfinic acids. Occasionally the sulfonic acid may be formed (Eq. 1). The antiseptic, 2-pyridinethiol *N*-oxide exemplifies the latter rare reaction, in that it is oxidized to the corresponding sulfonic acid after administration to rats.[70]

$$RSH \rightarrow RSOH \rightarrow RSO_2H \rightarrow RSO_3H \qquad \text{(Eq. 1)}$$

This elegant sequence of consecutive oxidations was first proposed sixty years ago (1932) after extensive chemical studies into the use of iodine as an oxidising agent.[71-73] It has been suggested more recently that the unstable sulfenic acids produced can either undergo disproportionation into sulfinic acids and thiols or react with excess thiols to form disulfides. The disulfides so generated, or other disulfide moieties within xenobiotics, can then be converted into sulfenic and sulfinic acids via the intermediate thiosulfenic and thiosulfinic acids.[74] Inference from reaction stoichiometry studies have led to the proposal of a similar sequence of reactions for dithioic acids.[75,76] However, the importance of this particular interconnected array of reactions within the biochemical situation is uncertain and their contribution to the metabolism of thiol drugs and other foreign thiols is not known.

Such extensive oxidation of the disulfide group to produce transitory sulfoxide and sulfone derivatives may simply serve to destabilise the disulfide moiety leading to S—S bond cleavage and the release of the appropriate acids. The end product of this exhaustive oxidation of thiols and disulfides is sulfate. This has been shown to occur with diallyl disulfide, a major constituent of garlic oil, where over half of the radiolabelled sulfur in this compound given to mice could be recovered as radioactive sulfate.[77] Penicillamine **2**, which readily forms a disul-

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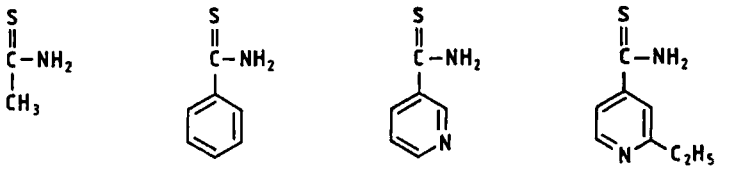
fide, is extensively degraded in man to produce inorganic sulfate which is excreted in the urine.[78,79]

Thiocarbamates (carbamothioates) also undergo *S*-oxidation. Sulfinic and sulfonic acid derivatives have been shown to be intermediate *S*-oxidation products during the metabolism of propylthiouracil in the rat[80] and 2-imidazolinyl sulfenate has been isolated and characterised as a major urinary metabolite in mice after the administration of ethylenethiourea.[81] The herbicide molinate also undergoes sulfoxidation in the rat.[82] Such reactions are not restricted to animals with drepamon, a pre-emergence herbicide, being converted to its sulfoxide and sulfone derivatives after exposure to rice and barngrass plants.[83]

Thioamides are also known to form sulfoxides. This has been shown to be the case for thioacetamide **16**,[84] thiobenzamide **17**,[85] thionicotinamide **18**[86] and ethionamide **19**.[42,87] This initial oxidation of thioamide sulfur is considered to be the first step in the formation of a highly reactive *S*,*S*-dioxide which has been implicated as being responsible for the observed toxicity of thioamides.[88]



16 **17** **18** **19**

(C) thioacetamide **16**, thiobenzamide **17**, thionicotinamide **18**, ethionamide **19**

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Enzymology of Sulfoxidation

The literature contains confusing and sometimes apparently contradictory statements concerning xenobiotic sulfoxidation and the enzymatic systems responsible. This confusion began to clear when it was realised that there were probably more than one enzyme system which could bring about what we perceive as the same reaction. However, although these reactions appear the same in their outcome, that is the addition of an oxygen to a sulfur, from the enzymatic level they may be strikingly different. The electromolecular surroundings of the sulfur moieties within the substrate molecules will dictate grossly differing environmental domains in which the sulfurs reside. These groups of domains, overlapping to varying degrees, but each with their own characteristics, are what the different enzymes recognise as their ideal sulfoxidizable substrate categories.

In simplistic terms, the microsomal cytochrome P-450 system will, as a general rule, oxidise 'carbon-like' sulfurs, that is sulfur atoms which reside within or adjacent to aromatic or heterocyclic ring systems, thereby losing partial control of their electronic shield through delocalisation. The P-450-independent microsomal flavin-containing monooxygenases (Ziegler's enzyme) favour those sulfur

atoms in aliphatic or alicyclic environments which tend to retain their electronic cover and are thus more nucleophilic in character.[13]

Several other enzyme systems undoubtedly contribute to the overall sulfoxidation of xenobiotic compounds. The cysteine oxygenases of the cell cytosol, mitochondria, and endoplasmic reticulum, enzymes whose prime function is to oxidatively degrade the thiol group of the amino acid cysteine, also sulfoxidize structurally related compounds.[89] Certain enzymes may employ drug substrates in co-oxygenation reactions where the sole function of the drug is to absorb the extra oxygen released during the reaction. For example, one oxygen atom from molecular oxygen would go to the true substrate of the enzyme system with the other surplus oxygen being discarded to the drug co-substrate. The mitochondrial prostaglandin synthetase complex can use sulfide as co-substrate reductant for the endoperoxidase precursors of prostaglandins and thromboxanes.[90,91]

Dopamine β-hydroxylase, a copper-containing monooxygenase present in a variety of mammalian tissues, is normally associated with neurotransmitter regulation where it has a crucial role in the conversion of dopamine to noradrenaline. This traditional activity of the enzyme can be corrupted, however, by substituting appropriately designed substrates were the β-methylene bridge, which occupies the site of hydroxylation in the β-phenylethylamine (phenethylamine) structure of dopamine, has been replaced by a divalent sulfur, thereby inducing the formation of a sulfoxide from a sulfide. In this manner, purified dopamine β-hydroxylase has been employed to catalyse the stereospecific sulfoxidation of phenyl 2-aminoethyl sulfide and several of its ring-substituted derivatives.[92,93]

Non-specific enzymes, such as catalase and peroxidases, which catalyse the transfer of active oxygen from hydrogen peroxide or organic peroxides to suitable acceptors may also inadvertently oxidise sulfide moieties to their corresponding sulfoxides. This mechanism has been shown to be possible for the antipsychotic agent chlorpromazine.[94]

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Removal of Oxygen

Sulfoxides can be reduced to sulfides by plants[95] and microorganisms.[96] The sulfoxide of the organophosphorus insecticide, fensulfothion, has been shown to be reduced in the bean plant[97] and the sulfoxides of disulfoton (dithiosystox) and phorate, also insecticides, are reduced to their corresponding sulfides in the soil.[98] Sulfoxide reduction also occurs in helminths.[99] Dimethyl sulfoxide **10** is metabolised to its sulfide **8** by a host of microorganisms but dimethyl sulfone **9** is not reduced (Fig. 3).[100,101] Sulindac **20** a non-steroidal antiinflammatory agent useful in the treatment of rheumatic arthritis, is a sulfoxide-containing compound which is reduced to the pharmacologically active sulfide **21** by many strains of human intestinal bacteria (Fig. 6).[102]

Sulphinpyrazone **22**, another sulfoxide-containing drug, is a compound which is used clinically in the prevention of platelet aggregation. Following oral or parenteral administration to man and other mammalian species, the sulfide **23** is

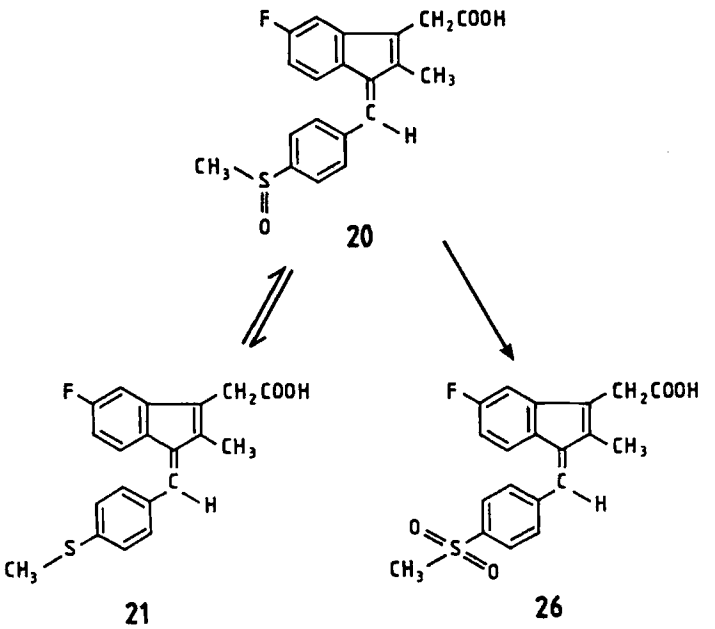

FIGURE 6. Oxidation and reduction of sulindac.

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the major circulating metabolite.[103] This is an important bioactivation pathway for this compound and it has been proposed that the gut microflora is the major site of reduction of sulphinpyrazone (Fig. 7).[104] Sulfoxide reduction also occurs in animal tissues. Sulindac is extensively reduced during incubation with rabbit liver[105] and after administration to man the coincident rapid occurrence of peak plasma levels of parent sulfoxide and sulfide metabolite, and the almost identical pharmacokinetics displayed by normal controls and ileostomy (removal of the colon) patients, confirm the involvement of the liver and/or other tissues in its reduction (Fig. 6).[106,107]

By contrast, the sulfone group is relatively resistant to metabolic reduction. Indeed certain sulfones (e.g. divinyl sulfone; 4,4′-difluoro-3,3′-dinitrophenyl sulfone) are useful as bifunctional reagents because of the inertness of the sulfone moiety joining the two reactive groups.[108] The sulfone group in dapsone, an antileprotic, and those in sulfonamides, antibacterial agents, are usually resistant to metabolic attack, these compounds being metabolised at other sites of the molecule. However, the sulfonamide moiety of 2-benzothiazole-sulfonamide **24** has been reported to be metabolised to a thiol function **25** after its intravenous administration to dogs, but this may not be via direct sulfone reduction[109–111] (Fig. 8).

Tissue studies have shown that the further oxidation of sulindac to its pharmacologically inactive sulfone **26** was irreversible and that administration of the sulfone to rats and monkeys did not result in detectable formation of sulindac.[43]

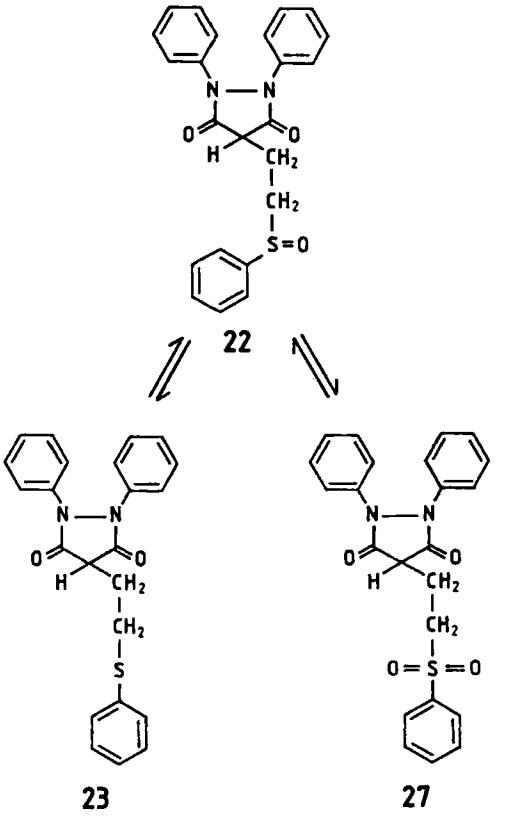

FIGURE 7. Oxidation and reduction of sulphinpyrazone.

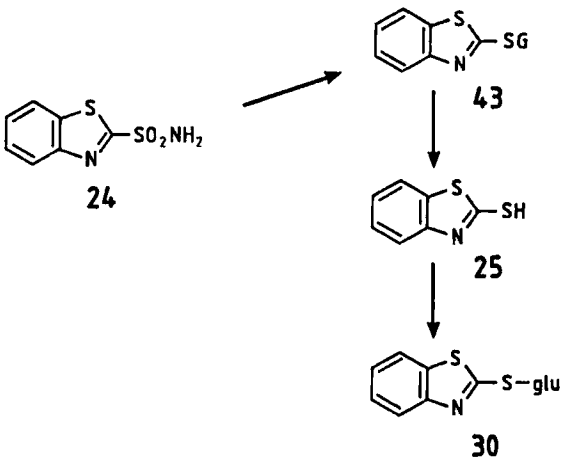

FIGURE 8. Metabolism of 2-benzothiazolesulfonamide.

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Similarly, sulfoxide or sulfide derivatives were not amongst the observed metabolites after tolmesoxide sulfone was administered to man and animals.[112] Sulfonic acids also appear resistant to reduction with many compounds, including ethanesulfonic acid and octanesulfonic acid, being excreted unchanged.[113,114]

Nevertheless, there are suggestions that sulfone reduction may occur. Plasma levels of sulphinpyrazone equivalent to a few percent reduction were observed in rats dosed with the sulfone metabolite **27**.[104] Also, the administration to rats of methyl pentachlorophenyl sulfone, the sulfone metabolite of pentachlorothioanisol, provided an array of metabolites similar to that obtained from the parent compound itself, thus suggesting extensive reduction back to the sulfoxide and sulfide.[115] However, these later metabolites, and those from 2-benzothiazolesulfonamide previously mentioned,[109-111] could have been produced by other, rather circuitous, routes of metabolism involving glutathione (Fig. 8).

Enzymology of Sulfoxide Reduction

The reduction of sulfoxides, and possibly sulfones, by mammalian tissues is a complex process which may involve both soluble (cytosol) and membrane-bound (microsomal) enzyme systems.[37] Various kinds of flavoenzymes such as NADPH-cytochrome c reductase (rat liver), NADH-cytochrome b_5 reductase (rat liver), xanthine oxidase (milk), lipoamide dehydrogenase (pig) and NADH dehydrogenase (pig) supplemented with their electron donors exhibited sulfoxide reductase activity in the presence of a soluble factor prepared from guinea pig liver cytosol.[116,117] Similar observations have been made with rat liver where the homogenates were purged with argon[118] and in helminths where sulfoxide reduction was inhibited by oxygen.[99] This may suggest that many systems, previously thought to have other functions, may be involved in sulfoxide reduction, especially when the surrounding oxygen tension is low. The existence of a thioredoxin-dependent sulfoxide reductase system has been demonstrated both in mammals and microorganisms and may have implication is terms of initial metabolism and in enterohepatic cycling.[119,120] Reduction by gut flora has also been reported[121] and the potential importance of this site of metabolic transformation must not be overlooked in the intact organism.

Exhaustive Oxidation to Sulfate

From early investigations into the metabolism and interconversion of the sulfur-containing amino acids and their essential role in the diet, it was realised that a number of compounds not normally encountered in intermediary metabolism and possessing either sulfide, sulfonium, thiol or disulfide groupings could undergo extensive degradation and their sulfur moieties could be oxidised to sulfate in the animal body.[13]

Unrelated research nad also reached similar conclusions. Ethanethiol, one of the first organosulfur compounds to be synthesized and characterised,[122,123] was shown almost a century ago to give rise to an increase in the output of urinary

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sulfate when administered to dogs.[124] Similar experiments with thioglycollic acid,[125] thiourethane,[126] thiouramil[127] and γ-thiopseudouric acid[127] all led to a similar increase in urinary sulfate. The inorganic ions, sulfide,[128,129] sulfite[130,131] and thiosulfate[132-134] as well as colloidal sulfur[135] are all oxidised to sulfate in living systems.

These observations, amidst others, firmly established the principle that the sulfur moiety of a xenobiotic can be detached, either before or after partial oxidation, and then completely oxidised in the animal body to yield sulfate, a metabolically stable and water-soluble ion.

CONJUGATION REACTIONS

Conjugation reactions involve the covalent linkage of a xenobiotic molecule through a functional group to an endogenous conjugating agent. The functional group on the foreign compound may have been introduced by previous metabolism (oxidation, reduction or hydrolysis) and the resulting conjugate is usually more water soluble than the original xenobiotic molecule, thereby enhancing its excretion from the body via the urinary system. Despite the fact that sulfur compounds exhibit a number of valency states and covalent bonding situations, very few sulfur compounds are involved in conjugation reactions. It would appear that only thiols (—SH) and dithioic acids (—CSSH) have protons which are sufficiently labile to permit their replacement by conjugating agents. Xenobiotics containing a disulfide linkage can only undergo conjugation after their initial reduction to the corresponding thiols.

Thiols and dithioic acids are rarely present in xenobiotics and are not generally created as a result of metabolism. Conjugation reactions involving these sulfur compounds include linkage with the glucuronic acid moiety (in insects and molluscs glucuronic acid is replaced by glucose). There are no references to the acetylation of xenobiotic organosulfur compounds in the literature and until recently there was no firm evidence for sulfate conjugation. Dithioic acids can undergo amino acid conjugation in plants but not in animal tissue.

S-*Glucuronidation*

Condensation with glucuronic acid is the most important conjugation mechanism and it occurs in all mammals and most vertebrates except fishes. It is only a minor pathway in the cat, however, since this species is deficient in UDP-glucuronyltransferase, the enzyme required to catalyse this reaction.

Thiophenol **28** was the first compound reported to be metabolised to its *S*-glucuronic acid conjugate. After the oral administration of benzene to rabbits, thiophenol *S*-glucuronide **29** was found in the urine (Fig. 9).[136] This was unexpected at the time but its formation has now been explained by the breakdown of the glutathione conjugate of benzene 1,2-oxide by the cysteine-β-lyase pathway. Similarly, an *S*-glucuronic acid conjugate of 2-benzothiazolesulfonamide was

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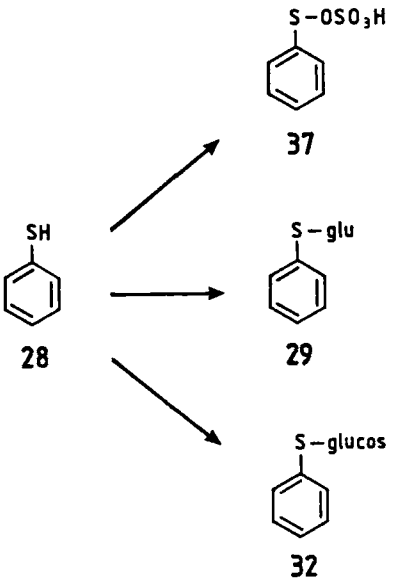

FIGURE 9. Metabolism of thiophenol.

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reported during a study which involved the evaluation of a series of carbonic anhydrase inhibitors.[110] 2-Benzothiazolesulfonamide **24** proved active *in vitro* but not *in vivo* because within the body the 2-sulfonamido group necessary for the desired biological effect was transformed to a thiol and the reduction product then eliminated via the urine as the *S*-glucuronide **30** (Fig. 8).

S-Glucuronides are also found in the metabolism of certain thiopurines and thiopyrimidines which have possible use as nucleic acid base analogues in chemotherapy. These compounds include 9-ethyl- and 9-*n*-butyl-6-mercaptopurine which are conjugated to glucuronic acid through the thiol group.[137] A β-glucuronidase-labile conjugate of 6-*n*-propyl-2-thiouracil has also been detected in small amounts in the urine, plasma and bile of rats and in the urine and plasma of man following the administration of the radiolabelled compound.[138,139] Recently, a range of *S*-glucuronides have been identified as minor metabolites arising from the breakdown of glutathione conjugates of haloaromatic compounds, mainly polychlorinated or polybrominated biphenyls.[140] In addition, the active metabolite of tetraethylthiuram disulfide **4**, the alcohol dehydrogenase inhibitor, is the dithioic acid, diethyldithiocarbamic acid **3**, which is itself metabolised to a glucuronic acid conjugate **31** (Fig. 2).[141]

The glucuronyl transferase enzymes responsible for the formation of glucuronic acid conjugates of thiophenols have been examined by comparison with those controlling the glucuronidation of phenolic hydroxyl groups. It was found that the enzymes behaved in a similar manner towards oxygen and sulfur moieties and that the two reactions exhibited the same characteristics. This suggests that

these enzymes have overlapping substrate specificities and perhaps cannot differentiate between the two elements.[142,143]

S-*Glucosidation*

In insects and other invertebrates glucoside formation replaces glucuronide formation.[144] The glucose used in the conjugation mechanism is derived from UDP-glucose and the transferase enzyme effecting the reaction is found in the microsomal fraction of the cell. It has been shown that intact gut cells and homogenates from the slug *Arion ater* carry out *S*-glucosidation of thiophenol **32** (Fig. 9) and of diethyldithiocarbamic acid **33**, (Fig. 2) in an essentially identical manner with the *O*-glucosidation of analagous substrates. In addition, it was found that the UDP-glucosyltransferase of *Arion ater* was inducible by phenobarbitone.[142,145] Studies concerning the glucosidation of thiols in insects have found that *S*-glucosidation of thiophenol and 5-thiouracil occurred *in vivo* in species of crickets and cockroaches.[146] Investigations *in vitro* showed that conjugation is effected by enzymes found in the fat bodies of the insects. The *S*-glucoside **34** of dimethyldithiocarbamate **35**, a fungicide, has also been reported to be present in plants (Fig. 10).[147]

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S-*Methylation*

Methylation is a common and important biochemical reaction encountered in intermediary metabolism which when applied to xenobiotic molecules results in a conjugate which may be less polar and less water soluble than the parent compound.

Methylation of inorganic sulfate was shown to occur when methanethiol and traces of hydrogen sulfide were produced by *Schizophylum commune* (a wood destroying fungus) which was grown on a nutrient medium containing glucose and sulfate as the only sulfur source.[148] The first reported example of metabolic methylation of a foreign thiol within a mammalian species appears to be that of

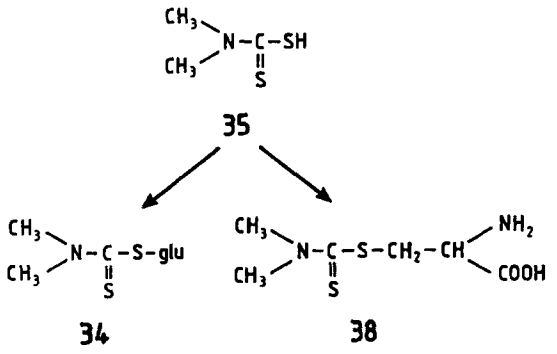

FIGURE 10. Metabolism of dimethyldithiocarbamic acid in plants.

thiouracil.[149] Following administration of this compound to rats, a small portion (8%) of the dose was recovered in the urine as an *S*-methyl conjugate. Other examples of this type of *S*-methylation, although quantitatively minor routes of biotransformation, occur with captopril **1** in the rat and man[150] and with penicillamine **2** in man.[151] The methyl ester **36** of diethyldithiocarbamic acid **3** has been isolated from the urine of rats following the administration of tetraethylthiuram disulfide **4** (Fig. 2). This was viewed as unusual and was thought to be an intermediate in a reaction sequence since the major metabolic pathway for the sulfur moiety of these compounds is exhaustive oxidation to inorganic sulfate.[152]

The nature of the enzymes catalysing the *S*-methylation of thiopurines and thiopyrimidines were amongst the first to be investigated.[153] It was shown that the methyl group transferred to the xenobiotic thiol was derived from *S*-adenosylmethionine and that the thiomethyltransferases present in the rat and mouse liver and kidney were generally non-specific. Further studies have led to the purification of a thiopurine-methyltransferase from the cytosolic fraction of human kidney and this enzyme was different from the already known thiomethyltransferases in terms of its subcellular distribution, substrate specificity and sensitivity to inhibitors.[154]

A novel pathway involved in the *S*-methylation of certain sulfides has been recently identified whereby the lone-pair of electrons in the divalent sulfur react with methyl groups to produce charged methylsulfonium ions as metabolites. The enzymes mediating these reactions are situated in the cytosol, use *S*-adenosylmethionine as the methyl donor and are known as sulfide-*S*-methyltransferases.[155,156]

It is possible that alkyl groups larger than methyl may be added to sulfur moieties. In plants, thiophenol derived from the insecticide dyfonate (fonofos), is methylated[157] and propanethiol metabolically liberated from the insecticide mocap undergoes ethylation.[158] Many more reactions involving larger alkyl groups may take place with subsequent degradation but this remains to be investigated.

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S-*Sulfation*

Until recently there was little evidence that organosulfur compounds formed sulfate conjugates. Early studies provided tentative evidence for the increase in the urinary excretion of 'ethereal sulfate' after administration of thiophenol to rabbits **37** (Fig. 9).[136,159] However, recent studies have shown that 4-nitrobenzenethiol was transformed to its *S*-sulfate conjugate by rat liver cytosol under anaerobic conditions when fortified with 3′-phosphoadenosine-5′-phosphosulfate (the biological sulfate donor) in the presence of ethylenediaminetetraacetic acid.[160] Further studies should hopefully ascertain the role of *S*-sulfation in the metabolism and excretion of xenobiotic thiols.

Thiosulfates of endogenous compounds play essential roles in intermediary metabolism. Rhodanese is an enzyme which has been implicated in the detoxi-

fication of cyanide. In the mitochondrial fraction of the liver and kidney, 3-mercaptopyruvate-sulfur-transferase catalyses the formation of persulfides from thiols. Rhodanese makes use of inorganic thiosulfate, thiosulfonates and persulfides as sulfur donors.[161] The differential substrate specificities of the two enzymes with respect to the thiols as sulfur acceptors has not been investigated but is expected to be broad.[162] The persulfides formed are labile and probably only exist transiently before they react with the thiol group of glutathione or that of other thiols in the cell.

Thioamide Formation

The dithioic acid group is the sulfur analogue of the carboxyl moiety. Generally, carboxylic acids are metabolised to glucuronic and amino acid conjugates.[163] There are many examples of thioester glucuronidation occurring in mammals but amino acid conjugation of dithioic acids in animal species has not been reported. In the potato however, it has been noted that the fungicide, dimethyldithiocarbamic acid **35**, was converted to the corresponding alanine thioamide conjugate **38**, although the major metabolite observed was the thioester glucoside **34** (Fig. 10).[164]

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MISCELLANEOUS REACTIONS

C—S Bond Cleavage

The removal of the acetylthio group from the diuretic, spironolactone **39**, to provide the non-sulfur compound, canrenone **40**, has been shown to be one of its major routes of metabolism but the details of this reaction are uncertain.[165] It is not known if the acetylthio group is cleaved as a complete unit or if the drug is first *S*-deacetylated **41**, another major route of metabolism,[166] and the remaining thiol group then removed, perhaps as methanethiol after being methylated **42** (Fig. 11).[167–170]

Ethanethiol has been shown to be liberated from a variety of thiol esters during incubation with homogenates of mouse liver and lung, and *Streptococcus faecalis* isolated from mouse gastrointestinal tract readily released ethanethiol from β-ethylthiopropionic acid and *S*-ethylcysteine.[171] The cleavage of cysteine itself and certain *S*-substituted cysteines to liberate hydrogen sulfide or the respective alkanethiol has been known for half a century.[172,173] These amine-containing sulfides are split by a 'thionase' activity found within the cytosolic fraction of the liver.[174,175] Similar C—S lyase activities have been observed in plants and bacteria, although the sulfoxides of the cysteine derivatives appeared to be the most favoured substrates.[176–179] More recent investigations have implicated an equivalent cytosolic activity, displayed by the enzyme, 'cysteine-β-lyase,' in the degradation of glutathione conjugates and the generation of toxic thiol metabolites.[180,181]

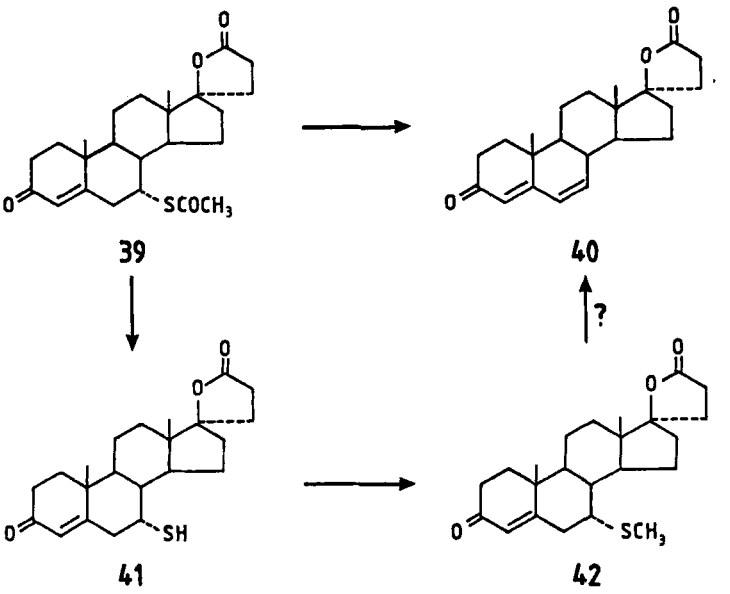

FIGURE 11. Degradation of spironolactone.

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The removal of complete *S*-alkyl groups also occurs in plants. An example of this is the metabolism of the 2-methyltriazine herbicides to 2-hydroxytriazine in peas, the suggested intermediates in this reaction were the sulfoxide and the sulfone.[182] The propylthio moiety of the insecticide mocap is enzymatically cleaved in corn and bean plants, the released fragment then forming a disulfide or being ethylated and oxidised to the sulfoxide.[158] This activity is not restricted to alkyl groups. The phenylthio group is removed during the metabolism of the fungicide, edifenphos, by animals, plants and fungi[183-185] and also during the breakdown by potatoes of dyfonate (fonofos), a cholinesterase inhibitor used as a soil insecticide.[157] The liberated thiophenol is then able to undergo methylation or oxidation to diphenyl disulfide.

The formation of a thiol metabolite from 2-benzothiazolesulfonamide with the apparent reduction of a sulfone to a sulfide is thought to occur indirectly via a glutathione intermediate **43**. Glutathione (or cysteine) reacts with the compound bringing about C—S bond cleavage and displacing the complete $—SO_2NH_2$ moiety, presumably as sulfur dioxide and ammonia. The resulting glutathione conjugate can then be metabolised to a mercapturic acid (*N*-acetylcysteine derivative) or to a thiol which is susceptible to glucuronidation (Fig. 8). Consequently, the sulfur atom within the resultant thiol group is derived from the exogenous conjugating agent and not from the original sulfonamide. This type of pathway appears to be restricted to imidazoles and thiazoles containing a 2-sulfonamido group.[109-111]

N—S Bond Cleavage

The cleavage of a xenobiotic nitrogen-sulfur bond is a rare occurrence but it has been reported for that contained within the sulfamate group of cyclohexylsulfamate **44** and 3-methylcyclopentylsulfamate.[186,187] The gut microflora is the principal and probably the only site of extensive cleavage, the enzymes within these microbes being thought to split the sulfamate by effectively adding water across the bond to produce inorganic sulfate and the resulting amine **45** (Fig. 12).[188,189] Contributions to this hydrolysis by animal tissues are thought to be negligible.

Substitution with Oxygen

A few examples exist where the sulfur moiety of a compound is metabolically replaced with oxygen. Parathion **46**, a biologically inert insecticide, depends upon metabolic activation in its target species for its effectiveness. The sulfur attached to the phosphorus atom is removed and replaced with oxygen to give the active metabolite, paraoxon **47** (Fig. 13). The livers of fish and other aquatic vertebrate species have been shown to catalyse this reaction for parathion and other related compounds such as malathion and guthion (azinphosmethyl).[190,191] Similarly, the fungicide pyrazophos can be metabolised to PO-pyrazophos, its oxygen analogue, by the fungus *Piricularia oryzae*[192] and this metabolite may also have been present in wheat leaves following pesticide treatment.[193]

Sulfur can also be replaced with oxygen when it is double-bonded to a carbon atom as well as to phosphorus. Oxidative desulfuration of the anaesthetic compound, thiopentone (pentothal), to pentobarbitone has been shown to occur in mammalian species including man.[194,195] This is also true for other related thiobarbiturates such as thiobarbitone, thialbarbitone and thiophenobarbitone which have been investigated in the rabbit.[196,197]

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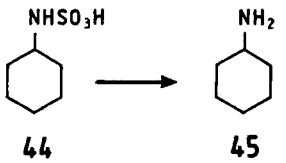
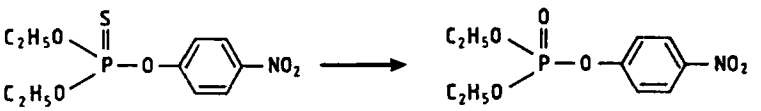


44 45

FIGURE 12. Hydrolysis of cyclohexylsulfamate (cyclamate).



46 47

FIGURE 13. Oxidative desulfuration of parathion.

CONCLUDING REMARKS

Sulfur appears to have been a prime mover in the initiation of chemical evolution. Simple hydrogen sulfide molecules probably acted as energy acceptors for long-wave ultraviolet radiation and providers of hot hydrogen atoms thereby enabling the formation of small volatile compounds and the eventual creation of complex organic molecules.[198] Such complex syntheses apparently have not been restricted to our planet alone.[199] The subsequent aggregation and coalescence of large molecules into ordered functional systems facilitated the continuum from chemical to biological evolution.

With the growth of these interdependent molecular soups, communication and coordination via (electro)chemical signalling became essential. Small reactive molecules became the messengers within living systems (e.g. H_2S, SO_2, CO_2, NO, etc.), or where permanent or continuous stationary recognition was required or where multiple assault in order to elicit a response was necessary, these small molecules were restrained and configured within a skeletal framework, usually carbon in nature, thereafter being designated as functional groups.

It is not suprising, therefore, as sulfur has been present since their inception, that living systems should have evolved a multitude of enzymes capable of catalysing chemical change to a sulfur centre existing in virtually any conceivable chemical environment. Although research into the biotransformation of sulfur within xenobiotics has lagged behind that undertaken on carbon and nitrogen centres, the evidence so far amassed suggests that this is the case. Indeed, only the sulfone group appears metabolically stable, even the exhaustively oxidised sulfate yields to the metabolic processes of plants and microbes.

Such a defensive armoury of catalytic reactions capable of chemically modifying invading xenobiotics, thereby decreasing potential metabolic activity which may deleteriously alter homeostasis and also enhancing their removal by increasing water solubility (although there are notable exceptions to both), is felicitous in light of the onslaught of sulfur-containing chemicals to which organisms are exposed. For example, the agricultural use of sulfur in 1985 accounted for 37 million tons (65% total world consumption)[200] and about one third of the organic pesticides in use today contain sulfur, often in an integral and active part of the molecule.[201,202] It is anecdotally accepted that about 10% of pharmaceutical preparations also contain sulfur, but a recent survey of a thousand substances used in medicine implied that this value may be as high as a quarter.[203] Added to the legion of sulfur compounds present in foods, the majority of which presumably have an anutrient function and are still unknown,[204,205] the total body burden of sulfur-containing compounds, consumed both deliberately and unintentionally, becomes immeasurable. Thankfully, nature through evolution has provided living systems with a chance to survive within their potentially deleterious chemical environments.

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