

**Biological Interactions of
Sulfur Compounds**

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Edited by STEVE MITCHELL

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Preface

Sulfur has fascinated people for a long time. Sulfurous smells frequently accompanied lightning, elevating it to empyrean status; a fiery means by which the wrath of the gods was manifest on earth. Indeed, the destruction of the twin cities of Sodom and Gomorrah, thought to be sited just south of the Dead Sea, was purported to be mediated by brimstone and fire, turning a once-fertile plain into a barren wilderness. Such brimstone, or '*burning stone*', may have been exploited by man in another sulfurous form, hewn from the depths of the earth. Realgar, bright red arsenic disulfide, which crumbles to yellow orpiment on exposure to light, and produces intense blue fire when oxidized with nitre, is a strong candidate for an ingredient of the mysterious 'Greek Fire' of Constantinople and the ancient world. Sulfur-containing gunpowder is more familiar and has spanned the last thousand years for fair means or foul. Wherever catastrophic events unfold and volcanoes belch forth, sulfurous fumes generated from the depths of the earth smother and poison everything in their wake, the nauseating hydrogen sulfide being comparable to cyanide in extinguishing oxygen-based life forms.

However, sulfur is not just associated with death and destruction. The purifying and beneficial properties of sulfur have been known for millennia. The Egyptians were familiar with a greasy paste containing sulfur applied for its mild antiseptic properties, and even today salves and ointments containing sulfur are still employed for topical application to the skin. During the earlier part of this century, colloidal sulfur injections were popular for the treatment of rheumatoid arthritis; even though this practice has now ceased, many sufferers today still claim relief and benefit from sulfurous spa waters. However, it is in combination with other elements, usually in organic form, that sulfur has had an impact. One-third of organic pesticides and probably a quarter of pharmaceutical products presently used contain sulfur. Ten years ago the production of organic pesticides accounted for two-thirds of the world sulfur consumption, and this is presumably rising. Given that the very life-forms upon which these compounds are designed to interact also employ sulfur in a myriad of roles including communication, defence, energy transfer and maintenance of their biological fabric, it can be appreciated that sulfur is an essential and irreplaceable part of all living systems.

Preface

So, sulfur has nurtured man and journeyed with him. From the divine and fearsome substance of the ancients, across the strange Chinese material which drained the atmosphere of its '*life-giving force*', through the mystical primary component of the alchemist's universe, to the Group 16 (VIB) element of our present scientific framework. And, when this modern post-Renaissance era changes, so too will the perception of sulfur.

This book is concerned with the biological interactions of sulfur compounds. Each contributor has taken a specific chemical combination and, with minimum intervention, has included those areas within their remit which they considered to be important. The contributors' individual styles of writing and modes of expression have been respected. Overlap has been discouraged but where it has occurred it has been because of approach from different viewpoints, making the overall text more fluid, and providing a fuller perspective of the entire problem. I thank the authors for their heroic efforts and hope that this work, with its chemically-oriented organization and wealth of referenced material, will impart information and inspire appreciation concerning the multiple roles of sulfur within our biosphere.

S. C. Mitchell
London, April 1995

Chemistry of Sulfur

J. D. WOOLLINS

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1.1 Introduction

Sulfur has been known since ancient times. The Greeks burnt sulfur to ‘purify the atmosphere’ and noted its usefulness in medicine. Brimstone was, in part, reported to be responsible for the destruction of the twin cities of Sodom and Gomorrah. From the thirteenth century, gunpowder or ‘black powder’ (made from potassium nitrate, charcoal and sulfur in 75:15:10 weight ratio) gained in importance in warfare, although it was later replaced by nitroglycerine and cordite. Metal sulfides are known amongst the earliest pigments (e.g. ultramarine). Sulfur is a minor constituent of a number of proteins and is an essential element for life; typical human consumption is ~100 mg per day. For many years the extent of the industrialization and gross national product (GNP) of the industrialized countries could be closely equated with their sulfuric acid production.

The physical properties of atomic sulfur are given in Table 1.1. The range of oxidation states for sulfur includes S(–2), S(0), S(II), S(IV) and S(VI) as summarized in Table 1.2. There is an extensive bibliography of sulfur chemistry available including entries in Beilstein, Gmelin and Mellor in addition to many

Table 1.1 Properties of atomic sulfur

Nuclide	Natural abundance (%)	Half-life	Nuclear spin
^{32}S	95.02	Stable	0
^{33}S	0.75	Stable	3/2
^{34}S	4.21	Stable	0
^{35}S	0	8.72 days (β decay)	3/2
^{36}S	0.02	Stable	0

Note: ^{33}S NMR: magnetogyric ratio = $2.0534 \times 10^7 \text{ rad T}^{-1} \text{ s}^{-1}$; quadrupole moment = $-0.05 \times 10^{-28} \text{ m}^2$; receptivity/ ^{13}C = 0.0973; reference: sulfate.

other chemistry texts (Cotton and Wilkinson 1988, Greenwood and Earnshaw 1984, Haiduc and Sowerby 1987, Nickless 1968, Woollins 1988).

Sulfur is relatively common and occurs naturally as the element in metal sulfides and in an oxidized state within sulfates such as gypsum. In addition, the sulfur compounds in natural gas and crude oil are now also becoming increasingly significant. The production of sulfur mirrors the development of chemistry. In the 1700s sulfur mining was undertaken on a steadily expanding scale. Demand for sulfur increased dramatically in the nineteenth century with volcanic-derived sulfur being the major source. The Frasch process ensured a stable supply from the turn of the century onwards. This method, used extensively in America and Iran, involves pumping superheated steam (160–170 °C) into the sulfur-rich rocks from where the molten sulfur is forced upwards using compressed air. Once pumped onto the surface the molten sulfur is allowed to cool to give solid blocks up to 18 m high. Nowadays, worldwide production of sulfur is estimated to be in excess of 60 million tonnes annually, the three main sources being the recovery from crude oil and gas, direct mining plus Frasch recovery of the element, and the roasting of metal sulfides such as pyrites. In addition, there is an increasingly large amount of sulfur obtained from sulfur dioxide in flue gases.

Qualitative and quantitative methods for the identification of sulfur compounds are well documented (Roy and Trudinger 1970, Vogel 1953) and sulfur compounds may easily be studied by vibrational spectroscopy. Both the oxides and oxyanions have characteristic spectra in the infrared (IR) range, whilst sulfanes and cyclo-sulfur compounds give good Raman spectra. The only potentially useful nuclear magnetic resonance (NMR) nucleus is ^{33}S which is quadrupolar and low in abundance (Table 1.1). Despite these difficulties, ^{33}S NMR has been used on an increasing scale in recent years and some solid-state ^{33}S NMR have even been reported (Belton *et al.* 1985, Belton and Woollins 1986, Wasylishen *et al.* 1984). Variable linewidths are observed and can be related to the symmetry of the substituents about the nucleus. Although these lines can be very broad, the chemical shift range is still useful.

1.2 Elemental sulfur

Sulfur vapour (generated from $\alpha\text{-S}_8$, HgS or electrochemically) contains a range of S_n species ($n = 1\text{--}10$). At higher temperatures significant amounts of $\text{S}_2\text{--S}_6$ are

Table 1.2 Typical oxidation states and geometries of sulfur

Oxidation number	Coordination number	Geometry	Examples
-2	2	Angular	H ₂ S
-1	2	Angular	H ₂ S ₂
0	2	Ring	S _n
I	2	Angular	S ₂ Cl ₂ , RSCl
II	2	Angular	Me ₂ S, H ₂ S
	3	Pyramidal	Me ₃ S ⁺
III	3	Angular	S ₂ O ₄ ²⁻
IV	2	Angular	SO ₂
	3	Pyramidal	SF ₃ ⁺ , OSF ₂ , SO ₃ ²⁻
	4	ψ -Trigonal-bipyramidal	SF ₄ , RSF ₃
		Tetrahedral	Me ₃ SO ⁺
V	6	Octahedral	S ₂ F ₁₀
VI	3	Trigonal-planar	SO ₃ (g)
	4	Tetrahedral	SO ₄ ²⁻ , SO ₃ (s), SO ₂ Cl ₂ , S ₂ O ₃ ²⁻
	5	Trigonal-bipyramidal	SOF ₄
	6	Octahedral	RSF ₅ , SF ₆

formed. Raman studies identified bands due to S₆–S₈, whilst ultraviolet/visible (UV/VIS) spectra have indicated that S₂ is an important constituent at high temperatures and low pressures.

Liquid sulfur is a very complex equilibrium system. For example, at its melting point it is a clear yellow colour but at its boiling point it is deep red and opaque. It undergoes viscosity changes as the temperature is raised, going through maxima at 159 and 185°C. There are no free radicals present at low temperatures but significant quantities at higher temperatures. At its melting point sulfur consists of mostly cyclo-S₈ rings; as the temperature is raised various chain-length sulfur species are formed (catena-S₈ through radical chains up to 10⁶ or more sulfur atoms).

There is a wide range of sulfur allotropes (Donohue 1974, Meyer 1965, Steudel 1982), and apart from the many different crystalline forms of S₈ several other sulfur rings have been reported. Historical names and properties of some of the common allotropes are given in Table 1.3.

Cyclo-S₈ crystallizes in orthorhombic (α) and two monoclinic (β , γ) forms. The α -form is stable up to 96°C; liquid sulfur above this temperature is usually cooled to give β -sulfur. However, if liquid sulfur is quenched the familiar plastic sulfur, believed to consist of S_n chains, is obtained. The crown structure of cyclo-octasulfur, S₈, is only one member of an homologous series of S_n ($n = 6$ –26) rings (Figure 1.1, Table 1.4). Additionally, cyclic sulfur oxides such as S₆O and S₇O₂, and mixed sulfur–selenium systems (with applications such as anti-dandruff shampoos, fireworks and polymerization inhibitors) are known.

All of the species consist (Figure 1.1) of puckered rings with variable bond angles and distances (Table 1.4). High-symmetry rings such as S₈ or S₆ have normal S–S

Table 1.3 Historically important names of allotropes of sulfur

Old and present names	Accepted name	Features
Rhombohedral sulfur Aten sulfur Engel sulfur ε -sulfur ρ -sulfur	Rhombohedral (ρ) sulfur	Crystalline cyclo-S ₆ Space group <i>R3</i>
Rhombic sulfur α -sulfur Orthorhombic sulfur Muthmann's sulfur I	Orthorhombic (α) sulfur	Crystalline cyclo-S ₈ Space group <i>Fddd</i>
Monoclinic sulfur Prismatic sulfur β -sulfur Muthmann's sulfur II	Monoclinic (β) sulfur	Crystalline cyclo-S ₈ Space group <i>P2₁/c</i>
γ -sulfur Nacreous sulfur Mother of pearl sulfur Muthmann's sulfur III Gernez's sulfur	Monoclinic (ν) sulfur	Crystalline cyclo-S ₈ Space group <i>P2₁/n</i>
Fibrous sulfur ψ -sulfur	Fibrous sulfur	Crystalline catena-S _x Space group not known
Plastic sulfur Elastic sulfur ν -sulfur η -sulfur	Mixture	Probably cyclo-S ₈ and catena-S _x
Insoluble sulfur Das sulfur	Insoluble (ω) sulfur, if crystallized	Probably catena-S X-ray pattern

Table 1.4 Molecular properties of sulfur allotropes

S _x	Colour	Space group	SS bond (pm)	Bond angles (°)	Torsional angles (°)	Melting point (°C)
S ₆	Orange-red	<i>R-3</i>	206.8	102.6	73.8	<i>d</i> > 50
γ -S ₇	Yellow	<i>P2₁/c</i>	199.8–217.5	101.9–107.4	0.4–108.8	<i>d</i> 39
δ -S ₇	Yellow	<i>P2₁/n</i>	199.5–218.2	101.5–107.5	0.3–108.0	<i>d</i> 39
α -S ₈	Yellow	<i>Fddd</i>	204.6–205.2	107.3–109.0	98.5	112.8
β -S ₈	Yellow	<i>P2₁/c</i>	204.7–205.7	105.8–108.3	96.4–101.3	119.6
γ -S ₈	Light yellow	<i>P2/c</i>	202.3–206.0	106.8–108.5	97.9–100.1	106.8
S ₁₀	Pale green	<i>C2/c</i>	203.3–207.8	103.3–110.2	75.4–123.7	<i>d</i> > 0
S ₁₂	Pale yellow	<i>Pnnm</i>	204.8–205.7	105.4–107.4	86.0–89.4	148
S ₁₈	Lemon yellow	<i>P2₁2₁2₁</i>	204.4–206.7	103.8–108.3	79.5–89.0	128 <i>m,d</i>
S ₂₀	Pale yellow	<i>Pbcn</i>	202.3–210.4	104.6–107.7	66.3–89.9	124 <i>m,d</i>

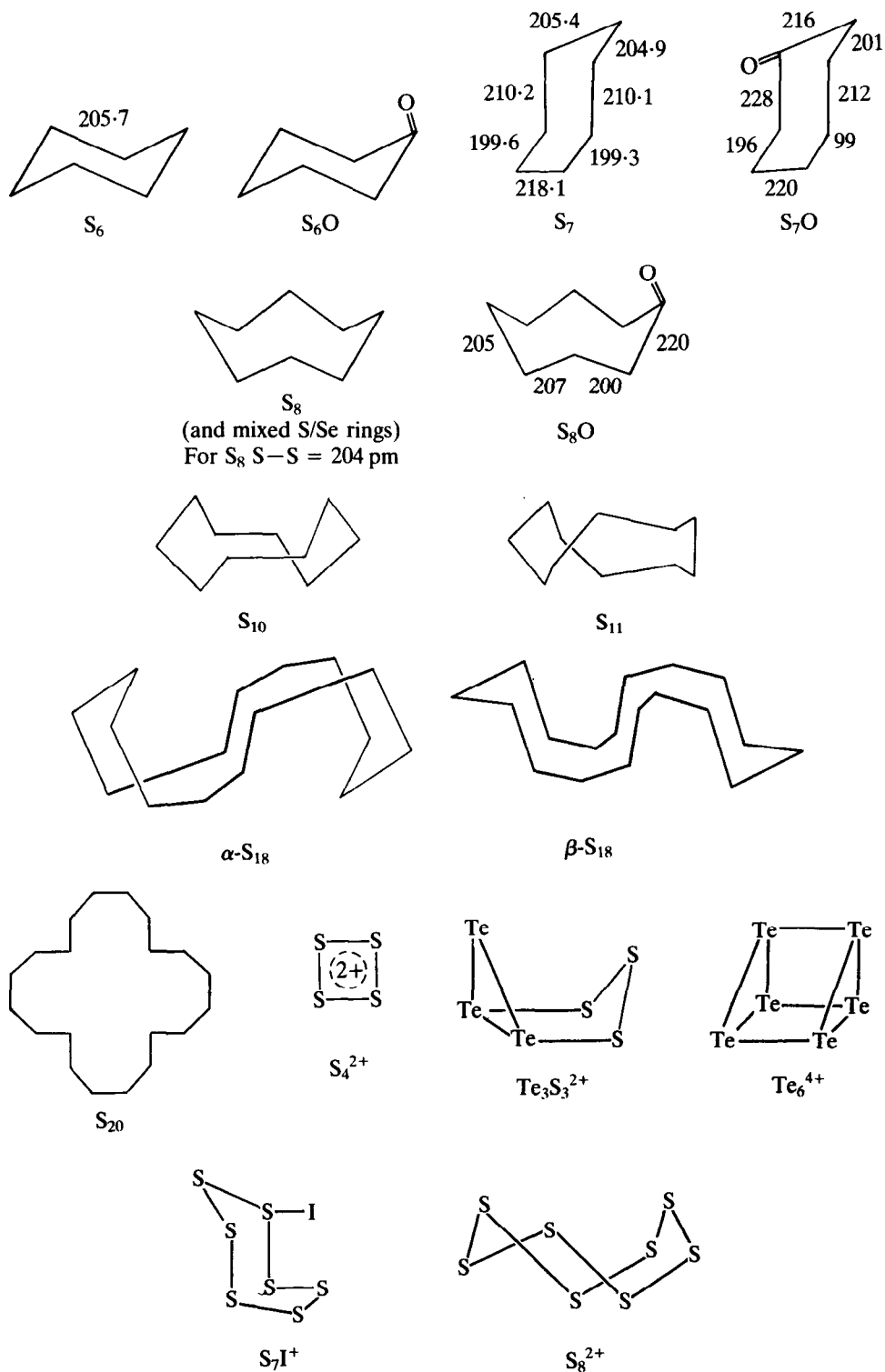


Figure 1.1 The structures of some S_n , S_n^{2+} and S_nO rings

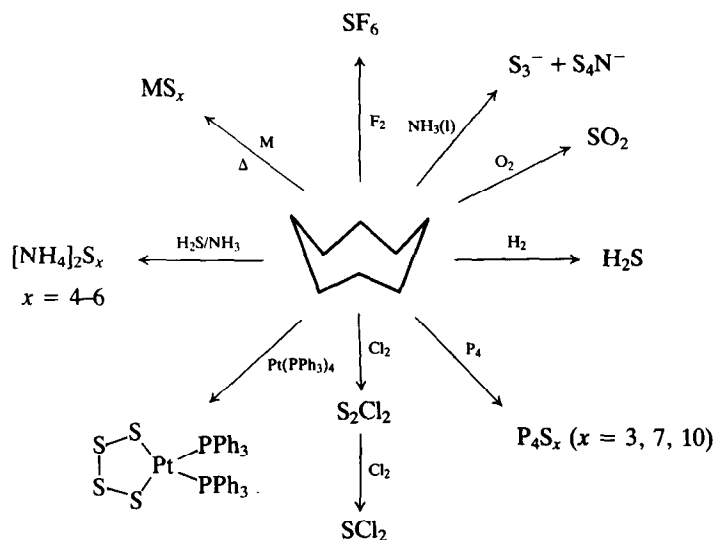


Figure 1.2 Reactions of S_8

bond distances ($\sim 2.05 \text{ \AA}$) and torsional angles ($\sim 85^\circ$) but lower symmetry species can be quite distorted. The most interesting example in this regard is S_7 which would be expected to have C_2 symmetry like cycloheptane but actually has C_s symmetry with four of the sulfur atoms in a plane and consequently one torsional angle of close to 0° . The S-S distances for the four coplanar sulfur atoms are unusually short and this may imply some π -bonding contributions.

Cyclosulfur rings are yellow or pale orange, low melting solids of variable stability. For example, S_9 decomposes above $0^\circ C$ whilst S_6 and S_7 are only moderately stable at room temperature. Their thermal stability decreases in the order S_{12}, S_{18}, S_{20} (years) $> S_6, S_9, S_{10}, S_{11}, S_{13}$ (days) $\gg S_7$ (minutes). Their interconversion reactions ($S_n \rightarrow S_m, m \neq n$) have been studied in some detail. Thermal, in addition to photochemical, reactions are possible and a number of mechanisms have been proposed. The various sulfur rings may be analysed by high-pressure liquid chromatography (HPLC) using a simple reserve-phase column and methanol as the eluent, the larger rings giving longer retention times. In addition, each ring has a very characteristic Raman spectrum which allows detection within a mixture.

Sulfur is a relatively reactive element, with the majority of reactions proceeding with cleavage of one or more S-S bonds (Figure 1.2) usually as a result of nucleophilic attack. Sulfur forms compounds by direct reaction with virtually every element (the exceptions being the noble gases, nitrogen, iodine, iridium, platinum and gold). It burns in air with a blue flame, in fluorine to give SF_6 and in chlorine to form S_2Cl_2 . Triplet sulfur (which is responsible for its blue flame) can be formed by the photochemical activation of CS_2 or COS . Sulfur is not especially reactive with water (which does not wet it) but does dissolve in liquid ammonia to give complex mixtures of anions. Cyclo- S_6 is known to be more susceptible to nucleophilic attack than is cyclo- S_8 and this feature may be utilized in organic synthesis. Oxidation reactions of sulfur rings have already been mentioned and the formation of cyclocations is discussed below.

In 1804 it was reported that sulfur reacts in oleum to give intense blue, red or yellow solutions depending upon the time of reaction and the acid concentration. Tellurium (carmine red) and selenium (green and yellow) also give rise to deeply coloured solutions in concentrated sulfuric acid. The identity of the species responsible for these colours was in doubt until quite recently. Apart from difficult synthetic chemistry, the various cations have thus far presented substantial problems theoretically, and the empirical treatments developed to explain their structure have been discussed in a review of this area (Gillespie 1979). The S_8^{2+} cation appears related to cuneane with three bonds broken and the structure of S_{19}^+ has recently been rationalized. The oxidizing power of S_n^{2+} cations has been demonstrated (Rosan 1985) and reaction of S_8^{2+} with hydrocarbons proceeds with insertion of sulfur into a C—H bond. Methane gives methanthiol, toluene is converted into a series of isomeric dimethylbiphenyls, and reaction with carbon monoxide gives COS.

1.3 Sulfur oxides

The sulfur oxides (Figure 1.3) include the simplest compounds (SO , SO_2 , SO_3) through to the very sulfur-rich species such as S_8O , although the dioxide and trioxide are undoubtedly of most commercial importance.

Sulfur monoxide, obtained by the reaction of sulfur dioxide and sulfur, has been observed in the gas phase but is very short lived (Schenk and Steudel 1965). The polymer, S_2O_2 , has a slightly longer lifetime (seconds) and disulfur monoxide (S_2O ; an orange solid), one of the decomposition products of sulfur monoxide, may also be prepared by burning sulfur under conditions of low oxygen pressure. Sulfur monoxide, disulfur monoxide and disulfur dioxide may be stabilized as ligands in metal complexes. Sulfur monoxide can coordinate in a number of modes including simple σ -donation (bent), bridging two or three metal centres (via sulfur or edge on). From X-ray crystal structural studies (Herberhold and Schmidkonz 1986, Herberhold *et al.* 1985) it appears that the coordinated S_2O ligand behaves as a π -acid as it has a substantially lengthened S—S compared to the free S_2O . The first example of an S_2O ligand was reported 20 years ago (Schmidt and Ritter 1975) and later extended to the corresponding S_2O_2 complex. A dinuclear complex of S_2O has also been prepared.

Sulfur dioxide is usually obtained commercially by the combustion of sulfur, typically by spraying fine droplets into a combustion chamber along with dry air. Both single- and two-stage combustion processes are used. Single-stage processes use an excess of oxygen/air whereas two-stage systems use ~90% of the required air in the first phase and additional oxygen in the second combustion. The two-stage system produces sulfur dioxide with virtually no contaminating nitrogen oxides. Sulfur dioxide is also prepared commercially from metal sulfide ores by roasting in fluidized bed furnaces at 650–1100°C.

Sulfur dioxide is a poisonous, colourless gas which condenses (-10°C) to give a colourless liquid and ultimately ($\sim -76^\circ\text{C}$) white crystals. It has a bent structure (S—O bond length 143.2 pm, O—S—O $\sim 119.5^\circ$ in the gas phase) and is only slightly associated in the liquid and forms an orthorhombic solid.

Sulfur dioxide is converted to sulfur trioxide and ultimately to sulfuric acid using the double-contact process which is based upon the equilibrium between sulfur

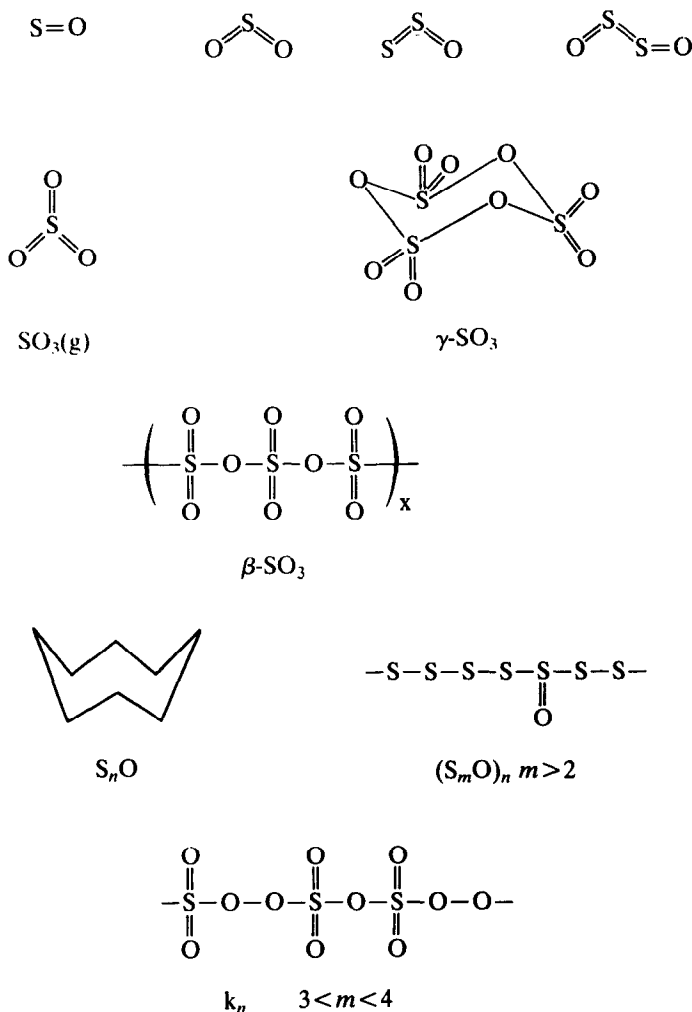


Figure 1.3 The range of sulfur oxides and the different forms of SO_3

dioxide and trioxide. Vanadium catalysts such as $\text{VO}(\text{SO}_4)$ supported on kieselguhr are used to push the equilibrium to the right-hand (SO_3) side. Since equilibrium shifts in favour of the starting materials with increasing temperature, as low a temperature as possible is employed together with the continuous removal of sulfur trioxide by absorption into water to give sulfuric acid.

The oxidation of the dioxide to the trioxide is the most important commercial reaction but there is quite an extensive chemistry of sulfur dioxide (Figure 1.4). In water SO_2 dissolves to form sulfurous acid which, according to Raman spectroscopy, contains large amounts of solvated sulfur dioxide in addition to HSO_3^- , H_3O^+ and $\text{S}_2\text{O}_5^{2+}$ ions. With ammonia and amines quite complex reactions (which are not fully understood) occur, and with tertiary amines SO_2 behaves as a Lewis acid to give simple adducts. A vast range of complexes of sulfur dioxide have now been prepared and examples of SO_2 coordination modes are given in Figure 1.5 (Mingos 1978, Ryan *et al.* 1981).

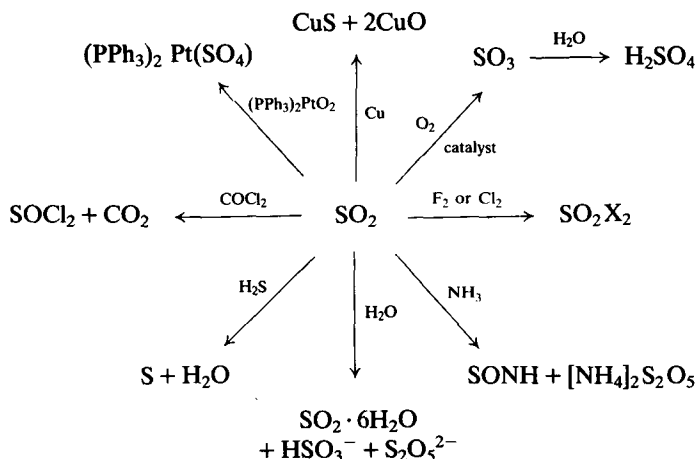


Figure 1.4 Reactions of sulfur dioxide

The environmental effects of airborne sulfur dioxide are a matter of public interest and debate (Ember 1981, Meyer 1977, Nriagu 1979). Some sulfur dioxide is released into the atmosphere from natural sources such as volcanoes but the majority (~200 million tonnes per year) is from either industrial processes or domestic use such as coal and oil burning. Two main methods for the abstraction of sulfur dioxide from flue gases can be employed: either the reduction of the dioxide to sulfur using the Claus process or alternatively neutralization using 'milk of lime' to give either calcium sulfite or, with complete oxidation, calcium sulfate (gypsum).

The commercial significance of sulfur trioxide in the preparation of sulfuric acid has already been mentioned and this is the most important aspect of its industrial chemistry. However large amounts of pure trioxide are also prepared for use in the manufacture of many compounds such as chlorosulfonic and fluorosulfonic acids (used for polishing lead crystal), thionyl chloride, and to act as a general sulfonylating agent.

Cyclic sulfur oxides S_nO ($n = 6, 8, 9, 10$) are normally prepared by oxidation of their parent sulfur rings using perfluoroacetic acid in an inert solvent such as carbon disulfide or methylene chloride, although in some cases the dioxide (e.g. S_2O_7) may also be obtained. These compounds are orange or dark yellow and decompose at or around room temperature to give sulfur and sulfur dioxide. A higher number of polymeric oxides are known and in general these are structurally related to $\beta\text{-SO}_3$ by random substitution of the oxo bridges by peroxo bridges. Monomeric SO_4 has been studied by matrix isolation and is believed to have a O_2SOO , peroxo structure.

1.4 Sulfur oxoacids

The oxoacids of sulfur are summarized in Figure 1.6. Industrially, sulfuric acid remains an extremely important material with a world production of ~150 million tonnes per year. Sulfuric acid has many applications, the largest amount being used

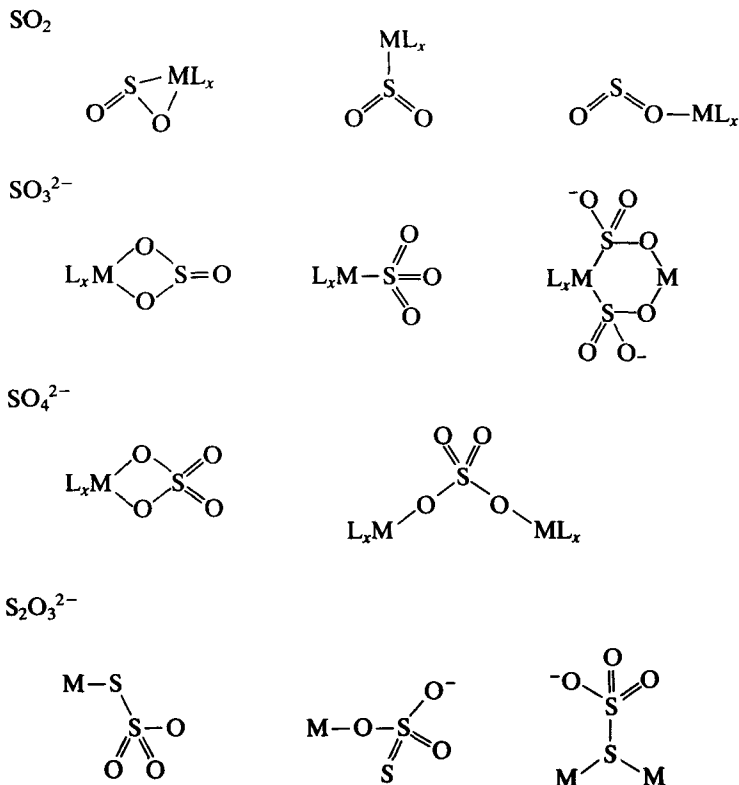


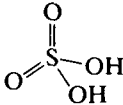
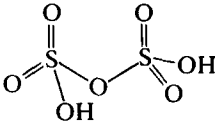
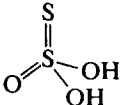
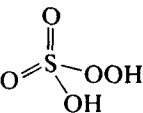
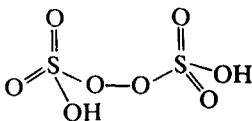
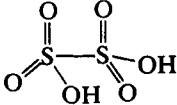
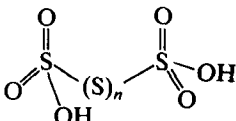
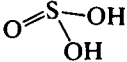
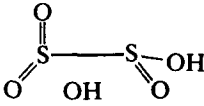
Figure 1.5 Coordination modes of SO_x ligands

in the manufacture of phosphorus- and nitrogen-based fertilizers. Sulfuric acid is also used in the manufacture of inorganic sulfates, hydrogen fluoride, boric anhydride and a range of organic compounds, and in the petrochemical industry for the refining of raw paraffin.

Aqueous sulfuric acid is a strong acid and forms salts with most metals by direct reaction and also by a variety of other routes such as neutralization of a metal oxide or hydroxide or decomposition of metal carbonates. In organic synthesis it is used with nitric acid for the nitration of aromatics via the NO_2^+ cation.

Apart from gypsum (CaSO_4) and Epsom salts ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), many other metal sulfates are known and often exist in nature. The sulfate ion is tetrahedral and has a large coordination chemistry, forming monodentate (O-coordinated) and bidentate (O,O'-chelating or O,O'-bridging) complexes (Figure 1.5).

Sulfurous acid exists in trace amounts in aqueous solutions of sulfur dioxide but has never been isolated. Nonetheless, metal sulfites are well known and stable. Sodium sulfite (obtained by reacting sulfur dioxide with sodium hydroxide) is used as a reducing agent in photography, for the preservation of foods, and in the textile and paper industries. The sulfite ion is pyramidal and displays a number of coordination modes. Interestingly, apart from monodentate coordination through sulfur and bidentate coordination through two oxygen atoms, binuclear compounds with $\text{S}=\text{O}$ coordination have recently been described (Ginn *et al.* 1993). Protonation of sulfite to give HSO_3^- appears to occur at the sulfur, rather than

Formula	Name	Oxidation states	Schematic structure ^a	Salt
H ₂ SO ₄	Sulfuric	VI		Sulfate, SO ₄ ²⁻ H-Sulfate, HOSO ₃ ⁻
H ₂ S ₂ O ₇	Disulfuric	VI		Disulfate, O ₃ SOSO ₃ ²⁻
H ₂ S ₂ O ₃	Thiosulfuric	IV, 0, (or VI, -II)		Thiosulfate, SSO ₃ ²⁻
H ₂ SO ₅	Peroxomonosulfuric	VI		Peroxomonosulfate, OOSO ₃ ²⁻
H ₂ S ₂ O ₈	Peroxodisulfuric	VI		Peroxodisulfate, O ₃ SOOSO ₃ ²⁻
H ₂ S ₂ O ₆	Dithionic ^a	V		Dithionate, O ₃ SSO ₃ ²⁻
H ₂ S _{n+2} O ₆	Polythionic	V, 0		Polythionate, O ₃ S(S) _n SO ₃ ²⁻
H ₂ SO ₃	Sulfurous ^a	IV		Sulfite, SO ₃ ²⁻ H-Sulfite, HOSO ₂ ⁻
H ₂ S ₂ O ₅	Disulfurous ^a	V, III		Disulfite, O ₃ SSO ₂ ²⁻
H ₂ S ₂ O ₄	Dithionous ^a	III		Dithionite, O ₂ SSO ₂ ²⁻

^aAcids only exist as salts.**Figure 1.6** The common oxoacids of sulfur

an oxygen, atom. The sulfite and bisulfite anions are both moderately strong reducing agents and are used in volumetric analysis.

Anhydrous thiosulfuric acid may be obtained at low temperatures and stable metal thiosulfates can be formed by the addition of sulfur to a sulfite, or the reduction of a sulfide. Structurally the thiosulfate anion is very similar to sulfate and has a range of coordination modes. Thiosulfates have a wide range of uses. Both sodium and ammonium thiosulfate are used for removing silver bromide (fixing) in photography. Silver thiosulfates may be used as flower preservatives and to inhibit ripening of fruit; it is believed that the thiosulfate disrupts the ethylene action which causes the onset of ripening. Sodium thiosulfate is also used to destroy excess chlorine (as a reducing agent) in the bleaching industry. Gold and silver thiosulfates are employed for the treatment of arthritis although the mechanism of action is not well understood (Laurence and Bennett 1992, Whitehouse and Walker 1978).

There are a range of S—O acids which contain S—S bonds reflecting, in part, the variety of oxidation states available to sulfur. Dithionic acid, $\text{H}_2\text{S}_2\text{O}_6$, can be obtained in quite concentrated aqueous solutions from barium dithionate. The metal dithionates are usually obtained by oxidizing the corresponding sulfite or metathesis. Sodium dithionate, $\text{Na}_2\text{S}_2\text{O}_4$, is well known and used as a reducing agent. There is also an emerging chemistry of the salts of the so-called polythionic acids, $\text{H}_2\text{S}_n\text{O}_6$ (Goehring and Feldmann 1948).

1.5 Sulfur hydrides

Hydrogen sulfide occurs in nature, in small concentrations, as a result of reduction of sulfate or sulfur-containing proteins by microorganisms. In crude oil and natural gas the hydrogen sulfide content can be as high as 10%. It is the only commercially important sulfane, and is used for the production of sulfides, hydrosulfides and organic sulfur compounds.

Hydrogen sulfide is usually prepared in the laboratory by the acid hydrolysis of sulfides (in the Kipp's apparatus), although industrially it is made by the reaction of hydrogen with molten sulfur at 350°C in the presence of a catalyst. The higher sulfanes, H_2S_x , are not thermodynamically stable and are obtained by other routes. Aqueous polysulfide solutions give, upon acidification, a crude oil which contains a range of polysulfanes (H_2S_x , $x = 2-6$), with H_2S_2 and H_2S_3 being obtained from this oil by careful distillation. Species with $x = 2-5$ can also be made by the acidification of K_2S_x with formic acid under anhydrous conditions (Feher and Berthold 1957) and crude polysulfanes with $x = 7-12$ are obtained in $\geq 50\%$ yield at the cathode by reduction of $\text{SO}_2/\text{H}_2\text{SO}_4$ solutions. For sulfanes with $x > 6$, systematic condensation reactions at -50°C may be employed (Feher and Berthold 1957, Feher *et al.* 1955).

The polysulfanes form an homologous series with well-defined physical properties including viscosity, molar volume and density. Hydrogen sulfide is bent with H—S—H being $\sim 90^\circ$ and H—S being 135 pm. As chain length increases the H_2S_x compounds exhibit deeper colours, indicating some delocalization of electron density. Raman spectra which are characteristic of chain length have been reported for $x = 2-8$. The spectrum of H_2S_2 suggests that this compound has a structure similar to that of H_2O_2 . The stereochemical freedom of the sulfur atom is observed

Table 1.5 Names and formulae for some common sulfide minerals

Name	Idealized formula	Name	Idealized formula
Molybdenite	MoS ₂	Cinnabar (vermillion)	HgS
Pyrite (fool's gold)	FeS ₂	Galena (Pb glance)	PbS
Marcasite	FeS ₂	Realgar	As ₄ S ₄
Pyrrhotite	Fe _{1-x} S	Orpiment	As ₂ S ₃
Chalcocite (Cu glance)	Cu ₂ S	Stibnite	Sb ₂ S ₃
Sphalerite (Zn blende)	ZnS		

in the structural chemistry associated with polysulfide (S_x^{2-}) anions (see below). Disulfide ligands (S_2^{2-}) can coordinate in a number of ways (edge on or bridging two or more central metal atoms) and larger rings can adopt a variety of geometries in the solid state and are often fluxional in solution (Burdett and Miller 1987, Draganjac and Rauchfuss 1985).

Hydrogen sulfide is a weak acid ($pK_a = 6.88$) whereas the other sulfanes are considerably stronger (for $x = 2, 3, 4$ and 5 the first dissociation constants are $5.0, 4.2, 3.8$ and 3.2 , respectively). Although they are stable under anhydrous conditions in neutral aqueous solution, the sulfanes decompose with the formation of sulfur and hydrogen sulfide.

Deprotonation of any of the sulfanes gives polysulfide anions, S_x^{2-} . Ammonium polysulfide solution, $[NH_4]_2S_x$ (mostly $x = 4$ and 5), is prepared by bubbling hydrogen sulfide through a suspension of sulfur in ammonium hydroxide. In lapis lazuli (lazurite which is made synthetically as ultramarine blue: $Na_8[Al_6Si_6O_{24}]S_x$, $x = 2-4$) the blue colour is due to the presence of S^{3-} . A range of polysulfide anions (S_x^{2-}) can also be obtained by reaction of sulfur with simple sulfides or by reaction of alkali metals with sulfur in liquid ammonia.

Many minerals exist as sulfides (Table 1.5). The alkali and alkali earth metals form simple ionic sulfides which are water soluble. Sulfides and polysulfides and their complexes have been extensively reviewed in articles on their structure and coordination chemistry (Burdett and Miller 1987, Draganjac and Rauchfuss 1985, Vahrenkamp 1975), their catalytic uses (Rakowski DuBois 1989) and their biological significance (Holm 1981, Holm *et al.* 1990, Kimura 1968, Trautwein *et al.* 1991). The monosulfide anion (S^{2-}) in particular has important coordination chemistry in biological systems (e.g. Fe-S proteins) where these cluster compounds facilitate electron transfer. The disulfide anion (S_2^{2-}) is able to bridge two or more central metal atoms and larger S_x^{2-} ligands exist, with complexes containing MS_4 and MS_5 units being well known. Perhaps the most familiar polysulfide complex is Cp_2TiS_5 .

Lightweight, high-capacity batteries based upon alkali metals and sulfur are known commercially; they consist of a solid electrolyte which separates the two liquid electrodes (molten sulfur is the anode and molten sodium the cathode). The advantage of the Na/S system is that it can store up to five times the energy of an equivalent-weight conventional battery, but they are more expensive to produce and operate at about $300^\circ C$ and so must be well insulated to maintain the molten electrodes when not in use (West 1988).

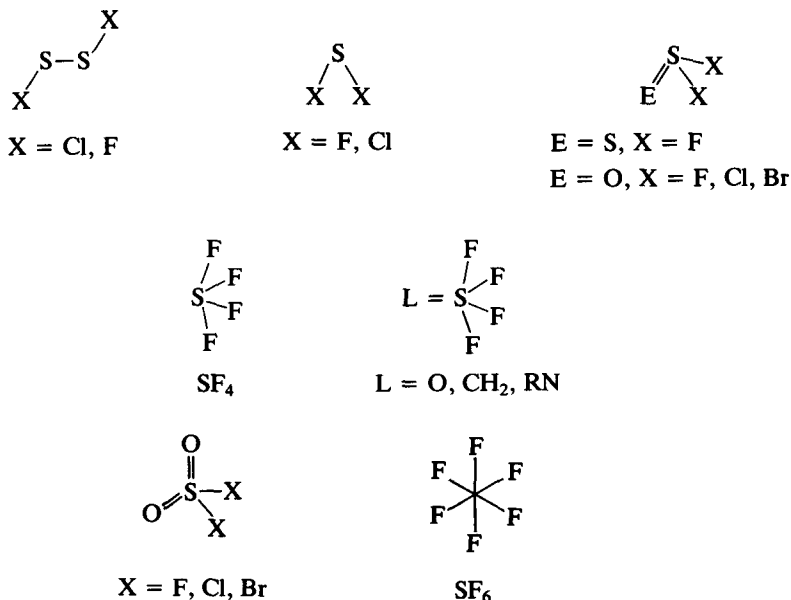


Figure 1.7 Structures of sulfur halides and oxohalides

1.6 Sulfur halides

There is an enormous range of compounds containing sulfur–halogen bonds (Figure 1.7). Here they are discussed on the basis of their oxidation states.

Apart from sulfur hexafluoride (and sulfonyl halides), the S(VI) oxidation state is also observed for SF_5R and SF_4R_2 species. All of these compounds are octahedral. Sulfur hexafluoride is obtained by burning sulfur in a fluorine atmosphere; it is a colourless, generally unreactive gas. This lack of reactivity has led to SF_6 being employed in tracer studies on flue gases from power stations and as an insulating gas in transformers. Some reactions are possible, such as treatment with sulfur trioxide to give sulfonyl fluoride, but SF_6 is not an especially useful starting material.

Disulfur decafluoride can be prepared in a similar fashion to SF_6 provided the reaction conditions are adjusted and carefully controlled. Alternatively it can be produced by photochemical means. Disulfur decafluoride is extremely toxic and much more reactive than is SF_6 . Structurally the two SF_5 groups are staggered with respect to each other and disproportionation of SF_4 and SF_6 occurs on heating. Disulfide decafluoride is a powerful oxidizing agent, reacting with ammonia to give NSF_3 , with chlorine and bromide to yield SF_5X , and with sulfur dioxide to form $\text{F}_5\text{SOSO}_2\text{F}$. There is a range of mixed halide species; SF_5Cl and SF_5Br are usually prepared by oxidation of SF_4 and are more reactive than are SF_6 or S_2F_{10} . They are both hydrolysed by aqueous alkali and the bromide will even react with water, whilst the chloride reacts both photochemically and thermally with organic olefins (Case *et al.* 1961a, b) with much of the chemistry of SF_5X compounds proceeding via free radicals ($\text{SF}_5^\cdot$).

A number of S(VI) oxohalides exist. SOF_4 (which has a trigonal-bipyramidal

structure) is obtained by fluorination of SOF_2 or by the oxidation of SF_4 , it is easily hydrolysed by water forming SO_2F_2 and reacts with ammonia to give $\text{NH}=\text{SOF}_2$. A very powerful oxidizing agent. SF_5OF undergoes facile decomposition to give free radicals which have been exploited in reactions with N_2F_4 to give SF_5ONF_2 . The sulfonyl halides, SO_2X_2 , are usually prepared directly by reaction of the halogen with sulfur dioxide. Sulfonyl bromide is not well characterized and is probably unstable at room temperature. Sulfonyl fluoride is relatively unreactive whereas sulfonyl chloride is extensively used in industry, readily dissociating into sulfur dioxide and chlorine hence its use in chlorination processes and for the introduction of SO_2Cl groups in organic chemical manufacture.

Amongst the S(IV) halides only SF_4 is stable at room temperature, although RSCl_3 compounds are known. Sulfur tetrafluoride is prepared on a laboratory scale by reaction of sulfur dichloride with sodium fluoride in acetonitrile (Tullock *et al.* 1960). It is violently hydrolysed by water to give SOF_2 and HF and great care must be exercised when using it as a reagent. It can be employed as a powerful fluorinating agent, forming adducts with main-group inorganic fluorides and converting carbonyl groups within organic molecules to difluorides. Sulfur tetrachloride is unstable at room temperature and has a postulated structure of $[\text{SCl}_3]\text{Cl}$ by analogy with phosphorus pentachloride. Organic sulfur trihalides (RSX_3 , $\text{X} = \text{Cl}, \text{F}$) are known (Brower and Douglass 1951); the chloro compounds, like SCl_4 , being less thermally stable than their fluoro analogues.

Several thionyl halides, SOX_2 ($\text{X} = \text{F}, \text{Cl}$ or Br), in addition to the mixed compound SOFCl , are known. Thionyl chloride is prepared commercially by reaction of either sulfur dioxide with chlorine and disulfur dichloride or reaction of phosphorus trichloride with disulfur dichloride. The other thionyl halides are obtained by halide exchange reaction of thionyl chloride with antimony trifluoride, sodium fluoride, potassium bromide and other appropriate compounds. The thionyl halides hydrolyse in water to give sulfur dioxide and hydrogen halide. Industrially, >50 000 tonnes per year of thionyl chloride is manufactured; it is used for the preparation of anhydrous metal halides and oxyhalides, and in organic transformations for the conversion of hydroxyl groups to chlorides. The corresponding fluoro compound has not been studied as extensively as has thionyl chloride.

The only well-established example of a S(II) halide is SCl_2 , but there are a fair number of S(I) halides. Sulfur dichloride is obtained by chlorination of S_2Cl_2 which can be readily prepared by reaction of the elements. Pure SCl_2 is difficult to obtain since it readily disproportionates to give S_2Cl_2 and Cl_2 . Industrially, S_2Cl_2 is continuously manufactured by passing chlorine into liquid sulfur (Buchner *et al.* 1989) and disulfur difluoride is prepared by reaction of sulfur with silver fluoride.

The sulfur chlorides are poisonous yellow/orange liquids. Both S_2Cl_2 and SCl_2 hydrolyse with water and burn in oxygen and are used in the manufacture of thionyl chloride and sulfur tetrafluoride, as catalysts for the chlorination of acetic acid, and in rubber vulcanization. The most notorious use of sulfur chlorides is in their reaction with ethylene during the preparation of the highly toxic 'mustard gases' (e.g. 2,2'-dichlorodiethyl sulfide). The simplicity of this reaction makes quite lethal chemical weapons available to so-called 'low technology' countries. Related to this chemistry (but not toxicity) are the sulfonyl chlorides, RSCl , which are obtained by chlorination of a disulfide (RSSR) with Cl_2 or SO_2Cl_2 . This class of compounds

has a large organic chemistry which has been extensively reviewed (Kuhle 1973, Patai 1990).

1.7 Non-metal sulfur compounds

Many main-group rings and cages containing sulfur exist (Gillespie 1979, Haiduc and Sowerby 1987, Woollins 1988) with phosphorus, nitrogen, silicon and carbon.

1.7.1 Phosphorus sulfur

There is an extensive chemistry of the phosphorus sulfides (Cotton and Wilkinson 1988, Haiduc and Sowerby 1987, Woollins 1988). Compounds such as P_4S_{10} , P_4S_7 and P_4S_3 are obtained by reaction of phosphorus with the appropriate quantity of sulfur at elevated temperatures. They are all hydrolysed by water to hydrogen sulfide and phosphoric acid (or other oxoacids), the ease of hydrolysis increasing in the order $P_4S_3 < P_4S_{10} < P_4S_7$.

Tetraphosphorus trisulfide became of importance at the turn of the century when white phosphorus in matches was banned and 'strike-anywhere' matches were made from P_4S_3 , chlorate and gum arabic. Tetraphosphorus decasulfide (also known as the pentasulfide, P_4S_{10}) is employed as a sulfur-transfer reagent in organic chemical manufacture where it readily replaces the oxygen atom in functional groups ($-\text{CO}$, $-\text{CO}_2\text{H}$, $-\text{CONH}$, $-\text{OH}$) with sulfur to give the corresponding sulfur analogue. When treated with alcohol, P_4S_{10} forms $(\text{RO})_2\text{PS}_2\text{H}$ which on neutralization with zinc oxide gives $\text{Zn}(\text{S}_2\text{P}(\text{OR})_2)_2$, a class of compound used as an anti-oxidant in engine oils (Klamann 1984).

1.7.2 Sulfur–nitrogen

There are many sulfur–nitrogen compounds (Cotton and Wilkinson 1988, Haiduc and Sowerby 1987, Woollins 1988), several of which are thermodynamically explosive and this can make their study very hazardous.

Tetrasulfur tetranitride has a cage structure with a square set of nitrogen atoms and a bisphenoid of sulfur atoms. It forms red crystals above 100°C which are thermochroic and become orange/yellow (25°C) to colourless at -190°C , and is prepared by reaction of ammonia with S_2Cl_2 or SCl_2 . It is used as a starting material in the synthesis of other sulfur–nitrogen compounds. It can be converted to disulfur dinitride, S_2N_2 , a colourless crystalline compound, by passing the vapour through silver wool or gauze. This latter compound is readily polymerized to polythiazyl, $(\text{SN})_x$, which has been known since 1910. However, the recent discovery of the metallic-type electrical conductance of this golden-bronze polymer has sparked renewed interest in this area.

Well-characterized sulfur–nitrogen anions include S_4N_5^- which is obtained from tetrasulfur tetranitride (as is S_3N_3^-) by the action of potassium or azide ions, and S_4N^- which is formed during the thermal decomposition of S_4N_5^- .

1.7.3 Sulfur–carbon/sulfur–silicon

There are many binary C–S compounds starting with CS₂ and leading through to complex charge-transfer materials. Both these and C–Si species fall outside the scope of this text. However, organic sulfur-containing systems are an essential part of many biological processes as the following chapters will reveal.

1.8 References

- BELTON, P. S. & WOOLLINS, J. D., 1986, ³⁵S and ¹⁴N nuclear magnetic resonance spectra of some sulphur–nitrogen compounds. *Magnetic Resonance in Chemistry*, **24**, 1080–1082.
- BELTON P. S., COX, I. J. & HARRIS, R. J., 1985, Experimental sulphur-33 nuclear magnetic resonance spectroscopy. *Journal of the Chemical Society, Faraday Transactions 2*, **81**, 63–75.
- BROWER, K. R. & DOUGLASS, I. B., 1951, Methylsulfur trichloride, *Journal of the American Chemical Society*, **73**, 5787–5789.
- BUCHNER, W., SCHLIEBS, R., WINTER, G. & BUCHEL, K., 1989, *Industrial Inorganic Chemistry* (translated by Terrell, D. R.), Weinheim: VCH.
- BURDETT, J. K. & MILLER, G. J., 1987, Polyhedral clusters in solids: the electronic structure of pentlandite, *Journal of the American Chemical Society*, **109**, 4081–4091.
- CASE, J. R., RAY, N. & ROBERTS, H. L., 1961a, Sulphur chloride pentafluoride: reaction with unsaturated hydrocarbons, *Journal of the Chemical Society*, 2066–2070.
- 1961b, Sulphur chloride pentafluoride: reaction with fluoro-olefins, *Journal of the Chemical Society*, 2070–2075.
- COTTON, F. A. & WILKINSON, G., 1988, *Advanced Inorganic Chemistry*, 5th Edn, pp. 402–404, 491–543, 1355–1358, New York: John Wiley.
- DONOHUE, J., 1974, *The Structures of the Elements*, New York: John Wiley.
- DRAGANJAC, M. & RAUCHFUSS, T. B., 1985, Transition metal polysulfides: coordination compounds with purely inorganic chelate ligands. *Angewandte Chemie (International Edition in English)*, **24**, 742–757.
- EMBER, L. R., 1981, Acid pollutants: hitchhikers ride the wind, *Chemical and Engineering News*, **59** (September 14), 20–31.
- FEHER, F. & BERTHOLD, R., 1957, Beiträge zur Chemie des Schwefels. XXXVI. Über die Umsetzung von definierten höheren Alkalisulfiden mit wasserfreier Ameisensäure, *Zeitschrift für Anorganische und Allgemeine Chemie*, **290**, 251–257.
- FEHER, F., LAUE, W. & KRAEMER, J., 1955, Beiträge zur Chemie des Schwefels. XXII. Die Reaktion zwischen Sulfanen H₂S_n und Chlorosulfanen S_mCl₂, *Zeitschrift für Anorganische und Allgemeine Chemie*, **281**, 151–160.
- GILLESPIE, R. J., 1979, Ring, cage, and cluster compounds of the main group elements, *Chemical Society Reviews*, **8**, 315–352.
- GINN, V. C., KELLY, P. F., PAPADIMITRIOU, C., SLAWIN, A. M. Z., WILLIAMS, D. J. & WOOLLINS, J. D., 1993, Hydrolysis reaction of [Pt(NSO)₂(PMe₂Ph)₂]. Crystal structures of [Pt(SO₃)(PMe₂Ph)₃]·H₂O and [{Pt(SO₃)(PMe₂Ph)₂]₂·O₅CHCl₃, *Journal of the Chemical Society, Dalton Transactions*, 1805–1809.
- Gmelin Handbook of Inorganic Chemistry* (Heidelberg: Springer) includes the following: Sulfur A1 (History, 1942); A2 (Occurrence, Technology, 1953); A3 (The Element, 1953); B1 (Hydrides and Oxides, 1953); B2 (Sulfur–Oxygen Acids, 1960); B3 (Compounds, 1963); Sulfur Suppl. Vol. 1 (Thionyl Halides, 1978); Sulfur Suppl. Vol. 2. (Sulfur Halides, 1978); Sulfur Suppl. Vol. 2. (Sulfur Oxides, 1980); Sulfur Suppl. Vol. 4a/b (Sulfanes, 1983); in addition to several volumes on S–N chemistry.

- GOEHRING, M. & FELDMANN, U., 1948, Neue Verfahren zur Darstellung von Kalium-pentathionat und von Kaliumhexathionat, *Zeitschrift für Anorganische und Allgemeine Chemie*, **257**, 223–226.
- GREENWOOD, N. N. & EARNSHAW, A., 1984, *Chemistry of the Elements*, Oxford: Pergamon Press.
- HAIDUC, I. & SOWERBY, D. B., 1987, *The Chemistry of Inorganic Homo and Heterocycles*, Vols 1 and 2, London: Academic Press.
- HERBERHOLD, M. & SCHMIDKONZ, B., 1986, Untersuchungen zur oxidation des dischwefel-liganded in $(\eta^5\text{-C}_5\text{Me}_5)\text{M}(\text{CO})_2\text{S}_2$ (M = Mn, Re), *Journal of Organometallic Chemistry*, **308**, 35–45.
- HERBERHOLD, M., SCHMIDKONZ, B., ZIEGLER, M. L. & ZAHN, T., 1985, Disulfur monoxide as complex ligand – preparation and molecular structure of $[(\eta^5\text{-C}_5\text{Me}_5)\text{Mn}(\text{CO})_2(\text{S}_2\text{O})]$, *Angewandte Chemie (International Edition in English)*, **24**, 515–516.
- HOLM, R. H., 1981, Metal clusters in biology: quest for a synthetic representation of the catalytic site of nitrogenase, *Chemical Society Reviews*, **10**, 455–490.
- HOLM, R. H., CIURLI, S. & WEIGEL, J. A., 1990, Subsite-specific structures and reactions in native and synthetic [4Fe–4S] cubane-type clusters, *Progress in Inorganic Chemistry (Bioinorganic Chemistry)*, **38**, 1–74.
- KIMURA, T., 1968, Biochemical aspects of iron sulfur linkage in non-heme iron proteins with special reference to “adrenodoxin”, *Structure and Bonding*, **5**, 1–40.
- KLAMANN, D., 1984, *Lubricants and Related Products*, Weinheim: VCH.
- KUHLE, E., 1973, *The Chemistry of Sulfenic Acids*, Stuttgart: Georg Thieme.
- LAURENCE, D. R. & BENNETT, P. N., 1992, *Clinical Pharmacology*, 7th Edn, pp. 222–223, Singapore: Churchill Livingstone.
- MEYER, B. (Ed.), 1965, *Elemental Sulfur, Chemistry and Physics*, New York: Wiley Interscience.
- 1977, *Sulfur, Energy and the Environment*, Amsterdam: Elsevier.
- MINGOS, D. M. P., 1978, Sulphur dioxide complexes of the platinum metals, *Transition Metal Chemistry (Weinheim)*, **3**, 1–15.
- NICKLESS, G., 1968, *Inorganic Sulfur Chemistry*, Amsterdam: Elsevier.
- NRIAGU, J. O. (Ed.), 1979, *Sulfur in the Environment*, Chichester: John Wiley.
- PATAI, S. (Ed.), 1990, *The Chemistry of Sulphenic Acids and their Derivatives*, Chichester: John Wiley.
- RAKOWSKI DUBOIS, M., 1989, Catalytic applications of transition-metal complexes with sulfide ligands, *Chemical Reviews*, **89**, 1–9.
- ROSAN, A. M., 1985, Reactivity of chalcogen cluster polycations, *Journal of the Chemical Society, Chemical Communications*, 377–378.
- ROY, A. B. & TRUDINGER, P. A., 1970, *The Biochemistry of Inorganic Compounds of Sulfur*, Cambridge: Cambridge University Press.
- RYAN, R. R., KUBAS, G. J., MOODY, D. C. & ELLER, P. G., 1981, Structure and bonding of transition metal-sulfur dioxide complexes, *Structure and Bonding (Inorganic Chemistry)*, **46**, 47–100.
- SCHENK, P. W. & STEUDEL, R., 1965, New findings in the chemistry of the lower oxides of sulfur [1], *Angewandte Chemie (International Edition in English)*, **4**, 402–409.
- SCHMIDT, G. & RITTER, G., 1975, Disulfur monoxide as complex ligand, *Angewandte Chemie (International Edition in English)*, **14**, 645–646.
- STEUDEL, R., 1982, Homocyclic sulfur molecules, *Topics in Current Chemistry*, **102**, 149–178.
- TRAUTWEIN, A. X., BILL, E., BOMINAAR, E. L. & WINKLER, H., 1991, Iron-containing proteins and related analogs – complementary Mössbauer, EPR and magnetic susceptibility studies, *Structure and Bonding (Bioinorganic Chemistry)*, **78**, 1–95.
- TULLOCK, C. W., FAWCETT, F. S., SMITH, W. C. & COFFMAN, D. D., 1960, The

- chemistry of sulfur tetrafluoride. 1. The synthesis of sulfur tetrafluoride, *Journal of the American Chemical Society*, **82**, 539–542.
- VAHRENKAMP, H., 1975, Sulfur atoms as ligands in metal complexes, *Angewandte Chemie (International Edition in English)*, **14**, 322–329.
- VOGEL, A. I., 1953, *A Textbook of Macro and Semimicro Qualitative Inorganic Analysis*, 4th Edn, London: Longman.
- WASYLISHEN, R. E., CONNOR, C. & FRIEDRICH, J. O., 1984, ^{33}S and ^{17}O nuclear magnetic resonance study of liquid sulfur dioxide, *Canadian Journal of Chemistry*, **62**, 1181–1183.
- WEST, A. R., 1988, *Basic Solid State Chemistry*, p. 325, Chichester: John Wiley.
- WHITEHOUSE, M. W. & WALKER, W. R., 1978, Copper and inflammation, *Agents and Action*, **8**, 85–90.
- WOOLLINS, J. D., 1988, *Non-Metal Rings, Cages and Clusters*, Chichester: John Wiley.

Biology of Sulfur

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2.1 The evolution of life

It is now generally accepted that living systems appeared on the earth after a long chemical evolution. Astrophysical data have indicated that sulfur took part in stellar evolution and can be detected in the interstellar medium in simple combination with other elements (H_2S , CS, NS, SO, SO_2 , SiS, HNCS, CH_2S , CH_3SH) (Herbst and Klemperer 1976, Winnewisser and Herbst 1987). The

spectral lines of our sun show no indication of sulfur whereas those from some of the hotter stars and nebulae strongly suggest its presence (Bowen 1929, Lockyer 1907). Free sulfur (1.24% by weight) was found during the chemical analysis of a meteorite which landed near Alais, France in 1806 and to a lesser extent in other heavenly bodies that have fallen to earth, although usually in combination with iron as pyrites (Roscoe 1863). From such information and the geological evidence that large quantities of sulfur are now present upon earth, it is reasonable to assume that sulfur was included early in the primitive medium during the formation of the earth but probably only as the reduced form of sulfides, notably hydrogen sulfide (Holland 1962, 1974).

An atmosphere virtually devoid of oxygen would have provided no ozone layer to filter out UV radiation and this would have been a major energy source on primitive earth (Becker *et al.* 1974, Hong *et al.* 1974). Hydrogen sulfide has been reported as an acceptor of longwave UV light (Khare and Sagan 1971, Sagan and Khare 1971) and this primary energy acceptor could, mainly through the production of hot hydrogen atoms, allow prebiological organic syntheses to occur. Conversely, hydrogen sulfide could also play a role as a free radical scavenger, inhibiting organic syntheses. The overall outcome would result from a balance of these two activities with the former abiotic syntheses prevailing at the lower concentrations of hydrogen sulfide thought to exist in primeval times (Raulin and Toupance 1977).

Experiments which have attempted to mimic the conditions on primitive earth have subjected sulfur-containing compounds (e.g. H_2S , CH_3S , NH_4S , FeS , FeS_2), mixed with other simple molecules (e.g. H_2 , N_2 , CO_2 , CH_4 , C_2H_6 , C_2H_2 , $(\text{CH}_3)_2\text{CO}$, NH_4Cl , NH_3), to excitatory stimuli which may have existed at that time (thermal, UV, X-rays, electron beams and electrical discharges). Amongst the identified products were the amino acids cysteine/cystine and methionine and other sulfur-containing compounds such as cysteic acid, cystamine, taurine, thioacetamide together with sulfides and thiols (Raulin and Toupance 1977). These compounds, once formed, can interact with the myriad of other simple non-sulfur-containing molecules generated under similar conditions. For example, it has been suggested that the prebiotic synthesis of methionine occurs via the addition of methanethiol to acrolein (Miller 1974, van Trump and Miller 1972). There are suggestions that other molecules such as thioethers and iminothioesters (ancestors of thioesters) could be formed in a similar fashion (Raulin and Toupance 1977).

Once initiated, the subsequent aggregation and coalescence of large molecules into ordered systems facilitated the continuum from chemical to biological evolution. With the growth of the interdependent molecular soups, communication and coordination via (electro)chemical signalling became essential. The small primitive 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, the small molecules were restrained and configured within a skeletal framework, usually carbon in nature, thereafter being designated as functional groups (Mitchell and Nickson 1993).

Indeed, sulfur may have played the quintessential role during the inception of life; a role usurped by oxygen some 1–2 billion (10^9) years later. 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 (Ault and Kulp 1959). 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 (Klein and Cronquist 1967, Pfennig 1967). These organisms, like the present-day green- and purple-sulfur bacteria, presumably used hydrogen sulfide rather than water as the hydrogen source for photoreduction of carbon dioxide to the carbohydrate level. Interestingly, even today such bacteria are very much a part of everyday life. *Thiobacillus concretivorus* poses continuous problems to civil engineers as it thrives on hydrogen sulfide in sewer systems, converting it into acid that rapidly disintegrates the concrete pipes into a soft paste. It is aptly named the 'concrete-eating sulfur rod'!

Ancestor tracing based on RNA sequence analysis has concluded that all living creatures originated in a sulfur-metabolizing thermophilic organism (Lake 1988, Penny 1988). These most primitive cells, the eocytes ('dawn cells'), may have lived as long as 3.8 billion years ago when sufficient thickening of the protocrust enabled a modest continental mass to form. Prior to this (Hadean period; 4.5–3.9 billion years ago), heat production from radioactive decay within the earth generated powerful convection currents throughout the mantle and effectively recycled any forming protocrust (Moorbath 1985), which resembled the dark plastic skin on a lava lake or slag floating atop molten iron in a furnace. It is interesting that similar primitive sulfur-organisms have recently been discovered surviving in remote environments not unlike those imagined in early geological times (McDuff and Edmond 1982, Tributsch 1984). These newly discovered bacteria live at very high temperatures (c. 350 °C) around geothermal vents known as 'Black Smokers', several of which have been discovered some 3500 m (>2 statute miles) down in the fracture zone of the mid-Atlantic ocean ridge (tectonic plate junction) (e.g. 'TAG', located 1985; Broken Spur, located 1993). They feed directly on sulfide chemicals in solution and support a food chain which flourishes in total darkness and extreme pressure amidst the warm water which leaks from the ocean floor.

An interesting idea exploiting sulfur-based organic growth has been proposed for the generation of potential food sources and fuel materials. The process of photosynthesis is known to be relatively inefficient, with half of the thermodynamically convertible energy (11–13%) being consumed by the life activities of the plant. Even during a favourable growing season energy conversion rarely exceeds 4% (Tributsch 1984). The oxidation of metal sulfides to sulfates is an exothermic reaction which yields $\sim 200 \text{ kcal mol}^{-1}$, the process occurring slowly in an oxygen atmosphere but more rapidly when certain bacteria are present. Indeed, those of the species *Thiobacillus ferrooxidans* are able to thrive on metal sulfides as their only energy source (Tributsch 1984, Trudinger, 1969). It has been proposed that the efficiency of energy conversion could be increased by an order of magnitude if solar radiation were employed to reduce metal sulfates to sulfides in order to grow bacterial biomass, instead of conventional trapping via photosynthesis. Unfortunately, technology is not yet available for the efficient solar thermal or solar electrochemical reduction of metal sulfates. However, much may still be learnt from the recent discoveries of bacterial ecosystems around deep ocean fumaroles which receive their energy from sulfide, apparently generated from

sulfate amidst a combination of heat and pressure (McDuff and Edmond 1982, Tributsch 1984).

2.2 Biogeochemical cycling

Sulfur is a major element for which a perfect biogeochemical cycling has evolved with intermediate exchange between the atmospheric, aquatic and terrestrial phases of the environment (Goldhaber and Kaplan 1974, Granat *et al.* 1976, Junge 1972, Trudinger 1969, Truper 1982). The primary sources of sulfur are the sulfides which are found throughout the earth's crust. These are converted to inorganic sulfate by climatic processes and by 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, and generally divalent sulfur is transformed into compounds possessing higher oxidation states and eventually to sulfate (Mitchell and Nickson 1993). This brief overview is relatively easy to understand, but the actual contributions of the different kinds of microorganisms involved and the overall global kinetics are still unclear (Kelly 1980).

Self-contained ecosystems exist in which sulfide is oxidized to sulfate by some microorganisms and then reduced back to sulfide by others (Kelly 1980). Similar cycling between sulfide and elemental sulfur is also seen. The growth of both oxidizing and reducing organisms in close physical proximity is termed a 'consortium' (Pfennig 1978a, b) and such an area where these microorganisms dwell is known as a 'sulfuretum' (Bass-Becking 1925). Sulfureta can exist in many inhospitable places (for man) such as quagmires, bogs, marshes, swamplands, wetlands, mudflats, ocean beds and volcanoes, and may even be approximated within the bowels of animals. The handful of black sludge dredged up from the outside traps of household drains brings with it the unmistakable odour of hydrogen sulfide, a miniature sulfuretum almost on the doorstep.

A key feature in all sulfureta is the existence of two zones: a lower anoxic section and an upper oxic part which has gaseous exchange with the atmosphere. Sulfide is produced in large quantities in the deeper regions where oxygen levels are extremely low, and diffuses upwards to a transition area where the higher oxygen concentrations permit oxidation to sulfate. The insertion of a silver rod into the putrefying centre of a marsh or bog enables the elegant visual demonstration of these two redox areas within a sulfuretum. The silver turns black in the lower anoxic area owing to the precipitation of silver sulfide, but near the surface it remains unchanged indicating that little sulfide actually diffuses out of the sulfuretum. The use of more elaborate methods can, of course, provide quantitative data (Huxtable 1986).

It has been proposed that, on a global scale, the trapping of oxygen in the form of sulfate which occurs within the upper regions of sulfureta is responsible for maintaining a constant atmospheric oxygen concentration undeterred by the ceaseless input of oxygen from the processes of photosynthesis. If the major oxygen sink is in fact the global sulfuretum, or the edge of it, we aerobes may simply live within the leased 'oxidized' section of an ecosystem of which the archaic and more efficient sulfur-based part is still in overall control. This is a somewhat vilifying thought (Board 1976, Postgate 1980).

2.3 The importance of sulfur compounds in living systems

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 large number of organisms and serves important structural functions in addition to providing charged anionic binding sites for movement, maintenance and regulation of appropriate charge separation (Mitchell and Nickson 1993).

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 these 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 ~ 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} (Huxtable 1986). For organisms using sulfate as a sole source of sulfur considerable energy must be invested in reducing it, whereas species which receive sulfur in the reduced state can derive appreciable amounts of energy from oxidizing it. However, within intermediary metabolism this energy may be largely wasted as the dioxygenases mainly involved in oxidizing sulfur from the -2 to $+4$ state appear not to be coupled to ATP synthesis (Huxtable 1986). 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 phosphorylation of ADP (Oshino and Chance 1975). In addition, energy released by the chemical oxidation (via bromine in pyridine) of the sulfur atom in xenobiotic sulfides (thioethers, thiolactones, thiazolidines) can be trapped via chemical systems resulting in the formation of ADP and ATP in excellent yields from AMP and Pi (Lambeth and Lardy 1969, Wieland and Aquila 1968, Wieland and Bauerlein 1967). Furthermore, it has been suggested that in living systems this process may be involved in the mechanism of oxidative phosphorylation (Lambeth and Lardy 1969). It would be intriguing if the presumed incidental oxidation of xenobiotic sulfides were not only beneficial in terms of their own potential detoxication, but also played a role in supplementing energy provision (Mitchell and Nickson 1993).

It may appear strange that both reductive and oxidative processes, opposite directions of chemical reaction, may be employed in gaining energy for microorganisms. Electron flow from an electron donor via several intermediate electron carriers towards an electron acceptor is the means by which biochemical energy is generated in most living organisms. These carriers are located in an orderly manner at and within membranes and the electron transport across these membranes gives rise to potential differences and proton-motive force, which in turn liberates energy for metabolic processes. Thus an organism needs suitable electron donors in addition to electron acceptors, and sulfur compounds act as admirable sinks for such reactions. Reduced sulfur compounds are excellent electron donors and oxidized sulfur compounds are excellent electron acceptors for a large number of microorganisms, with intermediates between sulfide and

sulfate (e.g. sulfite, thiosulfite, elemental sulfur) serving both processes dependent upon the situation (Truper 1984).

As mentioned earlier, sulfur is essential for the growth and proliferation of all living organisms, from microbes through algae and higher plants to animals. Certain sulfur compounds are ubiquitous such as the amino acids, methionine and cysteine. Other compounds may be unique, or at the present state of knowledge appear unique, to specific organisms or groups of organisms. However, a treasury of sulfur-containing molecules may still await identification, perhaps hidden amidst the bulk of the anutrient chemicals within food substances or amongst the tissue debris discarded during routine biochemical procedures. Still others, already isolated and identified, may be lurking in the background with their true roles masquerading behind erroneous assignments and misclassification. The role of sulfur compounds in biology is legion and the listing of such roles a gargantuan task. Within any classification system confusion is bound to arise as many functions overlap and many compounds perform more than one task. A complete listing, therefore, has not been attempted. Instead, three areas have been selected which will hopefully illustrate the amazing diversity of roles living systems have engineered for sulfur compounds.

2.4 The biological fabric

2.4.1 Nature and maintenance of structure

Sulfur has been found in the hooves of pigs, calves and oxen, in the feathers of birds, in sheep's wool and in the hair of rabbits, pigs, calves, horses and man. This sulfur accounted for 2.5–5.5% of the total tissue weight. Skin obtained from children contained 0.2% (by weight) sulfur, and that derived from a child who had died from sulfuric acid poisoning contained 0.15% in the unaffected areas and 0.37% in skin taken from the burned regions (Laborde 1992). In addition to the integument, sulfur is also present in other areas of the body. The amount of sulfur found in the dried organs obtained from a 39-year-old man varied from 0.57% in the brain to 1.03% in the jejunum. Other workers reported that human liver cells contained 2.41% sulfur and those from newly-born children 3.56%. Muscle tissue from various animals contained 0.86–1.33% of sulfur, the human aorta 0.47–0.67% and the human vena cava 0.28–0.73% (Schulz 1893). Overall, ~1% of man's body weight comprises sulfur and it is the eighth most abundant element in the human body after hydrogen, carbon, oxygen, nitrogen, calcium, phosphorus and potassium (Nickless 1968, Schmidt *et al.* 1975). Animals are not unique in this respect. Plant life also contains an array of sulfur compounds. Although the water content of plants accounts for 80–90% of their weight, sulfur is present in appreciable amounts, amongst the residues after combustion, with >90% of this sulfur initially in the chemical form of the sulfur-containing amino acids, methionine and cysteine (cystine).

Cysteine (cystine), in particular, is essential for the maintenance of protein systems, both in a structural and catalytic sense. The fibrous proteins, called keratins, which are major constituents of such biological tissues as hair, scales, horns, hooves, wool, beaks, nails, claws, and membranes of egg shells, owe their hardness to their high cystine content (human hair is 14% cystine).

Many proteins are cross-linked intramolecularly through the disulfide bonds of cystine. Cysteine, with its ability to dimerize via its thiol-containing sidechain, is unique amongst the amino acids in that it enables such cross-bridging to occur when built into the primary sequence of a linear polypeptide. The formation of a disulfide bridge reduces the entropy of the polypeptide chain (Florey 1956, Poland and Scheraga 1965) and when the disulfides form cross-overs, the energy contribution becomes even larger (Poland and Scheraga 1965). When all the disulfide bridges in a protein are reduced, both the structure and function are generally lost completely.

However, although disulfides clearly stabilize the final structures, all the available evidence on refolding suggests that the disulfides do not dictate the folding of the polypeptide chain, which is determined by the entire peptide sequence and subsequent total energy considerations (Chaverz and Scheraga 1980). After the protein chain has reached its optimal low-energy conformation, the disulfide bonds can increase the stability of this folded state in the fluctuating extracellular environment. Such disulfide bonds occur predominantly in extracellular proteins. Inside the cell, the thiol group of cysteine is maintained in the reduced state by glutathione, but extracellularly, in the presence of oxygen, naked thiols are unstable relative to disulfide bridges (Fahey *et al.* 1977).

In contrast to the reduced sulfur existing within cysteine, sulfate is used by living systems to form sulfate esters of polysaccharides, steroids and other organic compounds through a series of activation and transfer reactions. Algae produce sulfated polysaccharides as structural wall constituents, frequently in copious amounts. These gelatinous materials may have evolved in the algae as adaptations to periodic wetting and drying as water-conservation mechanisms, particularly problematic for benthic and terrestrial algae.

Sulfate esters of organic compounds occur within animals in the form of steroid sulfates, phenol sulfates, and polysaccharide sulfates such as chondroitin sulfate. In the higher animal, these sulfated polysaccharides are used to provide a matrix for cellular structures such as cartilage and skin (Schiff 1980). The hydration sphere surrounding a sulfate residue is important in attaining the slippery nature of such polymers and helps to maintain hydration of the skin and the ease with which joint surfaces glide over one another.

Thus, in addition to the calcium- and phosphorus-based skeletal system in vertebrates and the silicon-assisted structures in plants, sulfur plays a major role in the fabric of the organism, particularly within the softer parts of structure.

2.4.2 Mechanism of energy flow – metabolism and catalytic assistance

The reactivity of the sulfur atom, in its variety of oxidation states and within various molecular configurations, is the subject which is addressed in greater detail within the following chapters of this book. Certain bioactive substances are known to contain sulfur as their essential active component and undoubtedly more will be discovered as research progresses. If it can be summarized, these reactive molecules interface within intermediary metabolism as catalytic assistants, permitting reactions to occur more favourably by the production, stabilization and transfer of reactive intermediates and as conduits for the passage of electrons in redox situations. A few of these important molecules are described below.

2.4.2.1 Coenzyme A

Coenzyme A is biochemically constructed by a series of soluble enzymes within numerous organisms from the vitamin, pantothenic acid, the amino acid, L-cysteine and the high-energy compound, adenosine triphosphate (ATP), in the presence of magnesium ions. It is a relatively large molecule ($M_r = 767.6$) in which a 4'-phosphopantetheine moiety is attached covalently to an adenyl group. In addition to its own activities, coenzyme A is also the obligatory precursor of the 4'-phosphopantetheine portion of acyl carrier protein where this prosthetic group is bonded to a serine hydroxyl function. This acyl carrier protein is the functional unit in the fatty acid synthetase system (Elovson and Vagelos 1968). The 4'-phosphopantetheine group has also been found bound to other proteins which catalyse the activation and polymerization of amino acids to polypeptide antibiotics.

Coenzyme A serves as a carrier of activated acyl groups which may be transferred to or from amides, esters or anhydrides (Bruce and Benkovic 1966). A wide diversity of enzyme systems use coenzyme A or the acyl carrier protein and include the hydrolases, isomerases (epimerase, mutase, racemase), lyases (decarboxylase, hydratase, synthase), lygases (carboxylase, synthetase), oxidoreductases (dehydrogenase, reductase) and transferases (acetyl, acyl and carboxyltransferases) (Abiko 1975). Its role as an acyl group activator makes coenzyme A essential in the metabolism of fatty acids and carbohydrates. Other important functions of coenzyme A esters are in the formation of acetylcholine, acyl carnitines, triglycerides and other fatty substances such as lysophospholipids, phospholipids and sphingosines (Huxtable 1986).

Reactions involving coenzyme A depend on the special properties conferred on the molecule by the presence of sulfur. The thiol group of the β -mercaptoethylamine (cysteamine) moiety is the functional group that is directly involved in the enzymatic reactions. This group is esterified with acyl functions to yield a variety of thioesters. These thioesters are known as 'high-energy' compounds since they 'activate' the acyl groups and permit facile cleavage of acyl-sulfur bonds by hydrolysis or acyl transfer. The formation of thioesters also enables transformations (oxidation-reduction, hydration-dehydration, isomerization) to occur within acyl groups, as mentioned previously. The thioesters are good acylating agents because the orbital overlap between carbon and sulfur is less than that between the corresponding oxygen and carbon in oxyesters. The unpaired electrons on sulfur show little tendency for carbon-sulfur double bond formation and hence thioesters have less delocalization stability than do oxyesters. The carbon-sulfur bond of thioethers is thus longer but not stronger than the carbon-oxygen bond of oxyesters. The electrophilic nature of the thioester carbonyl carbon makes it more sensitive to nucleophilic attack and the release of thiolate ions from thioesters should be favoured.

Whilst the pantetheine thiol function has the appropriate chemical properties for activating acyl groups, this is not unique. In a number of processes, coenzyme A may be replaced, albeit with a loss of substrate affinity, by other thiols, notably N-acyl derivatives of cysteamine. The sulfur chemistry, therefore, does not by itself explain the importance of these coenzymes. Coenzyme A has many binding determinants in its large structure, especially in the nucleotide moiety which serve specificity functions in the attachment of coenzyme A esters by enzymes. In this

way anchorage points are provided which permit the 4'-phosphopantetheine portion to function as a flexible thiol-bearing arm in situations where the acyl group must have some mobility in the catalytic site (e.g. fatty acid synthesis).

2.4.2.2 *Lipoic acid*

Lipoic acid (or 1,2-dithiolane-3-valeric acid, the internal disulfide of 6,8-dithioctanoic acid) is widely distributed in living organisms where it is tightly bound to protein. The only well-defined function of lipoic acid is that of a coenzyme used in fatty acid and carbohydrate metabolism occurring in the highly organized multienzyme complexes, pyruvate dehydrogenase and α -ketoglutarate dehydrogenase (Baddiley 1955, Lipmann 1953, Reed and Cox 1966, Snell and Brown 1953).

Similar to coenzyme A, lipoate is able to form 'high energy' thioester bonds which permit acyl transfer and it assists in biological oxidations by oscillating between the reduced thiol and oxidized disulfide forms. By being attached to the lysine moiety of a protein chain it is able to swing from one active site to the next. As it moves from site to site the charge on the lipoyl moiety alters in accordance with the change in the number of ionizable thiol groups. The cycle of reactions the lipoyl group undergoes involves acyl acceptance, acyl transfer and electron transfer. Lipoic acid thereby combines two major functions of the thiol group: those of electron transfer and the generation of 'high energy' thioester bonds (Reed 1960, 1966).

The biological activity of lipoic acid depends upon the unusually high reactivity of the disulfide bond within the five-membered dithiolane ring. This increased reactivity above that observed in six-membered 1,2-dithianes or open-chain disulfides is due to the strain caused by the repulsion of the lone pair of electrons on adjacent sulfur atoms. Lipoic acid suffers around 14 kJ mol^{-1} of strain in the disulfide ring as it is constrained in an envelope conformation with the 'flap' angled at some 26° from the plane of the ring compared to the dihedral angle in open-chain disulfides of $\sim 90^\circ$ (Foss and Tjomsland 1958, Sunner 1955). This results in a pale-yellow hue for lipoic acid as opposed to the lack of colour observed from other disulfides (Barttrop *et al.* 1954, Calvin 1954).

2.4.2.3 *Thiamine*

Thiamine (vitamin B₁) occurs in mammals as a mixture of the free form and the monophosphate, diphosphate (major form) and triphosphate derivatives (Sable and Gubler 1982). It functions in nonredox enzyme reactions that involve proton transfer, usually aided by simple acid-base catalysis. It is a coenzyme for reactions that make or break carbon-carbon bonds; such reactions include aldol condensation and both oxidative and nonoxidative decarboxylations.

Thiamine pyrophosphate behaves as a nucleophile, activating a carbonyl group for further reaction. The adduct formed can stabilize a charge either on the oxygen or, via a tautomeric shift, on the carbon. Thiamine, in general, acts as an electron source and an electron sink and the versatility of electron shifts allowed by the thiazolium nucleus permits the smooth completion of the overall reactions. The sulfur itself is not directly involved in these catalytic machinations. However, if the sulfur atom is replaced by a nitrogen, the heterocyclic ring remains too acidic.

The diazo ring is pseudo-aromatic, whereas the sulfur-containing heterocycle is not. Consequently, the sulfur is essential in maintaining the electron distribution and flow necessary for successful biological activity (Huxtable 1986).

2.4.2.4 Iron-sulfur clusters

Microorganisms are able to metabolize sulfur compounds in many different ways which involve either oxidation or reduction, or both. A means of electron shuttling is therefore necessary for these reactions to occur and this need is fulfilled by the electron transfer proteins. Some examples of such electron carrier proteins are the cytochromes, sirohemes, and nonheme iron-sulfur proteins, like rubredoxin (containing one iron atom) and ferredoxins or high-potential iron-sulfur protein (HIP) containing iron clusters (Cotton and Wilkinson 1988, Fischer 1984).

Rubredoxins are relatively low molecular weight proteins ($M_r \approx 6000$) found in anaerobic bacteria which participate in biological redox reactions. From investigations into rubredoxin from the bacterium, *Clostridium pasturianum*, it is generally accepted that the one or more active centres consist of an iron atom (normally Fe^{3+}) surrounded by a distorted tetrahedron of four cysteinyl sulfur atoms serving as ligands. Bacterial ferredoxins and high-potential iron-sulfur proteins (HIP) are iron-sulfur proteins ($M_r = 6000\text{--}12\,000$) with an equal number of nonheme iron and acid-labile sulfur atoms; they function mainly as electron carriers. The proteins may contain two or four iron atoms in single or twin clusters. Ferredoxins normally have low midpoint redox potentials whereas HIPs have very positive redox potentials ($E_0 = +350\text{ mV}$) (Cotton and Wilkinson 1988, Sweeney and Rabinowitz 1980, Ueyama and Nakamura 1992).

2.5 Maintenance of integrity

To remain alive and functioning at optimum efficiency is a constant battle and many organisms fail to achieve this state. However, many manage to survive despite the perpetual barrage of injurious conditions which range from the biochemical level of highly-reactive species leaking from the energetically dangerous processes of intermediary metabolism or generated from unwanted xenobiotics to the ever-present threat of dissolution, disintegration and wholesale digestion by external parasites and predators. Damage limitation and subsequent repair are essential features of successful living organisms and a number of sulfur-containing molecules have evolved which are capable of assisting these processes.

2.5.1 Internal defence

Several molecules exist within the body which are able to react with and offset the damage that would otherwise be caused by toxic chemicals and metabolically generated reactive species. Glutathione and metallothioneine have been chosen as two examples to discuss in greater detail.

2.5.1.1 Glutathione

The tripeptide, glutathione (γ -glutamylcysteinyl glycine), is the most ubiquitous and abundant of the nonprotein thiols which is present in high concentrations (c.

5 mM) in plants, aerobic bacteria and many mammalian tissues (Colowick *et al.* 1954, Crook 1959, Larsson *et al.* 1983, Meister and Anderson 1983). The γ -glutamyl linkage imparts to the molecule resistance to the normal peptidases found in the cell. The thiol function which is located on the cysteine residue has a redox potential of ~ -230 mV which makes it the primary nucleophile in the cell, capable of transferring electrons to a variety of molecules including cellular constituents in addition to electrophilic xenobiotics (Huxtable 1986). The glutathione–glutathione disulfide interconversion forms the major thiol redox system within the cell and it is actively involved in the maintenance of cell redox state, this in turn being a modulator of many biochemical processes.

Glutathione is capable of participating in both one- and two-electron reactions. The one-electron events result in the scavenging of most carbon-, nitrogen-, and oxygen-centred radicals through hydrogen abstraction. The generated thiyl radical within the glutathione molecule in turn reacting with another thiyl radical to form glutathione disulfide.

As a result of living in an oxygen-rich environment, organisms encounter many types of reactive oxygen species including ozone, singlet oxygen and superoxide. These highly reactive oxygen species can initiate deleterious reactions with biological molecules through one-electron or free radical mechanisms. The result is the generation of reactive peroxides such as hydrogen peroxide or organic hydroperoxides, which can lead to cell damage and death. Organic hydroperoxides are also generated via reactive oxygen species during the normal processes of intermediary metabolism, and can be produced during the metabolism of a wide variety of xenobiotic molecules (Huxtable 1986). Glutathione fulfils a laudable role in preferentially reacting with such free radicals and preventing subsequent toxic injury.

The two-electron processes involve nucleophilic displacement and addition reactions and may well proceed via a thiolate ion, as this species is more nucleophilic and hence more reactive. Such reactions with halide-containing xenobiotics are well known and usually result in the urinary excretion of N-acetylcysteine conjugates of the xenobiotic, the glutathione moiety being degraded during its passage through the body. However, conjugation with glutathione may not always lead to a reduction in toxicity, as has been demonstrated with hexachlorobutadiene and trichloroethylene (Timbrell 1991).

2.5.1.2 Metallothionein

Metallothioneins (metallothioneins) are low-molecular-weight proteins which are found in all organs but in highest concentrations in the kidney and liver. They contain a large proportion of sulfur with $\sim 30\%$ of the total amino acid complement being cysteine residues. Aromatic amino acids are absent (Suzuki 1982).

The physiological function of metallothioneins appears to be in storage and regulation of the availability of zinc which is required by other apoenzymes within the body. Heavy metal ions, such as cadmium and mercury, are able to exchange with the chelated zinc and may themselves become avidly bound to the thiols within the protein (Boulanger *et al.* 1983, Otvos and Armitage 1980). In this way metallothionein can serve a protective function in offsetting the potential toxicity of the heavy metal ions. Indeed, it has been suggested that excess platinum ions which arise from the administration of platinum complexes during the treatment of

tumours may be detoxified by metallothionein. Repeated or constant low-level exposure to heavy metals such as cadmium has been reported to increase the amount of metallothionein available for binding, especially within the kidney (Suzuki 1982). However, this may be an accumulation of metallothionein perhaps stabilized by cadmium chelation and not available for normal metabolic turnover.

2.5.2 External defence

Many organosulfur natural products are known to have pesticidal activity (and more than one-third of the total number of synthetic organic pesticides contain sulfur). Many plants have evolved with such compounds being able to fulfil a protective role. For example, the turnip, cabbage, radish and several other plants produce 2-phenylethylisothiocyanate and related compounds which cause sharp irritating odours and blistering of the skin whilst possessing insecticidal and antibiotic properties which protect the plant in its constant war against parasitic invaders. (Lichtenstein 1966, Robinson 1963). The polycyclic antibiotics such as bacitracin, gliotoxin, cephalosporin and penicillin produced by microorganisms also provide protection within their own environments and can be exploited by man to ward off microbial infection. A marine annelid worm (*Lumbrineris heteropoda*) produces 4-N,N-dimethyl-amino-1,2-dithiolane (nereistoxin) which is fatal to predatory insects (Sakai and Sato 1972).

Many other compounds, some of which have been assigned apparently trivial functions such as taste and smell, undoubtedly have more important roles to play. For example, asparagusic acid (1,2-dithiolane-4-carboxylic acid) and its derivatives, which are structurally related to the cofactor, lipoic acid (thioctic acid), and which impart the characteristic flavour and aroma to the vegetable, have been implicated in the role of defence chemicals. Asparagusic acid, present in concentrations of ≤ 35 ppm in the roots of asparagus (*Asparagus officinalis*), is active against plant parasitic nematodes and is considered a major factor in the survival of the plant, preventing the entry of invading organisms into the root tissues (Takasugi *et al.* 1975). *Trans*-(+)-S-(1-propenyl)-L-cysteine sulfoxide which is present in the intact cells of onions in an odourless combined form (e.g. as γ -glutamyl peptides) is converted to volatile lachramatory substances (isomeric propanethial S-oxides) via rearrangement of the intermediate 1-propene sulfenic acid by the enzyme, alliinase, which is released when the cells are ruptured (Block *et al.* 1979). This well-known phenomenon which causes us to cry whilst slicing onions may have a more important role in the plant of deterring potential predators once initial injury to the tissue has taken place (Boelens *et al.* 1971). Similarly, the odourless alliin (S-allyl-L-cysteine sulfoxide), a major constituent of garlic, is broken down enzymatically to allicin when the bulb is damaged. This powerfully odorous compound is bacteriocidal, providing immediate defence at the site of tissue injury (Block and O'Connor 1974). Larvicidal activity is also bestowed upon garlic oil by the presence of diallyl disulfide and diallyl trisulfide (Amonkar and Banerji 1971).

2.6 Communication

Communication between cells and tissues has been essential for multicellular growth. The construction of a neural network, thus allowing rapid response, has

been one of the factors which has enabled species to evolve into relatively efficient biological machines. Communication between species has permitted existence in relatively ordered social groupings, hence the importance of being able to recognize like species with which they should be safe and can mate and distinguish foreign creatures which may be harmful. Sulfur compounds are again prevalent amongst the chemicals employed for these purposes.

2.6.1 Neurocommunication

Amino acids have been implicated as neurotransmitters in the central nervous system (CNS) for many years (Balderessarini and Karobath 1973, Curtis and Watkins 1960, Hebb 1970, De Robertis 1971) although glutamate, aspartate and glycine are perhaps the most familiar (Coyle *et al.* 1977, Iversen 1978; Krogsgaard-Larsen and Honore 1983). However, the sulfoxidized products of cysteine ($-\text{SH}$), cysteinesulfinic acid ($-\text{SO}_2\text{H}$) and cysteic acid ($-\text{SO}_3\text{H}$), have been shown to be able to excite neurones when administered by microiontophoresis into mammalian brains (Curtis and Watkins 1960, 1963, Curtis *et al.* 1960, 1961) and have been proposed as neurotransmitters (Griffiths 1990, Iwata and Baba 1983). This is a role that these substances may well play provided that sufficiently high local concentrations can be achieved in an intermittent manner.

Indeed, it has been demonstrated that release of cysteinesulfinic acid from preloaded rat brain cortical slices and crude synaptosomal fractions was partially calcium dependent (Baba *et al.* 1983, Iwata *et al.* 1982). Demonstration of a depolarization-induced, calcium-dependent release would support the hypothesis that these compounds are able to act as neurotransmitters. It has also been shown that a high-affinity uptake system exists for cysteinesulfinic acid in neuronal and glial primary cell cultures and cell lines (Abele *et al.* 1983) and in synaptosomes (Grieve *et al.* 1990, 1991, Iwata *et al.* 1982, Recasens *et al.* 1982) where cysteic acid is also imported (Wilson and Pastuszko 1986). The presence of distinct carriers for excitatory sulfur amino acids in the plasma membranes of both neurones and astrocytes has recently been established (Grieve *et al.* 1991) in alignment with current ideas on the physiological inactivation of amino acid transmitter action (Drejer *et al.* 1982, Griffiths *et al.* 1992).

Cysteinesulfinic acid and cysteic acid have excitatory effects upon neuronal firing whereas their decarboxylation products, hypotaurine and taurine, possess neuroinhibitory properties (Baba *et al.* 1982, 1985). It is interesting that cysteinesulfinic acid is similar in structure to aspartate, an excitatory neurotransmitter (Nadler *et al.* 1978), with the sulfinic acid group replacing the sidechain carboxyl of aspartic acid. These observations, therefore, conform well with the idea that dicarboxylic amino acids produce excitation whilst the monocarboxylic acids produce inhibition (Kelly and Beart 1975).

The enzyme cysteine dioxygenase catalyses the rate-limiting step in the oxidative metabolism of cysteine to yield cysteine sulfinic acid (Yamaguchi and Hosokawa 1987). This sulfinic acid may then be decarboxylated to hypotaurine with subsequent oxidation to taurine or may be initially oxidized, possibly nonenzymatically, to cysteic acid which is then decarboxylated directly to taurine (Blaschko *et al.* 1953). Transamination to yield pyruvate and sulfite via the unstable β -sulfinylpyruvate is also another major catabolic pathway for cysteinesulfinic acid (Singer and Kearney 1954, 1956).

Cysteine dioxygenase activity is unequally distributed within the CNS, being highest in the thalamus and superior colliculus and lowest in the hippocampus (Baba *et al.* 1980, Misra and Olney 1975, Pasantos-Morales *et al.* 1977). This places the enzyme, and its metabolic products, in a position to fulfil a regulatory role. The neuroexcitatory actions of cysteinsulfinic acid and the antagonistic effects of taurine suggests a means by which excitatory and inhibitory amino acids may interact to modulate neuronal excitability and this may be significant for neuronal functions (Baba *et al.* 1982, 1983, 1985, Iwata and Baba 1983).

Hallervorden-Spatz disease, a rare neurological disorder inherited in an autosomal recessive fashion which gives rise to choreic or athetoid movements with general bradykinesia, rigidity and progressive mental deterioration, has been linked with cysteine accumulation and cysteine dioxygenase deficiency in the globus pallidus and substantia nigra areas of the brain (Elejalde *et al.* 1979, Perry *et al.* 1985).

Errors in the metabolism of the sulfur amino acids within the CNS have been found to be relatively common and may be accompanied by serious neuropsychiatric disorders, with unusual sulfur-amino acids being observed in urine samples obtained from such patients (Ampola *et al.* 1969, Irreverre *et al.* 1967, Mudd and Levy 1983, Olney *et al.* 1975). Cysteine, if permitted to accumulate within the body, is known to be neurotoxic, as are cysteinsulfinic and cysteic acids; neuronal degeneration results from an overactivation of receptors. The possibility of impairment in sulfur-amino acid metabolism has been linked with various chronic late-onset neurodegenerative diseases, and it has been stated that metabolic disturbances (environmental, genetic) which result in elevated blood levels of cysteine and subsequent excitotoxicity may be prime factors in the pathogenicity of these disorders (Olney 1985, 1989, 1990a, b, c, Woodruff *et al.* 1988). Conversely, low levels of cysteine in specific brain regions have been implicated in mental dysfunction and retardation (Key and White 1970, Scriver *et al.* 1970). It appears that a fine balance has to be maintained in order to ensure neurological health.

2.6.2 Organoleptic properties

Organisms seem to be acutely sensitive to volatile sulfur-containing molecules which are thereby able to transmit information, sometimes over long distances. The human nose can detect simple thiols and other small-molecular-weight sulfur compounds at extremely low concentrations (e.g. methanethiol, 0.02 ppb; 2-methyl-2-butanethiol, 0.0007 ppb) and insects appear to respond at such great dilutions that only a few molecules may be present.

As mentioned previously, plants are able to exploit the properties of sulfur compounds for protective functions. However, the phenomenon is not all one-sided. Alkyl disulfides, such as di-*n*-propyl disulfide, produced by the onion has become an attractant for predatory insects such as the onion maggot and black blowfly and can stimulate egg laying (Matsumoto 1970). Chemical constituents of the mustard oils (isothiocyanates), which occur within many Brassica species, have been found to be attractants for the vegetable weevil in which it initiates biting. This is particularly pronounced following exposure to the allyl and *n*-butyl derivatives (Matsumoto 1970). Similar findings have been made with the flea beetle.

The spray of the skunk contains several sulfur compounds which include evil-smelling thiols such as trans-2-butenethiol, 3-methylbutanethiol and 2-quinolone-methanethiol. As a reinforcement, the corresponding thioacetates of these thiol compounds are also ejected within the spray. These thioesters hydrolyse slowly on exposure to moisture, releasing more of the foetid thiols. These substances are obviously very effective in repelling potential predators and also have the ability to remind the victim of the unfortunate encounter for several days (Emsley 1990). Other organosulfur compounds involved in communication between animals may be exemplified by the presence of Δ^3 -isopentyl- and 2-phenylethyl-methyl sulfides as urine scent markers in the red fox and the occurrence of 5-thiomethyl-2,3-pentadione as a volatile secretion from the anal gland of the striped hyena (Wheeler *et al.* 1975). A mixture of thietanes and dithiolane derivatives are secreted by members of the *Mustela* species, the mink (Schildknecht *et al.* 1976, Sokolov *et al.* 1980, Wheeler *et al.* 1975), the ferret (Crump 1980a) and the stoat (Crump 1978, 1980b), and probably also function as scent markers. Dimethyl disulfide has also been identified as an attractant pheromone within the vaginal secretion of the hamster (Singer *et al.* 1976). The venting of hydrogen sulfide amidst flatus by humans is also regarded as offensive when in polite company.

2.7 Reflections

In his book, *The Periodic Table*, Primo Levi assigned human characteristics to the elements and told of sulfur (B41) as a dark and fiery beast, full of energy ready to burst forth but able to be controlled and channelled for the overall good (Levi 1975). Have similar thoughts always plagued the mind of thinking man? Sulfur, together with salt and mercury, was one of the *tria prima* (Spagyric or Hypostatical Principles) which the later followers of Paracelsus (1493–1541) blended into the four 'Peripatetic' elements of the ancients. Sulfur became the principle of inflammability, the expulsion of sulfurous ingredients enabling combustion. Oil of vitriol (sulfuric acid) was regarded by many alchemists as the *sulphur philosophorum*, or at least as a substance that would lead to the acquirement of the *materia prima*. Its violent properties could strip bodies of their enveloping qualities so as to leave pure and noble matter. Such utterances are now a virtual heresy within our modern impersonal conception of the universe. However, being aware of the important roles which sulfur plays within the biological arena it appears unfair to constantly malign the substance. The museful states induced in travellers and attendants who ventured between the pillars and entered the confines of the Oracle of Apollo at Delphi have been thought to arise, at least in part, from sulfurous fumes creeping up through the rocks below. A divine and sagacious role indeed!

This short introduction to the biological aspects of sulfur has hopefully whetted the appetite. Sulfur already waiting at the genesis to take on multiple tasks, first as an energy trap and then, once this quintessential role was handed over to oxygen, to consolidate positions in communication, defence, energy transfer and even the maintenance of the biological fabric itself. In this way sulfur has become an essential and irreplaceable part of all living systems.

2.8 References

- ABELE, A., BORG, J. & MARK, J., 1983, Cysteine sulfinic acid uptake in cultured neuronal and glial cells, *Neurochemical Research*, **8**, 889–902.
- ABIKO, Y., 1975, Metabolism of coenzyme A, in Greenberg, D. M. (Ed.), *Metabolism of Sulfur Compounds: Metabolic Pathways*, Vol. 7, 3rd Edn, pp. 1–25, London: Academic Press.
- AMONKAR, S. V. & BANERJI, A., 1971, Isolation and characterisation of larvicidal principal of garlic, *Science*, **174**, 1343–1344.
- AMPOLA, M. G., EFRON, M. L., BIXBY, E. M. & MESHORER, E., 1969, Mental deficiency and a new aminoaciduria. *American Journal of Diseases in Children*, **117**, 66–70.
- AULT, W. V. & KULP, J. L., 1959, Isotopic geochemistry of sulphur. *Geochimica Cosmochimica Acta*, **16**, 201–235.
- BABA, A., YAMAGUCHI, S., MIZUO, H. & IWATA, H., 1980, Microassay of cysteine sulfinic acid by an enzymatic cycling method, *Analytical Biochemistry*, **101**, 288–293.
- BABA, A., LEE, E., TATSUNO, T. & IWATA, H., 1982, Cysteine sulfinic acid in the central nervous system: antagonistic effect of taurine on cysteine sulfinic acid-stimulated formation of cyclic AMP in guinea pig hippocampal slices, *Journal of Neurochemistry*, **38**, 1280–1285.
- BABA, A., TATSUNO, T., KUMAGAE, Y. & IWATA, H., 1983, Inhibitory effect of taurine on the formation of cyclic AMP in guinea pig hippocampus, in Kuriyama, K., Huxtable, R. J. & Iwata, H. (Eds), *Sulfur Amino Acids: Biochemical and Clinical Aspects*, pp. 161–168, New York: Alan R. Liss.
- BABA, A., MORIMOTO, H. & IWATA, H., 1985, Neurochemical relations between excitatory and inhibitory amino acids in hippocampus, in Oja, S. S., Ahtee, L., Kontro, P. & Paasonen, M. (Eds), *Taurine: Biological Actions and Clinical Perspectives*, pp. 271–278, New York: Alan R. Liss.
- BADDILEY, J., 1955, The structure of coenzyme A, *Advances in Enzymology*, **16**, 1–21.
- BALDESSARINI, R. J. & KAROBATH, M., 1973, Biochemical physiology of central synapses, *Annual Review of Physiology*, **35**, 273–304.
- BARLTROP, J. A., HAYES, P. M. & CALVIN, M., 1954, The chemistry of 1,2-dithiolane (trimethylene disulfide) as a model for the primary quantum conversion act in photosynthesis, *Journal of the American Chemical Society*, **76**, 4348–4367.
- BASS-BECKING, L. G. M., 1925, Studies on sulphur bacteria, *Annals of Botany*, **39**, 613–650.
- BECKER, R. S., HONG, K. Y. & HONG, T. H., 1974, Hot hydrogen atoms reactions of interest in molecular evolution and interstellar chemistry, *Journal of Molecular Evolution*, **4**, 157–172.
- BLASCHKO, H., DATTA, S. P. & HARRIS, H., 1953, Pyridoxin deficiency in the rat: liver L-cysteic acid decarboxylase activity and urinary amino acids, *British Journal of Nutrition*, **7**, 364–371.
- BLOCK, E. & O'CONNOR, J., 1974, The chemistry of alkyl thiosulfinate esters. VI. Preparation and spectral studies, *Journal of the American Chemical Society*, **96**, 3921–3929.
- BLOCK, E., PENN, R. E. & REVELLE, L. K., 1979, Structure and origin of the onion lachrymatory factor. A microwave study. *Journal of the American Chemical Society*, **101**, 2200–2201.
- BOARD, P. A., 1976, Bacterial sulphate reduction and the anaerobic regulation of atmospheric oxygen. *Atmosphere and Environment*, **10**, 339–342.

- BOELEN, M., DE VALOIS, P. J., WOBLEN, H. J. & VAN DER GEN, A., 1971, Volatile flavour compounds from onions, *Journal of Agricultural and Food Chemistry*, **19**, 984-991.
- BOULANGER, Y., GOODMAN, C. M., FORTE, C. P., FESIK, S. W. & ARMITAGE, I. M., 1983, Model for mammalian metallothionein structure, *Proceedings of the National Academy Sciences, USA*, **80**, 1501-1505.
- BOWEN, I. S., 1929, The presence of sulphur in the gaseous nebulae, *Nature*, **123**, 450.
- BRUCE, T. C. & BENKOVIC, S., 1966, Thioesters, in *Bioorganic Mechanisms*, Vol. 1, pp. 259-297, New York: W. A. Benjamin, Inc.
- CALVIN, M., 1954, Chemical and photochemical reactions of thioctic acid and related disulfides, *FASEB Proceedings*, **13**, 697-711.
- CHAUVERZ, L. G. & SCHERAGA, H. A., 1980, Folding of ribonuclease, S-protein, and Des(121-124)-ribonuclease during glutathione oxidation of the reduced proteins, *Biochemistry*, **19**, 996-1004.
- COLOWICK, S., LAZAROW, A., RACKER, E., SCHWARZ, D. R., STADTMAN, E. & WAELSCH, H. (Eds), 1954, *Glutathione*, 341 pp. New York: Academic Press.
- COTTON, F. A. & WILKINSON, G., 1988, *Advanced Inorganic Chemistry*, 5th Edn, pp. 1355-1358, New York: John Wiley & Sons.
- COYLE, J. T., SCHWARCZ, R., BENNET, J. P. & CAMPOCHIARO, P., 1977, Clinical neuropathological and pharmacological aspects of Huntington's disease: correlates with a new animal model, *Progress in Neuro-psychopharmacology*, **1**, 13-30.
- CROOK, E. M. (Ed.), 1959, *Glutathione*, Biochemical Society Symposium 17, Cambridge: Cambridge University Press.
- CRUMP, D. R., 1978, 2-Propylthietane, the major malodorous substance from the anal gland of the stoat, *Tetrahedron Letters*, 5233-5234.
- 1980a, Anal gland secretion of the ferret (*Mustela putorius*, forma *furo*), *Journal of Chemical Ecology*, **6**, 837-844.
- 1980b, Thietanes and dithiolanes from the anal gland of the stoat (*Mustela erminea*), *Journal of Chemical Ecology*, **6**, 341-348.
- CURTIS, D. R. & WATKINS, J. C., 1960, The excitation and depression of spinal neurones by structurally related amino acids. *Journal of Neurochemistry*, **6**, 117-141.
- 1963, Acidic amino acids with strong excitatory actions on mammalian neurones. *Journal of Physiology*, **166**, 1-14.
- CURTIS, D. R., PHILLIS, J. W. & WATKINS, J. C., 1960, The chemical excitation of spinal neurones by certain acidic amino acids, *Journal of Physiology*, **150**, 656-682.
- 1961, Actions of amino acids on the isolated hemisectioned spinal cord of the toad, *British Journal of Pharmacology*, **16**, 262-283.
- DE ROBERTIS, E., 1971, Molecular biology of synaptic receptors, *Science*, **171**, 963-971.
- DREJER, J., LARSSON, O. M. & SCHOUSBOE, A., 1982, Characterization of L-glutamate uptake into and release from astrocytes and neurons cultured from different brain regions, *Experimental Brain Research*, **47**, 259-269.
- ELEJALDE, B. R., MERCEDES, M., DE ELEJALDE, J. & LOPEZ, F., 1979, Hallervorden-Spatz disease, *Clinical Genetics*, **16**, 1-18.
- ELOVSON, J. & VAGELOS, P. R., 1968, Acyl carrier protein, *Journal of Biological Chemistry*, **243**, 3603-3611.
- EMSLEY, J., 1990, The seven deadly smells of a skunk, *New Scientist*, **4 August**, 31.
- FAHEY, R. C., HUNT, J. S. & WINDHAM, G. C., 1977, On the cysteine and cystine content of proteins. Differences between intracellular and extracellular proteins, *Journal of Molecular Evolution*, **10**, 155-160.
- FISCHER, U., 1984, Cytochromes and iron sulfur proteins in sulfur metabolism of phototrophic sulfur bacteria, in Muller, A & Krebs, B. (Eds), *Sulfur, its Significance*

- for Chemistry, for the Geo-, Bio- and Cosmosphere and Technology: *Studies in Inorganic Chemistry*, Vol. 5, pp. 383–407, Amsterdam: Elsevier.
- FLOREY, P. J., 1956, Theory of elastic mechanisms in fibrous proteins, *Journal of the American Chemical Society*, **78**, 5222–5235.
- FOSS, O. & TJOMSLAND, O., 1958, The crystal and molecular structure of 1,2-dithiolane-4-carboxylic acid, *Acta Chemica Scandinavica*, **12**, 1810–1818.
- GOLDHABER, M. B. & KAPLAN, I. R., 1974, Sulfur cycle, in Hill, M. N., Maxwell, A. E. & Goldberg, E. D. (Eds), *The Sea*, Vol. 5, pp. 569–655. New York: John Wiley.
- GRANAT, L., HALLBERG, R. O. & RODHE, H., 1976, The global sulphur cycle, in Svensson, B. H. & Soderlund, R. (Eds), *Nitrogen, Phosphorus and Sulphur – Global Cycles: Ecological Bulletin (Stockholm)*, **20**, 89–134.
- GRIEVE, A., CAMERON, D. & GRIFFITHS, R., 1990, Characterization of cysteine sulphinate transport by intact rat brain cerebrocortical synaptosome fractions, *Biochemical Society Transactions*, **18**, 426–427.
- GRIEVE, A., DUNLOP, J., SCHOUSBOE, A. & GRIFFITHS, R., 1991, Kinetic characterisation of excitatory amino acid transport in synaptosomes and in primary cultures of different brain cells, *Biochemical Society Transactions*, **19**, 5S.
- GRIFFITHS, R., 1990, Cysteine sulphinate (CSA) as an excitatory amino acid candidate in the mammalian central nervous system, *Progress in Neurobiology*, **35**, 313–323.
- GRIFFITHS, R., BUTCHER, S. P. & OLVERMAN, H. J., 1992, Sulphur-containing excitatory amino acids, in Krogsgaard-Larsen, P. & Hansen, J. J. (Eds), *Excitatory Amino Acid Receptors: Design of Agonists and Antagonists*, pp. 162–182, Chichester: Ellis Horwood.
- HEBB, C., 1970, CNS at the cellular level: identity of transmitter agents, *Annual Review of Physiology*, **32**, 165–192.
- HERBST, E. & KLEMPERER, W., 1976, The formation of interstellar molecules, *Physics Today*, **29**, 32–39.
- HOLLAND, H. D., 1962, Model for the evolution of the earth's atmosphere, in Engel, A. E. J., James, H. L. & Leonard, B. F. (Eds), *Petrologic Studies: A Volume to Honor A. F. Buddington*, pp. 447–477, New York: Geological Society of America.
- 1974, Aspects of the geologic history of seawater, *Origins of Life*, **5**, 87–91.
- HONG, K. Y., HONG, J. H. & BECKER, R. S., 1974, Hot hydrogen atoms: initiators of reactions of interest in interstellar chemistry and evolution, *Science*, **184**, 984–987.
- HUXTABLE, R. J., 1986, *Biochemistry of Sulfur*, pp. 4–6, 17, 231, 279, 415, 423, New York: Plenum.
- IRREVERRE, F., MUDD, S. H., HEIZER, W. D. & LASTER, L., 1967, Sulfite oxidase deficiency, *Biochemical Medicine*, **1**, 187–217.
- IVERSEN, L. L., NICOLL, R. A. & VALE, W. W., 1978, Neurobiology of peptides, *Neuroscience Research Program Bulletin*, **16**, 214–370.
- IWATA, H. & BABA, A., 1983, Neurochemical basis of cysteine sulfinic acid in the central nervous system, in Kuriyama, K., Huxtable, R. J. & Iwata, H. (Eds), *Sulfur Amino Acids: Biochemical and Clinical Aspects*, pp. 141–150, New York: Alan R. Liss.
- IWATA, H., YAMAGAMI, S., MIZUO, H. & BABA, A., 1982, Cysteine sulfinic acid in the central nervous system: uptake and release of cysteine sulfinic acid by a rat brain preparation, *Journal of Neurochemistry*, **38**, 1268–1274.
- JUNGE, C., 1972, Sulphur supplies of atmospheric origin, in *Symposium International sur le Soufre en Agriculture, Annales Agronomie, Numero hors série*: 235–247.
- KELLY, D. P., 1980, The sulphur cycle: definitions, mechanisms and dynamics, in Elliot, K. & Whelan, J. (Eds), *Sulphur in Biology*, CIBA Foundation Symposium 72, pp. 3–18, Amsterdam: Excerpta Medica.
- KELLY, J. S. & BEART, P. M., 1975, Amino acid receptors in CNS. II. GABA in

- supraspinal regions, in Iversen, L. L., Iversen, S. D. & Snyder, S. H. (Eds), *Handbook of Psychopharmacology*, Vol. 4, Sect. 1, pp. 129–209, New York: Plenum.
- KEY, B. J. & WHITE, R. P., 1970, Neuropharmacological comparison of cystathionine, cysteine, homoserine and alpha-ketobutyric acid in cats, *Neuropharmacology*, **9**, 349–357.
- KHARE, B. N. & SAGAN, C., 1971, Synthesis of cystine in simulated primitive conditions, *Nature*, **232**, 577–579.
- KLEIN, R. M. & CRONQUIST, A., 1967, A consideration of the evolutionary and taxonomic significance of some biochemical, micromorphology, and physiological characters in the thallophytes, *Quarterly Review of Biology*, **42**, 105–296.
- KROGSGAARD-LARSEN, P. & HONORE, T., 1983, Glutamate receptors and new glutamate agonists, *Trends in Pharmacological Sciences*, **4**, 31–33.
- LABORDE, E., 1922, Properties of sulphur in the skin of one-year infants, *Bulletin de la Societe de Chimie Biologique*, **4**, 584–585.
- LAKE, J. A., 1988, Origin of the eucaryotic nucleus determined by rate-invariant analysis of rRNA sequences, *Nature*, **331**, 184–186.
- LAMBETH, D. O. & LARDY, H. A., 1969, The oxidation of thioethers by bromine, A model system for oxidative phosphorylation, *Biochemistry*, **8**, 3395–3402.
- LARSSON, A., ORRENIUS, S., HOLMGREN, A. & MANNERVIK, B. (Eds), 1983, *Functions of Glutathione: Biochemical, Physiological, Toxicological and Clinical Aspects*, 393 pp., New York: Raven.
- LEVI, P., 1975, *The Periodic Table*, Turin: Giulio Einaudi Editore SpA. (Translated into English by Rosenthal, R., 1984, Shocken Books Inc., 1989, London: Abacus, Sphere Books Ltd.)
- LICHTENSTEIN, E. P., 1966, Insecticides occurring naturally in crops, in Crosby, D. G. (Ed.), *Natural Pest Control Agents*, pp. 34–38, Washington: American Chemical Society.
- LIPMANN, F., 1953, On the chemistry and function of coenzyme A, *Bacteriological Reviews*, **17**, 1–16.
- LOCKYER, J. N., 1907, On the presence of sulphur in some of the hotter stars, *Proceedings of the Royal Society*, **80A**, 50–56.
- MATSUMOTO, Y., 1970, Volatile organic sulfur compounds as insect attractants with special reference to host selection, in Nakajima, M. (Ed.), *Control of Insect Behaviour by Natural Products*, pp. 133–160, New York: Academic Press.
- MCDUFF, R. E. & EDMOND, J. M., 1981, On the fate of sulfate during hydrothermal circulation at mid-ocean ridges, *Earth and Planetary Science Letters*, **57**, 117–132.
- MEISTER, A. & ANDERSON, M. E., 1983, Glutathione, *Annual Reviews in Biochemistry*, **52**, 711–760.
- MILLER, S. L., 1974, The atmosphere of the primitive earth and the prebiotic synthesis of amino acids, *Origins of Life*, **5**, 139–151.
- MISRA, C. H. & OLNEY, J. W., 1975, Cysteine oxidase in brain, *Brain Research*, **97**, 117–126.
- MITCHELL, S. C. & NICKSON, R. M., 1993, Metabolism of sulfur-containing xenobiotics, *Sulfur Reports*, **13**, 161–195.
- MOORBATH, S., 1985, Crustal evolution in the early Precambrian, *Origins of Life*, **15**, 251–261.
- MUDD, S. H. & LEVY, H. L., 1983, Disorders of transsulfuration, in Stanbury, J. B., Wyngaarden, J. B., Fredrickson, D. S., Goldstein, J. L. & Brown, M. S. (Eds), *The Metabolic Basis of Inherited Disease* 5th Edn, pp. 522–559, New York: McGraw-Hill.
- NADLER, J. V., WHITE, W. F., VACA, K. W., PERRY, B. W. & COTMAN, C. W., 1978, Biochemical correlates of transmission mediated by glutamate and aspartate, *Journal of Neurochemistry*, **31**, 147–155.

- NICKLESS, G., 1968, *Inorganic Sulphur Chemistry*, Amsterdam: Elsevier.
- OLNEY, J. W., 1985, Excitatory transmitters and epilepsy-related brain damage, *International Review of Neurobiology*, **27**, 337–362.
- 1989, Excitatory amino acids and neuropsychiatric disorders, *Biological Psychiatry*, **26**, 505–525.
- OLNEY, J. W., 1990a, Excitotoxin-mediated neuron death in youth and old age, *Progress in Brain Research*, **86**, 37–51.
- 1990b, Excitotoxicity: an overview, *Canada Diseases Weekly Report*, **16** (Suppl. 1E), 47–58.
- 1990c, Excitotoxic amino acids and neuropsychiatric disorders, *Annual Reviews in Pharmacology and Toxicology*, **30**, 47–71.
- OLNEY, J. W., MISRA, C. H. & DE GUBAREFF, T., 1975, Cysteine-S-sulfate: brain damaging metabolite in sulfite oxidase deficiency, *Journal of Neuropathology and Experimental Neurology*, **34**, 167–177.
- OSHINO, N. & CHANCE, B., 1975, The properties of sulfite oxidation in perfused rat liver: interaction of sulfite oxidase with the mitochondrial respiratory chain, *Archives of Biochemistry and Biophysics*, **170**, 514–528.
- OTVOS, J. D. & ARMITAGE, I. M., 1980, Structure of the metal clusters in rabbit liver metallothionein, *Proceedings of the National Academy Sciences, USA*, **77**, 7094–7098.
- PASANTES-MORALES, H., LORLETTE, C. & CHATAGNER, F., 1977, Regional and subcellular distribution of taurine synthesizing enzymes in the rat central nervous tissue, *Neurochemical Research*, **2**, 671–680.
- PENNY, D., 1988, What was the first living cell?, *Nature*, **331**, 111–112.
- PERRY, T. L., NORMAN, M. G., YOUNG, V. S., WHITING, S., CRICHTON, J. U., HANSEN, S. & KISH, S. J., 1985, Hallervorden–Spitz disease: cysteine accumulation and cysteine dioxygenase deficiency in the *globus pallidus*, *Annals of Neurology*, **18**, 482–489.
- PFENNIG, N., 1967, Photosynthetic bacteria, *Annual Review of Microbiology*, **21**, 285–324.
- 1978a, General physiology and ecology of photosynthetic bacteria, in Clayton, R. K. & Sistrom, W. R. (Eds), *The Photosynthetic Bacteria*, pp. 3–18, New York: Plenum.
- 1978b, Syntrophic associations and consortia with photosynthetic bacteria, *Twelfth International Congress of Microbiology, Munich, Abstract(S11.6)*, 16.
- POLAND, D. C. & SCHERAGA, H. A., 1965, Statistical mechanics of noncovalent bonds in polyamino acids. VIII. Covalent loops in proteins, *Biopolymers*, **3**, 379–399.
- POSTGATE, J. R., 1980, in the discussion section of Kelly, D. P., 1980, The sulphur cycle: definitions, mechanisms and dynamics, in Elliot, K. & Whelan, J. (Eds), *Sulphur in Biology*, CIBA Foundation Symposium 72, p. 13, Amsterdam: Excerpta Medica.
- RAULIN, F. & TOUPANCE, G., 1977, The role of sulphur in chemical evolution, *Journal of Molecular Evolution*, **9**, 329–338.
- RECASENS, M., VARGA, V., NANOPOULOS, D., SAADOUN, F., VINCENDON, G. & BENDAVIDES, J., 1982, Evidence for cysteine sulfinic acid as a neurotransmitter, *Brain Research*, **239**, 153–173.
- REED, L. J., 1960, Lipoic acid, in Boyer, P. D., Lardy, H. & Myrback, K., (Eds), *The Enzymes*, Vol. 3, 2nd Edn, pp. 195–223, New York: Academic Press.
- 1966, Chemistry and function of lipoic acid, in Florkin, M. & Stotz, E. H. (Eds), *Comprehensive Biochemistry*, Vol. 14, pp. 99–126, Amsterdam: Elsevier.
- REED, L. J. & COX, D. J., 1966, Macromolecular organisation of enzyme systems, *Annual Review of Biochemistry*, **35**, 57–84.
- ROBINSON, T., 1963, *The Organic Constituents of Higher Plants*, Minneapolis: Burgess Publishing Company.
- ROSCOE, H. E., 1863, On the existence of a crystalline carbon compound and free sulphur

- ROSCOE, H. E., 1863, On the existence of a crystalline carbon compound and free sulphur in Alais meteorite, *Philosophical Magazine (4th Series)*, **25**, 319–320.
- SABLE, H. Z. & GUBLER, C. J., 1982, Thiamine: twenty years of progress, *Annals of the New York Academy of Sciences*, **378**, 1–470.
- SAGAN, C. & KHARE, B. N., 1971, Long-wavelength ultraviolet photoproduction of amino acids on primitive earth, *Science*, **173**, 417–420.
- SAKAI, M. & SATO, Y., 1972, Metabolic conversion of the nereistoxin-related compounds into nereistoxin as a factor of their insecticidal action, in Tahori, A. S. (Ed.), *Insecticides. Proceedings of the Second International IUPAC Congress on Pesticide Chemistry*, Vol. 1, pp. 455–467, New York: Gordon and Breach.
- SCHIFF, J. A., 1980, Pathways of assimilatory sulphate reduction in plants and microorganisms, in Elliot, K. & Whelan, J. (Eds), *Sulphur in Biology*, CIBA Foundation Symposium 72, pp. 49–69, Amsterdam: Excerpta Medica.
- SCHILDKNECKT, H., WILZ, I., ENZMANN, F., GRUND, N. & ZIEGLER, M., 1976, Mustelan, the malodorous substance from the anal gland of the mink, *Mustela vison*, and the polecat, *M. putorius*, *Angewandte Chemie (International Edition in English)*, **15**, 242–253.
- SCHMIDT, M., SIEBERT, W. & BAGNELL, K. W., 1975, The chemistry of sulphur, selenium, tellurium and polonium, *Comprehensive Inorganic Chemistry*, **15**, 795–1008.
- SCHULZ, H., 1893, Ueber den Schwefelgehalt Menschlicher und Thierischer Gewebe, *Pflugers Archiv fur die gesamte Physiologie des Menschen und der Tiere*, **54**, 555–573.
- SCRIVER, C. R., WHELAN, D. T., CLOW, C. L. & DALLAIRE, L., 1970, Cystinuria: increased prevalence in patients with mental disease, *New England Journal of Medicine*, **283**, 783–786.
- SINGER, A. G., AGOSTA, W. C., O'CONNELL, R. J., PFAFFMANN, C., BOWEN, D. V. & FIELD, F. H., 1976, Dimethyl disulphide: an attractant pheromone in hamster vaginal secretion, *Science*, **191**, 948–950.
- SINGER, T. P. & KEARNEY, E. B., 1954, Pathways of L-cysteine sulfinic acid metabolism in animal tissues, *Biochimica et Biophysica Acta*, **14**, 570–573.
- 1956, Intermediary metabolism of L-cysteinesulfinic acid in animal tissues, *Archives of Biochemistry and Biophysics*, **61**, 396–409.
- SNELL, E. E. & BROWN, G. M., 1953, Pantethine and related forms of the *Lactobacillus bulgaricus* factor (LBF), *Advances in Enzymology*, **14**, 49–71.
- SOKOLOV, V. E., ALBONE, E. S., FLOOD, P. F., HEAP, P. F., KAGAN, M. Z., VASILIEVA, V. S., ROZNOV, V. V. & ZINKEVICH, E. P., 1980, Secretion and secretory tissues of the anal sac of the mink, *Mustela vison*, Chemical and histological studies, *Journal of Chemical Ecology*, **6**, 805–825.
- SUNNER, S., 1955, Strain in 6,8-thioctic acid, *Nature*, **176**, 217.
- SUZUKI, K. T., 1982, Induction and degradation of metallothioneins and their relation to the toxicity of cadmium, in Foulkes, E. C. (Ed.), *Biological Roles of Metallothionein*, pp. 215–235, Amsterdam: Elsevier.
- SWEENEY, W. V. & RABINOWITZ, J. C., 1980, Proteins containing 4Fe–4S clusters: an overview, *Annual Reviews in Biochemistry*, **49**, 139–161.
- TAKASUGI, M., YACHIDA, Y., ANETAI, M., MASAMUNE, T. & KEGASAWA, K., 1975, Identification of asparagusic acid as a nematocide occurring naturally in the roots of asparagus, *Chemical Letters*, 43–44, *Chemical Abstracts*, **82**, 121683.
- TIMBRELL, J. A., 1991, *Principles of Biochemical Toxicology*, 2nd Edn, pp. 314–316, London: Taylor & Francis.
- TRIBUTSCH, H., 1984, Metallic sulfides in photovoltaic, photoelectrochemical and solar biological energy conversion, in Muller, A. & Krebs, B. (Eds), *Sulfur, its Significance for Chemistry, for the Geo-, Bio- and Cosmosphere and Technology: Studies in Inorganic Chemistry*, Vol. 5, pp. 277–310, Amsterdam: Elsevier.

- VAN TRUMP, J. E. & MILLER, S. L., 1972, Prebiotic synthesis of methionine, *Science*, **178**, 859–860.
- TRUDINGER, P. A., 1969, Assimilatory and dissimilatory metabolism of inorganic sulphur compounds by microorganisms, *Advances in Microbial Physiology*, **3**, 111–158.
- TRUPER, H. G., 1982, Microbial processes in the sulfur cycle through time, in Holland, H. D. & Schidlowski, M. (Eds), *Mineral Deposits and the Evolution of the Biosphere*, pp. 5–30, Berlin: Springer.
- 1984, Microorganisms in the sulfur cycle, in Muller, A. & Krebs, B. (Eds), *Sulfur, its Significance for Chemistry, for the Geo-, Bio- and Cosmosphere and Technology: Studies in Inorganic Chemistry*, Vol. 5, pp. 2351–2365, Amsterdam: Elsevier.
- UEYAMA, N. & NAKAMURA, A., 1992, Sulfur ligands in metalloproteins and metallo-enzymes, in Oae, S. & Okuyama, T. (Eds), *Organic Sulfur Chemistry: Biochemical Aspects*, pp. 45–70, Boca Raton: CRC Press.
- WHEELER, J. W., VON ENDT, D. W. & WEMMER, C., 1975, 5-Thiomethylpentane-2-3-dione. A unique natural product from the striped hyena, *Journal of the American Chemical Society*, **97**, 441–442.
- WIELAND, T. & AQUILA, H., 1968, Modellversuche zur oxydativen Phosphorylierung. IX. Thiazolidone als Vermittler einer oxydativen Phosphorylierung von Adenosinmonophosphat und -diphosphat, *Chemische Berichte*, **101**, 3031–3036.
- WIELAND, T. & BAUERLEIN, E., 1967, Modellversuche zur oxydativen Phosphorylierung. VII. N-Acetyl-homocysteinethiolacton als Vermittler einer oxydativen Synthese von Adenosindiphosphat und Adenosintriphosphat aus Adenosinmonophosphat und Orthophosphat, *Chemische Berichte*, **100**, 3869–3876.
- WILSON, D. F. & PASTUSZKO, A., 1986, Transport of cysteate by synaptosomes isolated from rat brain: evidence that it utilizes the same transporter as aspartate, glutamate, and cysteine sulfinic acid, *Journal of Neurochemistry*, **47**, 1091–1097.
- WINNEWISER, G. & HERBST, E., 1987, Organic molecules in space, *Topics in Current Chemistry* **139**, pp. 119–172, Berlin: Springer.
- WOODRUFF, G. N., FOSTER, A. C., WONG, E. H. F., GILL, R., KEMP, J. A. & IVERSEN, L. L., 1988, Excitatory amino acids and neurodegenerative disorders: possible therapeutic indications, in Lodge, D. (Ed.), *Excitatory Amino Acids in Health and Disease*, pp. 379–389, Chichester: John Wiley & Sons.
- YAMAGUCHI, K. & HOSOKAWA, Y., 1987, Cysteine dioxygenase, in Jakoby, W. B. & Griffiths, O. W. (Eds), *Sulfur and Sulfur Amino Acids: Methods in Enzymology*, Vol. **143**, pp. 395–403, London: Academic Press.

Sulfur–Oxygen Compounds

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3.1 Introduction

There are a vast number of compounds in which one or more oxygen atoms are linked to a sulfur centre. The chemical and biological properties of the compound depend on the nature of the substituents on the sulfur and oxygen atoms, and thus the potential scope of this chapter is almost limitless. In consequence the chapter concentrates on the interactions of the main classes of organic sulfur–oxygen compounds and their metabolic precursors.

The different oxidation states of sulfur are of major importance in determining the polarity of the compound and hence its interaction with biological systems (Figure 3.1). A major determinant of polarity in sulfur–oxygen compounds is the presence of an ionizable hydrogen atom which may be associated with either the

Valency	Compound	Structure	Compound	Structure
-2	Thiol	R-SH	Thioether	R-S-R
-1	Disulfide	R-S-S-R		
0	Sulfenic acid	R-S-OH	Sulfoxide	$\begin{array}{c} \text{O} \\ \\ \text{R-S-R} \end{array}$
+2	Sulfinic acid	$\begin{array}{c} \text{R-S-OH} \\ \\ \text{O} \end{array}$	Sulfone	$\begin{array}{c} \text{O} \\ \\ \text{R-S-R} \\ \\ \text{O} \end{array}$
+4	Sulfonic acid	$\begin{array}{c} \text{O} \\ \\ \text{R-S-OH} \\ \\ \text{O} \end{array}$	Sulfamate	$\begin{array}{c} \text{O} \\ \\ \text{H} \\ \\ \text{R-N-S-OH} \\ \\ \text{O} \end{array}$
+6	Sulfate ester	$\begin{array}{c} \text{O} \\ \\ \text{R-O-S-OH} \\ \\ \text{O} \end{array}$		

Figure 3.1 Oxidation states of sulfur

sulfur atom or more usually an adjacent oxygen or sometimes nitrogen atom. The polarity of a sulfur–oxygen compound is an important property which determines its fate in the body, in particular its potential for absorption and for elimination as the parent compound.

A further property of sulfur-oxygen compounds, and one which will be a focus for this chapter, is the potential for redox interconversion of the valency state of the sulfur atom between analogues. This is illustrated in Figure 3.1 and will be discussed where relevant in the text.

The chapter covers a very wide subject area with chemicals possessing vastly different physicochemical and biological properties. Therefore it has been subdivided into groups of structurally-related compounds.

3.2 Sulfoxides and sulfones

The sulfoxide group is part of an interconvertible redox series involving the sulfide (or thioether), sulfoxide and sulfone (Figure 3.2). The sulfoxide group is present in numerous molecules which show a diversity of biological activity; examples include pharmaceuticals such as sulindac and flosequinan, veterinary drugs such as albendazole, and numerous pesticides (Figure 3.3). In addition, a sulfoxide group, which acts as a site of weak polarity within a molecule, may be introduced into a molecule as a result of oxidation of a thioether. Therefore, the sulfoxide group is extremely widespread.

Oxidation of a sulfoxide yields a sulfone which is commonly found as a metabolite

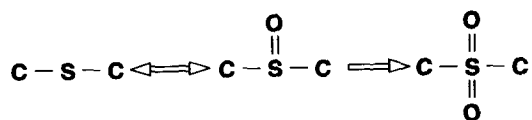
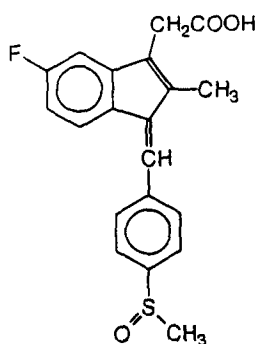
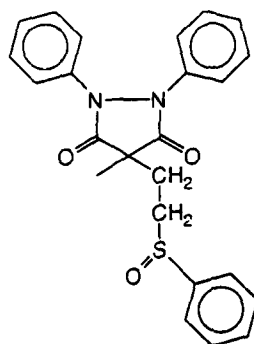


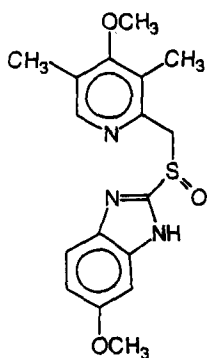
Figure 3.2 The interconversion of thioether, sulfoxide and sulfone



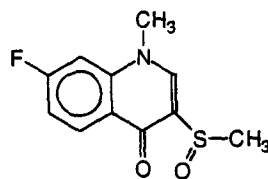
Sulindac



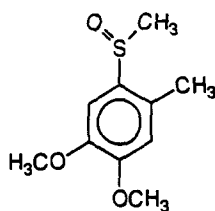
Sulfonpyrazone



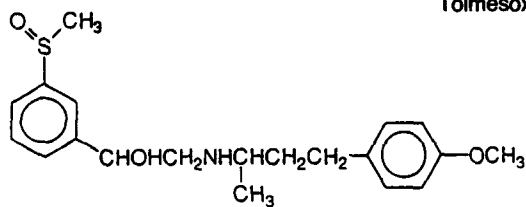
Omeprazole



Flosequin



Tolmesoxide



Sulfinalol

Figure 3.3 Therapeutic drugs containing a sulfoxide group

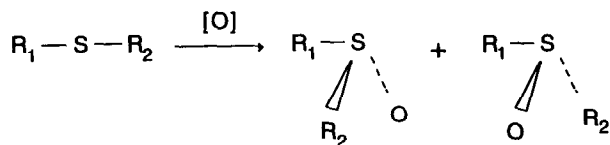


Figure 3.4 Oxidation of a thioether to produce a chiral sulfur centre

of sulfoxides but is less important as an active chemical entity. The major biological activity of $-\text{SO}_2-$ groups is seen when one of the carbon atoms in the sulfone structure in Figure 3.2 is replaced by a nitrogen atom. This gives rise to whole classes of biological compounds such as sulfonamides and sulfonylureas (see later).

In addition to metabolic oxidation of thioether to sulfoxide and to sulfone, there is the possibility of reduction of the sulfoxide back to the thioether (Figure 3.2). In consequence, it is not always possible to determine if the *in vivo* activity of a chemical resides in the parent drug or in one of the interconvertible redox forms. Further complications are that some compounds contain more than one thioether group capable of oxidation to a sulfoxide, and that sulfoxidation reactions may lead to a chiral sulfoxide with the potential for stereochemical selectivity in activity and further metabolism (Figure 3.4).

Sulfoxide and sulfone groups may be introduced into nonsulfur xenobiotics following their conjugation with glutathione and subsequent processing of the conjugate by hydrolysis reactions and C–S lyase. The xenobiotic product of the C–S lyase enzyme is a thiol which undergoes methylation to a thioether which can be oxidized to the sulfoxide and sulfone (Figure 3.5). Nonsulfur compounds excreted as thiomethyl ethers and/or the sulfoxide and sulfone include the herbicide, propachlor (Bakke *et al.* 1980, Larsen and Bakke 1981, Rafter *et al.* 1983), *m*-dichlorobenzene (Kato *et al.* 1986), caffeine (Rafter and Nilsson 1981), 2,2',5,5'-tetrachlorobiphenyl, probably via glutathione conjugation of a 3–4 epoxide intermediate (Preston *et al.* 1984) and other polychlorinated biphenyls (Mio and Sumino 1985), and paracetamol (Gemborys and Mudge 1981, Mikov *et al.* 1988, Warrander *et al.* 1985). In addition, the N-methylcysteine conjugate may undergo sulfoxidation (Larsen and Bakke 1981, Rafter *et al.* 1983).

3.2.1 Biological activity

The biological activity of thioethers, sulfoxides and sulfones is summarized in Table 3.1. It is clear that this group of chemicals displays a wide diversity of actions and shows differences between different redox forms of the same basic structure. Possibly the best examples are sulindac, where the active compound is produced by reduction of the inactive, sulfoxide parent compound (a prodrug), and sulphinpyrazone, where the parent sulfoxide has uricosuric properties and its thioether analogue inhibits platelet aggregation.

The sulfoxide group is critical to the action of omeprazole. Omeprazole and structural analogues such as pantoprazole are inhibitors of the gastric proton pump and act via the formation of a covalently binding irreversible form of the drug under acidic conditions (Keeling *et al.* 1985). This reaction probably involves protonation of the sulfoxide followed by elimination of water to form a reactive sulfenium ion (Rackur *et al.* 1985), or a cyclic cationic sulfenamide (Figure 3.6). This compound

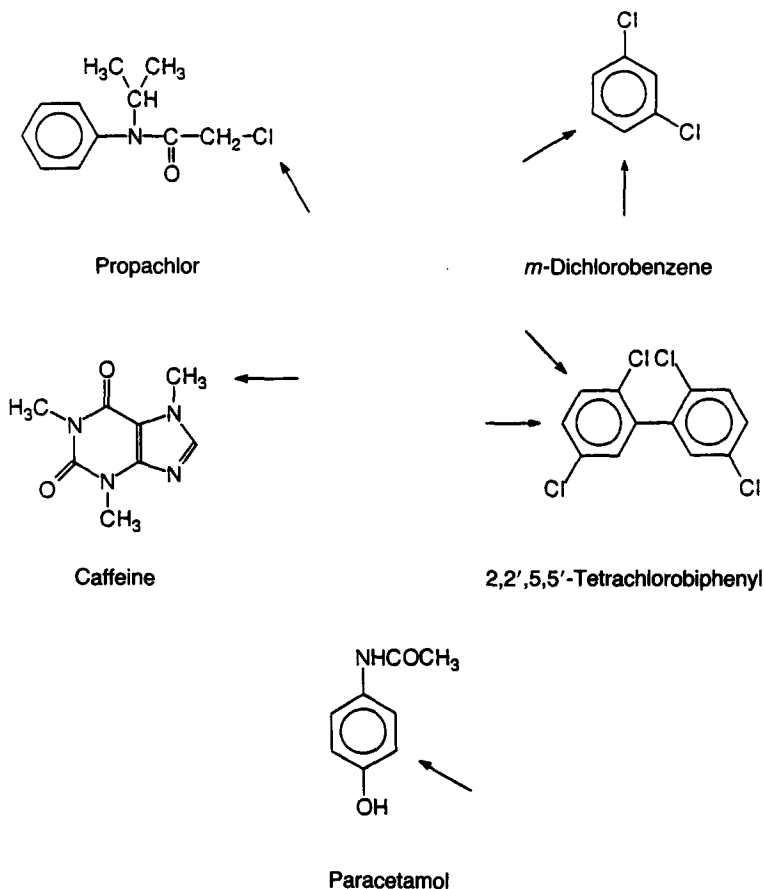


Figure 3.5 Introduction of thiomethyl groups as sites of sulfoxidation in nonsulfur chemicals (position of thiomethyl group indicated by an arrow)

binds to the $H^+/K^+-ATPase$, probably by forming mixed disulfides with enzyme cysteine groups (Besancon *et al.* 1993, Shin *et al.* 1993), which blocks phosphorylation of the enzyme by ATP, thereby blocking the pump action.

Omeprazole may undergo both sulfoxidation to the sulphone and reduction to the sulfide. The oxidation of omeprazole to the corresponding sulfone by isolated perfused rat liver preparations is dependent on the oxygen supply, while reduction to the sulfide was only detected under hypoxic conditions (Angus *et al.* 1989). The sulfide shows weak inhibition of the proton pump compared with omeprazole (Fryklund and Wallmark 1986). Binding of pantoprazole occurs at cysteine residues 813 and 822 of hog gastric $H^+/K^+-ATPase$ and blocks the conformational change necessary for phosphorylation of the enzyme by ATP. Similar binding was found for omeprazole (Besancon *et al.* 1993).

3.2.2 Enzymes involved in the metabolic interconversion of thioethers, sulfoxides and sulfones

There are numerous different enzymes which show the ability to perform oxidation or reduction reactions at sulfur atoms. These are discussed most logically under

Table 3.1 The activity of sulfoxides, thioethers and sulfones

Compound	Activity	Reference(s)
<i>Thioethers oxidized to active sulfoxides</i>		
Albendazole	Anthelmintic	Averkin <i>et al.</i> (1975) Marriner and Bogan (1980)
Enoximone	Positive inotropy	Dage and Okerhjolm (1990)
O-Ethyl-O-[4-(methylthio)-phenyl]S-propyl phosphorodithioate	Insecticide	Bull <i>et al.</i> (1976)
Insecticides (various)	Insecticide	Kulkarni and Hodgson (1980)
Thioridazine	Cardiotoxicity via 5-sulfoxide	Hale and Poklis (1984), Heath <i>et al.</i> (1985)
Thioalkyl-substituted 4,5-diarylimidazoles	Anti-arthritic activity (all redox forms show some activity)	Sharpe <i>et al.</i> (1985)
Disulfiram (via S-methyl N,N-diethylthio carbamate)	Aldehyde dehydrogenase inhibition	Johansson (1992) Madan and Faiman (1994)
DCVC (S-(1,2-dichlorovinyl)-L-cysteine)	Nephrotoxicity	Lash <i>et al.</i> (1994)
Phosfolan and mephosfolan	Insecticide/irreversible acetylcholinesterase inhibitor	Gorder <i>et al.</i> (1985)
Metribuzin	Hepatotoxicity	Bleeke <i>et al.</i> (1985)
<i>Sulfoxides reduced to active thioethers</i>		
Dimethyldisulfide sulfoxide	Haemolytic anaemia	Smith (1980)
Sulindac	Anti-inflammatory	Duggan (1981), Duggan <i>et al.</i> (1977)
Sulphinpyrazone	Anti-aggregatory on platelets	Kirstein Pedersen and Jakobsen (1979) Ritschel and Kuo (1987) Hanna <i>et al.</i> (1990)
6,7-Dihydro-2-(4-(methylsulfinyl)phenyl)-3-(4-pyridyl)-5(H)-pyrrolo(1,2-a)imidazole	Inhibition of 5-lipoxygenase and prostaglandin synthase	
<i>Sulfoxides and sulfones with inherent activity</i>		
Dapsone (di(p-aminophenyl)-sulphone)	Anti-leprosy	Ellard (1966), Venkatesan (1989)
Omeprazole	Proton pump inhibition (via sulfenamide)	Fryklund and Wallmark (1986)
Sulfinpyrazone	Uricosuric	Burns <i>et al.</i> (1957)

the reaction catalysed. Most of the data relate to the oxidation or reduction of simple sulfoxides and the enzymes have been well characterized. In contrast, there is little information available on the enzymatic redox interconversion of other sulfur–oxygen centres such as sulfenamides. The following enzymes may or may not be involved in redox reactions at more complex sulfur–oxygen centres.

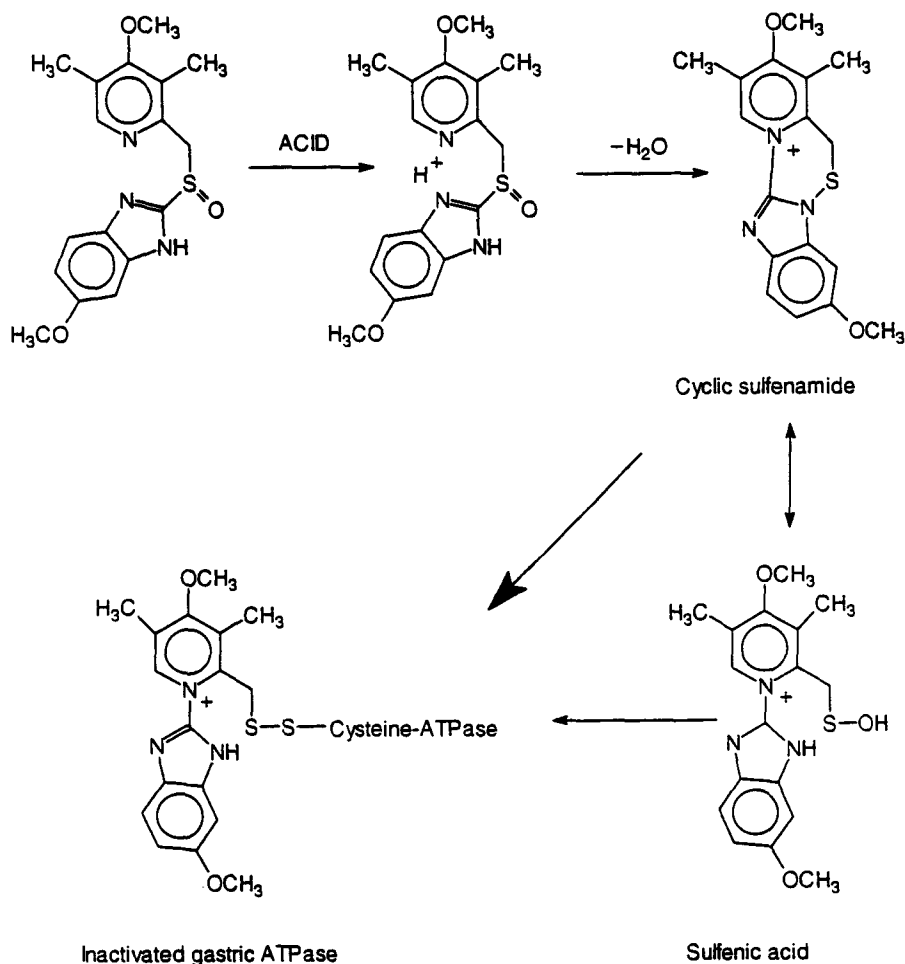


Figure 3.6 The mechanism of action of omeprazole

3.2.2.1 Oxidation of thioethers to sulfoxides

The major enzyme systems involved in xenobiotic oxidation are the cytochrome P450 superfamily (Souhali *et al.* 1987) and the flavin-monooxygenases (Hodgson and Levi 1992, Levi and Hodgson 1988, Ziegler 1989).

The sulfoxide of carbocysteine (S-carboxymethyl-L-cysteine) has been reported to be a major urinary metabolite in some subjects. The formation of the sulfoxide is probably via a cytochrome P450 isoenzyme since its production *in vivo* is inhibited by co-administration of cimetidine (Mitchell *et al.* 1981). The wide interindividual variations are probably under genetic control (Mitchell *et al.* 1984), although more recent studies which have used a more specific HPLC assay method have failed to confirm the reported polymorphism (Brockmoller *et al.* 1991).

Poor sulfoxidation of garlic sulfur constituents has been associated with their elimination in sweat and a problem of contact contamination in the workplace (Harris *et al.* 1986).

3.2.2.2 Reduction of sulfoxides to thioethers

There are three important sites of sulfoxide reduction *in vivo*: the liver, the kidney and the gut microflora. The tissue enzymes capable of reducing sulfoxides *in vitro* are primarily aldehyde oxidase (Tatsumi *et al.* 1982, 1983, Yoshihara and Tatsumi 1985a, b) and thioredoxin and its reductase (Anders *et al.* 1980, 1981). *In vitro* studies using tissue homogenates with various cofactors and inhibitors have shown that the principal enzyme involved in the liver is aldehyde oxidase, whereas thioredoxin is important in the kidney (Lee and Renwick 1995a). The former enzyme was mostly active under anaerobic conditions while thioredoxin-linked sulfoxide reduction occurred under both aerobic and anaerobic conditions. The possible role of aldehyde oxidase *in vivo* may be determined by the degree of hypoxia. Studies using an isolated perfused guinea pig liver under both normal and hypoxic conditions showed that cytochrome P450 catalysed oxidation of both diphenylsulfide and diphenylsulfoxide predominated under aerobic conditions (Yoshihara and Tatsumi 1990). Under hypoxic conditions oxidation of diphenylsulfoxide to the sulfone was decreased and trace amounts of diphenylsulfide were formed. Coinfusion of the sulfoxide with 2-hydroxypyrimidine and benzaldehyde, which are electron donors for aldehyde oxidase, resulted in increased reduction, whereas coinfusion with xanthine did not increase the formation of diphenylsulfide. The importance of the kidney and thioredoxin *in vivo* under normoxic conditions is indicated by the decreased reduction of sulindac in patients with end-stage renal disease as compared to controls (Gibson *et al.* 1987).

The gut microflora combined with the anaerobic environment of the lower bowel can be an important, and sometimes the sole, site of reduction of sulfoxides. Sulfapyrazone is a sulfoxide drug which is reduced to a thioether analogue with platelet anti-aggregatory activity. Studies in germ-free animals or animals treated with antibiotics to suppress the gut microflora indicated that the gut bacteria were the main, or possibly the sole, site of sulfoxide reduction *in vivo* (Renwick *et al.* 1982, Strong *et al.* 1984b). Studies in humans who had undergone an ileostomy (Strong *et al.* 1984a) or who had been treated with antibiotics (Strong *et al.* 1986) indicated that the anaerobic bacteria of the lower bowel were essential for the reduction of sulfapyrazone to its active thioether.

Sulindac is a sulfoxide prodrug which requires reduction to the active anti-inflammatory thioether analogue. *In vitro* studies have shown extensive reduction of sulindac to its active metabolite by tissues such as the liver and kidneys. The intestinal bacteria are also able to reduce sulindac *in vitro* (Strong *et al.* 1985) and the thioether is a major circulating metabolite *in vivo*. The contribution of the intestinal microflora to the reduction of sulindac in humans was demonstrated by data from normal subjects and patients who had undergone an ileostomy. The results suggested that the tissues produced an initial peak (0–12 h after the dose) corresponding to 45% of the total thioether, and the intestinal bacteria produced a secondary phase (12–72 h) corresponding to 55% of the total thioether (Strong *et al.* 1985).

Studies with > 200 isolated strains of bacteria from human faeces (Strong *et al.* 1987) demonstrated significant sulfoxide reduction by many aerobic organisms such as *Escherichia coli* under anaerobic conditions. Few strict anaerobes show high reducing activity *in vitro* but they are probably essential *in vivo* in providing the

anaerobic environment in which the aerobes are able to express their sulfoxide reductase activity (Strong *et al.* 1987).

The enzymes involved in microbial xenobiotic sulfoxide reduction have not been defined. Early studies on sulfoxide reductase by *E. coli* utilized the sulfoxides of normal cell constituents such as methionine (Sourkes and Trano 1953) and biotin (Cleary and Dykhuizen 1974), the sulfoxides of which are probably reduced by different enzyme systems since some mutants of *E. coli* are able to utilize one of the sulfoxides but not the other (Zinder and Brock 1978). The enzyme(s) responsible for the reduction of methionine sulfoxide is (are) not the same enzyme(s) as that (those) responsible for reducing this entity when it exists within peptide chains (Brot *et al.* 1981). Recent studies using xenobiotics as substrates have indicated the presence of a number of different soluble enzymes in *E. coli* capable of sulfoxide reduction, one of which appeared to be a NADPH-linked thioredoxin system; only one of the enzymes was able to reduce the more hindered sulfoxide groups of sulfinpyrazone or flosequinan (Lee and Renwick 1995b). Chiral inversion of the enantiomers of the sulfoxide flosequinan involves redox cycling with stereoselective reduction to the thioether via the intestinal bacteria, which are the sole site of reduction in rats, and reoxidation back to the sulfoxide (Kashiyama *et al.* 1994).

The interconversion of albendazole and its sulfoxide have been studied *in vitro* under anaerobic conditions with the ruminal and ileal contents of cattle and sheep (Lanusse *et al.* 1992). The oxidation of albendazole to its sulfoxide was greater in cattle than in sheep while the opposite was found for the reduction of the sulfoxide; the sulfone was metabolically stable. The sulfoxide metabolite of cimetidine is reduced by faecal preparations *in vitro* (Mitchell *et al.* 1982), but the significance of this reaction *in vivo* is undefined.

3.2.2.3 Oxidation of sulfoxides to sulfones

The oxidation of sulfoxides to the corresponding sulfones occurs with both tissues and microorganisms. The oxidation of the sulfoxide, sulindac, to its inactive sulfone is a major metabolic pathway in humans (Strong *et al.* 1985) and in microbes such as *Nocardia* under aerobic conditions (Davis and Guenther 1985). The extent of microbial oxidation was dependent upon the degree of aeration, and this may explain the negligible oxidation of sulfoxides but extensive reduction to the thioether with incubations of mixed intestinal bacteria under anaerobic conditions (Renwick *et al.* 1982). The enzymes involved in the oxidation of sulfoxides to sulfones have not been identified in either mammalian tissues (probably cytochrome P450; Levi and Hodgson 1988) or in microbes (Davis and Guenther 1985).

3.2.2.4 Reduction of sulfones to sulfoxides

Sulfones are generally stable to metabolism both *in vivo* and *in vitro*, e.g. dapsone (4,4'-diamino-diphenylsulfone) (Ellard 1966), tolmesoxide sulfone (Greenslade *et al.* 1981) or sulindac sulfone (Duggan *et al.* 1978). Sulindac sulfone is not reduced by organisms such as *Aspergillus*, *Arthrobacter* and *Nocardia* (Davis and Guenther 1985) or by mixed intestinal organisms (Strong and Renwick, unpublished data), indicating that the microbial sulfoxide reductases (Lee and

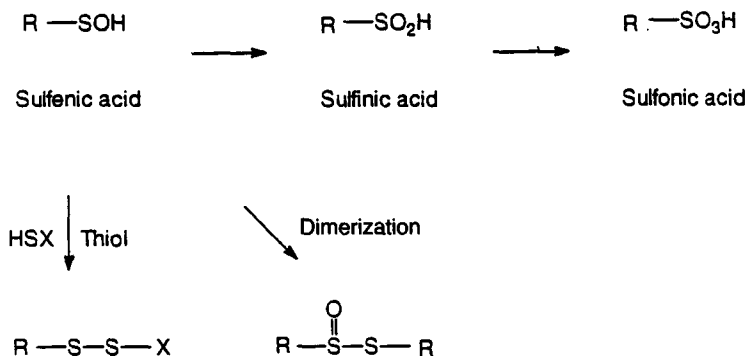


Figure 3.7 The fates of sulfenic acids

Renwick 1995b) are unable to reduce xenobiotic sulfones. Similarly, *E. coli* did not reduce dimethylsulfone (Zinder and Brock 1978) or methionine sulfone (Ejiri *et al.* 1979) *in vitro* and these sulfones did not inhibit the reduction of the corresponding sulfoxides to their thioethers. In contrast, mutants of *E. coli* have been isolated which express a novel sulfone-oxidizing enzyme which is active against heterocyclic sulfones and aliphatic sulfones (Juhl and Clark 1990).

3.3 Sulfenic acids

Sulfenic acids (R—S—OH) are formed by mild oxidation of thiols but are unstable and readily undergo further oxidation to sulfinic and sulfonic acids or combine with nucleophiles (Figure 3.7). Electrochemical oxidation of 6-thiopurine to the corresponding sulfenic and sulfinic acids occurs at a potential of 0.4–0.8 V but oxidation through to purine-6-sulfonic acid occurs at >0.8 V (Zhu and BrajterToth 1990). The sulfenic acid group of purine-6-sulfenic acid is chemically reactive and binds covalently to proteins *in vivo* (Abraham *et al.* 1983). Additional reactions of sulfenic acids include dimerization with elimination of water to produce R—SO—S—R , and oxidation to the corresponding sulfinic (RSO_2H) and sulfonic (RSO_3H) acids.

Oxidation of the thiol group of cysteine in proteins can give stabilized sulfenic acid groups which are critical to the active site of enzymes such as papain and glyceraldehyde-3-phosphate dehydrogenase (Claiborne *et al.* 1993). Sulfenic acid groups have been identified in the active sites of streptococcal NADH peroxidase (Parsonage *et al.* 1993, Stehle *et al.* 1993) and flavoprotein NADH oxidase (by sequence homology; Ross and Claiborne 1992). It is possible that the local environment of the active site stabilizes the sulfenic acid group and prevents reaction with other thiols. Organisms such as *Streptococcus faecalis*, which do not synthesize haem and lack peroxide-metabolizing enzymes such as catalase, use these two flavoproteins to reduce H_2O_2 and O_2 . In both enzymes, the nonflavin redox centre is believed to be a cysteine sulfenic acid (Ahmed and Claiborne 1989, Poole and Claiborne 1989), rather than a cystine disulfide, which is interconverted between sulfenic acid and thiol. The glutathione reductase and thioredoxin reductase of *E. coli* show significant sequence homology with the streptococcal

NADH peroxidase, and the active cysteine of the glutathione reductase shows a similar environment (Ross and Claiborne 1991). Thus redox cycling between thiol and sulfenic acid may be critical to the catalytic activity of a number of bacterial redox enzymes.

3.3.1 Sulfenic acids as active/reactive metabolites

Sulfenic acids have been postulated as reactive covalently binding metabolites of thiocarbamides, produced by both cytochrome P450 and FMN enzymes, and which bind to thiol groups in microsomal proteins including cytochrome P450 (Decker and Doerge 1992). Propyluracil-2-sulfenic acid has been proposed as a reactive metabolite of propylthiouracil which may be related to the hypersensitivity reactions, such as agranulocytosis, seen with this drug (Waldhauser and Utrecht 1991).

Deacetylation of spironolactone produces a thiol, oxidation of which is via an unstable sulfenic acid to sulfinic and sulfonic acids (Figure 3.8); this oxidation is involved in irreversible inactivation of cytochrome P450 (Decker *et al.* 1989) which has been implicated in gynaecomastia linked to the use of this diuretic.

Cytochrome P450 oxidation of 6-thiopurine results in a reactive entity, probably purine-6-sulfenic acid, which forms disulfide bonds with cysteine residues in microsomal proteins (Abraham *et al.* 1983).

3.4 Sulfinic acids

The sulfinic acid group ($R-SO_2H$) is an intermediate redox state between sulfenic and sulfonic acids. Endogenous sulfinic acids include cysteine sulfinic acid which is released during neuronal stimulation (Klancnik *et al.* 1992) and may be involved in synaptic transmission in the hippocampus. Cysteine- and homocysteine-sulfinic acids are excitatory and cytotoxic acidic amino acids (Frandsen *et al.* 1993). Cysteine sulfinic acid probably acts as a neurotransmitter in the hippocampus since it undergoes specific uptake and is released in response to depolarization (Baba 1987).

Cysteine sulfinic acid undergoes decarboxylation which is important in the synthesis of taurine (Jenkins and Steele 1991a) (Figure 3.9). Cysteine sulfinic acid decarboxylase responds rapidly and reversibly to changes in the nature of dietary proteins (Jenkins *et al.* 1989) and sulfur amino acid (Jenkins and Steele 1991b).

3.5 Sulfonic acids

The sulfonic acid group ($R-SO_3H$) is a highly polar centre and gives very high water solubility to the molecule. Therefore the sulfonic acid group is not present on many drugs since it would result in very slow or incomplete absorption from the gastrointestinal tract and very rapid elimination in urine. In addition, the polar centre would limit distribution of the chemical to the tissues so that very low tissue concentrations would occur *in vivo*.

These properties of the sulfonic acid group have been utilized in the drug

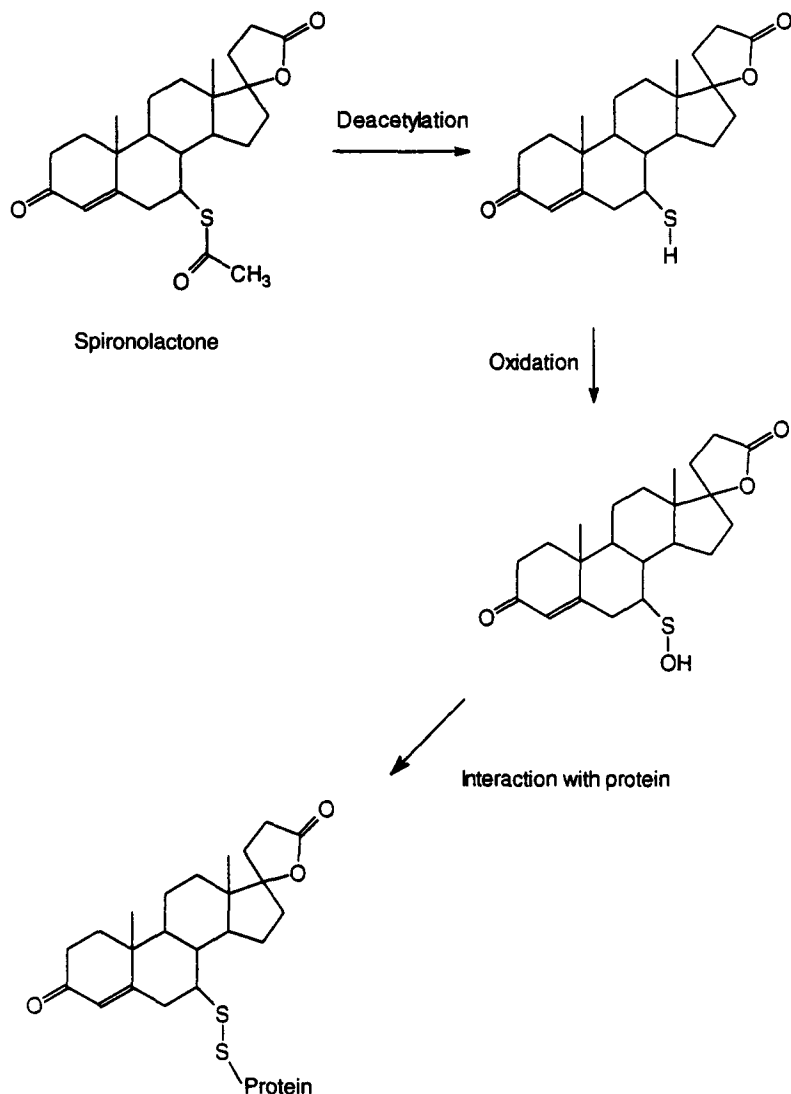


Figure 3.8 Metabolic activation of spironolactone

MESNA (mercaptoethane sulfonic acid) which is given in conjunction with cytotoxic chemotherapy in order to deliver a cytoprotective thiol (mercapto) group to the urinary bladder in order to reduce unwanted toxicity at this site. MESNA is usually given by intravenous injection because of its poor and erratic absorption.

The other major occurrence of sulfonic acids in xenobiotics is their presence as the polar end of detergents. In consequence, the main data on the biodegradation of alkyl and aryl sulfonic acids relates to environmental organisms. The sulfonic acid group of taurine (Figure 3.9) is important in the emulsifying action of bile salts when it is conjugated to cholic acid. Taurine is synthesized from cysteine, via oxidation to cysteine sulfinic acid, by the enzyme cysteine dioxygenase

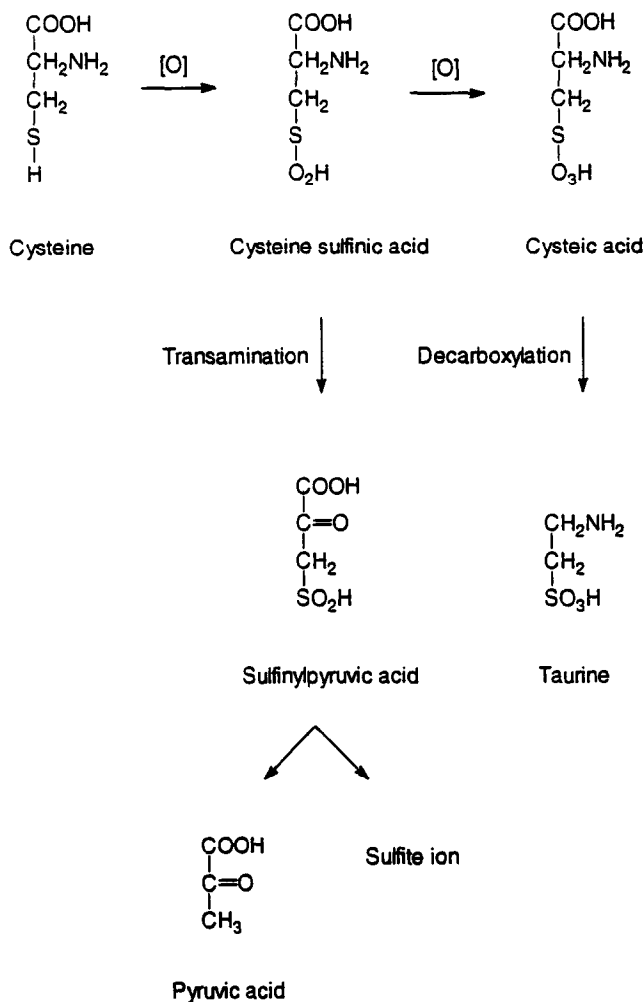


Figure 3.9 The fates of cysteine sulfenic acid

(Figure 3.9). Apart from its importance in the synthesis of bile salts, taurine may serve a transmitter or neuromodulator role in the retina (Lin *et al.* 1985), hippocampus (Taber *et al.* 1986), cerebellum (Magnusson *et al.* 1988) and pyramidal cells (Magnusson *et al.* 1989). The synthesis of taurine in the CNS is controlled by the action of cysteine dioxygenase (Ida *et al.* 1985). Although taurine has clear effects *in vitro*, there are questions over its importance *in vivo* (Taber *et al.* 1986).

An alternative metabolic pathway for cysteine sulfenic acid is transamination to β -sulfinylpyruvate and hence to sulfite, which is a toxic inorganic form of sulfur (Westley 1989). Sulfite oxidation to sulfate is via the enzyme, sulfite oxidase, the importance of which is shown by the fatal neurological and respiratory symptoms reported in patients with the rare deficiency in the enzyme inherited as a recessive autosomal trait (Vianey Liand *et al.* 1988).

In general, sulfonic acids are stable to metabolism (Williams 1959), and many

water-soluble sulfonated azo-dyes which are poorly absorbed and reach the gut flora are not metabolized at the sulfonic acid moiety but at other sites such as the azo bond (Walker 1970). Desulfonation was not found *in vivo* with simple alkyl sulfonates (Taylor *et al.* 1978), with branched-chain sulfonates (Michael 1968) or with the 1- and 6-sulfonic acid derivatives of 2-naphthylamine (Batten 1979). In contrast to these studies with animals, desulfonation has been found *in vitro* on incubation of alkyl sulfonates with environmental organisms such as the algae *Chlorella fusca* (Biedlingmaier and Schmidt 1983) and *Pseudomonas* (Thyssen and Wanders 1974). The mechanism of desulfonation of *n*-alkane sulfonates involves an initial oxidation on C1 to form a 1-hydroxy-alkane sulfonate, which is equivalent to an aldehyde bisulfite complex and which decomposes to the aldehyde and bisulfite ion. The desulfonation reactions were often found under conditions in which the sulfonate was the sole source of carbon and sulfur. The mechanisms of desulfonation of aromatic sulfonates (Ripin *et al.* 1971) and alkyl benzene sulfonates (Willettts and Cain 1972) are not known. Some intestinal bacteria, e.g. *E. coli*, *Enterobacter aerogenes* and *Serratia marcescens*, are able to utilize sulfonates as sulfur sources under aerobic, but not anaerobic, conditions (Uria Nickelsen *et al.* 1983). The reaction may involve the release of sulfite, but not sulfate, since studies in mutant strains have shown that sulfite reductase is essential for the utilization of alkylsulfonates.

3.6 Sulfate esters

Sulfate esters ($R-O-SO_3H$) are encountered most frequently following conjugation of alcoholic or phenolic hydroxyl groups with sulfate which is one of the most common phase II reactions. The conjugation reaction requires high-energy sulfate (3'-phosphoadenosine-5'-phosphosulfate or PAPS) and a transferase enzyme (Figure 3.10). Sulfate conjugation occurs primarily in the gut wall and liver and the products are highly-ionic, water-soluble metabolites which are frequently excreted largely in the urine. Sulfate conjugates are rapidly eliminated by renal tubular secretion such that renal clearance exceeds the glomerular filtration rate.

The bile is an important route by which sulfate conjugates can pass to the gut microflora, but there is a molecular weight threshold (~ 500 in humans and ~ 325 in rats) below which there is negligible biliary excretion of anions. Sulfate conjugation increases the molecular weight of an hydroxy compound by 80, whereas glucuronidation causes an increase of 176. In consequence, the enterohepatic circulation of xenobiotics usually involves glucuronic acid, rather than sulfate, conjugates.

Biliary excretion of sulfate conjugates is most important for larger substrates (i.e. of $M_r \geq 300$) such as triiodothyronine (Rutgers *et al.* 1989) and steroids. The sulfate conjugate may undergo hydrolysis by the bacteria present in the lower bowel. Hydrolysis of the sulfate conjugates of iodothyronine is catalysed by rat caecal anaerobes such as *Lactobacillus* (DeHerder *et al.* 1985). The 3-sulfate ester of sulfated bile acids is removed by anaerobic bacteria in human faeces (Pacini *et al.* 1987) and rat microflora (Huijghebaert *et al.* 1982). Bile acid sulfates are hydrolysed by a strictly anaerobic strain of gut bacteria, probably a *Clostridium* (Huijghebaert *et al.* 1982). The activity of the enzyme towards xenobiotic sulfates has not been

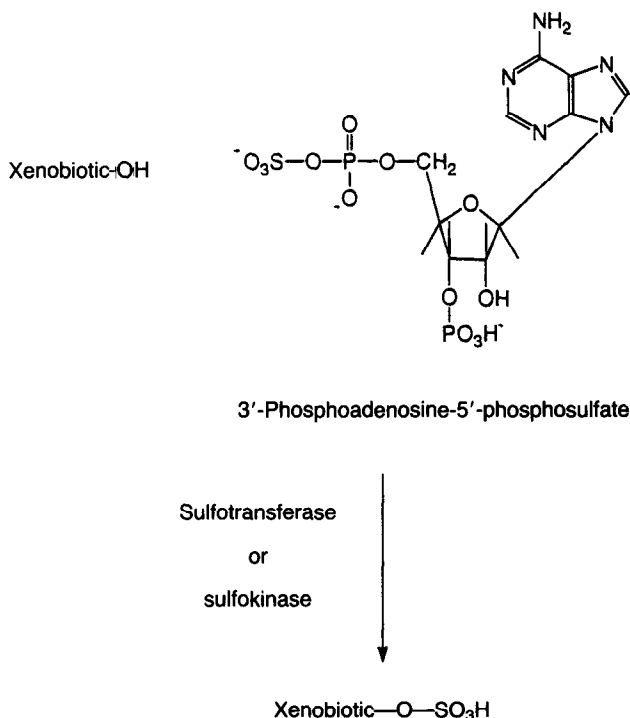


Figure 3.10 Phase II xenobiotic conjugation with sulfate

defined. Hydrolysis of sulfate conjugates eliminated in the bile, by the gut microflora, is an important part of the enterohepatic circulation of a number of xenobiotics, e.g. benzo[a]pyrene (Boroujerdi *et al.* 1981), carbenoxolone (Iveson *et al.* 1971), oxyphenisatin (Sund *et al.* 1979) and vanillin/isovanillin (Strand and Scheline 1975); however, in many cases glucuronidation and microbial β -glucuronidase are also involved in the enterohepatic cycling. In most cases the hydrolysis of sulfate esters releases an active compound such that the reaction can be regarded as an activation or intoxication process.

Although mammalian tissues do not reduce inorganic sulfate, the sulfate released by the gut microflora sulfatase may be reduced by sulfate-reducing bacteria (Gibson *et al.* 1993a); there is wide interindividual variability in the presence of bacterial sulfate-reducing activity in humans which shows an inverse relationship with methane-producing organisms (Gibson *et al.* 1993b). A large proportion (~25%) of humans do not show sulfate-reducing activity despite a wide distribution of sulfate-reducing activity, e.g. *Desulfovibrio* spp., *Desulfobacter* spp., *Desulfobulbus* spp. and *Desulfomonas* spp. The situation is analogous to that of cyclamate hydrolysis (see later), and like cyclamate hydrolysis, the sulfate-reducing activity is inducible by the substrate (Christl *et al.* 1992).

Although phase II sulfate conjugates are generally inactive excretory products, there are a number of examples where formation of a sulfate ester is important for activity. Steroid sulfates represent important transport forms in the general circulation (Briggs and Brotherton 1970) and are activated by hydrolysis back to the parent steroid by tissue sulfatases (Farooqui 1980, Farooqui and Mandel 1977,

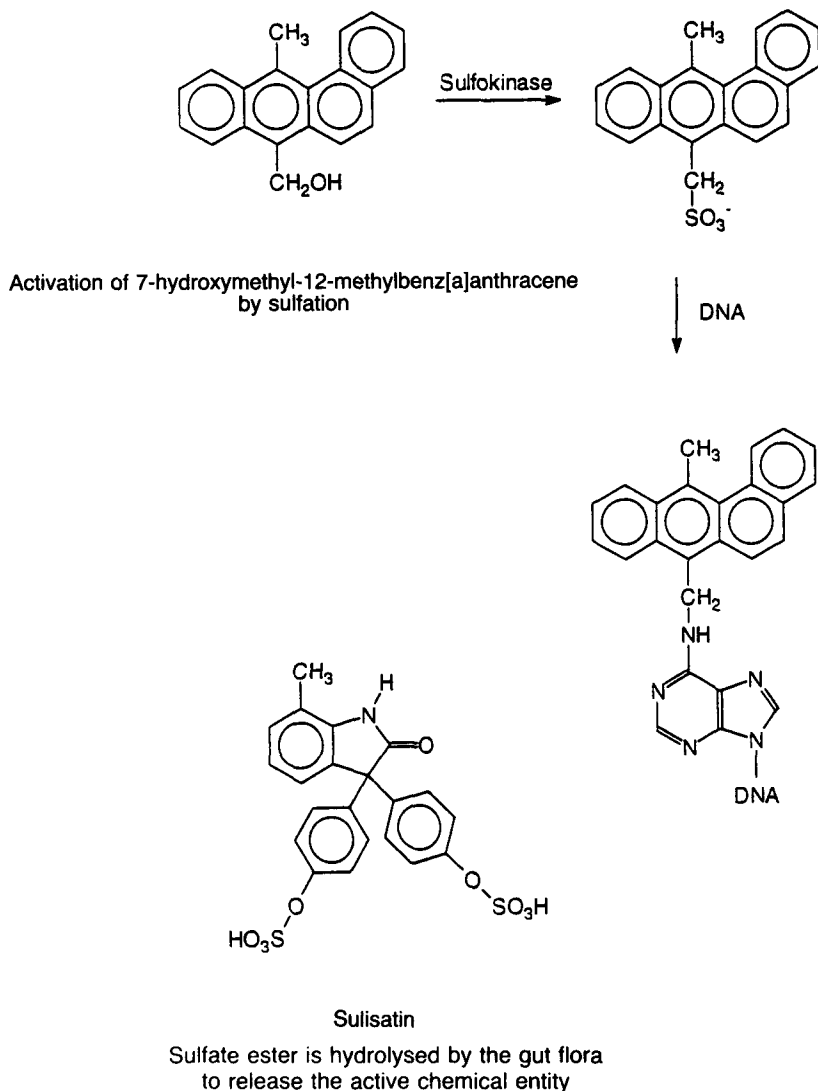


Figure 3.11 Active sulfate esters

Moriyasu and Ito 1982, Moriyasu *et al.* 1982, Roy 1970). The gut microflora play an important role in the enterohepatic circulation of steroid sulfates by hydrolysis of both the sulfate moiety and also the glycosidic link of double conjugates such as oestriol-3-sulfate-16-glucuronide (Levitz and Katz 1968).

For some carcinogens, conjugation with sulfate to form CO— and NO— sulfate esters results in activation to a reactive species which is critical to the biological activity of the parent compound. Examples include N-hydroxyacetylaminofluorene (Grantham *et al.* 1970, Smith *et al.* 1987, Williams *et al.* 1970), polycyclic aromatic hydrocarbons (Boroujerdi *et al.* 1981, Watabe *et al.* 1985) and the 1-hydroxy metabolite of alkylbenzenes (Randerath *et al.* 1984) (Figure 3.11).

Exogenous sulfate esters are rarely encountered as therapeutic drugs because

of their high polarity and poor absorption as discussed above for sulfonic acids. However, an exogenous sulfate ester could represent a prodrug capable of undergoing hydrolysis within the bowel by the gut flora to liberate an active entity. This is the mechanism of action of sulfate esters given as laxatives, e.g. 4,4'-dihydroxydiphenyl(pyridyl-2-)methane disulfate (Forth *et al.* 1972), sulisatin (Moreto *et al.* 1977) and oxyphenisatin (Sund *et al.* 1979).

Exogenous sulfates, in common with sulfonic acids, are also used as detergents, although they can undergo *in vivo* hydrolysis to liberate sulfate. Primary alkylsulfate esters undergo ω - and β -oxidation to yield primarily butanoate-4-sulfate and propionate-3-sulfate which can be degraded nonenzymatically to sulfate (Burke *et al.* 1976, Denner *et al.* 1969, Merits 1975, Ottery *et al.* 1970). Secondary alkyl sulfate esters undergo more complex metabolism but the sulfate ester group is retained (Bains *et al.* 1987, Maggs *et al.* 1982).

3.7 Sulfamic acids

The sulfamic acid group ($-\text{NHSO}_3\text{H}$) is a highly polar ionizable group which is the nitrogen equivalent of a sulfate ester ($-\text{OSO}_3\text{H}$). The group is widely distributed in nature as a polar functional group in heteropolysaccharides. The sulfamate group is also produced metabolically as a phase II conjugation reaction of xenobiotic amino compounds (Iwasaki *et al.* 1986, Turesky *et al.* 1986) including the food-borne carcinogens, IQ, MeIQ and MeIQX (Alexander *et al.* 1989, Inamasu *et al.* 1989, Turesky *et al.* 1988, 1993). The N-sulfamate is a urinary metabolite of the β_2 -adrenoceptor agonist, clenbuterol (Schmid *et al.* 1990).

The sulfamate group in amino sugars can be hydrolysed both by mammalian enzymes (Freeman and Hopwood 1986) and by microorganisms such as *Flavobacterium heparinum* (Bruce *et al.* 1987). In contrast, xenobiotic sulfamates appear to be stable to tissue hydrolysis but may be hydrolysed by an inducible bacterial enzyme system (see below).

An interesting property exhibited by some sulfamates is that of sweetness (Spillane and McGlinchey 1981, Spillane *et al.* 1983) which was originally observed accidentally for N-cyclohexylsulfamate (Audrieth and Sveda 1944). This sulfamate was developed as the intense sweetener, cyclamate, which has been the source of most of our knowledge and understanding of the fate of xenobiotic sulfamates in the body.

The introduction of a sulfamate group into a molecule produces a more water-soluble chemical and blocks a potentially active amino group; but this does not necessarily cause inactivation, for example, ribavirin-5'-sulfamate is an antiviral agent (Smee *et al.* 1988). It is not clear whether such compounds are active *per se* or following hydrolysis by tissue sulfamate sulfohydrolase active against glycosulfamates (Freeman and Hopwood 1986).

The lowering of blood lipids, including cholesterol, is another interesting property of some sulfamates, which is shared by benzylsulfonamides and cyclic sulfonamides like saccharin (Wynik *et al.* 1984). However, despite their low toxicity, this property has not been exploited in the production of hypolipidaemic agents.

The fate of xenobiotic sulfamates in the body is dominated by the polarity of the group. Although most of the data relate to the simple sulfamate, cyclamate, with a limited nonpolar region, it is likely that other larger sulfamates would share

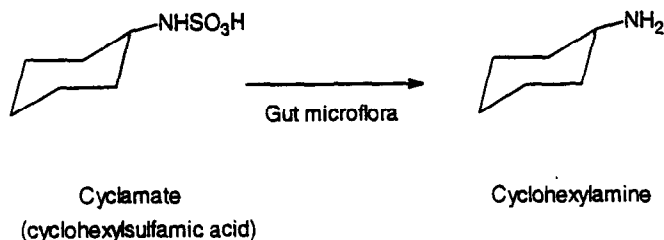


Figure 3.12 Metabolism of cyclamate to cyclohexylamine

many of its properties. There is incomplete absorption of cyclamate after oral administration, and >50% of an oral dose passes down the gut to the microflora of the lower bowel. Almost all information on the microbial hydrolysis of sulfamates has been developed in relation to cyclamate and its structural analogues. The hydrolysis of cyclamate to cyclohexylamine (Figure 3.12) also provides a number of interesting insights into the metabolism of sulfur xenobiotics by the intestinal microflora.

Cyclamate has been used as an intense sweetener since the mid-1950s (Bopp *et al.* 1986) but in 1969 it was banned in a number of countries when bladder tumours were detected in a study in which rats were given high dietary concentrations of a 10:1 cyclamate:saccharin mixture supplemented with cyclohexylamine, the metabolite of cyclamate (Price *et al.* 1970). Many countries did not ban cyclamate, because of the complexity of the study; recent studies have not confirmed the original finding (Bopp *et al.* 1986). The metabolism of cyclamate to cyclohexylamine is central to the establishment of safe use levels of the sweetener since the metabolite is considerably more toxic than is the parent compound (Renwick 1990). Metabolism was not detected in early studies on cyclamate (Miller *et al.* 1966, Taylor *et al.* 1951), but in 1966 cyclohexylamine was reported as a urinary metabolite of the sweetener in humans (Kojima and Ichibagase 1966). This discovery was rapidly confirmed by other researchers and resulted in the decision to add cyclohexylamine to the rat diet half-way through the carcinogenicity study of Price *et al.* (1970).

The site of cyclamate hydrolysis *in vivo* is the gut microflora since negligible hydrolysis is found after parenteral administration of cyclamate to rats (Bickel *et al.* 1974, Renwick and Williams 1972, Sonders *et al.* 1969), guinea pigs (Asahina *et al.* 1972b) and pigs (Collings 1971). In addition, large oral doses of antibiotics suppress the extent of cyclamate metabolism in animal species (Asahina *et al.* 1972b, Bickel *et al.* 1974, Collings 1971, Sonders *et al.* 1969) and also in humans (Collings 1971). These *in vivo* data are supported by the *in vitro* conversion of cyclamate to cyclohexylamine by mixed caecal or faecal organisms (Asahina *et al.* 1972a, b, Bickel *et al.* 1974, Collings 1971, Drasar *et al.* 1972, Golberg *et al.* 1969, Mallet *et al.* 1985, Matsui *et al.* 1981, Renwick 1976, Tesoriero and Roxon 1975, Tokeida *et al.* 1979, Tsuchiya 1981). Many of these *in vitro* studies also investigated isolated strains of intestinal bacteria from different mammalian hosts and reported cyclamate hydrolysing activity in *Clostridia*, *Pseudomonas*, *Corynebacterium*, *Enterobacterium*, *Campylobacteria*, *E. coli*, *Enterococci*, *Streptococcus faecalis* and *Bacillus*. Given this apparent widespread occurrence of cyclamate sulfamate activity, it is surprising that there is negligible metabolizing activity *in vivo* prior

to chronic cyclamate administration and also that there is very wide interindividual variability in both animals and humans.

Single doses of cyclamate undergo negligible hydrolysis (Renwick and Williams 1972), and cyclohexylamine formation increases during chronic cyclamate intake to reach a relatively constant level. In humans it takes ~2 weeks for cyclamate metabolism to reach a plateau (Buss *et al.* 1992, Davis *et al.* 1969, Renwick and Williams 1972). In contrast, induction of cyclamate metabolism in rats may take considerably longer (Renwick 1986), and some rat colonies never developed cyclamate metabolism during long-term chronic intake (Collings 1971, Dalderup *et al.* 1970). After a period of induction the extent of cyclamate metabolism *in vivo* shows considerable day-to-day fluctuations during regular daily dosing in both rats and humans (Buss *et al.* 1992, Collings 1971, Davis *et al.* 1969, Litchfield and Swan 1971, Renwick 1976, Renwick and Williams 1972). Bowel function is partly responsible for the fluctuations in metabolism since there is an increase in the extent of metabolism within an individual during periods of constipation (Davis *et al.* 1969). The fluctuations probably arise also from variations in enzyme content of the gut flora. There is little consistency in cyclamate metabolizing ability when subjects are restudied after an interval of several months (Buss *et al.* 1992).

Factors known to affect cyclamate metabolism *in vitro* include the presence of a nutrient medium (Mallett *et al.* 1985), sulfur amino acids (Tesoriero and Roxon 1975), glucose (Dalderup *et al.* 1970) and cyclohexylamine (Drasar *et al.* 1972), but the cause of the intraindividual variability during chronic cyclamate intake has not been defined. Cyclamate metabolism *in vivo* is affected by constipation, coprophagy in animals (Dalderup *et al.* 1970), by neonatal exposure (Renwick 1976) and by short-term cessation of intake (Bickel *et al.* 1974, Renwick and Williams 1972).

Cyclamate sulfamatase activity shows extremely wide interindividual variability *in vivo* in experimental animals (Bickel *et al.* 1974, Collings 1971, Oser *et al.* 1968, Renwick 1976, 1986, Renwick and Williams 1972, Sonders *et al.* 1969) and also in humans (Buss *et al.* 1992, Collings 1971, Davis *et al.* 1969, Leahy *et al.* 1967, Litchfield and Swan 1971, Renwick and Williams 1972, Wills *et al.* 1981). About 80% of a human population do not excrete detectable amounts of cyclohexylamine during chronic intake, whereas 3–4% of the population can metabolize >20% of the daily dose. The same proportion was found in a study in diabetic patients in the UK in 1989 after a period of 20 years without cyclamate in the diet (Buss *et al.* 1992) (Figure 3.13). Therefore, there is probably some natural substrate (possibly a heteropolysaccharide) which maintains the potential for cyclamate hydrolysis within the microbial flora of the lower bowel.

The cyclamate sulfamatases in rat caecal contents (Renwick 1976), in *Pseudomonas* (Niimura *et al.* 1974, Tsuchiya 1981) and in an extract of rat faeces (McGlinchey *et al.* 1982) show a wide substrate specificity for alkylsulfamates, and simple aryl sulfamates, but there is no hydrolysis of the sulfamate ion, cyclohexanol sulfate or sulfanilamide by *Pseudomonas*, or of saccharin by rat caecal contents (Ball *et al.* 1977). *In vivo* studies have confirmed the wide specificity for simple alkylsulfamates (McGlinchey *et al.* 1982, Renwick 1976). It is not known if cyclamate sulfamatase can hydrolyse sulfamate groups in heteropolysaccharides.

The toxicological consequences of cyclamate hydrolysis relate to the safety of cyclamate as a food additive since cyclohexylamine can produce cardiovascular effects (Eichelbaum *et al.* 1974) and produces testicular toxicity when given to rats

Number of subjects

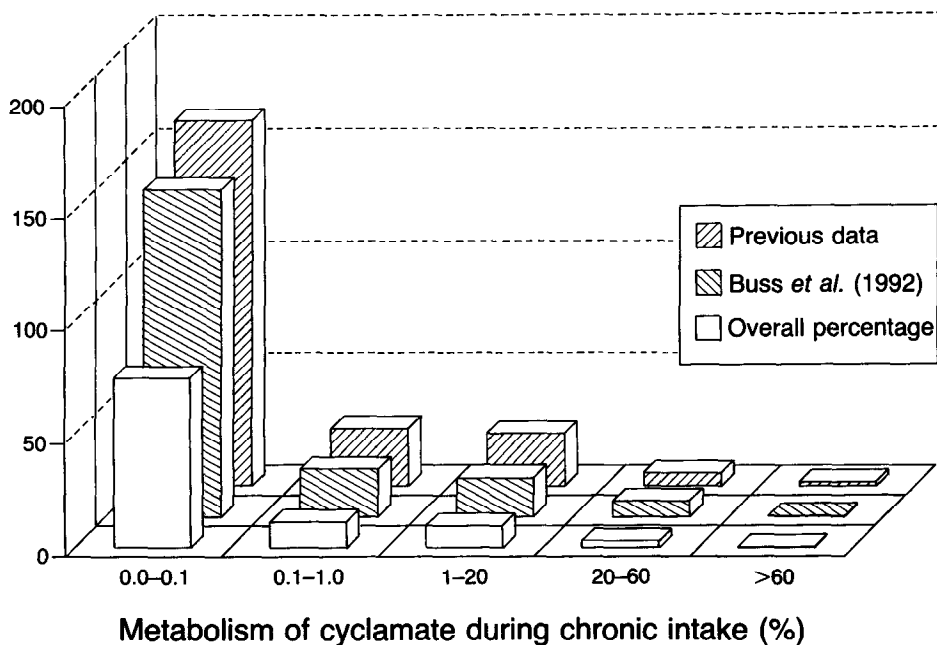


Figure 3.13 Interindividual variability in cyclamate metabolism in humans

at high doses (Bopp *et al.* 1986). Hydrolysis of cyclamate to cyclohexylamine *in vivo* did not cause cardiovascular effects in humans, despite the high plasma concentrations of cyclohexylamine produced in some subjects (Buss *et al.* 1992). The absence of an effect is explained by the indirect mechanisms of action of cyclohexylamine (Buss and Renwick 1992) and the slow build-up of cyclamate metabolizing ability. The testicular toxicity of cyclohexylamine is the basis for the current acceptable daily intake (ADI) allocated to cyclamate (Renwick 1990).

3.8 Sulfenamides and sulfinamides

There has been interest recently in these compounds which represent lower redox equivalents of the long-established sulfonamides and their structural analogues.

Sulfenosine (2-amino-9- β -D-ribofuranosylpurine-6-sulfenamide) and the higher sulfur redox analogues, sulfinosine and sulfonosine, have useful antitumour activities. Although there is the possibility of *in vivo* conversion of sulfenosine to sulfonosine, it is likely that each compound has independent activity since sulfonosine was highly active against a leukemia resistant to sulfenosine (Finch *et al.* 1990).

Nucleoside derivatives of purine-6-sulfenamides, sulfinamides and sulfonamides have been developed for their possible antitumour activity (Revankar *et al.* 1990a, b); the redox state of the sulfur atom was not a major determinant of activity (Revankar *et al.* 1990b). Changes in the carbohydrate and pyrimidine base were

of more importance than was the redox status of the sulfur atom in a series of pyrimidine analogues of sulfenosine (Ramasamy *et al.* 1990).

N-Oxydiethylene thiocarbamyl-N-oxydiethylene sulfenamide is a rubber accelerator of relatively low long-term toxicity (Hinderer *et al.* 1986).

3.9 Sulfonamides

The sulfonamide group ($-\text{SO}_2\text{NH}-$) is one of the most common sulfur-oxygen groups encountered in therapeutic drugs (Figure 3.14). This functional group rose to prominence with the discovery of the sulfonamide antibiotics (Domagk 1935, Fournau *et al.* 1936), although the chemical properties of the group had been under investigation for many years previously. For example, studies on the oxidation of isomeric toluene sulfonamides led to the accidental discovery of the sweetener, saccharin, by Fahlberg in 1879. The benzothiadiazide (thiazide) diuretics were also discovered 'accidentally' during studies to improve the action of the carbonic anhydrase inhibitor, acetazolamide.

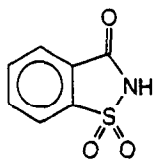
The hydrogen atom on the sulfonamide group is ionizable and increases the polarity, especially when a second electron-withdrawing group is adjacent to the nitrogen atom as in saccharin (Figure 3.14).

The main therapeutic action of sulphonamides is as antimicrobial agents. Structural requirements for antimicrobial activity are that the $-\text{SO}_2-\text{NH}_2$ has to be attached to a benzene ring [although the NH_2 group (N1) is not essential] and there needs to be an amino group (N4) *para* to the sulfonamide moiety as shown for sulfanilamide in Figure 3.15. The range of different sulfonamides available today arises from structural diversity largely at the N1 position. The developments of this chemical diversity spawned other 'sulfonamide' classes with different therapeutic properties.

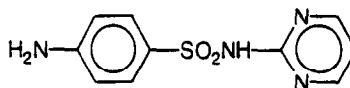
Sulfonamides exert their antibacterial action by inhibiting the incorporation by the bacteria of *p*-aminobenzoic acid into folic acid (Woods 1940). The selectivity of sulfonamides was revolutionary and arose from the fact that mammals, but not bacteria, can utilize preformed folic acid and indeed folic acid is a vitamin for humans. In consequence, sulfonamides do not affect the folate status of the host but prevent the growth and replication of bacteria.

Although sulfonamides have been replaced in recent years for many indications, they are still a drug of choice for urinary tract infections. This arises because sulfonamides are sufficiently water-soluble to be excreted unchanged and because there is limited metabolism *in vivo*. Pathways of metabolism include conjugation of both the N1 and N4 nitrogens with glucuronic acid and/or an acetyl group (Figure 3.15). Glucuronidation of the N4 site is possible but produces an unstable product (Bridges 1963), whereas N1-glucuronidation gives a stable excretory product which is the major urinary metabolite in humans and primates (Adamson *et al.* 1970). Acetylation of the N4 site is a major reaction for most therapeutic sulfonamides (Parke 1968).

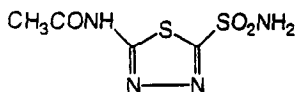
The primary sulfonamide group ($\text{R}-\text{SO}_2\text{NH}_2$) is an important part of carbonic anhydrase inhibitors such as acetazolamide (Figure 3.14) (Preisig *et al.* 1987). Carbonic anhydrase inhibitors have limited clinical use and have been replaced by more recently developed alternatives – many of which retain the sulfonamido group.



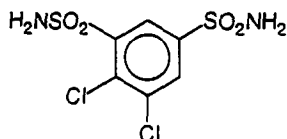
Saccharin
(sweetener)



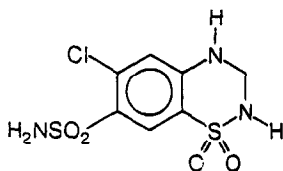
Sulfadiazine
(antibacterial)



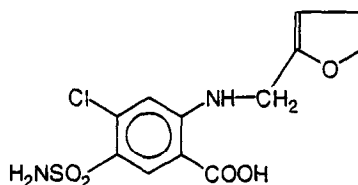
Acetazolamide
(carbonic anhydrase inhibitor)



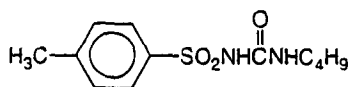
Dichlorophenamide
(carbonic anhydrase inhibitor)



Chlorothiazide
(diuretic)



Frusemide
(loop diuretic)



Tolbutamide
(hypoglycaemic)

Figure 3.14 Sulfonamido-compounds

The thiazide diuretics were synthesized in 1957 as potential carbonic anhydrase inhibitors but it was recognized in the earliest studies that chlorothiazide (Figure 3.14) produced increased excretion of chloride (Beyer 1958) which was not related to an action on carbonic anhydrase [see Orloff and Berliner (1961) for an early review]. Thiazides contain a sulfonamide group which is probably responsible for their weak carbonic anhydrase activity. Dichlorophenamide (Figure 3.14) is a noncyclic thiazide which is a 300-fold more potent inhibitor of carbonic anhydrase than is hydrochlorothiazide, but which has a much weaker natriuretic action (Velazquez 1987) and is classified as a carbonic anhydrase inhibitor (Preisig *et al.*

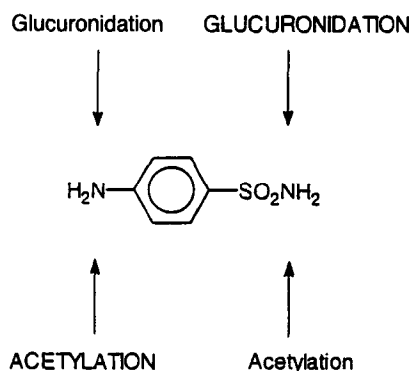


Figure 3.15 Sites of conjugation of sulfanilamide

1987). The mechanisms of action of thiazides include inhibition of intracellular carbonic anhydrase, but are mainly attributable to inhibition of the apical sodium/chloride cotransporter of the distal convoluted tubule. The ionic gradient across the cell is maintained by the basolateral Na^+/K^+ -ATPase and sodium and chloride are cotransported from the urine to the intracellular fluid down their concentration gradients. Simple thiazides, such as chlorothiazide and hydrochlorothiazide, are well absorbed from the gastrointestinal tract, but are excreted in the urine unchanged because of their high water solubility and the metabolic stability of the sulfonamide group (Beermann *et al.* 1976, Brettell *et al.* 1960). The introduction of sidechains into the benzothiazine structure allows metabolism in these sites (Wiseman *et al.* 1962). Other cyclic sulfonamides such as bentazon and saccharin undergo little or no metabolism (Hathway 1975, Renwick 1985).

The sulfonamide group is also present on the more recently introduced loop diuretics such as frusemide (Figure 3.14) and bumetanide. The sulfonamide group is an important structural element together with anionic and basic sites (Gregor and Wangemann 1987). Loop diuretics are high-ceiling diuretics and, as their name implies, act on the loop of Henlé to allow a far greater natriuresis and diuresis. In common with the thiazides, the site of action is on the apical membrane transporters, in this case the sodium/potassium/2 chloride cotransporter of the thick ascending limb of the loop of Henlé.

The sulfonylurea hypoglycaemics were developed in 1955 when Franke and Fuchs demonstrated a hypoglycaemic action in maturity-onset diabetes (Karam *et al.* 1975). Tolbutamide (Figure 3.14) is one of the most commonly used sulfonylureas, which act by increasing the release of insulin from the pancreas (Fain 1977). The site of action of sulfonylureas is the ATP sensitive- K^+ channel in the pancreatic β -cells. Closure of this channel is involved in the glucose-mediated release of insulin and closure of the channel is also produced via a high-affinity sulfonylurea receptor (Boyd 1988, Panten *et al.* 1989, Schmid-Antomarchi *et al.* 1987).

Diazoxide acts as a hypotensive agent by opening the ATP-sensitive K^+ -channels (Standen *et al.* 1989). Its use is now largely restricted to hypertensive crises since it produces profound hyperglycaemia by inhibiting insulin release; it acts at a different site to sulfonylureas on pancreatic cells (Panten *et al.* 1989).

It is clear from the above account that the sulfonamido group has been one of the most fertile sources of therapeutic agents, and that it is present in a wide variety of drug and chemical classes.

3.10 References

- ABRAHAM, R. T., BENSON, L. M. & JARDINE, I., 1983, Synthesis and pH dependent stability of purine-6-sulfenic acid, a putative reactive metabolite of 6-thiopurine, *Journal of Medicinal Chemistry*, **26**, 1523–1526.
- ADAMSON, R. H., BRIDGES, J. W., KIBBY, M. R., WALKER, S. R. & WILLIAMS, R. T. 1970, The fate of sulphadimethoxine in primates compared with other species, *Biochemical Journal*, **118**, 41–46.
- AHMED, S. A. & CLAIBORNE, A., 1989, The streptococcal NADH oxidase. I. Evidence linking NADH oxidase and NADH peroxidase cysteinyl redox centers, *Journal of Biological Chemistry*, **264**, 19856–19863.
- ALEXANDER, J., HOLME, J. A., WALLIN, H. & BECKER, G., 1989, Characterisation of the metabolites of food mutagens 2-amino-3-methylimidazo (4,5-f)quinoline and 2-amino-3,4-dimethylimidazo(4,5-f)quinoline formed after incubation with isolated rat liver, *Chemico-Biological Interactions*, **72**, 125–142.
- ANDERS, M. W., RATNAYAKE, J. H., HANNA, P. E. & FUCHS, J. A., 1980, Involvement of thioredoxin in sulfoxide reduction by mammalian tissues, *Biochemical and Biophysical Research Communications*, **97**, 846–851.
- 1981, Thioredoxin-dependent sulfoxide reduction by rat renal cytosol, *Drug Metabolism and Disposition*, **9**, 307–310.
- ANGUS, P. W., MIHALY, G. W., MORGAN, D. J. & SMALLWOOD, R. A. 1989, Oxygen dependence of omeprazole clearance and sulfone and sulfide metabolite formation in the isolated perfused rat liver, *Journal of Pharmacology and Experimental Therapeutics*, **250**, 1043–1047.
- ASAHINA, M., NIIMURA, T., YAMAHARA, T. & TAKAHASHI, T., 1972a, Formation of cyclohexylamine and cyclohexanone from cyclamate by microorganisms isolated from the feces of guinea pigs, *Agricultural and Biological Chemistry*, **36**, 711–718.
- ASAHINA, M., YAMAHARA, T., SARRAZIN, G. & WATANABE, K., 1972b, Conversion of cyclamate to cyclohexylamine in guinea pigs, *Chemical and Pharmaceutical Bulletin*, **20**, 102–108.
- AUDRIETH, L. F. & SVEDA, M., 1944, Preparation and properties of some N-substituted sulfamic acids, *Journal of Organic Chemistry*, **9**, 89–101.
- AVERKIN, E. A., BEARD, C. C., DVORAK, C. A., EDWARDS, J. A., FRIED, J. H., KILIAN, J. G., SCHILTZ, R. A., KISTNER, T. P., DRUDGE, J. H., LYONS, E. T., SHARP, M. L. & CORWIN, R. M., 1975, Methyl 5(b)-phenylsulfinyl-2-benzimidazolecarbamate, a new potent anthelmintic, *Journal of Medicinal Chemistry*, **18**, 1164–1166.
- BABA, A., 1987, Neurochemical characterisation of cysteine sulfinic acid, an excitatory amino acid in hippocampus, *Japanese Journal of Pharmacology*, **43**, 1–7.
- BAINS, S. K., OLAVESSEN, A. H., BLACK, J. G., HOWES, D., CURTIS, C. G. & POWELL, G. M., 1987, Metabolism in the rat of potassium nonan-5-sulphate, a symmetrical anionic surfactant, *Xenobiotica*, **17**, 709–723.
- BAKKE, J. E., GUSTAFSSON, J. A. & GUSTAFSSON, B. E., 1980, Metabolism of propachlor by the germ free rat, *Science*, **210**, 433–435.
- BALL, L. M., RENWICK, A. G. & WILLIAMS, R. T., 1977, The fate of [¹⁴C]saccharin in man, rat and rabbit and of 2-sulphamoyl[¹⁴C]benzoic acid in the rat, *Xenobiotica*, **7**, 189–203.

- BATTEN, P. I., 1979, Metabolism of 2-naphthylamine sulphonic acids, *Toxicology and Applied Pharmacology*, **48**, A171.
- BEERMANN, B., GROSCHINSKY-GRIND, M. & ROSEN, A., 1976, Absorption, metabolism and excretion of hydrochlorothiazide, *Clinical Pharmacology and Therapeutics*, **19**, 531-537.
- BESANCON, M., SHIN, J. M., MERCIER, F., MUNSON, K., MILLER, M., HERSEY, S. & SACHS, G., 1993, Membrane topology and omeprazole labeling of the gastric H^+ , K^+ -adenosinetriphosphatase, *Biochemistry*, **32**, 2345-2355.
- BEYER, K. H., 1958, The mechanism of action of chlorothiazide, *Annals of the New York Academy of Sciences*, **71**, 363-379.
- BICKEL, M. H., BURKARD, B., MEIER-STRASSER, E. & VAN DEN BROEK-BOOT, M., 1974, Enterobacterial formation of cyclohexylamine in rats ingesting cyclamate, *Xenobiotica*, **4**, 425-439.
- BIEDLINGMAIER, S. & SCHMIDT, A., 1983, Alkylsulfonic acids and some sulfur-containing detergents as sulfur sources for growth of *Chlorella fusca*, *Archives of Microbiology*, **136**, 124-130.
- BLEEKE, M. S., SMITH, M. T. & CASIDA, J. E., 1985, Metabolism and toxicity of metribuzin in mouse liver, *Pesticide Biochemistry and Physiology*, **23**, 123-130.
- BOPP, B. A., SONDEERS, R. C. & KESTERSON, J. W., 1986, Toxicological aspects of cyclamate and cyclohexylamine, *CRC Critical Reviews in Toxicology*, **16**, 213-306.
- BOROUJERDI, M., KUNG, H., WILSON, A. G. E. & ANDERSON, M. W., 1981, Metabolism and DNA binding of benzo(a)pyrene *in vivo* in the rat, *Cancer Research*, **41**, 951-957.
- BOYD, A. E., 1988, Sulfonylurea receptors, ion channels and fruit flies, *Diabetes*, **37**, 847-850.
- BRETTELL, H. R., AIKAWA, J. K. & GORDON, G. S., 1960, Studies with chlorothiazide tagged with radioactive carbon (^{14}C) in human beings, *Archives of Internal Medicine*, **106**, 57-63.
- BRIDGES, J. W., 1963, Aspects of the metabolism of aromatic amines, particularly sulphonamide drugs, PhD Thesis, University of London.
- BRIGGS, M. H. & BROTHERTON, J., 1970, *Steroid Biochemistry and Pharmacology*, pp. 80-81, London: Academic Press.
- BROCKMOLLER, J., STAFFELDT, B. & ROOTS, I., 1991, Evaluation of proposed sulfoxidation pathways of carbocysteine in man by HPLC quantification, *European Journal of Clinical Pharmacology*, **40**, 387-392.
- BROT, N., WEISSBACH, L., WORTH, J. & WEISSBACH, H., 1981, Enzymatic reduction of protein-bound methionine sulfoxide, *Proceedings of the National Academy of Sciences, USA*, **78**, 2155-2158.
- BRUCE, J. S., MCLEAN, M. W., LONG, W. F. & WILLIAMSON, F. B., 1987, Flavobacterium heparinum sulphamidase for D-glucosamine sulphamate. Purification and characterisation, *European Journal of Biochemistry*, **165**, 633-638.
- BULL, D. L., WHITTEN, C. J. & IVIE, G. W., 1976, Fate of O-ethyl O-[4-(methylthio)phenyl]S-propyl phosphorodithiolate (BAY NTN 9306) in cotton plants and soil, *Journal of Agriculture and Food Chemistry*, **24**, 601-605.
- BURKE, B., OLAVESAN, A. H., CURTIS, C. G. & POWELL, G. M., 1976, The biodegradation of the surfactant undecyl sulphate, *Xenobiotica*, **6**, 667-678.
- BURNS, J. J., YU, T. F., RITTERBAND, A., PEREL, J. M., GUTMAN, B. & BRODIE, B. B., 1957, A potent new uricosuric agent, the sulfoxide metabolite of the phenylbutazone analogue, G-25671, *Journal of Pharmacology and Experimental Therapeutics*, **119**, 418-426.
- BUSS, N. E. & RENWICK, A. G., 1992, Blood pressure changes and sympathetic function in rats given cyclohexylamine by intravenous infusion, *Toxicology and Applied Pharmacology*, **115**, 211-215.

- BUSS, N. E., RENWICK, A. G., DONALDSON, K. M. & GEORGE, C. F., 1992, The metabolism of cyclamate to cyclohexylamine and its cardiovascular consequences in human volunteers, *Toxicology and Applied Pharmacology*, **115**, 199–210.
- CHRISTL, S. U., GIBSON, G. R. & CUMMINGS, J. H., 1992, Role of dietary sulphate in the regulation of methanogenesis in the human large intestine, *Gut*, **33**, 1234–1238.
- CLAIBORNE, A., MILLER, H., PARSONAGE, D. & ROSS, R. P., 1993, Protein–sulfenic acid stabilisation and function in enzyme catalysis and gene regulation, *FASEB Journal*, **7**, 1483–1490.
- CLEARY, P. P. & DYKHUIZEN, D., 1974, Enzymatic reduction of D-biotin-D-sulfoxide with cell-free extracts of *Escherichia coli*, *Biochemical and Biophysical Research Communications*, **56**, 629–634.
- COLLINGS, A. J., 1971, The metabolism of sodium cyclamate, in Birch, G. C., Green, L. F. & Coulson, C. B. (Eds), *Sweetness and Sweeteners*, pp. 51–68, London: Applied Science.
- DAGE, R. C. & OKERHJOLM, R. A., 1990, Pharmacology and pharmacokinetics of enoximone, *Cardiology*, **77**(Suppl. 3), 2–13.
- DALDERUP, L. M., KELLER, G. H. M., & SCHOETEN, F., 1970, Cyclamate and cyclohexylamine, *Lancet*, **1**, 845.
- DAVIS, P. J. & GUENTHNER, L. E., 1985, Sulindac oxidation/reduction by microbial cultures; microbial models for mammalian metabolism, *Xenobiotica*, **15**, 845–857.
- DAVIS, T. R. A., ADLER, N. & OPSAHL, J. C., 1969, Excretion of cyclohexylamine in subjects ingesting sodium cyclamate, *Toxicology and Applied Pharmacology*, **15**, 106–116.
- DECKER, C. J. & DOERGE, D. R., 1992, Covalent binding of ^{14}C - and ^{35}S -labeled thiocarbamates in rat hepatic microsomes, *Biochemical Pharmacology*, **43**, 881–888.
- DECKER, C. J., RASHED, M. S., BAILLIE, T. A., MALTBY, D. & CORREIA, M. A., 1989, Oxidative metabolism of spironolactone: evidence for the involvement of electrophilic thioesteroid species in drug-mediated destruction of rat hepatic cytochrome P450, *Biochemistry*, **28**, 5128–5136.
- DEHERDER, W. W., HAZENBERG, M. P. & OTTEN, M. H., 1985, Hydrolysis of iodothyronine sulfates by sulfatase activity of anaerobic bacteria from the rat intestinal flora, *FEMS Microbiology Letters*, **27**, 79–83.
- DENNER, W. H. B., OLAVESSEN, A. H., POWELL, G. M. & DODGSON, K. S., 1969, The metabolism of potassium dodecyl [^{35}S]sulphate in the rat, *Biochemical Journal*, **111**, 43–51.
- DOMAGK, G., 1935, Chemotherapy of bacterial infections, *Deutsche Medizinische Wochenschrift*, **61**, 250–253.
- DRASAR, B. S., RENWICK, A. G. & WILLIAMS, R. T., 1972, The role of the gut flora in the metabolism of cyclamate, *Biochemical Journal*, **129**, 881–890.
- DUGGAN, D. E., 1981, Sulindac: therapeutic implications of the prodrug/pharmacophore equilibrium, *Drug Metabolism Reviews*, **12**, 325–337.
- DUGGAN, D. E., HOOKE, K. F., RISLEY, E. A., SHEN, T. Y. & VAN ARMAN, C. G., 1977, Identification of the biologically active form of sulindac, *Journal of Pharmacology and Experimental Therapeutics*, **201**, 8–13.
- DUGGAN, D. E., HOOKE, K. F., NOLL, R. M., HUCKER, H. B. & VAN ARMAN, C. G., 1978, Comparative disposition of sulindac and metabolites in five species, *Biochemical Pharmacology*, **27**, 2311–2320.
- EICHELBAUM, M., HENGSTMANN, J. H., ROST, H. D., BRECHT, T. & DENGLER, H. J., 1974, Pharmacokinetics, cardiovascular and metabolic actions of cyclohexylamine in man, *Archiv fur Toxikologie*, **31**, 243–263.
- EJIRI, S. I., WEISSBACH, H. & BROTH, N., 1979, Reduction of methionine sulfoxide to methionine by *Escherichia coli*, *Journal of Bacteriology*, **139**, 161–164.
- ELLARD, G. A., 1966, Absorption, metabolism and excretion of di(*p*-aminophenyl)-

- sulphone (dapsons) and di(*p*-aminophenyl)sulphoxide in man, *British Journal of Pharmacology*, **26**, 212–217.
- FAIN, J. N., 1977, Mode of action of oral hypoglycaemic drugs. Introductory remarks, *Federation Proceedings*, **36**, 2712–2713.
- FAROOQUI, A. A., 1980, Sulfatases, sulfate esters and their metabolic disorders, *Clinica Chimica Acta*, **100**, 285–299.
- FAROOQUI, A. A. & MANDEL, P., 1977, On the properties and role of arylsulphatases A, B and C in mammals, *International Journal of Biochemistry*, **8**, 685–691.
- FINCH, R. A., VASQUEZ, K. M., HANNA, N. B., REVANKAR, G. R., ROBINS, R. K. & AVERY, T. L., 1990, Oxidation of 2-amino-9-beta-D-ribofuranosylpurine-6-sulfenamide to the corresponding sulfonamide facilitates changes in biologic characterization that include activity against thiopurine-refractory experimental leukemia, *Cancer Letters*, **50**, 63–70.
- FORTH, W., NELL, G., RUMMEL, W. & ANDRES, H., 1972, The hydragogue and laxative effect of the sulfuric acid ester and the free diphenol of 4,4'-dihydroxy-diphenyl-(pyridyl-2)-methane, *Naunyn-Schmeideberg's Archives of Pharmacology*, **274**, 46–53.
- FOURNEAU, E., TRÉFOUEL, J., NITTI, F. & BOVET, D., 1936, Chemotherapy of Streptococci infections by derivatives of *p*-aminophenylsulphonamide, *Comptes Rendue Société Biologie*, **122**, 258–259.
- FRANDSEN, A., SCHOUSBOE, A. & GRIFFITHS, R., 1993, Cytotoxic actions and effects on intracellular Ca²⁺ and cGMP concentrations of sulphur-containing excitatory amino acids in cultured cerebral cortical neurons. *Journal of Neuroscience Research*, **34**, 331–339.
- FREEMAN, C. & HOPWOOD, J. J., 1986, Human liver sulphamate sulphohydrolase. Determinations of native protein and subunit *M_r* values and influence of substrate aglycone structure on catalytic properties, *Biochemical Journal*, **234**, 83–92.
- FRYKLUND, J. & WALLMARK, B., 1986, Sulfide and sulfoxide derivatives of substituted benzimidazoles inhibit acid formation in isolated gastric glands by different mechanisms, *Journal of Pharmacology and Experimental Therapeutics*, **236**, 248–253.
- GEMBORYS, M. W. & MUDGE, G. H., 1981, Formation and disposition of the minor metabolites of acetamidophen in the hamster, *Drug Metabolism and Disposition*, **9**, 340–351.
- GIBSON, G. R., MACFARLANE, G. T. & CUMMINGS, J. H., 1993a, Sulphate reducing bacteria and hydrogen metabolism in the human large intestine, *Gut*, **34**, 437–439.
- GIBSON, G. R., MACFARLANE, S. & MACFARLANE, G. T., 1993b, Metabolic interactions involving sulphate-reducing and methanogenic bacteria in the human large intestine, *FEMS Microbiology Letters*, **12**, 117–125.
- GIBSON, T. P., DOBRINSKA, M. R., LIN, J. H., ENTWISTLE, L. A. & DAVIES, R. O., 1987, Biotransformation of sulindac in end-stage renal disease, *Clinical Pharmacology and Therapeutics*, **42**, 82–88.
- GOLDBERG, L., PAREKH, C., PATTI, A. & SOIKE, K., 1969, Cyclamate degradation in mammals and *in vitro*, *Toxicology and Applied Pharmacology*, **14**, 654.
- GORDER, G. W., HOLDEN, I., RUZO, L. O. & CASIDA, J. E., 1985, Phosphinyliminodithiolane insecticides: oxidative bioactivation of phospholan and mephosfolan, *Bioorganic Chemistry*, **13**, 344–352.
- GRANTHAM, P. H., HORTON, R. E., WEISBURGER, E. K. & WEISBURGER, J. H., 1970, Metabolism of the carcinogen N-2-fluorenylacetamide in germ-free and conventional rats, *Biochemical Pharmacology*, **19**, 163–171.
- GREENSLADE, D., HAVLER, M. E., HUMPHREY, M. J., JORDAN, B. L., LEWIS, C. J. & RANCE, D. J., 1981, Biotransformation of tolmesoxide in animals and man, *Xenobiotica*, **11**, 89–96.
- GREGOR, R. & WANGEMANN, P., 1987, Loop diuretics, *Renal Physiology*, **10**, 174–183.

- HALE, P. W. & POKLIS, A., 1984, Thioridazine-5-sulphoxide cardiotoxicity in the isolated, perfused rat heart, *Toxicology Letters*, **21**, 1-8.
- 1986, Cardiotoxicity of thioridazine and two stereoisomeric forms of thioridazine-5-sulfoxide in the isolated perfused rat heart, *Toxicology and Applied Pharmacology*, **86**, 44-55.
- HANNA, N., MARSHALL, P. J., NEWTON, J., SCHWARTZ, L., KIRSH, R., DIMARTINO, M. J., ADAMS, J., BENDER, P. & GRISWOLD, D. E., 1990, Pharmacological profile of SK and F 105809, a dual inhibitor of arachidonic acid metabolism, *Drugs and Experimental Clinical Research*, **16**, 137-147.
- HARRIS, C. M., MITCHELL, S. C., WARING, R. H. & HENDRY, G. L., 1986, The case of the black speckled dolls: an occupational hazard of unusual sulphur metabolism, *Lancet*, **1**, 492-493.
- HATHWAY, D. E., 1975, Biotransformations, in Hathway, D. E. (Ed.), *Foreign Compound Metabolism in Mammals*, Vol. 3, pp. 201-448, London: Chemical Society.
- HEATH, A., SVENSSON, C. & MARTENSSON, E., 1985, Thioridazine toxicity - an experimental cardiovascular study of thioridazine and its major metabolites in overdose, *Veterinary and Human Toxicology*, **27**, 100-110.
- HINDERER, R. K., LANKAS, G. R., KNEZERICH, A. L. & AULETTA, C. S., 1986, The effects of long term dietary administration of the rubber accelerator, N-oxydiethylene thiocarbamyl-N-oxydiethylene sulfenamide, to rats, *Toxicology and Applied Pharmacology*, **82**, 521-531.
- HODGSON, E. & LEVI, P. A., 1992, The role of the flavin-containing monooxygenases (EC 1.14.13.8) in the metabolism and mode of action of agricultural chemicals, *Xenobiotica*, **22**, 1175-1183.
- HUIJGHEBAERT, S. M., MERTENS, J. A. & EYSEN, H. J., 1982, Isolation of a bile salt sulfatase producing *Clostridium* strain from rat intestinal microflora, *Applied and Environmental Microbiology*, **43**, 185-192.
- IDA, S., OHKUMA, S., KIMORI, M., KURIYAMA, K., MORIMOTO, N. & IBATA, Y., 1985, Regulatory role of cysteine dioxygenase in cerebral biosynthesis of taurine. Analysis using cerebellum from 3-acetylpyridine-treated rats, *Brain Research*, **344**, 62-69.
- INAMASU, T., LUKI, H., VAVREK, M. T. & WEISBURGER, J. H., 1989, Metabolism of 2-amino-3-methylimidazo (4,5-f)quinoline in the male rat, *Food and Chemical Toxicology*, **27**, 369-376.
- IVESON, P., LINDUP, W. E., PARKE, D. V. & WILLIAMS, R. T., 1971, The metabolism of carbenoxolone in the rat, *Xenobiotica*, **1**, 79-95.
- IWASAKI, K., SHIRAGA, T., TADA, K., NODA, K. & NOGUCHI, H., 1986, Age- and sex-related changes in amine sulphoconjugations in Sprague-Dawley strain rats. Comparison with phenol and alcohol sulphoconjugations, *Xenobiotica*, **16**, 717-723.
- JERKINS, A. A. & STEELE, R. D., 1991a, Cysteine sulfinic acid decarboxylase activity in response to thyroid hormone administration in rats, *Archives of Biochemistry and Biophysics*, **286**, 428-432.
- 1991b, Dietary sulfur amino acid modulation of cysteine sulfinic acid decarboxylase, *American Journal of Physiology, Endocrinology and Metabolism*, **261**, E551-E555.
- JERKINS, A. A., BOBROFF, L. E. & STEELE, R. D., 1989, Hepatic cysteine sulfinic acid decarboxylase activity in rats fed various levels of dietary casein, *Journal of Nutrition*, **119**, 1593-1597.
- JOHANSSON, B., 1992, A review of the pharmacokinetics and pharmacodynamics of disulfiram and its metabolites, *Acta Psychiatrica Scandinavica*, **86**(Suppl.), 15-26.
- JUHL, M. J. & CLARK, D. P., 1990, Thiophene-degrading *Escherichia coli* mutants possess sulphone oxidase activity and show altered resistance to sulphur-containing antibiotics, *Applied and Environmental Microbiology*, **56**, 3179-3185.
- KARAM, J. H., MATIN, S. B. & FORSHAM, P. H., 1975, Antidiabetic drugs after the

- University Group Diabetes Program (UGDP), *Annual Review of Pharmacology*, **15**, 351–366.
- KASHIYAMA, E., YOKOI, T., TODAKA, T., ODOMI, M. & KAMATAKI, T., 1994, Chiral inversion of drug: role of intestinal bacteria in the stereoselective sulfoxide reduction of flosequinan, *Biochemical Pharmacology*, **48**, 237–243.
- KATO, Y., KOGURE, T., SATA, M., MURATA, T. & KIMURA, R., 1986, Evidence that methylsulfonyl metabolites of *m*-dichlorobenzene are causative substances of induction of hepatic microsomal drug-metabolising enzymes by the parent compound in rats, *Toxicology and Applied Pharmacology*, **82**, 505–511.
- KEELING, D. J., FALLOWFIELD, C., MILLINER, K. J., TINGLEY, S. K., IFE, R. J. & UNDERWOOD, A. H., 1985, Studies on the mechanism of action of omeprazole, *Biochemical Pharmacology*, **34**, 2967–2973.
- KIRSTEIN PEDERSEN, A. & JAKOBSEN, P., 1979, Two new metabolites of sulfinpyrazone in the rabbit: a possible cause of the prolonged *in vivo* effect, *Thrombosis Research*, **16**, 871–876.
- KLANCNIK, J. M., CUENOD, M., GAHWILER, B. H., JIANG, Z. P. & DO, K. Q., 1992, Release of endogenous amino acids, including homocysteic acid and cysteine sulphinic acid, from rat hippocampal slices evoked by electrical stimulation of Schaffer collateral–commissural fibres, *Neuroscience*, **49**, 557–570.
- KOJIMA, S. & ICHIBAGASE, H., 1966, Studies on synthetic sweetening agents. VIII. Cyclohexylamine, a metabolite of sodium cyclamate, *Chemical and Pharmaceutical Bulletin*, **14**, 971–974.
- KULKARNI, A. P. & HODGSON, E., 1980, Metabolism of insecticides by mixed function oxidase, *Pharmacology and Therapeutics*, **8**, 379–475.
- LANUSSE, C. E., NARE, B., GASCON, L. H. & PRICHARD, R. K., 1992, Metabolism of albendazole and albenzazole sulfoxide by ruminal and intestinal fluids of sheep and cattle, *Xenobiotica*, **22**, 419–426.
- LARSEN, G. L. & BAKKE, J. E., 1981, Enterohepatic circulation in formation of propachlor (2-chloro-*N*-isopropylacetanilide) metabolite in the rat, *Xenobiotica*, **11**, 473–480.
- LASH, L. H., SAUSEN, P. J., DUESCHER, R. J., COOLEY, A. J. & ELFARRA, A. A., 1994, Roles of cysteine conjugate beta-lyase and S-oxidase in nephrotoxicity: studies with S-(1,2-dichlorovinyl)-L-cysteine and S-(1,2-dichlorovinyl)-L-cysteine sulfoxide, *Journal of Pharmacology and Experimental Therapeutics*, **269**, 374–383.
- LEAHY, J. S., TAYLOR, T. & RUDD, C. J., 1967, Cyclohexylamine excretors among human volunteers given cyclamate, *Food and Cosmetics Toxicology*, **5**, 595–596.
- LEE, S. C. & RENWICK, A. G., 1995a, Sulfoxide reduction by rat and rabbit tissues *in vitro*, *Biochemical Pharmacology*, **49**, 1557–1565.
- 1995b, Sulfoxide reduction by rat intestinal flora and by *Escherichia coli* *in vitro*, *Biochemical Pharmacology*, **49**, 1567–1576.
- LEVI, P. E. & HODGSON, E., 1988, Stereospecificity in the oxidation of phorate and phorate sulfoxide by purified FAD-containing mono-oxygenase and cytochrome P-450 isozymes, *Xenobiotica*, **18**, 29–39.
- LEVITZ, M. & KATZ, J., 1968, Enterohepatic metabolism of estriol-3-sulfate-16-glucosiduronate in women, *Journal of Clinical Endocrinology and Metabolism*, **28**, 862–868.
- LIN, C. T., SONG, G. X. & WU, J. Y., 1985, Is taurine a neurotransmitter in rabbit retina?, *Brain Research*, **337**, 293–298.
- LITCHFIELD, M. H. & SWAN, A. A. B., 1971, Cyclohexylamine production and physiological measurements in subjects ingesting sodium cyclamate, *Toxicology and Applied Pharmacology*, **18**, 535–541.
- MADAN, A. & FAIMAN, M. D., 1994, Diethyldithiocarbamate methyl ester sulfoxide, an inhibitor of rat liver mitochondrial low K_m aldehyde dehydrogenase and putative

- metabolite of disulfiram, *Alcoholism: Clinical and Experimental Research*, **18**, 1013–1017.
- MAGGS, J. L., POWELL, G. M., DODGSON, K. S., HOWES, D., BLACK, J. G. & OLAVESSEN, A. H., 1982, Metabolism in the rat of potassium DL-octan-2-sulphate, a secondary alkylsulphate, *Xenobiotica*, **12**, 101–109.
- MAGNUSSON, K. R., MADL, J. E., CLEMENTS, J. R., WU, J. Y., LARSON, A. A. & BEITZ, A. J., 1988, Colocalization of taurine and cysteine sulfinic acid decarboxylase-like immunoreactivity in the cerebellum of the rat with monoclonal antibodies against taurine, *Journal of Neuroscience*, **8**, 4551–4564.
- MAGNUSSON, K. R., CLEMENTS, J. R., WU, J. Y. & BEITZ, A. J., 1989, Colocalization of taurine and cysteine sulfinic acid decarboxylase-like immunoreactivity in the hippocampus of the rat, *Synapse*, **4**, 55–69.
- MALLETT, A. K., ROWLAND, I. R., BEARNE, C. A., PURCHASE, R. & GANGOLLI, S. D., 1985, Metabolic adaptation of rat faecal microflora to cyclamate *in vitro*, *Food and Chemical Toxicology*, **23**, 1029–1034.
- MARRINER, S. E. & BOGAN, J. A., 1980, Pharmacokinetics of albendazole in sheep, *American Journal of Veterinary Research*, **41**, 1126–1129.
- MATSUI, M., TANIMURA, A. & KURATA, H., 1981, Identification of cyclamate-converting bacteria. (Studies on the metabolism of food additives by microorganisms inhabiting the gastrointestinal tract. VI.) *Journal of the Food Hygiene Society of Japan*, **22**, 215–222.
- MCGLINCHY, G., COAKLEY, C. B., GESTAUSTUS-TANSEY, V. GAULT, J. & SPILLANE, W. J., 1982, *In vivo* and *in vitro* studies with sulfamate sweeteners, *Journal of Pharmaceutical Science*, **71**, 661–665.
- MERITS, I., 1975, The metabolism of labelled hexadecyl sulphate salts in rat, dog and human, *Biochemical Journal*, **148**, 219–228.
- MICHAEL, W. R., 1968, Metabolism of linear alkylate sulfonate and alkyl benzene sulfonate in albino rats, *Toxicology and Applied Pharmacology*, **12**, 473–485.
- MIKOV, M., CALDWELL, J., DOLPHIN, C. T. & SMITH, R. L., 1988, The role of intestinal microflora in the formation of methylthio adduct metabolites of paracetamol. Studies in neomycin-pretreated and germ-free mice, *Biochemical Pharmacology*, **37**, 1445–1449.
- MILLER, J. P., CRAWFORD, L. E. M., SONNERS, R. C. & CARDINAL, E. V., 1966, Distribution and excretion of ^{14}C -cyclamate sodium in animals, *Biochemical and Biophysical Research Communications*, **25**, 153–157.
- MIO, T. & SUMINO, K., 1985, Mechanism of biosynthesis of methylsulfones from PCBs and related compounds, *Environmental Health Perspectives*, **59**, 129–135.
- MITCHELL, S. C., WARING, R. H., IDLE, J. R. & SMITH, R. L., 1981, Cimetidine pretreatment decreases carbocysteine sulfoxidation in man, *IRCS Medical Science*, **9**, 1028–1029.
- MITCHELL, S. C., IDLE, J. R. & SMITH, R. L., 1982, The metabolism of (^{14}C)cimetidine in man, *Xenobiotica*, **12**, 283–292.
- MITCHELL, S. C., WARING, R. H., HALEY, C. S., IDLE, J. R. & SMITH, R. L., 1984, Genetic aspects of the polymodally distributed sulfoxidation of S-carboxymethyl-L-cysteine in man, *British Journal of Clinical Pharmacology*, **18**, 507–521.
- MORETO, M., GONALONS, E., MYLONAKIS, N., GIRALDEZ, A. & TORRALBA, A., 1977, Enterohepatic circulation of sodium sulisatin and its effects on glucose absorption in the rat, *Journal of Pharmacy and Pharmacology*, **29**, 446–448.
- MORIYASU, M. & ITO, A., 1982, Transmembranous disposition of arylsulphatase C in microsomal membranes of rat liver, *Journal of Biochemistry*, **92**, 1197–1204.
- MORIYASU, M., ITO, A. & OMURA, T., 1982, Purification and properties of arylsulfatase C from rat liver microsomes, *Journal of Biochemistry*, **92**, 1189–1195.
- NIIMURA, T., TOKEIDA, T. & YAMAHARA, T., 1974, Partial purification and some properties of cyclamate sulfamatase, *Journal of Biochemistry*, **75**, 407–417.

- ORLOFF, J. & BERLINER, R. W., 1961, Renal pharmacology, *Annual Review of Pharmacology*, **1**, 287–314.
- OSER, B. L., CARSON, S., VOGIN, E. E. & SONDEERS, R. C., 1968, Conversion of cyclamate to cyclohexylamine in rats, *Nature*, **220**, 178–179.
- OTTERY, J., OLAVESON, A. H. & DODGSON, K. S., 1970, Metabolism of dodecyl sulphate in the rat: non-enzymatic liberation of sulphate and γ -butyrolactone from the major metabolite, butyric acid 4-sulphate, *Life Sciences*, **9**, 1335–1340.
- PACINI, N., ALBINI, E., FERRARI, A., ZANCHI, R., MARCA, G. & BANDIERA, T., 1987, Transformation of sulfated bile acids by human intestinal microflora, *Arzneimittel-Forschung/Drug Research*, **37**, 983–987.
- PANTEN, U., BURGFELD, J., GOERKE, F., RENNICKE, M., SCHWANSTECHE, M., WALLASCH, A., ZÜNKLER, B. & LENZEN, S., 1989, Control of insulin secretion by sulfonylureas, meglitinide and diazoxide in relation to their binding to the sulfonylurea receptor in pancreatic islets, *Biochemical Pharmacology*, **38**, 1217–1229.
- PARKE, D., 1968, *The Biochemistry of Foreign Compounds*, Vol. 5, *International Series of Monographs in Pure and Applied Biology*, pp. 180–184, Oxford: Pergamon.
- PARSONAGE, D., MILLER, H., ROSS, R. P. & CLAIBORNE, A., 1993, Purification and analysis of streptococcal NADH peroxidase expressed in *Escherichia coli*, *Journal of Biological Chemistry*, **268**, 3161–3167.
- POOLE, L. B. & CLAIBORNE, A., 1989, The non-flavin redox center of the streptococcal NADH peroxidase. II. Evidence for a stabilised cysteine-sulfenic acid, *Journal of Biological Chemistry*, **264**, 12330–12338.
- PREISIG, P. A., TOTO, R. D. & ALPERN, R. J., 1987, Carbonic anhydrase inhibitors, *Renal Physiology*, **10**, 136–159.
- PRESTON, B. D., MILLER, J. A. & MILLER, E. C., 1984, Reactions of 2,2',5,5'-tetrachlorobiphenyl 3,4-oxide with methionine, cysteine and glutathione in relation to the formation of methylthio-metabolites of 2,2',5,5'-tetrachlorobiphenyl in the rat and mouse, *Chemico-Biological Interactions*, **50**, 289–312.
- PRICE, J. M., BIAVA, C. G., OSER, B. L., VOGIN, E. E., STEINFELD, J. & LEY, H. L., 1970, Bladder tumors in rats fed cyclohexylamine or high doses of a mixture of cyclamate and saccharin, *Science*, **167**, 1131–1132.
- RACKUR, G., BICKEL, M., FEHLHABER, H. W., HERLING, A., HITZEL, V., LANG, H. J., RÖSNER, M. & WEYER, R., 1985, 2-((-2 pyridylmethyl)sulfinyl)benzimidazoles: acid sensitive suicide inhibitors of the proton transport system in the parietal cell, *Biochemical and Biophysical Research Communications*, **128**, 477–484.
- RAFTER, J. J. & NILSSON, L., 1981, Involvement of the intestinal microflora in the formation of sulphur containing metabolites of caffeine, *Xenobiotica*, **11**, 771–778.
- RAFTER, J. J., GUSTAFSSON, J. A., BAKKE, J. E., LARSEN, G. E., NORIN, K. E. & GUSTAFSSON, B. E., 1983, Studies on the re-establishment of the intestinal microflora in germ-free rats with special reference to the metabolism of N-isopropyl- α -chloroacetanilide (propachlor), *Xenobiotica*, **13**, 171–178.
- RAMASAMY, K., IMAMURA, N., HANNA, N. B., FINCH, R. A., AVERY, T. L., ROBINS, R. K. & REVANKAR, G. R., 1990, Synthesis and antitumour evaluation in mice of certain 7-deazapurine(pyrrolo(2,3-d)pyrimidine) and 3-deazapurine(imidazo(4,5-c)pyridine) nucleosides structurally related to sulfenosine, sulfinosine and sulfonosine, *Journal of Medicinal Chemistry*, **33**, 1220–1225.
- RANDERATH, K., HAGLUND, R. E., PHILLIPS, D. H. & REDDY, M. V., 1984, Phosphorus 32 post labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally-occurring alkenylbenzenes: 1. Adult female CD-1 mice, *Carcinogenesis*, **5**, 1613–1622.
- RENWICK, A. G., 1976, Microbial metabolism of drugs, in Parke, D. V. & Smith, R. L. (Eds), *Drug Metabolism from Microbe to Man*, pp. 169–189, London: Taylor & Francis.

- RENWICK, A. G., 1985, The disposition of saccharin in animals and man – a review, *Food and Chemical Toxicology*, **23**, 429–435.
- 1986, The metabolism of intense sweeteners, *Xenobiotica*, **16**, 1057–1071.
- 1990, Acceptable daily intake and the regulation of intense sweeteners, *Food Additives and Contaminants*, **7**, 463–475.
- RENWICK, A. G. & WILLIAMS, R. T., 1972, The fate of cyclamate in man and other species, *Biochemical Journal*, **129**, 869–879.
- RENWICK, A. G., EVANS, S. P., SWEATMAN, T. W., CUMBERLAND, J. & GEORGE, C. F., 1982, The role of the gut flora in the reduction of sulphinpyrazone in the rat, *Biochemical Pharmacology*, **31**, 2649–2656.
- REVANKAR, G. R., HANNA, N. B., IMAMURA, N., LEWIS, A. F., LARSON, S. B., FINCH, R. A., AVERY, T. L. & ROBINS, R. K., 1990a, Synthesis and *in vivo* antitumour activity of 2-amino-9H-purine-6-sulfenamide, -sulfenamide and -sulfonamide and related purine ribonucleosides, *Journal of Medicinal Chemistry*, **33**, 121–128.
- REVANKAR, G. R., HANNA, N. B., RAMASAMY, K., LARSON, S. B., SMEE, D. F., FINCH, R. A., AVERY, T. L. & ROBINS, R. K., 1990b, Synthesis and *in vivo*, antitumour and antiviral activities of 2'-deoxyribofuranosyl and arabinofuranosyl nucleosides of certain purine-6-sulfenamides, sulfenamides and sulfonamides, *Journal of Heterocyclic Chemistry*, **27**, 909–918.
- RIPIN, M. J., NOON, K. F. & COOK, T. M., 1971, Bacterial metabolism of arylsulfonates 1. Benzene sulfonate as growth substrate for *Pseudomonas testosteroni*, H-8, *Applied Microbiology*, **21**, 495–499.
- RITSHEL, W. A. & KUO, B. S., 1987, Correlation between inhibitory effect on platelet aggregation and disposition of sulfinpyrazone and its metabolites in rabbits, part I. Single dose study, *Biopharmaceutics and Drug Disposition*, **8**, 1–9.
- ROSS, P. R. & CLAIBORNE, A., 1991, Cloning, sequence and overexpression of NADH peroxidase from *Streptococcus faecalis* 10C1. Structural relationship with the flavoprotein disulfide reductases, *Journal of Molecular Biology*, **221**, 857–871.
- 1992, Molecular cloning and analysis of the gene encoding the NADH oxidase from *Streptococcus faecalis* 10C1. Comparison with NADH peroxidase and the flavoprotein disulfide reductase, *Journal of Molecular Biology*, **227**, 658–671.
- ROY, A. B., 1970, Enzymological aspects of steroid conjugation, in Bernstein, S. & Solomon, S. (Eds), *Chemical and Biological Aspects of Steroid Conjugation*, pp. 74–130, Berlin: Springer.
- RUTGERS, M., HEUSDENS, F. A., BONTHUIS, F., DEHERDER, W. W., HAZENBERG, M. P. & VISSER, T. J., 1989, Enterohepatic circulation of triiodothyronine (T3) in rats: importance of the microflora for the liberation and reabsorption of T3 from biliary T3 conjugates, *Endocrinology*, **125**, 2822–2830.
- SCHMID, J., PROX, A., ZIMMER, A., KECK, J. & KASCHKE, S., 1990, Biotransformation of clenbuterol, *Fresenius Journal of Analytical Chemistry*, **337**, 121.
- SCHMID-ANTOMARCHI, H., DEWILLE, J., FOSSET, M. & LAZDUNSKI, M., 1987, The receptor for antidiabetic sulfonylureas controls the activity of the ATP-modulated K⁺channel in insulin-secreting cells, *Journal of Biological Chemistry*, **262**, 15840–15844.
- SHARPE, T. R., CHERKOFKY, S. C., HEWES, W. E., SMITH, D. H., GREGORY, W. A., HABER, S. B., LEADBETTER, M. R. & WHITNEY, J. G., 1985, Preparation and antiarthritic and analgesic activity of 4,5-diaryl-2-(substituted thio)-1H-imidazoles and their sulfoxide and sulfones, *Journal of Medicinal Chemistry*, **28**, 1188–1194.
- SHIN, J. M., BESANCON, M., SIMON, A. & SACHS, G., 1993, The site of action of pantoprazole in the gastric H⁺/K⁺-ATPase. *Biochimica et Biophysica Acta: Biomembranes*, **1148**, 223–233.
- SMEE, D. F., ALAGHAMANDAN, H. A., KINI, G. D. & ROBINS, R. K., 1988, Antiviral activity and mode of action of ribavirin 5'-sulfamate against Semliki Forest virus,

Antiviral Research, **10**, 253–262.

- SMITH, B. A., SPRINGFIELD, J. R. & GUTMANN, H. R., 1987, Solvolysis and metabolic degradation, by rat liver, of the ultimate carcinogen, N-sulfonyl-2-acetylaminofluorene, *Molecular Pharmacology*, **31**, 438–445.
- SMITH, R. H., 1980, Kale poisoning: the brassica anaemia factor, *Veterinary Record*, **5 July**, 12–15.
- SONDERS, R. C., NETWAL, J. C. & WIEGAND, R. G., 1969, Site of conversion of cyclamate to cyclohexylamine, *Pharmacologist*, **11**, 241.
- SOUHAILI, EL AMRI, H., FARGETTON, X., DELATOUR, P. & BATT, A. M., 1987, Sulphoxidation of albendazole by the FAD-containing and cytochrome P-450 dependent mono-oxygenases from pig liver microsomes, *Xenobiotica*, **17**, 1159–1168.
- SOURKES, T. L., & TRANO, Y., 1953, Reduction of methionine sulfoxide by *Escherichia coli*, *Archives of Biochemistry and Biophysics*, **42**, 321–326.
- SPILLANE, W. J. & MCGLINCHY, G., 1981, Structure–activity studies on sulfamate sweeteners II: Semiquantitative structure–taste relationship for sulfamate (RNHSO_3^-) sweeteners – the role of R, *Journal of Pharmaceutical Sciences*, **70**, 933–935.
- SPILLANE, W. J., MCGLINCHY, G., MUIRCHEORTAIGH, I. O. & BENSON, G. A., 1983, Structure–activity studies on sulfamate sweeteners III: Structure–taste relationships of heterosulfamates, *Journal of Pharmaceutical Sciences*, **72**, 852–856.
- STANDEN, N. B., QUAYLE, J. M., DAVIES, N. W., BRAYDEN, J. E., HUANG, Y. & NELSON, M. T., 1989, Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle, *Science*, **245**, 177–180.
- STEHLE, T., CLAIBORNE, A. & SCHULZ, G. E., 1993, NADH binding site and catalysis of NADH peroxidase, *European Journal of Biochemistry*, **211**, 221–226.
- STRAND, L. P. & SCHELINE, R. R., 1975, The metabolism of vanillin and isovanillin in the rat, *Xenobiotica*, **5**, 49–63.
- STRONG, H. A., OATES, J., SEMBI, J., RENWICK, A. G. & GEORGE, C. F., 1984a, Role of gut flora in the reduction of sulphinpyrazone in humans, *Journal of Pharmacology and Experimental Therapeutics*, **230**, 726–732.
- STRONG, H. A., RENWICK, A. G. & GEORGE, C. F., 1984b, The site of reduction of sulphinpyrazone in the rabbit, *Xenobiotica*, **14**, 815–826.
- STRONG, H. A., WARNER, J., RENWICK, A. G. & GEORGE, C. F., 1985, Sulindac metabolism: the importance of an intact colon, *Clinical Pharmacology and Therapeutics*, **38**, 387–393.
- STRONG, H. A., ANGUS, R., OATES, J., SEMBI, J., HOWARTH, P., RENWICK, A. G. & GEORGE, C. F., 1986, Effects of ischaemic heart disease, Crohn's disease and anti-microbial therapy on the pharmacokinetics of sulphinpyrazone, *Clinical Pharmacokinetics*, **11**, 402–411.
- STRONG, H. A., RENWICK, A. G., GEORGE, C. F., LIU, Y. F. & HILL, M. J., 1987, The reduction of sulphinpyrazone and sulindac by intestinal bacteria, *Xenobiotica*, **17**, 685–696.
- SUND, R. B., HOL, L. & STROBRATEN, A., 1979, Studies in the rat on the absorption biliary excretion, laxative action and interference with intestinal transport of some oxyphenisatin derivatives, *Acta Pharmacologica et Toxicologica*, **44**, 251–259.
- TABER, K. H., LIN, C. T., LIU, J. W., THALMANN, R. H. & WU, J. Y., 1986, Taurine in hippocampus: localization and postsynaptic action, *Brain Research*, **386**, 113–121.
- TATSUMI, K., KITAMURA, S. & YAMADA, H., 1982, Involvement of liver aldehyde oxidase in sulfoxide reduction, *Chemical and Pharmaceutical Bulletin*, **30**, 4585–4588.
- 1983, Sulfoxide reductase activity of liver aldehyde oxidase, *Biochimica et Biophysica Acta*, **747**, 86–92.
- TAYLOR, A. J., OLAVESSEN, A. H., BLACK, J. G. & HOWES, D., 1978, The metabolism of the surfactants dodecylsulfonate and hexadecylsulfonate in the rat, *Toxicology and*

Applied Pharmacology, **45**, 105–117.

- TAYLOR, J. D., RICHARDS, R. K. & DAVIN, J. C., 1951, Excretion and distribution of radioactive S³⁵-cyclamate sodium (sucaryl sodium) in animals, *Proceedings of the Society of Experimental Biology and Medicine*, **78**, 530–533.
- TESORIERO, A. A. & ROXON, J. J., 1975, [³⁵S]Cyclamate metabolism: incorporation of ³⁵S into protein of intestinal bacteria *in vitro* and production of volatile ³⁵S containing compounds, *Xenobiotica*, **5**, 25–31.
- THYSSE, G. J. E. & WANDERS, T. H., 1974, Initial steps in the degradation of *n*-alkane-1-sulphonates by *Pseudomonas*, *Antonie van Leeuwenhoek*, **40**, 25–37.
- TOKEIDA, T., NIIMURA, T., YAMAHARA, T., HASEGAWA, T. & SUZUKI, T., 1979, Anaerobic deamination of cyclohexylamine by intestinal microorganisms in rabbits, *Agricultural and Biological Chemistry*, **43**, 25–32.
- TSUCHIYA, T., 1981, Studies on the metabolism of sodium cyclamate by intestinal bacteria, *Memoirs Tokyo University of Agriculture*, **23**, 1–55.
- TURESKY, R. J., SKIPPER, P. L., TANNENBAUM, S. R., COLES, B. & KETTERER, B., 1986, Sulfamate formation is a major route for detoxification of 2-amino-3-methylimidazo[4,5-*f*]quinoline in the rat, *Carcinogenesis*, **7**, 1483–1486.
- TURESKY, R. J., AESCHBACHER, H. U., WURZNER, H. P., SKIPPER, P. L. & TANNENBAUM, S. R., 1988, Major routes of metabolism of the food-borne carcinogen 2-amino-3,8-dimethylimidazo(4,5-*f*)quinoxaline in the rat, *Carcinogenesis*, **9**, 1043–1048.
- TURESKY, R. J., STILLWELL, W. G. S., SKIPPER, P. L. & TANNENBAUM, S. R., 1993, Metabolism of food-borne carcinogens 2-amino-3-methylimidazo(4,5-*f*)quinoline and 2-amino-3,8-dimethylimidazo(4,5-*f*)quinoxaline in the rat as a model for human biomonitoring, *Environmental Health Perspectives*, **99**, 123–128.
- URIA NICKELSEN, M. R., LEADBETTER, E. R. & GODCHAUX, W., 1983, Sulphonate utilisation by enteric bacteria, *Journal of General Microbiology*, **139**, 203–208.
- VELAZQUEZ, H., 1987, Thiazide diuretics, *Renal Physiology*, **10**, 184–197.
- VENKATESAN, K., 1989, Clinical pharmacokinetic considerations in the treatment of patients with leprosy, *Clinical Pharmacokinetics*, **16**, 365–386.
- VIANEY LIAND, C., DESJACQUES, P., GAULME, J., DORCHE, C., VANLIEFERINGHEN, P., DECHELOTTE, P. & DIVRY, P., 1988, A new case of isolated sulphite deficiency with rapid fatal outcome, *Journal of Inherited Metabolic Disease*, **11**, 425–426.
- WALDHAUSER, L. & UETRECHT, J., 1991, Oxidation of propylthiouracil to reactive metabolites by activated neutrophils: Implications for agranulocytosis, *Drug Metabolism and Disposition*, **19**, 354–359.
- WALKER, R., 1970, The metabolism of azo compounds: a review of the literature, *Food and Cosmetics Toxicology*, **8**, 659–676.
- WARRANDER, A., ALLEN, J. M. & ANDREWS, R. S., 1985, Incorporation of radiolabelled amino acids into the sulphur-containing metabolites of paracetamol by the hamster, *Xenobiotica*, **15**, 891–897.
- WATABE, T., FUJIEDA, T., HIRATSUKA, A., ISHIZUKE, T., HAKAMATA, Y. & OGURA, K., 1985, The carcinogen, 7-hydroxymethyl-12-methylbenz[a]anthracene, is activated and covalently binds to DNA via a sulphate ester, *Biochemical Pharmacology*, **34**, 3002–3005.
- WESTLEY, J., 1989, Inorganic sulphur compounds, in Damani, L. A. (Ed.), *Sulphur-Containing Drugs and Related Compounds: Chemistry, Biochemistry and Toxicology*, Vol. 1, Part A. *Metabolism of Sulphur Functional Groups*, pp. 113–134, Chichester: Ellis Horwood.
- WILLETTTS, A. J. & CAIN, R. B., 1972, Microbial metabolism of alkylbenzene sulphonates. Bacterial metabolism of undecylbenzene-*p*-sulphonate and dodecylbenzene-*p*-sulphonate, *Biochemical Journal*, **129**, 389–402.
- WILLIAMS, J. R., GRANTHAM, P. H., MARSH, H. H., WEISBURGER, J. H. &

- WEISBURGER, E. K., 1970, Participation of liver fractions and of intestinal bacteria in the metabolism of N-hydroxy-N-2-fluorenylacetamide in the rat, *Biochemical Pharmacology*, **19**, 173–188.
- WILLIAMS, R. T., 1959, *Detoxication Mechanisms. The Metabolism and Detoxication of Drugs, Toxic Substances and Other Organic Compounds*, pp. 497–500, London: Chapman & Hall.
- WILLS, J. H., SERRONE, D. M. & COULSTON, F., 1981, A 7-month study of ingestion of sodium cyclamate by human volunteers, *Regulatory Toxicology and Pharmacology*, **1**, 163–176.
- WISEMAN, E. H., SCHREIBER, E. C. & PINSON, R., 1962, Studies of N-dealkylation of some aromatic sulphonamides, *Biochemical Pharmacology*, **11**, 881–886.
- WOODS, D. D., 1940, Relation of *p*-aminobenzoic acid to mechanism of action of sulphanilamide, *British Journal of Experimental Pathology*, **21**, 74–90.
- WYNIK, S. D., HALL, I. H. & DUBEY, A., 1984, Structure–activity studies on antihyperlipidemic N-benzoylsulfamates, N-benzylsulfamates, and benzylsulfonamides, *Journal of Pharmaceutical Sciences*, **73**, 374–377.
- YOSHIHARA, S. & TATSUMI, K., 1985a, Sulfoxide reduction catalysed by guinea pig liver aldehyde oxidase in combination with one electron reducing flavoenzymes, *Journal of Pharmacobio-Dynamics*, **8**, 996–1005.
- 1985b, Guinea pig liver aldehyde oxidase as a sulfoxide reductase: its purification and characterisation, *Archives of Biochemistry and Biophysics*, **242**, 213–224.
- 1990, Metabolism of diphenyl sulfoxide in perfused guinea pig liver; involvement of aldehyde oxidase as a sulfoxide reductase, *Drug Metabolism and Disposition*, **18**, 876–881.
- ZHU, S. M. & BRAJTERTOOTH, A., 1990, Liquid chromatographic determination of 6-thiopurine metabolites formed *in vitro*, in electrochemical and enzymatic oxidative activation, *Analytica Chimica Acta*, **237**, 305–310.
- ZIEGLER, D. M., 1989, S-Oxygenases. I. Chemistry and biochemistry, in Damani, L. A. (Ed.), *Sulphur-Containing Drugs and Related Organic Compounds. Chemistry, Biochemistry and Toxicology*, Vol. 2, Part A, *Analytical, Biochemical and Toxicological Aspects of Sulphur Xenobiochemistry*, pp. 53–66, Chichester: Ellis Horwood.
- ZINDER, S. H. & BROCK, T. D., 1978, Dimethylsulphoxide reduction by micro-organisms, *Journal of General Microbiology*, **105**, 335–342.

Sulfur–Carbon Compounds

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4.1 Introduction

The immense number of carbon–sulfur compounds in use today, both in medicine and agriculture, means that any review of the area can be best served only by the use of examples to highlight the general trends that exist in the various groups that comprise the collection of compounds that are classified as carbon–sulfur compounds. These include the dithioic acids, thioamides, thioethers (or sulfides), thioketones, thiocarbamides, thiocarbamates (which includes the carbamothioates), dithiocarbamates (or carbamodithioates) and sulfur heterocycles. The sulfur atom, which has the ability to expand electronically into the *d*-orbitals, exists in a number of varying oxidation states ranging from -2 to $+6$ (Mitchell and Nickson 1993). The carbon–sulfur groups of compounds all exist in the -2 oxidation state. To enable the reader to come to terms with this vast group of compounds this chapter has been divided into sections – thiones, sulfur heterocycles and thioethers – and then further subdivided into the various classes of compounds again. Each class of compound is reviewed in terms of its applications, metabolism and toxicity.

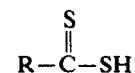
4.2 Thiones

This group of compounds includes the dithioic acids, thioamides, thiocarbamides and thiocarbamates (carbamothioates), and the structurally related dithiocarbamates (carbamodithioates). The biochemistry and biological interactions of these various compounds will be reviewed on an individual and group basis to enable the reader to understand fully the diverse nature of this grouping of chemicals.

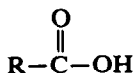
4.2.1 Dithioic acids

4.2.1.1 Applications

Dithioic acids are sulfur analogues of carboxylic acids (Figure 4.1) and this class of compounds was one of the earliest discovered in sulfur organochemistry (Debus, 1850). The main basis of this class of sulfur compound is the large number of dithiocarbamates and related compounds that have found utilization in the fields of agriculture and medicine (Thorn and Ludwig 1962).



Dithioic acid



Carboxylic acid

Figure 4.1 Dithioic acid

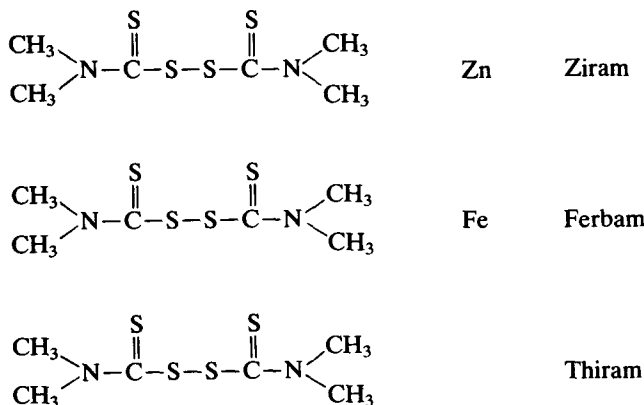


Figure 4.2 Dithiocarbamates

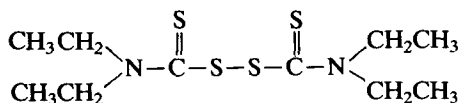


Figure 4.3 Disulfiram

4.2.1.2 Metabolism

The dithioic acids are primarily converted into sulfenic acid and sulfinic acid by S-oxygenation (Damani 1989) which are phase I reaction products. The phase II metabolic products are the S-glucuronide, glucoside and methyl conjugates. Since limited numbers of free dithioic acids have been investigated in detail, the remainder of the discussion will be devoted to the dithiocarbamates (carbamodithioates), thiocarbamates (carbamothioates), thiocarbamides and thioamides.

4.2.2 Dithiocarbamates

4.2.2.1 Applications

The most important fungicides and bactericides in use today are the zinc metallic complex of N,N-dimethyldithiocarbamate (Ziram, a fungicide) and the iron metallic complex of N,N-dimethyldithiocarbamate (Ferbam, bactericide) together with N,N-dimethyldithiocarbamate disulfide (Thiram) (Figure 4.2), the oxidation product formed from both Ziram and Ferbam. However, the best-known example of this class of compounds is the drug, disulfiram. This compound is in fact a disulfide of the dithiocarbamate, N,N-diethyldithiocarbamate (Figure 4.3), which has been in use for over 40 years as an adjuvant in the treatment of chronic alcohol abuse. It was in fact Hald and Jacobsen (1948) who described the experiments using diethylthiram disulfide. They investigated the effects of the disulfide in doses of 0.5–1.5 g in individuals who were then allowed to drink alcohol. They noticed a spectrum of characteristics which were dose-dependent, and which at low

quantities of alcohol included: (i) feeling of heat in the face, (ii) intense flushing located principally in the face, (iii) dilation of the scleral vessels and (iv) palpitations followed sometimes by dyspnoea. These clinical features are also seen when large quantities of alcohol are consumed but in addition nausea and vomiting often develop. The active metabolite of the tetraethylthiram disulfide (N,N-diethyldithiocarbamate) is believed to be liberated *in vivo* by disulfide reduction via plasma proteins since incubation of the disulfide with human plasma and also bovine serum albumin (BSA) causes rapid loss of the disulfide from the sample (Agarwal *et al.* 1983). The dithiocarbamate is believed to act as an inhibitor of the mitochondrial enzyme, acetaldehyde dehydrogenase, which converts acetaldehyde to acetate in the body. The clinical features of the 'alcohol toxicity' associated with this compound and alcohol ingestion is the build-up of acetaldehyde in the body of the individual.

The metabolism of N,N-diethyldithiocarbamate results in the formation of minor amounts of diethylamine and carbon disulfide but the major metabolite produced is the S-glucuronide conjugate of the dithiocarbamate (Zeigler 1982). However, a study by Gessner and Jakubowski (1972) showed that in male blue spruce mice (HA:ICR strain) and Holtzman rats S-methyl-N,N-diethyldithiocarbamate was also a metabolite of antabuse and that administration of the ^{35}S -methyl metabolite to the animals resulted in the urinary excretion of 60% of the administered dose as ^{35}S -inorganic sulfate. The conclusion drawn from this is that the S-methyl metabolite was an intermediate in the production of inorganic sulfate. The exact mechanism of this reaction is unknown but oxidative desulfuration by the flavin monooxygenase (FMO) system is probably the main route of metabolism (Zeigler 1982).

In terms of agrochemicals, of the dithiocarbamate class of compounds the chemical Thiram is representative of the N-dimethyldithiocarbamate group. The disulfide is reduced in plants and the N-dimethyldithiocarbamate anion is then conjugated with glucose and incorporated into amino acids and also proteins (Vonk 1983). The dithiocarbamates are in fact metabolic poisons, causing similar acute effects to those caused by carbon disulfide. The carbon disulfide together with dimethylamine probably both arise from the initially formed N,N-dimethyldithiocarbamic acid metabolite. The N-dimethyldithiocarbamate group is of less importance in the compounds Maneb, Zineb and Mancozeb (Figure 4.4) which

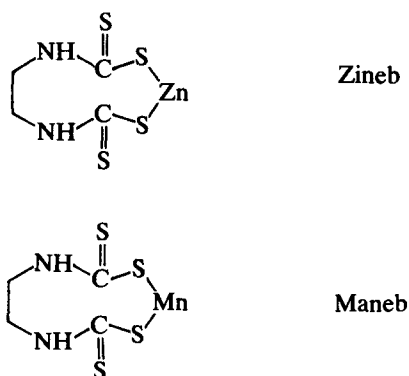


Figure 4.4 Zineb and Maneb

are based around the ethylene bisdithiocarbamate group. These seed dressing and foliar application compounds are insoluble, polymeric coordination complexes containing manganese, zinc and zinc–manganese, respectively. In water they readily degrade to fungitoxic products that are also found in treated plants. Ethylenethiourea and ethylene diamine are formed in plants and animals; plants additionally produce ethylene bisisothiocyanate (Vonk 1983). The picture is however further complicated by the formation of both elemental sulfur and hydrogen sulfide, both of which are known toxic compounds.

4.2.2.2 Toxicity

The tetraethylthiram disulfide is in fact relatively nontoxic, with an LD_{50} in rats of 3 g kg^{-1} body weight. In experiments on rats and rabbits (Hald and Jacobsen 1948), doses of 10 and 60 mg daily of the drug given over a period of >3 months had no discernible effect on the animals in question. This was extended to the clinical trials where single doses of 0.25–0.6 g of antabuse were given for several months without any side effects being reported or detected.

The vast majority of the dithiocarbamates are agricultural chemicals that are widely used in crop protection as either nematocides, fungicides, herbicides or bactericides, their broad spectrum of activity being dependent on the substituent that is present on the nitrogen and sulfur atoms in the compounds. The ethylene bisdithiocarbamates, Zineb, Maneb and Mancozeb, all produce ethylenethiourea on metabolism in both plants and animals. This toxic compound can also arise as a contaminant of the fungicide. The LD_{50} (oral) of ethylenethiourea in rat is 900 mg kg^{-1} and the compound is a known carcinogen and teratogen (Khera 1973). The LD_{50} (oral) of Zineb in the rat has been reported to be $>5200 \text{ mg kg}^{-1}$ (Worthing and Walker 1987) and also 1000 mg kg^{-1} (Hassall 1982).

4.2.3 Thiocarbamates

4.2.3.1 Applications

The thiocarbamates are used extensively as soil herbicides (Table 4.1) against a wide spectrum of noncommercial plants. The thiocarbamate, diallate (S-(2,3-dichloroallyl)diisopropylthiocarbamate) has been in use as a selective herbicide for nearly 40 years in the control of wild oats in both wheat and oat pastures. The exact mechanism of action of this thiocarbamate was investigated in terms of its action on gibberellin biosynthesis (Wilkinson 1986). It was observed that the uptake of diallate through the underground portion of shoots or coleoptiles was devastating to the plant but that uptake through the roots was not (Appleby *et al.* 1965, Friesen *et al.* 1962). The selective toxicity to the wild oats and the commercial crop occurs due to the wheat/commercial oat stem apex moving through the treated layer of soil at a later stage than in the wild oat. Other biochemical mechanisms of action of diallate have been put forward and these include: (i) decreased epicuticular wax synthesis (Still *et al.* 1970, Wilkinson 1974) and (ii) inhibition of protein synthesis (Mann *et al.* 1965). However, the main morphological and biochemical responses to diallate are almost identical to those caused by EPTC (S-ethyl dipropylthiocarbamate) which is known to inhibit

Table 4.1 Commercial use of thiocarbamates in soil application herbicides

Trade name	Common name	Uses	Control
Eptam	EPTC	Corn	Wild oat
Sutan	Butylate	Corn	Green foxtail, cyperus
Ro-neet	Cycloate	Sugar beet, spinach	Broad-leaved weeds
Ordram	Molinate	Rice	Germinating weeds
Avadex	Diallate	Beet, potato, corn, beans	Wild oat, black grass
BW Far-Go	Triallate	Spring barley, Spring wheat	Wild oat, black grass
Saturn	Benthioncarb	Rice, soybean, peanut	Germinating weeds (paddy)

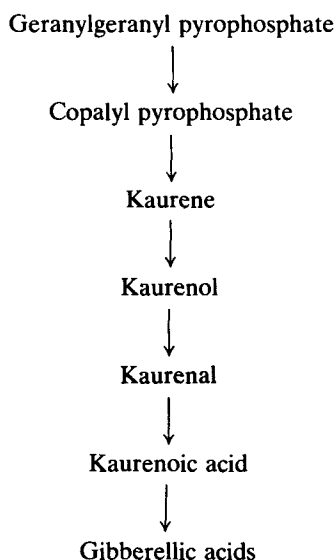


Figure 4.5 Gibberellin biosynthesis

gibberellin precursor biosynthesis (Dawson 1963, Wilkinson 1983a, 1985). The metabolic pathway that results in gibberellin synthesis can be seen (Figure 4.5) and is blocked at kaurene synthesis by EPTC.

Wilkinson (1986) found that 4 μM diallate resulted in 88% inhibition of *Sorghum* growth in terms of height, with the primary leaves failing to penetrate the coleoptiles, but at sublethal concentrations of 0.25–0.5 μM after a period of growth inhibition, growth resumed by the process of bud development along the side of the coleoptile. In the gibberellin precursor biosynthesis studies *in vitro*, diallate at 1 nM was seen to be ineffective in terms of inhibition of gibberellin production. However, at concentrations of 0.1 μM diallate, a fivefold increase of *trans*-geranylgeraniol from *trans*-geranylgeranyl pyrophosphate was seen (indicating a block at this point of the pathway), but this was only accompanied by a small decrease in kaurene concentration. When the concentration of herbicide was

increased to $10\ \mu\text{M}$ a significant decrease in kaurene concentration was achieved, but this indicated that kaurene synthetase inhibition takes place only at diallate concentrations in excess of normal field rate application. Thus, if the *in vitro* data are an indication of the *in vivo* enzyme activity, then inhibition of kaurene synthetase is probably not the primary mode of action of diallate. The conversion of kaurenal into kaurenoic acid by kaurene oxidase was significantly inhibited by diallate in the range of the herbicide normally found in plants at normal field application concentrations and this probably is the major mode of action of diallate.

4.2.3.2 Metabolism

As opposed to their nonsulfur analogues, the carbamates which are mostly acetylcholinesterase inhibitors (used as insecticides), the thiocarbamates are propesticides which need to undergo metabolic biotransformation via oxidation. The main activation reaction that increases the reactivity of thiocarbamates in general is sulfoxidation which increases the carbamoylation activity of the compounds (Casida 1983, Unai and Tomizawa 1986). This activation process may enhance the selectivity of the compounds (Casida *et al.* 1974) and decrease mammalian toxicity (Lay *et al.* 1975, Lay and Casida 1976). Metabolic studies indicate that thiocarbamates are rapidly degraded in both plants and mammals. The major metabolite is the thiocarbamate sulfoxide (both in microsomal preparations and in mammals *in vivo*), and these sulfoxide metabolites are the carbamoylating agents for tissue sulfydryls such as glutathione, cysteine and coenzyme A, and are more potent herbicides than is the parent thiocarbamate.

The metabolism of S-ethyl-dipropylthiocarbamate by mouse liver microsomes *in vitro* was investigated by Chen and Casida (1978). At this time the compound was known to undergo both sulfoxidation and N-depropylation. The sulfoxide metabolite is then conjugated by the glutathione S-transferases to yield the S-(dipropylcarbamoyl)-glutathione metabolite. The possible formation of the sulfone metabolite, the mechanism of ester cleavage not involving the sulfoxide metabolite, and the metabolites that arise from N-propyl and S-ethyl substituents require further clarification. The outcome of this study was that the sulfoxide was identified as the major metabolite, with little if any sulfone being formed. The decreasing order of metabolic transformation being, at sulfur, α -C of C_2H_5 , α -, β - and γ -C of $\text{N-C}_3\text{H}_7$ groups and β -C of $\text{S-C}_2\text{H}_5$. The hydroxylated metabolites of the carbons α to the nitrogen and sulfur decompose at physiological pH to yield S-ethyl-N-propylthiocarbamate and carbonyl sulfide and acetaldehyde, respectively. This study continued the earlier work of Hubbell and Casida (1977) who studied the metabolic fate of the N,N-dialkylcarbamoyl moiety of the thiocarbamate herbicides in both rats and corn. Again the S-ethyl-N,N-dipropylthiocarbamate was investigated, together with the compound butylate (S-ethyl-N,N-diisobutylthiocarbamate) plus their sulfoxide metabolites *in vivo* in the rat. The metabolic profile of the two thiocarbamates (the dipropyl and diisobutyl derivatives) were somewhat similar, the major metabolites being the sulfoxides and glutathione conjugates (cysteinyl and mercapturic acid conjugates) which account for 34–45% of the S-ethyl-dipropylthiocarbamate and 27–32% of the S-ethyl-diisobutylthiocarbamate. The metabolic profiles obtained when the sulfoxide metabolites of the two thiocarbamates were dosed to rats were those of

Table 4.2 Mouse intraperitoneal LD₅₀ values for EPTC, butylate and their metabolites

Compound	LD ₅₀ (mg kg ⁻¹ at day indicated after treatment)	
	Day 1	Day 7
EPTC	>500	58
EPTC sulfoxide	325	215
EPTC sulfone	130	40
Butylate	>500	365
Butylate sulfoxide	440	250
Butylate sulfone	200	80

the glutathione (cysteinyl and mercapturic acid) conjugates. The observation in this study that the sulfone and sulfoxide metabolites of both S-ethyl-N,N-dipropyl and S-ethyl-N,N-diisobutylthiocarbamates are more toxic than are their respective parent compounds is of interest. That the sulfone metabolites are the more toxic of all the compounds investigated in the mouse intraperitoneal LD₅₀ study is of interest (Table 4.2). Finally, the metabolism of S-ethyl-N,N-dipropylthiocarbamate and its sulfoxide metabolite were investigated in corn. The thiocarbamate was converted to the sulfoxide, and hence the glutathione and mercapturic acid conjugates, whilst the sulfoxide metabolite of the thiocarbamate was converted to its corresponding glutathione and mercapturic acid conjugates.

The metabolism of thiocarbamates in plants has also been under investigation. They are also converted to glutathione conjugates *in vivo* (Lamoureux and Frear 1979) in addition to sulfoxide metabolites (Ikeda *et al.* 1986, Wilkinson 1983b). The exact mechanism of sulfur oxidation in plants is not clearly understood (Owen 1987). The work of Casida *et al.* (1974) on the metabolic fate of S-ethyl-N,N-dipropylthiocarbamate and vernolate (S-propyl-N,N-dipropylthiocarbamate) intimated that sulfur oxidation is one of the initial steps in the detoxification of thiocarbamates under a variety of environmental conditions. The metabolism of benthioncarb (S-4-chlorobenzyl-N,N-diethylthiocarbamate) and orbencarb (S-2-chlorobenzyl-N,N-diethylthiocarbamate) has been investigated in rice (Nakamura *et al.* 1977), soybean (Unai *et al.* 1986a) and wheat and corn (Ikeda *et al.* 1986). The pattern of metabolites of orbencarb is orbencarb sulfoxide, mono- and diethylated orbencarb plus numerous minor metabolites such as 2-chlorobenzyl thiol, S-methyl-2-chlorobenzyl thiol, 2-chlorobenzyl alcohol and 2-chlorobenzoic acid.

An important pathway of thiocarbamate metabolism in plants involves the glutathione S-transferases since the sulfoxide metabolites produced during the phase I reactions are the major metabolites. Soybean plants (Unai *et al.* 1986b) oxidize benthioncarb and orbencarb to their respective sulfoxide metabolites which are then further degraded to S-(2-chlorobenzyl)-L-cysteine, S-(chlorobenzyl)-N-malonyl-L-cysteine and their sulfoxides. The S-substituted N-malonyl cysteine metabolites, which are also found in the metabolism of glutathione conjugates in higher plants (Lamoureux and Rusness 1981), appear to be the equivalent of mercapturic acid conjugates in the animal kingdom.

4.2.3.3 Toxicity

Sulfur-containing functional groups are found in approximately one-third of all organic pesticides (Schuphan and Casida 1983), and oxidation is an essential bioactivation process for these pesticides which contributes greatly to their selectivity, potency, type of reaction at their target site, and mode of action. The two major metabolic pathways of thiocarbamates are sulfoxidation and S-methylene hydroxylation (Distlerath *et al.* 1985). The herbicidal activity of the thiocarbamate sulfoxide is probably a result of the carbamoylation of essential sulfydryls. However, in mammals this increased reactivity may result in reduced toxicity. In terms of acute toxicity studies in rat the thiocarbamates can be classed as slightly toxic with LD₅₀ (oral) values ranging from 2550 mg kg⁻¹ for S-ethyl N,N-dipropylthiocarbamate to 720 mg kg⁻¹ for molinate (S-ethyl perhydroazepine-1-thiocarbamate) (Buchel 1983). However, the thiocarbamates tend on the whole to show moderate [LD₅₀ (oral) 50–500 mg kg⁻¹] toxicity to birds.

In terms of chronic effects, the major research has been to investigate the mutagenicity and carcinogenicity of the thiocarbamates. Studies by Innes *et al.* (1969) concluded that diallate (S-(2,3-dichloroallyl)diisopropylthiocarbamate) was an active carcinogen and further studies (Sikka and Floczyk 1978) showed that diallate was also a mutagen with activity at 1 µg/plate. This mutagenic activity was only seen following S9 fraction bioactivation and only in TA 100 and TA 1535 strains (base pair substitution mutants) and not the TA 98 and TA 1538 (frame shift mutants) strains of the histidine-requiring *Salmonella typhimurium*. The other thiocarbamate investigated was triallate (S-(2,2,3-trichloroallyl)diisopropylthiocarbamate) which was also found to be mutagenic, but again only following metabolic activation and only in the TA 100 and TA 1535 strains at a concentration of 50 µg/plate. Indeed the mutagenicity of these two thiocarbamates is primarily due to the 2-chloropropenyl group present in each. The thiocarbamates, butylate, S-ethyl N,N-dipropylthiocarbamate and molinate, which lack the 2-chloropropenyl group, are not mutagenic but still show considerable herbicidal and fungicidal activity (De Lorenzo *et al.* 1978).

The mechanism by which the promutagen, diallate, is activated was investigated by Schuphan *et al.* (1979). The S-2-chloropropenyl thiocarbamate sulfoxides are unstable due to their spontaneous conversion into the sulfenate ester via a [2,3] sigmatropic rearrangement reaction which is then followed by a 1,2 elimination reaction resulting in the formation of 2-chloropropenal (the ultimate mutagen). Now, since diallate is not a mutagen without bioactivation by the sulfoxide metabolite, the herbicidal activity of diallate may be due either to the sulfoxide's ability to act as a carbamoylating agent with certain tissue sulfydryls or to the release of 2-chloropropenal.

Finally, it should be noted that diallate and also triallate (Fisher and Metcalf 1983, Hansen *et al.* 1985) reported delayed neurotoxicity in hens. Oral administration of diallate (at 312 mg kg⁻¹) produced reproducible ataxia in hens with resulting leg weakness and an inclination to sit or stand with closed eyes. Certain hens suffered a severe ataxia with drastic weight loss and also muscular atrophy which was closely followed by paralysis and death within a few days. The mechanism of action is unclear but triallate produces only mild ataxia. In general, the thiocarbamates show higher levels of neurotoxicity than those shown by their corresponding carbamates, but weaker than those of the organophosphates.

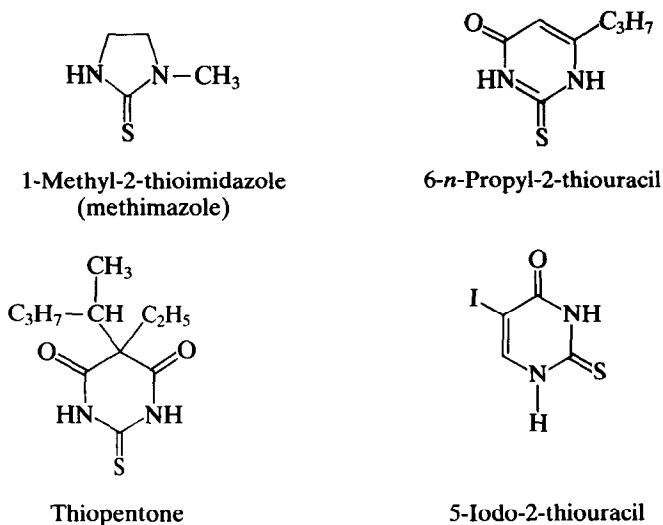


Figure 4.6 Thiocarbamides

4.2.4 Thiocarbamides

4.2.4.1 Applications

The simplest of the thiocarbamides is the compound thiourea which is the originator of this class of compounds. The biological interactions of thiourea have been extensively reviewed (Mitchell and Steventon 1994) and so this section will concentrate heavily on the other thiocarbamide compounds. The only naturally occurring thiocarbamide is the compound, ergothioneine. It is present in ergot and is also found in large quantities in mammalian blood cells and also in the seminal fluid of certain species (Stowell 1961). In contrast, the synthetic thiocarbamides have a wide and extensive usage from intermediates in organic reactions to therapeutic agents.

The thiourea class of thiocarbamides are used as rodenticides (1-naphthylthiourea; Richter 1945), bactericides (N-methyl-N'-(hydroxymethyl)thiourea; Pickard 1972), which acts as a disguised formaldehyde compound in use as a lavaging agent following abdominal surgery, and in the compounding of rubber (ethylenethiourea), while the thiohydantoins have found use in the sequencing of amino acids in proteins (Edman 1950). The next two types of thiocarbamides are used primarily in the treatment of hyperthyroidism. These are the 2-thioimidazoles (1-methyl-2-thioimidazole; Solomon 1986) and the 2-thiouracils (6-n-propyl-2-thiouracil; Solomon 1986). However, the 2-thioimidazole (2-thiobenzimidazole) is used in rubber processing (El Dareer *et al.* 1984) and the 2-thiouracil (5-iodo-2-thiouracil) is employed in the diagnosis of melanoma (Franken *et al.* 1986). The final class of thiocarbamides to find use is the 2-thiobarbiturates, of which thiopentone is a good example, as anaesthetics (Morgan *et al.* 1981). These compounds are all represented in Figure 4.6.

4.2.4.2 Metabolism

The major metabolite of ^{35}S -phenylthiourea is ^{35}S -inorganic sulfate in the rat (Scheline *et al.* 1961), and because phenylcarbamic acid and phenylurea are also produced it was postulated that the desulfuration was via a reductive mechanism with the subsequent production of hydrogen sulfide, a toxic metabolite. However, subsequent studies implicated the S-oxides as the potentially reactive metabolites (Poulson *et al.* 1979). This is because labile sulfenic and sulfinic acids were identified as products of both phenylthiourea and also ethylenethiourea following metabolism by the hepatic flavin monooxygenase (FMO) system. In a further study, 2-imidazolyl sulfenate was identified as the major metabolite of ethylenethiourea (Savolainen and Pyysalo 1979) in mice. The sulfenic acids of thiocarbamides are sensitive to further oxidation to the more stable sulfinic acids which then can be hydrolysed to the corresponding urea or converted into a formamidine or urea.

Desulfuration of methimazole (1-methyl-2-thioimidazole) is via assumed sulfenic and sulfinic acid intermediates which are labile and impossible to isolate, with the latter being hydrolysed to the imidazole (Poulson *et al.* 1974). It was proposed that the S-monoxide of methimazole decomposes to the imidazole carbene and sulfur monoxide (Neal 1980), and that the sulfur monoxide disproportionates to yield sulfur dioxide and highly reactive atomic sulfur. Poulson *et al.* (1979) concluded that if the thiocarbamide S-dioxides were the reactive metabolites then their relative stabilities may explain the differing toxicities of these compounds in experimental animals. Thiocarbamide desulfuration to give its oxygen analogue may result from nucleophilic attack on the S-dioxides since the sulfur dioxide is a relatively good leaving group. However, it has also been hypothesized that for 1-naphthylthiourea, S-monoxide formation following nucleophilic attack by the oxygen atom at the thiocarbonyl carbon atom causes an oxathiirane to be formed which subsequently rearranges to produce the 1-naphthylurea and atomic sulfur (Neal 1980). In conclusion to this section on phase I oxidative metabolism, the following generalizations can be made: (i) The thioureas are capable of desulfuration to their corresponding oxygen analogues and to formamidine derivatives and (ii) The 2-thioimidazoles and 2-thiopyrimidine derivatives are seemingly only desulfurated to their corresponding imidazoles and to date no oxygen analogues have been reported as metabolites.

The metabolism of the thiocarbamides would be incomplete without the description of the phase II or conjugation reactions of these compounds. Glucuronidation is reported to be a major metabolic pathway for the 2-thiouracils in man (Marchant *et al.* 1978, McGinty *et al.* 1984) and rat (Lindsay *et al.* 1974, Sitar and Thornhill 1972). In this reaction there is a nucleophilic attack of the sulfur functional group at the C1 position of the glucuronic acid in uridine diphosphate glucuronic acid. This reaction is catalysed by the microsomal family of enzymes, the UDP-glucuronosyl transferases. This reaction does show species variation since 2-thiouracil and propylthiouracil (2-thiopyrimidine derivatives) are S-glucuronidated, while methimazole and thiourea are not when incubated with guinea-pig microsomes *in vitro* (Lindsay *et al.* 1977). This discrepancy in metabolism is probably due to the relative differences in the ionization of the thiocarbamides, as the 2-thiouracils are more acidic than are thiourea and

methimazole (Foye and Lo 1972, Ganellin and Durant 1981, Stanovnik and Tisler 1964a, b). However, the S-glucuronide of methimazole was reported to be excreted by the rat (Sitar and Thornhill 1973).

The compound 2-thiouracil is structurally similar to its naturally occurring pyrimidine, uracil, which is incorporated into RNA in many organisms including man. The 2-thiouracil is initially N-ribosylated to form the nucleotides, 2-thiouridine (Strominger and Friedkin 1954) or 2-thio-5'-uridine monophosphate (Lindsay *et al.* 1972). The compounds methylthiouracil, propylthiouracil and methimazole are not, however, substrates for the enzyme, UMP-pyrophosphorylase, which synthesizes the formation of 2-thiouridine from 2-thiouracil (Lindsay *et al.* 1972). Also the compounds 2-thiouridine and 2-thio-5'-uridine monophosphate are not as effective an antithyroid agent in the rat as is the parent compound, 2-thiouracil (Yu *et al.* 1973).

The S-methylation of the thiocarbamides is well documented, with both 2-thiouracil and propylthiouracil (Lindsay *et al.* 1974b, Sarcione and Sokal 1958) finding the S-methyl metabolites in the rat. The exact organ location of the S-methyl transferase is different in the rat and mouse, with rat showing hepatic S-methylation of methimazole, 2-thiobenzimidazole and propylthiouracil (Weisiger and Jakoby 1979), while the mouse showed kidney S-methylation of 2-thiouracil and propylthiouracil but no S-methylation of methimazole (Lindsay *et al.* 1975).

4.2.4.3 Toxicity

The thiocarbamides accumulate in the thyroid gland (Marchant *et al.* 1972) where they inhibit thyroid peroxidase with the consequence that the biosynthesis of the thyroid hormones, thyroxine (T_4) and tri-iodothyronine (T_3). Thus, the thiocarbamides are nearly all goitrogenic (Astwood 1943, Astwood *et al.* 1945, MacKenzie and MacKenzie 1943, Searle *et al.* 1951). The enzyme, thyroid peroxidase (tyrosine iodinase), is located in the membranes at or near to the apical surface of the thyroid cell. This enzyme catalyses the iodination step in the biosynthesis of both T_4 and T_3 , the so-called 'organification reaction' of iodine (Marchant *et al.* 1978, Taurog 1986). The thiocarbamide prevents the iodide anion from being converted into iodine and/or the reactive intermediate before or during its attachment to one of the tyrosine residues in the thyroglobulin glycoprotein. The degree of toxicity to thiocarbamides is species-dependent (Neal *et al.* 1977, Neal and Halpert 1982) but the most common toxic reactions observed in patients being treated with 2-thioimidazole and 2-thiouracil are hypersensitivity reactions (Bouillon 1980). Drug-induced agranulocytosis is found to occur in <1% of patients treated with these drugs.

The N-substituted derivatives of 1-phenylthiourea (Richter and Clisby 1942) 1-naphthylthiourea (Latta 1947, Meyrich *et al.* 1972) and thiourea, in addition to being goitrogenic, produce pulmonary oedema and pleural effusion of varying intensities in animals. The toxic response is produced by metabolic activation of the thiourea moiety of the compound (Boyd and Neal 1976, Lee *et al.* 1980) and is probably related to the extent of the desulfuration of the compound (Smith and Williams 1961, Williams 1961).

The compound ethylenethiourea is known to show a wide range of toxicities including goitrogenic, carcinogenic, teratogenic and also to induce liver tumours

(Graham *et al.* 1973, Innes *et al.* 1969, Khera and Inverson 1981, Ulland *et al.* 1972) in rats and mice but cat and rabbit (Khera 1973, Khera and Inverson 1978) are not susceptible to the teratogenic effects. This may relate to the fact that the major pathways of metabolism in the rat are oxidative whereas in the cat, S-methylation predominates. This may result in species differences in susceptibility to ethylenethiourea toxicity.

4.2.5 Thioamides

4.2.5.1 Applications

The thioamide-containing compounds, especially the nonphysiological variety, are distributed widely throughout the environment. The thioamide function group is present in many drugs, chemicals and agrochemicals and exposure of man and animals to these compounds results in inadvertent toxicity. A number of clinically useful drugs containing the thioamide group have limited application due to the toxic side effects associated with this class of compounds. The thioamide, Prefex (2,6-dichlorothiobenzamide), has been used as a herbicide (Griffiths *et al.* 1966); a new benzodiazepine, quazepam (7-chloro-1-(2,2,2-trifluoroethyl)-5-(*o*-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-thione), has clinical efficacy as a hypnotic (Teitz *et al.* 1981); ethionamide is used as an antitubercular agent (Shepard *et al.* 1985); and thiobenzamide (Figure 4.7) has antibacterial (Waisser *et al.* 1982) and immunosuppressant properties (Pasquinelli *et al.* 1985). The latter compound is also useful in terms of its antitubercular activity against strains of *Mycobacterium tuberculosis* that are resistant to ethionamide, isoniazid and thioacetazone (Drasata *et al.* 1981).

4.2.5.2 Metabolism

The associated toxicity of the thioamides has been linked to their oxidative metabolism. The simple thioamide, thioacetamide (a carcinogen, teratogen and hepatotoxin; McCann *et al.* 1975, Neal and Halpert 1982), is oxidized to thioamide S-oxide and then to the S,S-dioxide which is a highly reactive acylating intermediate with sufficient chemical reactivity to bind covalently to cellular macromolecules. The cytochrome P450 (Porter and Neal 1978) and flavin monooxygenase (Zeigler 1980) are believed to be responsible for this biotransformation. The anti-tubercular

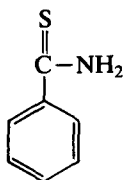


Figure 4.7 Thiobenzamide

drug, ethionamide, is metabolized to its corresponding S-oxide metabolite in a number of experimental animals (dog, rat and mouse) in addition to the main metabolite, 2-ethylisonicotinamide (Johnston *et al.* 1967). Ethionamide S-oxide, in common with other thioamide S-oxides, has a greater biological activity than has the parent compound (Prema and Gopinathan 1976).

The herbicide, Prefix, is metabolized to 2,6-dichlorobenzonitrile which implicates oxidative desulfuration (Griffiths *et al.* 1966) as the major metabolic pathway in the metabolism of this thioamide. In addition, rats dosed with ^3H -thioacetamide produced thioacetamide S-oxide, acetamide and inorganic sulfate (Rees *et al.* 1966). These were not the only products detected since labelled protein, nucleic acid and phospholipid were also isolated in rats following radioactive thioacetamide administration. This observation was followed by the work of Ammon *et al.* (1967) which indicated that cysteine can reduce thioacetamide S-oxide to thioacetamide *in vitro*. This probably explains the mechanism of cysteine protection of hepatotoxicity if preadministered before thioacetamide (de Ferreyra *et al.* 1979). In the presence of the microsomal fraction, hydrogen peroxide oxidation of thioacetamide S-oxide to a reactive intermediate results in the acylation of the ϵ -amino group of lysine in proteins to form N- ϵ -acetyllysine. The reactive intermediate is believed to be thioacetamide S,S-dioxide which can also acetylate water to produce acetamide (Dryoff & Neal 1981). The metabolism of ethionamide is believed to follow similar metabolic pathways to thioacetamide (for a more extensive review of ethionamide metabolism see Waring *et al.* 1989).

4.2.5.3 Toxicity

Thiobenzamide is hepatotoxic (Chieli *et al.* 1979) while the *p*-methoxythiobenzamide is far less toxic to the liver (Hanzlik *et al.* 1978); the difference in toxicity is believed to be due to the electronic substituent group effects on (i) rates of metabolic activation and (ii) the relative reactivities of toxic metabolites. In a series of experiments on the substitution of thiobenzamide at the *meta* and *para* position (Figure 4.7) and its effect on hepatotoxicity as measured by hepatic transaminase release, the following observation was made: that toxicity increased with increasing power of electron donation from the substituent to the aromatic ring (Cashman *et al.* 1983, Hanzlik *et al.* 1980). This pattern of toxicity is consistent with the idea that enzymatic S-oxidation is an essential step in the activation of this class of compounds. This is complicated somewhat by the observation that Prefix is not hepatotoxic in rats; however, this may be due to the electron-withdrawing effects of the two chlorine substituents or to steric hindrance of the bioactivation process (Lowry and Richardson 1976).

Both ethionamide and prothionamide cause adverse reactions, with ethionamide being the more toxic of the two (Gupta *et al.* 1975, Patteyn *et al.* 1984). The picture in patients is of gastric intolerance and hepatic damage; the exact toxic agent (parent compound or metabolite) is unclear. However, investigation in the rat points to the inhibition of lipoprotein synthesis and thus the consequential build-up of lipids in the liver being due to direct damage to the hepatocytes by ethionamide. This results in the condition known as fatty liver (Inoue *et al.* 1973). The potential desulfuration of ethionamide and also prothionamide is believed to be responsible for the hepatotoxicity observed. Other reported side-effects of ethionamide include

induction of goitrous hypothyroidism (Moulding and Fraser 1970) and peripheral neuropathy, insomnia, depression, mania and drowsiness (Lansdown *et al.* 1967, Narang 1972).

4.3 Sulfur heterocycles

The presence of the sulfur atom in medicinal and other xenobiotic compounds is widespread. The number of three- or four-membered sulfur compounds in use is limited and so this section will be devoted to the five-, six- and seven-membered heterocycles. The use of compounds of this series is very extensive; examples of drugs or chemicals in their respective groups will be provided to enable the reader to obtain the flavour of these compounds in their various roles in medicine and agriculture.

4.3.1 Thiophenes (five-membered sulfur heterocycles)

These compounds are all analogues of thiophene (Figure 4.8); detailed reviews of the chemistry of thiophene are available in the literature (Rajappa 1984). The reactions in terms of their biotransformation are based around the oxidation of the sulfur atom and electrophilic substitution reactions of the aromatic ring.

4.3.1.1 Applications

The anthelmintics, thenium (N,N-dimethyl-N-2-phenoxyethyl-N-2'-thenyl-ammonium)-*p*-chlorobenzenesulfonate), pyrantel (1,4,5,6-tetrahydro-1-methyl-2-(*trans*-2)thienyl-vinyl-pyrimidine) and morantel (1,4,5,6-tetrahydro-1-methyl-2-(*trans*-2)-3-methyl-2-theinyl-pyrimidine) (see Figure 4.9) are used as nematocides in veterinary practice. Thenium closylate is used primarily for the treatment of canine hookworm infections. It is highly effective against adult and immature forms in addition to fourth-stage larvae of the canine *Ancylostoma caninum* (Miller 1966). The drug shows moderate effectiveness against canine ascarids but little potency against feline parasites *Toxocara cati* or *Trichuris vulpis*.

Pyrantel is used as its pamoate salt and is a broad-spectrum nematocide which is effective against *Ascaris lumbricoides*, *Enterobius vermicularis* and *Ancylostoma duodenale*. The drug is believed to cause depolarization of muscle cells and contraction of *Ascaris suum* at one-hundredth of the concentration of acetylcholine required to produce a similar effect (Aubry *et al.* 1970). Thus it appears that pyrantel acts by inducing paralysis of the worm which leads to dislodgement from the gut by the peristaltic movement of the gastrointestinal tract. Finally, the thiophene drug, morantel, has shown anthelmintic potency in ruminant animals and is believed to act via a similar mechanism to pyrantel.

Other drugs that contain a thiophene ring are suprofen (an anti-inflammatory drug), tienilic (a naturetic compound), tiquizium bromide (anticholinergic agent) (Figure 4.10), while the drugs based on the thiazole ring constitute another group of anthelmintic compounds (diathiazanine, levamisole, niridazole and thiabendazole) (Figure 4.11), the anti-inflammatory drug, sudoxicam, and the immunoregulator, fanetizole.

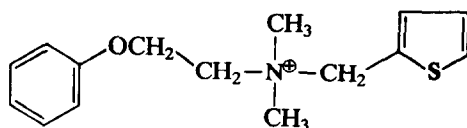


Figure 4.8 Thiophene

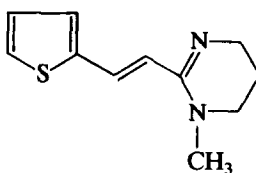
4.3.1.2 Metabolism

There is little if any literature on the metabolism of thenium, whereas pyrantel has been extensively studied in sheep, ox, pig, dog and rat (Faulkner *et al.* 1972). The liver is the main site of oxidative metabolism which follows two main metabolic routes. The first is the cleavage of the carbonyl chain which results in the formation of thienylic acids, in particular thiophene carboxylic acid. The second is the hydroxylation of the thiophene ring at the 5-position (Fidgor *et al.* 1978). This results in the subsequent opening of the thiophene ring and the conversion of the resulting thioketone to an oxoketone which is finally converted to levulinic acid (Fidgor *et al.* 1978). The pyrimidyl moiety of the compound is transformed into the N-methyl-1,3-propanediamine and is found frequently as a urinary metabolite. In humans only limited information is available concerning the metabolism of pyrantel pamoate. Faecal excretion of an orally administered dose of pyrantel results in 60–94% recovery, with the urinary metabolites being identified as thiophene carboxylic acid, levulinic acid and N-methyl-1,3-propanediamine (Kimura and Kume 1971).

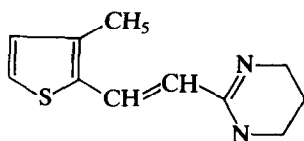
The metabolism of morantel in sheep is similar to that of pyrantel (O'Brien 1970), and it has also been shown that ~17% of a dose is excreted in the urine



Thenium

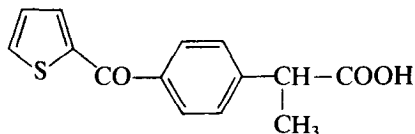


Pyrantel

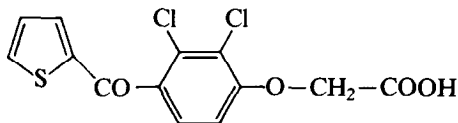


Morantel

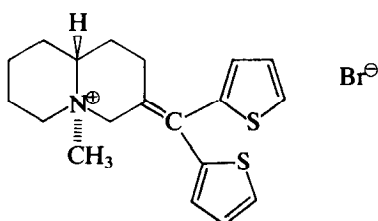
Figure 4.9 The anthelmintics



Suprofen



Tienilic acid

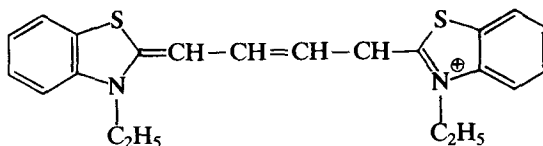


Tiquizium bromide

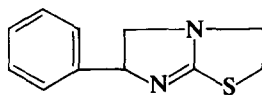
Figure 4.10 Thiophene ring compounds

as metabolites in the first 96 h, with the remaining amount being excreted in the faeces.

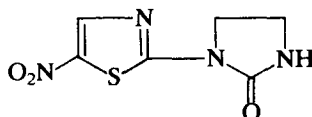
The anthelmintics based on the thiazole ring are dithiazanine, levamisole, niridazole and thiabendazole. Dithiazanine and levamisole have the thiazole inserted into a benzothiazole or an imidazothiazole, while niridazole has the thiazole linked directly to an imidazolidine. Finally, thiabendazole is closely related to a thio-substituted benzimidazole. Dithiazanine (3-ethyl-2-(5-(3-ethyl-2-(3*H*)benzothiazolydene)-1,3-pentadienyl)benzothiazolium) is used as its iodide salt but its metabolic fate appears not to have been investigated. However, the drug levamisole is the *L*-isomer of the racemic mixture *DL*-tetramisole (2,3,5,6-tetrahydro-6-phenylimidazo(2,1-*b*)thiazole). The drug is a rapid but short-acting compound with peak blood concentrations being reached within 2 h of a 50 mg oral dose in man (Graziani and De Martin 1977). The drug is virtually eliminated from the body within a few days. In rats receiving only one 7.5 mg kg⁻¹ oral dose of ³H-levamisole, the urine obtained 96 h post-dosing contained 68% of the dose in which the parent compound contributed only 6% of the radioactivity (Galtier *et al.* 1983). Levamisole is extensively metabolized in the liver and the possible metabolic routes of the drug in the rat have been investigated (Graziani and De Martin 1977). The metabolic biotransformations are: (i) oxidative introduction of a new double bond into the imidazole ring; (ii) hydrolysis of the thiazole, resulting in the formation of the thioxoimidazole acetic acid; (iii) *p*-hydroxylation of tetramisole and the formation of the glucuronide conjugate; and (iv) scission of



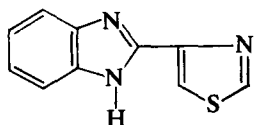
Dithiazine



Levamisole



Niridazole



Thiabendazole

Figure 4.11 Thiazole anthelmintic compounds

the thiazole, resulting in the production of the mercaptoethyl derivative which subsequently can undergo S-oxidation or methylation. In man, *p*-hydroxylation is an important metabolic pathway since it represents 1.6–12.4% of an oral dose of levamisole (Kouassi *et al.* 1986). The most important metabolic conversion of levamisole is the ring fission of the thiazole nucleus which results in the formation of 2-oxo-3-(2-mercaptoethyl)-5-phenylimidazole (OMPI) as reported by Janssen (1976). The immunomodulating properties of levamisole have been hypothesized to be due to OMPI (De Brabander *et al.* 1979). This metabolite has been shown to inhibit microtubule formation, probably via its interaction with certain critical sulfhydryl groups (de Brabander *et al.* 1978), to protect cultured cells against necrosis induced by glutathione depletion (De Brabander *et al.* 1979).

Niridazole (1-(5-nitro-2-thiazolyl)-2-imidazolidinone) is a schistosomicidal drug. The anthelmintic activity is probably due to the parent drug (Faigle and Keberle 1969) since the death of the parasites is directly related to their uptake of niridazole from the incubation medium containing the drug. The mechanism of action probably results from increased rates of glycogenolysis in the worm since niridazole inhibits phosphorylase phosphatase activity which will result in starvation for the worm (Bueding and Fisher 1970). The metabolism of niridazole is extensive, with six metabolites being identified in the serum of patients infected with *Schistosoma japonicum* (Valencia *et al.* 1984). The highest concentrations of metabolites seen

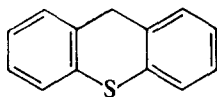
are the 4-hydroxy and 4-ketoniridazole whilst the reductive metabolite, 1-thiocarbamoyl-2-imidazolidinone, is found at the lowest concentrations. The hepatic cytochrome P450 system is probably responsible for the various hydroxylation reactions (Blummer *et al.* 1979) with either further hepatic or renal metabolism of the 4-hydroxyniridazole to the 4-ketoniridazole (Catto *et al.* 1984). The reductive metabolite, thiocarbamoyl-2-imidazolidinone, is probably formed in the mammalian intestine by the reductive cleavage of the nitrothiazole ring by gut microflora under anaerobic conditions (Tracy and Webster 1981).

Finally, the drug thiabendazole (2-(4'-thiazolyl)benzimidazole) is one of the most extensively used benzimidazoles against gut helminths of animals and humans. Thiabendazole inhibits both fumarate reductase and NADH oxidase from adult *Haemonchus contortus* (Prichard 1970) and thus it interferes with parasite energy metabolism. The most important oxidative pathway for the drug is the 5-hydroxylation of the benzimidazole ring, and in urine both the glucuronide and sulfate conjugates of the 5-hydroxythiabendazole have been identified (Tocco *et al.* 1966). All metabolites are devoid of any anthelmintic activity (Stone *et al.* 1965). Work by Wilson *et al.* (1973) has shown that thiabendazole has a type II binding spectrum in the presence of liver microsomes. This implicates the cytochrome P450 as the site of 5-hydroxylation.

4.3.1.3 Toxicity

There is very little information on the toxicity of pyrantel but the side-effects that have been reported include nausea, vomiting, anorexia, diarrhoea and dizziness and also elevation of aspartate aminotransferase values. The last value is indicative of hepatic damage and necrosis. Dogs receiving daily doses of 50–75 mg kg⁻¹ for 90 days have suffered sporadic symptoms which include prostration, retching and depressed respiration (O'Brien 1970). The information on thenium, morantel and dithiazanine toxicity is very sparse and contradictory in places and as such needs further investigation. However, the drug levamisole has had the following side-effects reported in man: nausea, abdominal pain, headache, agranulocytosis, leucopaenia and thrombocytopenia. Thus the drug is usually avoided in patients with advanced kidney or liver disease and also in patients with pre-existing blood disorders.

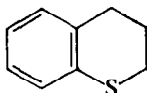
Niridazole, on the other hand, has several well-characterized side-effects. These include nervous system disorders, carcinogenicity and immunosuppression. Since the immunosuppressant action of niridazole *in vivo* is not reproduced *in vitro* in guinea-pig lymphocyte cultures (Mahmoud *et al.* 1975), it has been suggested that a metabolite of niridazole is responsible for this action (Daniels *et al.* 1975). Again when the effects of its nervous system disorders were investigated in an inbred strain of mice the parent drug was found not to be responsible for the toxicity (Blummer *et al.* 1980). The carcinogenic effects of niridazole (Urman *et al.* 1975) can probably be explained by the *in vitro* formation of a reactive epoxide metabolite (Blummer *et al.* 1979). This observation is further supported by the results of Mohtashamipur and Norpoth (1986) which found mutagenic derivatives of niridazole excreted in sweat and in faeces of humans following oral doses of niridazole. Finally, the side-effects of thiabendazole that are frequently encountered are anorexia, nausea and vomiting and these seem to correlate with high plasma concentrations of the 5-hydroxythiabendazole metabolite.



9H-Thioxanthene

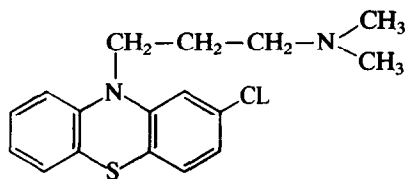


Tetrahydro-2H-thiin

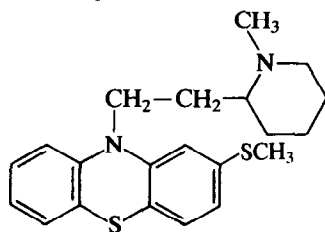


3,4-Dihydro-2H-benzothiin

Figure 4.12 Six-membered sulfur heterocycles



Chlorpromazine



Thioridazine

Figure 4.13 Phenothiazine drugs

4.3.2 9H-Thioxanthenes (fused six-membered sulfur heterocycles) and phenothiazines

The six-membered sulfur heterocycles could form the basis of a book in their own right let alone part of a chapter. They can exist as a fused or unfused ring system (Figure 4.12) but can be regarded as cyclic thioethers, alkylaryl and diaryl thioethers, respectively. Since the metabolism of the dibenzo-fused thiins (9H-thioxanthenes) has been studied in depth these compounds will form the basis of this section together with the structurally similar phenothiazines. The reader is referred to Rance (1989) for a detailed review of the 3,4-dihydro-2H-benzothiins and the tetrahydro-2H-thiins.

The 9H-thioxanthenes and phenothiazines are used extensively as antidepressants and neuroleptics and as such have numerous applications. The phenothiazines, however, have also been used extensively for other purposes (Mitchell 1989). The structural similarity between the compounds can be seen in Figure 4.13; the main difference is the presence of a nitrogen atom at position 10 of the b ring opposite the sulfur atom at position 5. This is not present in the 9H-thioxanthenes and as a result different metabolic products are produced during their biotransformation.

4.3.2.1 Metabolism

The 9H-thioxanthenes tend to form sulfoxide metabolites as their main metabolic oxidation products but not the sulfone, and earlier reports that thiothixine (Hobbs 1968), flupenthixol (Jorgensen *et al.* 1969) and clopenthixol (Khan 1969) are not subjected to ring oxidation indicated another difference between these compounds and the phenothiazines. However, Breyer-Pfaff *et al.* (1985) have identified hydroxylated metabolites of the thioxanthene, chlorprothixene, in the urine of both dog and man as conjugates. Both species hydroxylated at the 7- and 6-position but in dog the main hydroxylated metabolite was the 5-hydroxy chlorprothixene which was absent from human urine. The phenothiazine class of compounds is subject to a wide range of biotransformation reactions, with sulfoxide and sulfone metabolites being the predominant sulfur metabolites. The sulfoxide metabolites tend to predominate the sulfone as a general rule but exceptions can and do occur. Ring hydroxylations and oxidations of the N₁₀ side chain are also common sites for metabolic attack on this class of compounds.

This is best illustrated by the metabolism of the phenothiazine drugs, thioridazine and chlorpromazine (Figure 4.13) where both show side chain N-dealkylation, ring sulfoxidation and ring hydroxylation but only thioridazine shows side chain S-oxidation (Papadopoulos and Crammer 1986). The cytochrome P450 is probably central to the N-dealkylations and ring hydroxylation reactions but both cytochrome P450 and flavin monooxygenase (Breyer 1971, Prema and Gopinathan 1976) are involved in the sulfoxidation of the ring sulfur of chlorpromazine. This work was extended further by Gorrod *et al.* (1974) and Traficante *et al.* (1979) who showed that the less nucleophilic sulfur atom of the aromatic nucleus of chlorpromazine was readily oxidized to the sulfoxide by a microsomal NADPH-requiring monooxygenase. The microsomal flavin monooxygenase has also been shown capable of oxidizing the dialkyl and alkyaryl thioethers (Zeigler *et al.* 1980), but in thioridazine the first side chain S-oxidation reaction is performed by Cyp 2D6 (debrisoquine 4-hydroxylase) as shown by Meyer *et al.* (1990) and von Bahr *et al.* (1991).

4.3.2.2 Toxicity

The main site of action of the 9H-thioxanthenes and phenothiazines is the blockade of the dopamine-D₂ receptor in the brain. Thus these drugs have found wide usage as antidepressant and antipsychotic agents. The phenothiazines inhibit the dopamine-stimulated increase in cAMP production in rat striatal brain homogenates in addition to increasing dopamine turnover in the mouse brain. Phenothiazine drugs are able to assume a three-dimensional conformation, part

of which is similar to that of dopamine, and this is now believed to underlie their mode of action. The major toxicity problems observed with these compounds are a drug-induced Parkinsonian syndrome which is readily reversible upon cessation of treatment, and cholestatic jaundice, chlorpromazine being particularly guilty of the latter problem. Postural hypotension may also result from α -adrenoceptor antagonism along with sedation, visual disturbances, constipation and increased prolactin secretion (which in men results in gynaecomastia, lactation and painful breasts).

4.4 Thioethers

4.4.1 Histamine blockers and mucolytics

This section on thioethers (sulfides) will concentrate on the histamine H_2 -receptor antagonists cimetidine, ranitidine, famotidine and nizatidine, all based (initially) on the histamine molecule (Figure 4.14), and the mucolytic agents S-carboxymethyl-L-cysteine and S-methyl-L-cysteine which both contain the sulfydryl amino acid, L-cysteine.

4.4.1.1 Applications

The H_2 nomenclature was introduced to differentiate the histamine receptor subtype which mediated gastric acid secretion in the stomach (Black *et al.* 1972). The development of the histamine H_2 -antagonists has been extensively reviewed within the literature (Brittain 1982, Durant *et al.* 1977, Ganellin 1985). All the clinically useful H_2 -antagonists are based around the imidazole nucleus of histamine (with minor modifications) and possess differing side chain substituents (Figure 4.15). The four main H_2 -antagonists in use around the world are cimetidine, ranitidine, famotidine and nizatidine. They all competitively inhibit histamine-stimulated gastric acid secretion and are therefore used to treat conditions of excessive gastric acid production (Bianchi Porro *et al.* 1987, Brogden *et al.* 1982, Jones *et al.* 1987). They are all given orally or by injection but the dosing regimen is different in each case. Their primary use is in the treatment of duodenal ulcers which can occur in <20% of men in Western countries.

The thioether derivatives of L-cysteine (Figure 4.16) were initially introduced in the 1950s as therapeutic agents for reducing mucus congestion (Aylward *et al.* 1980). They are believed to help by reducing sputum viscosity and as such have found use as a second-line drug in the treatment of asthma (chronic) and bronchitis. However, their use has been extended to patients with cystic fibrosis

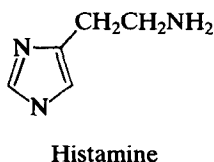
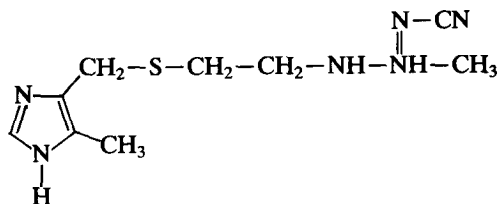
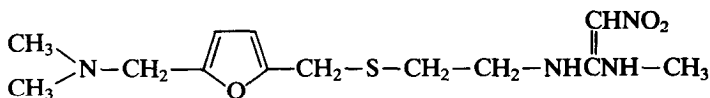


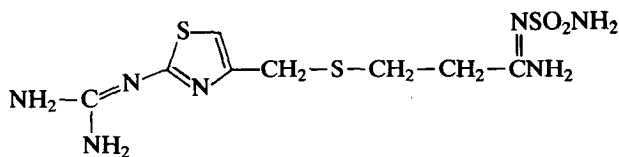
Figure 4.14 Histamine



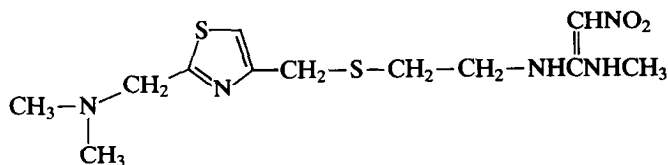
Cimetidine



Ranitidine

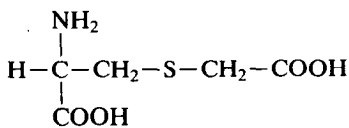


Famotidine

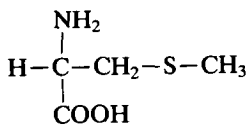


Nizatidine

Figure 4.15 H₂-receptor antagonists



S-Carboxymethyl-L-cysteine



S-Methyl-L-cysteine

Figure 4.16 Thioether derivatives of L-cysteine

and in gynaecological and urological problems (Benderev 1988, Malhi *et al.* 1987) where they are well tolerated.

4.4.1.2 *Metabolism*

The extent of metabolism of this group of H₂-antagonists varies considerably with the hydrophilic compounds, cimetidine, ranitidine and famotidine being cleared largely by renal excretion with little biotransformation. The sulfur-containing functional groups present in the four compounds under discussion are: (i) sulfonamide (famotidine), (ii) thioether (cimetidine, ranitidine, famotidine and nizatidine) and (iii) thiazole (famotidine and nizatidine). When radioactive cimetidine was administered to rats the major portion of the label recovered in urine was the parent compound (70% after intravenous and 65% following oral dosing). The major metabolite was cimetidine sulfoxide in both male and female rats. This accounted for 31% of the dose in male rats but only 13% of the dose in female rats. This is interesting since the parent compound was recovered in urine as 50–55% of the dose in male rats but 75% of the dose in female rats. Both the hydroxymethyl and guanylurea metabolites of cimetidine were excreted in the urine but these accounted for less than 10% of the urinary radioactivity (Mitchard *et al.* 1989). The urinary profile of radioactive metabolites in dogs was similar to that in female rats, with the route of administration causing little, if any, difference in urinary excretion of the drug and its metabolites. No sex difference was found in sulfoxide production in dogs as there was in the rat (Mitchard *et al.* 1989). However, when ¹⁴C-cimetidine was administered to normal individuals, only 10–15% of the dose was present in the urine as cimetidine sulfoxide with approximately 40% of the radioactivity present as an unidentified polar metabolite (Taylor *et al.* 1978). This was investigated further (Mitchell *et al.* 1982) and the metabolite was identified as cimetidine N-glucuronide.

The metabolism of ranitidine has been extensively investigated in a number of species (rat, rabbit, mouse, dog and man) (Bell *et al.* 1980, Martin *et al.* 1982). Following radiolabelled oral dosing of ranitidine to dogs, approximately 70% of the dose was excreted in the urine, but this fell to 30–40% of the dose in the mouse, rat and rabbit. In all the species investigated the radioactivity was mostly recovered in the first 24 h with a small fraction being excreted in the faeces. In fact in the rat, biliary cannulation studies showed that <17% of an intravenous dose was excreted into the bile during the first 44 h. The major metabolites of ranitidine found in the urine of the mouse, rat and rabbit were ranitidine S-oxide, N-oxide and desmethyl metabolites, with furoic acid being present also. Parent compound was by far the largest component of radioactivity present in the urine. Thus in terms of sulfoxidation, this plays a minor role in ranitidine metabolism.

Nizatidine is also relatively insensitive to sulfoxidation in terms of its metabolism. In the rat, nizatidine was converted to the N₂-oxide. This was also seen in man following a single oral dose of ¹⁴C-nizatidine, with the unchanged drug accounting for ~65% of the urinary radioactivity (Knadler *et al.* 1986). The N₂-oxide accounted for ~5% of the dose while the sulfoxide could not be positively identified. The metabolism of famotidine in healthy humans revealed that renal excretion of ¹⁴C-famotidine varied from 17 to 30% of the dose but that this accounted for ~82–89% of the radioactivity present in the urine. Only a small amount was excreted as the sulfoxide metabolite (Hucker *et al.* 1984).

Table 4.3 Recent studies on S-carboxymethyl-L-cysteine metabolism

Technique	Major metabolites found	Investigators
Paper chromatography	SCMC sulfoxide SCMC	Mitchell <i>et al.</i> (1984 ^a)
Paper chromatography and TLC	SCMC sulfoxide SCMC	Kupfer and Idle (1990 ^b)
¹³ C-NMR	TDA	Meese <i>et al.</i> (1990 ^c)
GC-MS	TDA sulfoxide	
¹³ C-NMR	TDA	Hofmann <i>et al.</i> (1991 ^d)
GC-MS	TDA sulfoxide	
HPLC	TDA and SCMC	Brockmoller <i>et al.</i> (1991 ^e)
HPTLC	SCMC and TDA	Meese <i>et al.</i> (1991 ^f)
¹³ C-NMR	TDA sulfoxide CMTC	
HPTLC	SCMC CMTC	Gregory <i>et al.</i> (1993 ^g)

Key: SCMC, S-carboxymethyl-L-cysteine; SCMC sulfoxide, S-carboxymethyl-L-cysteine sulfoxide; TDA, thiodiglycolic acid; TDA sulfoxide, thiodiglycolic acid sulfoxide; CMTC, S-carboxymethylthio-L-cysteine; TLC, thin-layer chromatography; NMR, nuclear magnetic resonance; GC–MS, gas chromatography – mass spectroscopy; HPLC, high-pressure liquid chromatography; HPTLC, high-performance thin-layer chromatography.

Notes:

^aA 750 mg dose and a 0–8-h urine collection ($n = 200$).

^bA 750 mg dose and a 0–8- and 8–16-h urine collection ($n = 40$).

^cA 750 mg dose and a 0–8-h urine collection.

^dA 1.5 g dose and a 0–8- and 8–72-h urine collection ($n = 11$); 375 and 750 mg dose and a 0–8-, 8–16- and 16–24-h urine collection ($n = 14$).

^eA 1.125 g dose and a 0–8-h urine collection ($n = 33$).

^fA 750 mg dose and a 0–72-h urine collection ($n = 2$).

^gA 750 mg dose and a 0–24-h urine collection ($n = 120$).

The metabolism of the mucolytic agents S-methyl-L-cysteine and S-carboxymethyl-L-cysteine has also been studied extensively. In the early 1930s the metabolism and interconversions of sulfur-containing compounds were investigated. A large number of workers reported that S-methyl-L-cysteine could not replace L-cysteine in the diet of rats or chickens (Armstrong and Lewis 1951, Block and Jackson 1932, Grau and Almquist 1943). So that *in vivo* demethylation does not occur to any significant extent. A study in rats by Barnsley (1964), followed by one by Sklan and Barnsley (1968) in hamsters and guinea pigs, gave the following results: (i) 50% of the ³⁵S-methyl-L-cysteine appeared in urine as inorganic sulfate and (ii) N-acetylation, S-oxidation and deamination were major metabolic pathways for S-methyl-L-cysteine.

It is well documented that N-acetylation of S-substituted cysteines is a major pathway in their metabolism (Barnsley *et al.* 1969). A further investigation into the metabolism of S-methyl-L-cysteine in rats and guinea pigs (Waring and McCormick, 1988) showed that 48 h following the administration of ³⁵S-labelled

methyl-L-cysteine, 20% of the dose was excreted in the urine. The use of a ^{14}C -labelled compound resulted in 30% of the radioactivity being expired as carbon dioxide. Again the major urinary metabolite was inorganic sulfate, with varying amounts of sulfoxides and N-acetyl metabolites being present in the urine also. The work on S-methyl-L-cysteine metabolism is similar in certain respects to that of S-carboxymethyl-L-cysteine.

Brand *et al.* (1936) showed that S-carboxymethyl-L-cysteine was not converted to L-cysteine but that inorganic sulfate was a metabolite. This work was further developed in the study of Turnbull *et al.* (1978) in rats and in dogs. The main finding in this study was that the parent compound and thiodiglycollic acid were the main metabolites, but Waring (1978) reported that N-acetylation, S-oxidation and decarboxylation were the main metabolic routes in rats. This observation was further supported by an *in vivo* study in man (Waring 1980). The use of ^{35}S -carboxymethyl-L-cysteine (Waring and Mitchell 1982) again confirmed the finding of the original studies that the main metabolic routes were S-oxidation of the thioether sulfur, N-acetylation of the amino group, and decarboxylation of the side chain carboxyl group. The presence of labelled inorganic sulfate in the urine demonstrated that the thioether can be degraded to this metabolite. The metabolic production of sulfoxide metabolites of S-carboxymethyl-L-cysteine in man showed a deficiency that was reported by Mitchell *et al.* (1984) as sulfoxidation polymorphism, with 2% of the population producing no sulfoxide metabolites and 30–35% of the population producing only limited quantities. However, this polymorphism has been recently questioned on the grounds that the production of S-oxides of S-carboxymethyl-L-cysteine is negligible and insignificant (Table 4.3). The results show some disagreement between the actual metabolic products excreted in urine as well as in the methods of analysis. A more detailed investigation of S-carboxymethyl-L-cysteine metabolism in man is required to compare the various methods of analysis, time of urine collections, and dose of compound given to the subjects under investigation since the variability of these parameters in the various studies to date has been considerable.

4.4.1.3 Toxicity

The thioethers described in this section have very limited toxicity to man and experimental animals, however S-carboxymethyl-L-cysteine can cause gut irritation and skin rashes in certain individuals (Waring 1989).

4.5 References

- AGARWAL, R. P., MCPHERSON, R. A. & PHILLIPS, M., 1983, Rapid degradation of disulfiram by serum albumin, *Research Communications in Chemical Pathology and Pharmacology*, **42**, 293–310.
- AMMON, R., BERNINGER, H., HAAS, H. S. & LAUDSBERG, I., 1967, Thioacetamid-sulfoxid, ein Stoffwechselprodukt des Thioacetamids, *Arzneimittel Forschung*, **17**, 521–525.
- APPLEBY, A. P., FURTICK, W. R. & FANG, S. C., 1965, Soil placement studies with EPTC and other carbamate herbicides in *Avena sativa*, *Weed Research*, **3**, 259–264.
- ARMSTRONG, M. D. & LEWIS, J. D., 1951, Growth experiments with thioether derivatives of cysteine and homocysteine, *Journal of Biological Chemistry*, **189**, 461–466.

- ASTWOOD, E. B., 1943, The chemical nature of compounds which inhibit the function of the thyroid gland, *Journal of Pharmacology*, **78**, 79-89.
- ASTWOOD, E. B., BISSELL, A. & HUGHES, 1945, Further studies on the chemical nature of compounds which inhibit the function of the thyroid gland, *Endocrinology*, **37**, 456-481.
- AUBRY, M. L., COWELL, P., DAVEY, M. J. & SHEVDE, S., 1970, Aspects of the pharmacology of the new anthelmintic: pyrantel, *British Journal of Pharmacology*, **35**, 332-344.
- AYLWARD, M., MADDOCK, J. & DEWLAND, P., 1980, Clinical evaluation of acetylcysteine in the treatment of patients with chronic obstructive bronchitis, *European Journal of Respiratory Disease*, **111** (Suppl.), 81-89.
- VON BAHR, C., MOVIN, G., SCI, M. P., NORDIN, C., LIDEN, A., HAMMARLUND-UENAES, M., HEDBERG, A., SCI, B., RING, H. & SJOQVIST, F., 1991, Plasma levels of thioridazine and metabolites are influenced by the debrisoquin hydroxylation phenotype, *Clinical Pharmacology and Therapeutics*, **49**, 234-240.
- BARNESLEY, E. A., 1964, Metabolism of S-methyl-L-cysteine in the rat, *Biochimica et Biophysica Acta*, **90**, 24-36.
- BARNESLEY, E. A., ESKIN, N. A. M., JAMES, S. P. & WARING, R. H., 1969, The acetylation of S-alkylcysteines by the rat, *Biochemical Pharmacology*, **18**, 2393-2401.
- BELL, J. A., DALLAS, F. A. A., JENNER, W. N. & MARTIN, L. E., 1980, The metabolism of ranitidine in animals and man, *Biochemical Society Transactions*, **8**, 93.
- BENDEREV, T. V., 1988, Acetylcysteine for urinary tract mucolysis, *Journal of Urology*, **139**, 353-354.
- BIANCHI PORRO, G., LAZZARONI, M., PARENTE, F. & PETRILLO, M., 1987, Medical treatment of duodenal ulcer: present status, in Bianchi Porro, G. & Bardhan, K. D. (Eds), *Topics in Peptic Ulcer Disease*, New York: Raven Press.
- BLACK, J. W., DUCAN, W. A. M., DURANT, C. J., GANELLIN, C. R. & PARSONS, E. M., 1972, Definition and antagonism of histamine H₂-receptors, *Nature*, **236**, 385-390.
- BLOCK, R. J. & JACKSON, R. W., 1932, The metabolism of cystine and methionine, *Journal of Biological Chemistry*, **97** (CVI-CVII Proceedings).
- BLUMMER, J. L., NOVAK, R. F., LUCAS, S. V., SIMPSON, J. M. & WEBSTER, L. T. J., 1979, Aerobic metabolism of niridazole by the rat liver microsomes, *Molecular Pharmacology*, **16**, 1019-1030.
- BLUMMER, J. L., SIMPSON, J. M., LUCAS, S. V. & WEBSTER, L. T. J., 1980, Toxicogenetics of niridazole in mice, *Journal of Pharmacology and Experimental Therapeutics*, **212**, 509-513.
- BOUILLON, R., 1980, Thyroid and antithyroid drugs, in Dukes, M. N. G. (Ed.), *Meyler's Side Effects of Drugs*, 10th Edn, pp. 782-794, Amsterdam: Excerpta Medica.
- BOYD, M. R. & NEAL, R. A., 1976, Studies on the mechanism of toxicity and of development of tolerance to the pulmonary toxin, α -naphththiourea (ANTU), *Drug Metabolism and Disposition*, **4**, 314-322.
- BRAND, E., BLOCK, R. J., KASSELL, B. & CAHILL, G. F., 1936, Metabolism of S-carboxymethyl-L-cysteine, *Proceedings of the Society of Experimental Biology and Medicine*, **35**, 501-503.
- BREYER, U., 1971, Metabolism of the phenothiazine drug perazine by liver and lung microsomes from various species, *Biochemical Pharmacology*, **20**, 3341-3351.
- BREYER-PFAFF, U., WIEST, E., PROX, A., WACHSMUTH, H., PROTIVA, M., SINDELAR, K., FRIEBOLIN, H., KRAUSS, D. & KUNZELMAN, P., 1985, Phenolic metabolites of chlorprothixene in man and dog, *Drug Metabolism and Disposition*, **13**, 479-489.
- BRITTAIN, R. T., 1982, Discovery and evolution of ranitidine, in Riley, A. J. & Salmon, P. R. (Eds), *Ranitidine: Proceedings of an International Symposium held in the Context*

of the Seventh World Congress of Gastroenterology, pp. 5–15, Amsterdam: Excerpta Medica.

- BROCKMOLLER, J., STAFFELDT, B., & ROOTS, I., 1991, Evaluation of proposed sulphoxidation pathways of carbocysteine in man by HPLC quantification, *European Journal of Clinical Pharmacology*, **40**, 387–392.
- BROGDEN, R. N., CARMINE, A. A., HEEL, R. C., SPIEGHT, T. M. & AVERY, G. C., 1982, Rantidine: a review of its pharmacology and therapeutic use in peptic ulcer disease and other allied diseases, *Drugs*, **24**, 267–303.
- BUCHER, K. H., 1983, *Chemistry of Pesticides*, pp. 273–282, New York: John Wiley.
- BUEDING, E. & FISHER, J., 1970, Biochemical effects of niridazole on *Schistosoma mansoni*, *Journal of Molecular Pharmacology*, **6**, 532–539.
- CASHMAN, J. R., PARIKH, K. K., TRAIGER, G. J. & HANZLIK, R. P., 1983, Relative hepatotoxicity of ortho and meta monosubstituted thiobenzamides in the rat, *Chemico-Biological Interactions*, **45**, 341–347.
- CASIDA, J. E., 1983, Propesticides: bioactivation in pesticide design and toxicological evaluation, in Miyamoto, J. and Kearney, P. C. (Eds), *IUPAC Pesticide Chemistry: Human Welfare and Environment*, pp. 239–246, New York: Pergamon.
- CASIDA, J. E., GRAY, R. A. & TILLES, H., 1974, Thiocarbamate sulphoxides: potent, selective and biodegradable herbicides, *Science*, **184**, 575–574.
- CATTO, B. A., VALENCIA, C. I., HAFEZ, K., FAIRCHILD, E. H. & WEBSTER, L. T. T., 1984, 4-Ketoniridazole: a major niridazole metabolite with central nervous system toxicity different than niridazole, *Journal of Pharmacology and Experimental Therapeutics*, **228**, 662–668.
- CHEN, Y. S. & CASIDA, J. E., 1978, Thiocarbamate herbicide metabolism: microsomal oxygenase metabolism of EPTC involving mono- and dioxygenation at the sulfur and hydroxylation at each alkyl carbon, *Journal of Agricultural and Food Chemistry*, **26**, 263–267.
- CHIELI, E., MALVALDI, G. & TONGGIANI, R., 1979, Early biochemical liver changes following thiobenzamide poisoning, *Toxicology*, **13**, 101–109.
- DAMANI, L. A., 1989, Thioethers, thiols, dithioic acids and disulphides: phase I reactions, in Damani, L. A. (Ed.), *Sulphur-Containing Drugs and Related Organic Compounds*, Vol. 1A, pp. 21–25, Chichester: Ellis Horwood.
- DANIELS, J. C., WARREN, K. S. & DAVID, J. R., 1975, Studies on the mechanism of suppression of delayed hypersensitivity by the antischistosomal compound niridazole, *Journal of Immunology*, **115**, 1414.
- DE BRABANDER, M., AERTS, F., GEUENS, G., VAN GINCKEL, R., VAN DE VEIRE, R. & VAN BELLE, H., 1978, DL-2-Oxo-3(mercaptoethyl)-5-phenylimidazolidine. A sulphhydryl metabolite of levamisole that interacts with microtubules, *Chemico-Biological Interactions*, **23**, 45–63.
- DE BRABANDER, M., VAN BELLE, H., AERTS, F., VAN DE VEIRE, R. & GEUENS, G., 1979, Protective effect of levamisole and its sulphhydryl metabolite OMPI against cell death induced by glutathione depletion, *International Journal of Immunopharmacology*, **1**, 93–100.
- DEBUS, H., 1850, Über die Verbindungen der sulfocarbaminsäure, *Annals of Chemistry (Leibigs)*, **73**, 26–30.
- DE LORENZO, F., STAIANO, N., SILENGO, L. & CORTESE, R., 1978, Mutagenicity of diallate, sulfallate and triallate and relationship between structure and mutagenic effects of carbamates used widely in agriculture, *Cancer Research*, **38**, 13–15.
- DISTLERATH, L., LOPER, J. C. & TABOR, M. W., 1985, Metabolic activation of 3-(2-chloroethoxy)-1,2-dichloropropene: a mutagen structurally related to diallate, triallate, and sulfallate, *Environmental Mutagenicity*, **7**, 303–312.
- DRASATA, J., LOSKOT, J. & WAISSER, K., 1981, Biological side effects of potential antitubercular agents, *Ceskoslovenska Farmacie*, **30**, 266–269.

- DRYOFF, M. C. & NEAL, R. A., 1981, Identification of the major protein adduct formed in the rat after thioacetamide administration, *Cancer Research*, **41**, 3430–3435.
- DURANT, G. J., EMMETT, J. C., GANELLIN, C. R., MILES, P. D., PARSONS, M. E., PRAIN, H. D. & WHITE, G. R., 1977, Cyanoguanidine–thiourea equivalence in the development of the histamine H₂-receptor antagonist, cimetidine, *Journal of Medicinal Chemistry*, **20**, 901–906.
- EDMAN, P., 1950, Method for determination of the amino acid sequence in peptides, *Acta Chemica Scandinavica*, **4**, 283–289.
- EL DAREER, S. M., KALIN, J. R., TILLERY, K. F. & HILL, D. L., 1984, Disposition of 2-mercaptobenzimidazole in rats dosed orally or intravenously, *Journal of Toxicology and Environmental Health*, **14**, 595–604.
- FAIGLE, J. W. & KEBERLE, H., 1969, Metabolism of niridazole in various species including man, *Annals of the New York Academy of Sciences*, **160**, 544–557.
- FAULKNER, J. K., FIDGOR, S. K., MONRO, A. M., SCHACH VON WITTENAU, M., STOPHER, P. A. & WOOD, B. A., 1972, The comparative metabolism of pyrantel in five species, *Journal of Science, Food and Agriculture*, **23**, 79–91.
- DE FERREYRA, E. C., DE FENOS, O. M., BERNACCHI, A. S., DE CASTRO, C. R. & CASTRO, J. A., 1979, Therapeutic effectiveness of cystamine and cysteine to reduce liver cell necrosis induced by several hepatotoxins, *Toxicology and Applied Pharmacology*, **48**, 221–229.
- FIDGOR, S. K., SCHACH VON WITTENAU, M. & LYNCH, M. J., 1978, Banminth and a common fragment assay, *Journal of the Association of the Official Analytical Chemist*, **61**, 1228–1231.
- FISHER, S. W. & METCALF, R. L., 1983, Production of delayed ataxia by carbamate acid esters, *Pesticide Biochemistry and Physiology*, **19**, 243–253.
- FOYE, W. O. & LO, J., 1972, Metal-binding abilities of antibacterial heterocyclic thiones, *Journal of Pharmaceutical Science*, **61**, 1209–1212.
- FRANKEN, N. A. P., VAN LANGEVELDE, A., PAUWELS, E. K. J., JOURNEE-DE KORVER, J. G., BAKKER, C. N. M. & OOSTERHUIS, J. A., 1986, Radioiodine-labelled-5-iodo-2-thiouracil: a potential radiopharmaceutical for establishing the viability of ocular melanoma after radiation therapy, *Nuclear Medicine Communications*, **7**, 797–809.
- FRIESEN, H. A., BANTING, J. D. & WALKER, D. R., 1962, The effect of placement and concentration of 2,3-DCDT on the selective control of wild oats in wheat, *Canadian Journal of Plant Sciences*, **42**, 91–98.
- GALTIER, P., COCHE, Y. & ALVINERIE, M., 1983, Tissue distribution and elimination of (³H)levamisole in the rat after oral and intramuscular administration, *Xenobiotica*, **7**, 407–413.
- GANELLIN, C. R., 1985, Discovery of cimetidine, in Roberts, S. M. & Price, B. J. (Eds), *Medicinal Chemistry: the Role of Organic Chemistry in Drug Research*, pp. 93–118, London: Academic Press.
- GANELLIN, C. R. & DURANT, G. J., 1981, Histamine H₂-receptor agonists and antagonists, in Wolf, M. E. (Ed.), *Burger's Medicinal Chemistry*, Part III, 4th Edn, pp. 487–551, New York: Wiley-Liss.
- GESSNER, T. & JAKUBOWSKI, M., 1972, Diethyldithiocarbamic and methyl ester a metabolite of Disulfiram, *Biochemical Pharmacology*, **21**, 219–230.
- GORROD, J. W., LAZARUS, C. R. & BECKETT, A. H., 1974, Some aspects of the *in vitro* oxidation of chlorpromazine, *Advances in Biochemistry and Pharmacology*, **9**, 191–200.
- GRAHAM, S. L., HANSEN, W. H., DAVIES, K. J. & PERRY, C. H., 1973, Effects of one-year administration of ethylenethiourea upon the thyroid of the rat, *Journal of Agriculture, Food and Chemistry*, **21**, 324–329.

- GRAU, C. R. & ALMQUIST, H. R., 1943, The utilisation of the sulphur amino acids by the chick, *Journal of Nutrition*, **26**, 631–640.
- GRAZIANI, G. & DE MARTIN, G. L., 1977, Pharmacokinetic studies of levamisole: on the pharmacokinetics and relative bioavailability of levamisole in man, *Drugs, Experimental and Clinical Research*, **2**, 235–240.
- GREGORY, W. L., JAMES, O. F. W., TURNER, I., MEESE, C. O. & IDLE, J. R., 1993, Re-evaluation of the metabolism of carbocysteine in a British white population, *Pharmacogenetics*, **3**, 270–274.
- GRIFFITHS, M. H., MOSS, J. A., ROSE, J. A. & HATHAWAY, D. E., 1966, The comparative metabolism of Prefix in the dog and the rat, *Biochemical Journal*, **98**, 770–781.
- GUPTA, D. K., MITAL, O. P. & AGARWALA, M. C., 1975, Hepatic injury due to ethionamide (1314 Th) and porthionamide (1312 Th), *Journal of the Association of Physicians of India*, **23**, 147–151.
- HALD, J. & JACOBSEN, E., 1948, A drug sensitising the organism to ethyl alcohol, *The Lancet*, Dec **25**, 1001–1004.
- HANSEN, L. G., FRANCIS, B., METCALF, R. L., REINDERS, J. H., 1985, Neurotoxicity of diallate and triallate when administered orally or topically to hens, *Environmental Science and Health*, Part B, **20**, 97–111.
- HANZLIK, R. P., YYAS, K. P. & TRAIGER, G. J., 1978, Substituent effects on the hepatotoxicity of thiobenzamide derivatives in the rat, *Toxicology and Applied Pharmacology*, **46**, 685–694.
- HANZLIK, R. P., CASHMAN, J. R. & TRAIGER, G. J., 1980, Relative hepatotoxicity of substituted thiobenzamide and thiobenzamide S-oxides in the rat, *Toxicology and Applied Pharmacology*, **55**, 685–694.
- HASSALL, K., 1982, *The Chemistry of Pesticides*, pp. 318–323, Deerfield Beach, FL: Verlag Chemie.
- HOBBS, D. C., 1968, Metabolism of thiothixene, *Journal of Pharmaceutical Science*, **57**, 105–111.
- HOFMANN, U., EICHELBAUM, M., SEEFRIED, S. & MEESE, C. O., 1991, Identification of thiodiglycolic acid, thiodiglycolic acid sulfoxide, and (3-carboxymethylthio)lactic acid as major human biotransformation products of S-carboxymethyl-L-cysteine, *Drug Metabolism and Disposition*, **19**, 222–226.
- HUBBELL, J. P. & CASIDA, J. E., 1977, Metabolic fate of the N,N-dialkylcarbamoyl moiety of thiocarbamate herbicides in rats and corn, *Journal of Agricultural and Food Chemistry*, **25**, 404–413.
- HUCKER, H. B., HUTT, J. E., CHREMOS, A. N. & ROTMENSCH, H., 1984, Disposition and metabolism of famotidine, a potent H₂-receptor blocker, *Federation Proceedings*, **43**, 655.
- IKEDA, M., UNIA, T., TOMIZAWA, C., 1986, Metabolism of herbicide orbencard in soybean, wheat, corn and crabgrass seedlings, *Weed Research (Japan)*, **31**, 238–243.
- INNES, J. R. M., ULLAND, B. M., VALERIO, M. G., PETRUCCELLI, L., FISHBEIN, L., HART, E. R., PALLOTTA, A. J., BATES, R. R., FALK, H. L., GART, J. J., KLEIN, M., MITCHELL, I. & PETERS, J., 1969, Bioassay of pesticides and industrial chemicals for tumorigenicity in mice; a preliminary note, *Journal of the National Cancer Institute*, **42**, 1101–1114.
- INOUE, B., YOSHIMURA, N. & WACHI, T., 1973, Experimental studies on the mechanism of fatty liver formation induced by ethionamide. V. Liver and serum total cholesterol in ethionamide administered rats, *Kekkaku*, **48**, 71–74.
- JANSSEN, P. A. J., 1976, The levamisole story, *Progress in Drug Research*, **20**, 347–383.
- JOHNSTON, J. P., KANE, P. O. & KIBBY, M. R., 1967, The metabolism of ethionamide and its sulfoxide, *Journal of Pharmacy and Pharmacology*, **19**, 1–9.

- JORGENSEN, A., HANSEN, V., LARSEN, U. D. & KHAN, A. R., 1969, Metabolism, distribution and excretion of flupenthixol, *Acta Pharmacologica Toxicologica*, **27**, 301–313.
- KHERA, K. S., 1973, Ethylenethiourea: teratogenicity study in rats and rabbits, *Teratology*, **7**, 243–252.
- KHERA, K. S. & INVERSON, F., 1978, Toxicity of ethylenethiourea in pregnant cats, *Teratology*, **18**, 311–314.
- 1981, Effects of pretreatment with SKF-525A, N-methyl-2-thioimidazole, sodium phenobarbital, or methyl cholanthrene on ethylenethiourea-induced teratogenicity in rats, *Teratology*, **24**, 131–137.
- KIMURA, Y. & KUME, M., 1971, Absorption, distribution, excretion and metabolism of pyrantel pamoate, *Oyo Yakuri*, **5**, 347–358.
- KNADLER, M. P., BERGSTROM, R. F., CALLAGHAN, J. T. & RUBIN, A., 1986, Nizatidine, an H₂-blocker. Its metabolism and disposition in man, *Drug Metabolism and Disposition*, **143**, 175–182.
- KOUASSI, E., CAILLE, G., LERY, L., LARIVIERE, L. & VEZINA, M., 1986, Noval assay and pharmacokinetics of levamisole and *p*-hydroxylevamisole in human plasma and urine, *Biopharmacy and Drug Disposition*, **7**, 71–89.
- KUPFER, A. & IDLE, J. R., 1990, False positive with current carbocysteine protocol for sulphoxidation phenotyping, *Lancet*, **335**, 1107.
- LAMOUREUX, G. L. & FREAR, D. S., 1979, in Paulson, G. D., Frear, D. S. & Marks, E. P., (Eds), *Xenobiotic Metabolism: In Vitro Methods*, Vol. 97, ACS Symposium Series, p. 102, Washington DC: American Chemical Society.
- LAMOUREUX, G. L. & RUSNESS, D. G., 1981, Catabolism of GSH conjugates of pesticides in higher plants, in Rosen, J. D., Magee, P. S. & Casida, J. E. (Eds), *Sulfur in Pesticide Action and Metabolism*, ACS Symposium Series, Vol. 158, pp. 133–164. Washington, DC: American Chemical Society.
- LANSDOWN, F. S., BERAN, M. & LITWAK, T., 1967, Psychotoxic reaction during ethionamide therapy, *American Review of Respiratory Diseases*, **95**, 1053–1055.
- LATTA, H., 1947, Pulmonary edema and pleural effusion produced by acute α -naphthylthiourea poisoning in rats and dogs, *Bulletin of Johns Hopkins Hospital*, **80**, 181–197.
- LAY, M. M. & CASIDA, J. E., 1976, Dichloroacetamide antidotes enhance thiocarbamate sulphoxide detoxification by elevating corn root GSH content and GSH S-transferase activity, *Pesticide Biochemistry and Physiology*, **6**, 442–456.
- LAY, M. M., HUBBELL, J. P. & CASIDA, J. E., 1975, Dichloroacetamide antidotes for thiocarbamate herbicides: mode of action, *Science*, **189**, 287–288.
- LEE, P. W., ARNAU, T. & NEAL, R. A., 1980, Metabolism of α -naphthiourea by rat liver and rat lung microsomes, *Toxicology and Applied Pharmacology*, **53**, 164–173.
- LINDSAY, R. H., TILLERY, C. R. & YU, M. W., 1972, Conversion of the antithyroid drug 2-thiouracil to 2-thio-5'-UMP by UMP pyrophosphorylase, *Archives of Biochemistry and Biophysics*, **148**, 466–474.
- LINDSAY, R. H., HILL, J. B., KELLY, K. & VAUGHN, A., 1974a, Excretion of propylthiouracil and its metabolites in rat bile and urine, *Endocrinology*, **94**, 1689–1698.
- LINDSAY, R. H., ABOUL-ENEIN, H. Y., MOREL, D. & BROWN, S., 1974b, Synthesis and antiperoxidase activity of 6-*n*-propylthiouracil derivatives and metabolites, *Journal of Pharmaceutical Sciences*, **63**, 1383–1386.
- LINDSAY, R. H., HULSEY, B. S. & ABOUL-ENEIN, H. Y., 1975, Enzymatic S-methylation of 6-*n*-propyl-2-thiouracil and other antithyroid drugs, *Biochemical Pharmacology*, **24**, 463–468.
- LINDSAY, R. H., CASH, A. G., VAUGHAN, A. W. & HILL, J. B., 1977, Glucuronide conjugation of 6-*n*-propyl-2-thiouracil and other antithyroid drugs by guinea pig liver

- microsomes in vitro, *Biochemical Pharmacology*, **26**, 617–623.
- LOWRY, R. H. & RICHARDSON, K. S., 1976, Some fundamentals of physical organic chemistry, in *Mechanism and Theory in Organic Chemistry*, pp. 60–71, New York: Harper & Row.
- MACKENZIE, C. G. & MACKENZIE, J. B., 1943, Effects of sulphonamides and thioureas on the thyroid gland and basal metabolism, *Endocrinology*, **32**, 185–209.
- MAHMOUD, A. A. F., MANDELM, A., WARREN, K. S. & WEBSTER, L. T. J., 1975, Niridazole, a potent long-acting suppressant of cellular hypersensitivity, *Journal of Immunology*, **114**, 279–283.
- MALHI, J. S., GARD, P. R., HANLON, G. W. & MARRIOTT, C., 1987, The effects of bromhexine and S-carboxymethyl-L-cysteine on guinea-pig uterine microflora, *Journal of Pharmacy and Pharmacology*, **39**, 1025–1028.
- MANN, J. D., JORDAN, L. S. & DAY, B. E., 1965, A survey of herbicides for their effect upon protein synthesis, *Plant Physiology*, **40**, 840–844.
- MARCHANT, B., ALLEXANDER, W. D., LAZARUS, J. H., LESS, J. & CLARK, D. H., 1972, The accumulation of ³⁵S-antithyroid drugs by the thyroid gland, *Journal of Endocrinology and Metabolism*, **34**, 847–851.
- MARCHANT, B., LEES, J. F. H. & ALEXANDER, W. D., 1978, Antithyroid drugs, *Pharmaceutical Therapeutics B*, **3**, 305–348.
- MARTIN, L. E., BELL, J. A., CAREY, P. F., DALLAS, F. A. A., DIXON, G. T. & JENNER, W. N., 1982, A review of pharmacokinetics and metabolism of ranitidine in animals and man, in *Clinical Use of Ranitidine*, pp. 23–31, Oxford: Medicine Publishing Foundation.
- MCCANN, J., CHOI, E., YAMASKI, E. & AMES, B. N., 1975, Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals, *Proceedings of the National Academy of Sciences, USA*, **72**, 5135–5139.
- MCGINTY, D. A., SHARP, E. A., DILL, W. A., RAWSON, B. S. & RAWSON, R. W., 1948, Excretion studies on thiouracil and its 6-benzyl, 6-*n*-propyl and 6-methyl derivatives in man, *Journal of Clinical Endocrinology*, **8**, 1043–1050.
- MEESE, C. O., HOFMANN, U. & EICHELBAUM, M., 1990, Polymorphic sulfoxidation of carbocysteine, *Lancet*, **335**, 1107.
- MEESE, C. O., FISCHER, C., KUPFER, A., WISSER, H. & EICHELBAUM, M., 1991, Identification of the “major” polymorphic carbocysteine metabolite as S-(carboxymethylthio)-L-cysteine, *Biochemical Pharmacology*, **42**, R13–R16.
- MEYER, J. W., WOGGON, B., BAUMANN, P. & MEYER, U. A., 1990, Clinical implications of slow sulfoxidation of thioridazine in poor metabolizer of the debrisoquine type, *European Journal of Clinical Pharmacology*, **39**, 613–614.
- MEYRICH, B., MILLER, J. & REID, L., 1972, Pulmonary edema induced by ANTU or by high or low oxygen-concentrations in rat — an electron microscopic study, *British Journal of Experimental Pathology*, **53**, 347–358.
- MILLER, T. A., 1966, Anthelmintic activity of thenium *p*-chlorobenzenesulfonate against various stages of *Ancylostoma caninum* in young dogs, *American Journal of Veterinary Research*, **101**, 205–208.
- MITCHARD, M., MCISAAC, R. L. & BELL, J. A., 1989, H₂-Receptor antagonists, in Damani, L. A. (Ed.), *Sulphur-Containing Drugs and Related Organic Compounds*, Vol. 3A, pp. 64–69, Chichester: Ellis Horwood.
- MITCHELL, S. C., 1989, Phenothiazine antipsychotics, in Damani, L. A. (Ed.), *Sulphur Containing Drugs and Related Organic Compounds*, Vol. 3A, pp. 19–52, Chichester: Ellis Horwood.
- MITCHELL, S. C. & NICKSON, R. M., 1993, Metabolism of sulfur-containing xenobiotics, *Sulfur Reports*, **13**, 161–195.
- MITCHELL, S. C. & STEVENTON, G. B., 1994, Thiourea and its biological interactions, *Sulfur Reports*, **16**, 117–137.

- MITCHELL, S. C., IDLE, J. R. & SMITH, R. L., 1982, The metabolism of [^{14}C]cimetidine in man, *Xenobiotica*, **12**, 283–292.
- MITCHELL, S. C., WARING, R. H., HALEY, C. S., IDLE, J. R. & SMITH, R. L., 1984, Genetic aspects of the polymodally distributed sulphoxidation of S-carboxymethyl-L-cysteine in man, *British Journal of Clinical Pharmacology*, **18**, 507–521.
- MOHTASHAMIPUR, E. & NORPOTH, K., 1986, Excretion of mutagens in sweat and faeces of man, and in serum, gastric juice and urine of rats after dosing of niridazole of metronidazole, *Mutagenesis*, **5**, 371–374.
- MORGAN, D. J., BLACKMAN, G. L., PAULL, J. D. & WOLF, L. J., 1981, Pharmacokinetics and plasma binding of thiopental II. Studies at cesarean section, *Anesthesiology*, **54**, 474–480.
- MOULDING, T. & FRASER, R., 1970, Hypothyroidism related to ethionamide, *American Review of Respiratory Diseases*, **101**, 90–94.
- NARANG, R. K., 1972, Acute psychotic reaction probably caused by ethionamide, *Tubercle*, **53**, 137–138.
- NEAL, R. A., 1980, Microsomal metabolism of thiono-sulfur compounds: mechanisms and toxicological significance, in Hodgson, E., Bend, R. R. & Philpot, R. M. (Eds), *Reviews in Biochemical Toxicology*, Vol. 2, pp. 131–171, New York: Elsevier.
- NEAL, R. A. & HALPERT, J., 1982, Toxicology of thiono-sulphur compounds, *Annual Reviews of Pharmacology and Toxicology*, **22**, 321–339.
- NEAL, R. A., KAMATAKI, T., HUNTER, A. L. & CATIGNANI, G., 1977, Monooxygenase catalysed activation of thiono-sulphur containing compounds to reactive intermediates, in Ullrich, V., Roots, I., Hildebrandt, A. & Estabrook, R. W. (Eds), *Microsomes and Drug Oxidations*, pp. 467–475, New York: Pergamon.
- O'BRIEN, J. J., 1970, Toxicological aspects of some modern anthelmintics, *Australian Veterinary Journal*, **46**, 297–300.
- OWEN, W. J., 1987, Herbicide detoxification and selectivity, in *British Crop Protection Conference: Weeds*, Vol. 1, p. 312, Brighton: BCPC.
- PAPADOPOULOS, A. S. & CRAMMER, J. L., 1986, Sulphoxide metabolites of thioridazine in man, *Xenobiotica*, **16**, 1097–1107.
- PASQUINELLI, P., BRUSCHI, F., SAVIOZZI, M. & MALVADI, G., 1985, Immunosuppressive effect and promotor activity for hepatic carcinogenesis of thiobenzamide, *Bollettino della Societa Italiana di Biologia Sperimentale*, **61**, 61–66.
- PATTYN, S. R., JANSSENS, L., BOURLAND, J., SAYLAN, T., DAVIES, E. M., GRILLONE, S. & FERACCI, C., 1984, Hepatotoxicity of the combination of rifampicin–ethionamide in the treatment of multibacillary leprosy, *International Journal of Leprosy and Other Mycobacterial Diseases*, **52**, 1–6.
- PICKARD, R. G., 1972, Treatment of peritonitis with per- and postoperative irrigation of the peritoneal cavity with noxythiolin solution, *British Journal of Surgery*, **59**, 642–648.
- PORTER, W. R. & NEAL, R. A., 1978, Metabolism of thioacetamide and thioacetamide S-oxide by rat liver microsomes, *Drug Metabolism and Disposition*, **6**, 379–388.
- POULSEN, L. L., HYSLOP, R. M. & ZIEGLER, D. M., 1974, S-oxidation of thiouracil catalysed by a microsomal flavoprotein mixed-function oxidase, *Biochemical Pharmacology*, **23**, 3431–3440.
- 1979, S-oxygenation of N-substituted thioureas catalysed by the pig liver microsomal FAD-containing monooxygenase, *Archives of Biochemistry and Biophysics*, **198**, 78–88.
- PREMA, K. & GOPINATHAN, K. P., 1976, Distribution, induction and purification of monooxygenase catalyzing sulfoxidation of drugs, *Biochemical Journal*, **25**, 1299–1307.
- PRICHARD, R. K., 1970, Mode of action of the anthelmintic thiabendazole in *Haemonchus contortus*, *Nature*, **228**, 684–685.

- RAJAPPA, S., 1984, Thiophenes and their benzo derivatives: (ii) reactivity, in Bird, C. W. & Cheeseman, G. W. H. (Eds), *Comprehensive Heterocyclic Chemistry*, Vol. 4, pp. 741–861, Oxford: Pergamon.
- RANCE, D. J., 1989, Sulphur heterocycles, in, DAMANI, L. A. (Ed.), *Sulphur-Containing Drugs and Related Organic Compounds*, Vol. 1B, pp. 217–268, Chichester: Ellis Horwood.
- REES, K. R., ROWLAND, G. F. & VARCOE, J. S., 1966, The metabolism of tritiated thioacetamide in the rat, *International Journal of Cancer*, **1**, 197–203.
- RICHTER, C. P., 1945, The development of and use of α -naphthiourea (ANTU) as a rat poison, *Journal of the American Medical Association*, **129**, 927–931.
- RICHTER, C. P. & CLISBY, K. H., 1942, Toxic effects of the bitter-tasting phenylthiocarbamide, *Archives of Pathology*, **33**, 46–57.
- SARCIONE, E. J. & SOKAL, J. E., 1958, Detoxification of thiouracil by S-methylation, *Journal of Biological Chemistry*, **231**, 605–608.
- SAVOLAINEN, K. & PYYSAALO, H., 1979, Identification of the main metabolite of ethylenethiourea in mice, *Journal of Agricultural and Food Chemistry*, **27**, 605–608.
- SCHELINE, R. R., SMITH, R. L. & WILLIAM, R. T., 1961, The metabolism of arylthioureas II. The metabolism of 14C- and 35S-labelled 1-phenyl-2-thiourea and its derivatives, *Journal of Medical Pharmaceutical Chemistry*, **4**, 109–135.
- SCHUPAN, I. & CASIDA, J. E., 1983, Metabolism and degradation of pesticides and xenobiotics: bioactivations involving sulfur containing substituents, in Miyamoto, J. (Ed.), *IUPAC Pesticide Chemistry: Human Welfare and Environment*, pp. 287–294, New York: Pergamon.
- SCHUPAN, I., ROSEN, J. D. & CASIDA, J. E. 1979, Novel activation mechanism for the promutagenic herbicide diallate, *Science*, **205**, 1013–1015.
- SEARLE, C. E., LAWSON, A. & MORELY, H. V., 1951, Antithyroid substances. 2. Some mercaptoglyoxalines, mercaptothiazoles and thiohydantoins, *Biochemical Journal*, **49**, 125–128.
- SHEPARD, C. C., JENNER, P. J., ELLARD, G. A. & LANCASTER, R. D., 1985, An experimental study of the antileprosy activity of a series of thioamides in the mouse, *International Journal of Leprosy*, **53**, 587–594.
- SIKKA, H. & FLOCZYK, P., 1978, Mutagenic activity of thiocarbamate herbicides in *Salmonella typhimurium*, *Journal of Agricultural and Food Chemistry*, **26**, 146–148.
- SITAR, D. S. & THORNHILL, D. P., 1972, Propylthiouracil: absorption, metabolism and excretion in the albino rat, *Journal of Pharmacology and Experimental Therapeutics*, **183**, 440–448.
- 1973, Methimazole: absorption, metabolism and excretion in the albino rat, *Journal of Pharmacology and Experimental Therapeutics*, **184**, 432–439.
- SKLAN, N. M. & BARNSLEY, E. A., 1968, The metabolism of S-methyl-L-cysteine, *Biochemical Journal*, **107**, 217–223.
- SMITH, R. L. & WILLIAMS, R. T., 1961, The metabolism of arylthioureas III. (a) The toxicity of hydrogen sulphide in relation to that of phenylthiourea. (b) The protection of rats against the toxic effects of phenylthiourea with 1-methyl-2-phenylthiourea, *Journal of Medicinal and Pharmacological Chemistry*, **4**, 137–146.
- SOLOMON, D. H., 1986, Treatment of Graves' hyperthyroidism, in Ingbar, S. H. & Braverman, L. E. (Eds), *Werner's The Thyroid: A Fundamental and Clinical Text*, 5th Edn, pp. 987–1014, Philadelphia: Lippincott.
- STANOVNIK, B. & TISLER, M., 1964a, Dissociation constants and structure of ergothioneine, *Analytical Biochemistry*, **9**, 68–74.
- 1964b, Contribution to the structure of heterocyclic compounds with thioamide groups, *Arzneimittel-Forschung*, **14**, 1004–1012.
- STILL, G. G., DAVIES, D. G., ZANDER, G. L., 1970, Plant epicuticular lipids: alterations by herbicidal carbamates, *Plant Physiology*, **46**, 307–311.

- STONE, O. J., MULLINS, J. F. & WILLIS, C. J., 1965, Comparison of thiabendazole and 5-hydroxythiabendazole, *Journal of Investigative Dermatology*, **45**, 132–133.
- STOWELL, E. C., 1961, Ergothioneine, in Kharasch, N. (Ed.), *Organic Sulfur Compounds*, Vol. I, pp. 462–490, New York: Pergamon.
- STROMINGER, D. M. & FRIEDKIN, M., 1954, Enzymatic synthesis of thiouracil ribosides and thiouracil desoxyriboside, *Journal of Biological Chemistry*, **208**, 663–668.
- TAUROG, A., 1986, Hormone synthesis: thyroid iodine metabolism, in Ingbar, S. H. & Braverman, L. E. (Eds), *Werner's The Thyroid: A Fundamental and Clinical Text*, 5th Edn, pp. 53–97, Philadelphia: Lippincott.
- TAYLOR, D. C., CRESSWELL, P. R. & BARTLETT, D. C., 1978, The metabolism and elimination of cimetidine, a histamine H_2 -receptor antagonist, in the rat, dog and man, *Drug Metabolism and Disposition*, **6**, 21–30.
- TEITZ, E. L., ROTH, T., ZORICK, F. J., PICCIONI, P. & KRAMER, M., 1981, The acute effects of quazepam in the sleep of chronic insomniacs, *Arzneimittel Forschung*, **31**, 1963–1966.
- THORN, G. D. & LUDWIG, R. A., 1962, *The Dithiocarbamates and Related Compounds*, Amsterdam: Elsevier.
- TOCCO, D. J., ROSENBLUM, C., MARTIN, C. M. & ROBINSON, H. J., 1966, Absorption, metabolism and excretion of thiabendazole in man and laboratory animals, *Toxicology and Applied Pharmacology*, **9**, 31–39.
- TRACY, J. W. & WEBSTER, L. T. J., 1981, The formation of 1-thiocarbamoyl-2-imidazolidine from niridazole in mouse intestine, *Journal of Pharmacology and Experimental Therapeutics*, **217**, 363–368.
- TRAFICANTE, L. J., SIEKIERSKI, J., SAKALIS, G. & GERSHON, S., 1979, Sulphoxidation of chlorpromazine and thioridazine by bovine liver: preferential metabolic pathways, *Biochemical Pharmacology*, **28**, 621–626.
- TURNBULL, L. B., TENG, L., KINZIE, J. M., PITTS, J. E., PINCHBEC, F. M. & BRUCE, R. B., 1978, Excretion and biotransformation of carboxymethylcysteine in rat, dog, monkey and man, *Xenobiotica*, **8**, 621–628.
- ULLAND, B. M., WEISBUGER, J. H., WEISBURGER, E. K., RICE, J. M. & CYPHER, R., 1972, Thyroid cancer in rats from ethylenethiourea intake, *Journal of the National Cancer Institute*, **49**, 583–584.
- UNAI, T. & TOMIZAWA, C., 1986, Metabolic fate of fenothiocarb sulfoxide in soils, *Journal of Pesticide Science*, **11**, 357–361.
- UNAI, T., TAMARU, M. & TOMIZAWA, C., 1986a, Translocation and metabolism of the acaricide fenothiocarb in citrus, *Journal of Pesticide Science*, **11**, 347–356.
- UNAI, T., IKEDA, M. & TOMIZAWA, C., 1986b, Metabolic fate of the chlorobenzyl moiety of orbencarb sulfoxide and benthocarb sulfoxide in soybean seedlings, *Weed Research, Japan*, **31**(3), 228–237.
- URMAN, H. K., BULAY, O., CLAYSON, D. B. & SHUBIK, P., 1975, Carcinogenic effects of niridazole, *Cancer Letters*, **1**, 69–74.
- VALENCIA, C. I., CATTO, B. A., FAIRCHILD, E. H., WILSON, S. B., MARAMBA, N. C. & WEBSTER, L. T. J., 1984, Concentration–time course of niridazole and six metabolites in the serum of four Filipinos with *Schistosoma japonicum*, *Journal of Pharmacology and Experimental Therapeutics*, **230**, 133–140.
- VONK, J. W., 1983, Metabolism of fungicides in plants, in Hutson, D. H. & Roberts, T. R. (Eds), *Progress in Pesticide Biochemistry and Toxicology*, Vol. 3, pp. 111–162, Chichester: John Wiley.
- WAISSER, K., CELADNIK, M., PALAT, K. & ODLEROVA, Z., 1982, Antituberculotics. The effect of test microbiological media on the evaluation of structure–antituberculous activity relationships of thiobenzamide, *Ceskoslovenska Farmacie*, **31**, 303–307.
- WARING, R. H., 1978, The metabolism of S-carboxymethyl-L-cysteine in rodents, marmosets and humans, *Xenobiotica*, **8**, 265–270.

- 1980, Human metabolism of S-carboxymethyl-L-cysteine, *European Journal of Drug Metabolism and Pharmacokinetics*, **5**, 49–52.
- 1989, Mucolytic agents: cysteine derivatives, in Damani, L. A. (Ed.), *Sulphur-Containing Drugs and Related Organic Compounds*, Vol. 3a, pp. 87–107, Chichester: Ellis Horwood.
- WARING, R. H. & MCCORMICK, L. A., 1988, A comparison of the metabolism of S-methyl-L-cysteine in the rat and the guinea-pig, *Medical Science Research*, **16**, 285–286.
- WARING, R. H. & MITCHELL, S. C., 1982, Metabolism and elimination of S-carboxymethyl-L-cysteine in man, *Drug Metabolism and Disposition*, **10**, 61–63.
- WARING, R. H., MITCHELL, S. C. & RUSE, M. J., 1989, Antituberculous drugs: ethionamide and related compounds, in Damani, L. A. (Ed.), *Sulphur-Containing Drugs and Related Organic Compounds*, Vol. 3b, pp. 165–180, Chichester: Ellis Horwood.
- WEISIGER, R. A. & JAKOBY, W. R., 1979, Thiol S-methyltransferase from rat liver, *Archives of Biochemistry and Biophysics*, **196**, 631–637.
- WILKINSON, J. E., 1974, Sicklespod surface wax response to photoperiod and S-(2,3-dichloroallyl)-disisopropylthiocarbamate (Diallate), *Plant Physiology*, **53**, 269–275.
- 1983a, Gibberellin precursor biosynthesis inhibition by EPTC and reversal by R-25788, *Pesticide Biochemistry and Physiology*, **19**, 321–329.
- 1983b, Metabolism of [¹⁴C]vernolate in soybean, *Pesticide Biochemistry and Physiology*, **20**, 347–353.
- 1985, CDAA inhibition of kaurene oxidation in etiolated sorghum coleoptiles, *Pesticide Biochemistry and Physiology*, **23**, 19–25.
- 1986, Diallate inhibition of gibberellin biosynthesis in sorghum coleoptiles, *Pesticide Biochemistry and Physiology*, **25**, 93–97.
- WILLIAMS, R. T., 1961, The metabolism and toxicity of arylthioureas, *Biochemical Journal (Proceedings)*, **80**, 1–2P.
- WILSON, C. G., PARKE, D. V. & CAWTHORNE, M. A., 1973, Investigation of the 5-hydroxylation of thiabendazole in rat liver microsomal preparations, *Biochemical Society Transactions*, **1**, 195–196.
- WORTHING, C. R. & WALKER, S. B., 1987, *The Pesticide Manual*, Thornton Heath: BCPC.
- YU, M. W., SEDLAK, J. & LINDSAY, R. H., 1973, Relative antithyroid effects of 2-thiouracil, 2-thiouridine and 2-thio-UMP, *Proceedings of the Society of Experimental Biology and Medicine*, **143**, 672–676.
- ZIEGLER, D. M., 1980, Microsomal flavin-containing monooxygenase. Oxygenation of nucleophilic nitrogen and sulfur compounds, in Jakoby, W. B. (Ed.), *Enzymic Basis of Detoxification*, Vol. 1, pp. 201–227, New York: Academic Press.
- 1982, Functional groups bearing sulphur, in Jakoby, W. B., Blend, J. R. & Caldwell, J. (Eds), *Metabolic Basis of Detoxification*, pp. 171–184, New York: Academic Press.

Sulfur–Hydrogen Compounds

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5.1 Endogenous thiols

Sulfur–hydrogen compounds include thiols and the thiol acids, RCOSH . Generally, however, in terms of biological interactions it is the thiol group which is the most significant and so this chapter will deal primarily with thiols. There

are a host of protein and nonprotein thiols. These include cysteine, cysteamine, coenzyme A and the tripeptide, glutathione (γ -L-glutamyl-L-cysteinylglycine, GSH). In proteins the thiolic functionality carries out a myriad of tasks, from metal binding in the ferridoxins to proteolytic cleavage in the thiol proteases. The thiol group can also be important in hydrogen bond donor/acceptor interactions and has even been discussed as providing an oxidizable switch for protein function.

Glutathione is the most important nonprotein thiol present in animal cells, most plants and bacteria. It is found intracellularly in mammals at millimolar concentrations, mostly in the free thiol form, however, mixed disulfides, such as GSS–protein, the symmetrical disulfide (GSSG) and thioethers (glutathione conjugates) are also present. Although mainly found in the cytosol, glutathione is also present in the mitochondria and nucleus. Both free thiol and symmetrical disulfide forms are also present at low concentrations in plasma, bile and the glomerular filtrate.

Glutathione and other thiols act as protectants against electrophiles, radical damage and oxidative stress due to their thiolic function. These processes are often enzyme catalysed, with glutathione S-transferases (GSTs), glutathione peroxidases and glutathione reductases playing especially important roles. The significance of these will be illustrated in the following sections.

5.2 Chemistry review

The chemistry of thiols has been well reviewed (Friedman 1973, Patai 1974) and can be summarized under the following headings: (i) acid–base equilibria, (ii) nucleophilicity, (iii) elimination, (iv) metal complexes and (v) redox reactions. All of these aspects are important in the biological interactions of sulfur–hydrogen compounds, and so each will be considered in turn.

5.2.1 Acid–base equilibria

The typical thiol group is mildly acidic, with a pK_a value of between 9 and 11. This is comparable to, but generally lower than, the pK_a value of the ammonium ion, RNH_3^+ . The actual pK_a value depends both on the structure and local environment of the thiol group including the effective local solvation. This acidity arises from the relative stability of the large thiolate anion. Table 5.1 gives some illustrative pK_a values. The enthalpy of deprotonation of thiolic species varies little, and thus the differences in the free energy of deprotonation, reflecting the pK_a values, are due to differences in the entropy term ΔS . Such differences may arise from ordering of solvent molecules around the resulting thiolate anion. The deprotonation profile of cysteine is worth considering as it contains up to two ionizable protons at basic pHs. At pH 7 the predominant form is $H_3N^+CH(CH_2SH)CO_2^-$, at pH 9.5 $H_3N^+CH(CH_2S^-)CO_2^-$ and $H_2NCH(CH_2SH)CO_2^-$ are in equilibrium (favouring the former). Deprotonation of the amino group is only significant above pH 10, and at pH 12 the thiolate $H_2NCH(CH_2S^-)CO_2^-$ dominates.

Although thiols can exhibit significant Brønsted–Lowry acidity, they are much poorer bases compared with analogous amino and hydroxylic functions. The thiolic function can, however, act as both hydrogen bond donor and acceptor. That

Table 5.1 pK_a Values of illustrative thiol compounds

Compound	pK_a of SH group
C_2H_5SH	10.61
Cysteine, cysteinyl residues	9.0 to -9.5
$^+NH_3CH_2CH_2SH$	8.23
CH_3COSH	3.62
RSH_2^+	-6.5 to -7.5

hydrogen bonding can significantly affect the acidity of the thiol can be deduced from the low pK_a value for the 2-mercaptoethylammonium ion (Table 5.1).

5.2.2 Nucleophilicity

Amino groups generally remain protonated below pH 10 and thus the major nucleophilic functionality available in biological systems is the thiol group. Alkylation, acylation and arylation of thiols proceed readily *in vitro* and *in vivo*, and are favoured by a basic environment in which the thiolate anion is formed. Thus a range of cysteine and glutathione conjugates can be readily synthesized (Gandolfi *et al.* 1981, Lyttle *et al.* 1994, McKinney *et al.* 1959, Parke and Williams, 1951). Biological thiols also provide a major defence against electrophilic species, such as chlorinated hydrocarbons, conjugation being either nonenzymatic or catalysed by enzymes such as the GSTs. It is worth noting in passing that while S-conjugation will provide the kinetically preferred product, migration onto nearby amino groups may provide a more thermodynamically stable product; this is especially likely in the case of S-acylation. One further example of the thiolate nucleophilicity is in thiol-disulfide exchange reactions; such processes are responsible for the mode of action of such enzymes as thioredoxin and the thioltransferase glutaredoxin.

5.2.3 Elimination reactions

There are many routes for substitution of the hydroxyl group, however there are very few analogous procedures for thiols, most attempts resulting in elimination reactions. For instance cysteine can be dehydrosulfated in the presence of boiling ethylamine, and cysteine S-conjugates can undergo α,β -elimination catalysed by pyridoxal phosphate-dependent enzymes. Indeed it is reported that α,β -elimination occurs on treatment of cysteine S-conjugates with pyridoxal phosphate alone or pyridoxal phosphate and a metal ion catalyst (Hayden *et al.* 1991b).

5.2.4 Metal complexes

The effective binding of thiols to transition metals is illustrated by the fact they are also known as mercaptans – a name arising from their affinity for mercury. Hence, thiols are often found at the metal binding site of very many metalloen-

zymes. Due to the ease of polarization of the sulfur atom's electron density, thiols and thiolate anions are classified as soft ligands, and generally form stable complexes with easily polarized, soft metal cations. These include Cu^+ , Ag^+ , Au^+ , Hg^{2+} , Zn^{2+} , Cu^{2+} and Fe^{2+} .

5.2.5 Redox reactions

Whereas alcohols oxidize to give carbonyl compounds (aldehydes, ketones and carboxylic acids), thiols are not readily oxidized to corresponding thiocarbonyls. This difference in reactivity arises because the larger size of the sulfur atom, compared with the oxygen atom, does not allow good p-orbital overlap with carbon, and therefore weak π -bonds are formed. In addition, the availability of sulfur d-orbitals allows expansion beyond divalent compounds to valencies of 4 and 6. Thus oxidation of the thiol group can lead to disulfides (RSSR'), sulfenic acids (RSOH), sulfinic acids (RSO_2H) or sulfonic acids (RSO_3H). In biological systems the major redox couple is thiol–disulfide exchange, which is important for the maintenance of cellular redox potential. A typical standard redox potential for thiol–disulfide exchange would be of the order of -0.22 V . This can be compared to redox potentials for ferredoxin (-0.43 V) and nicotinamide adenine dinucleotide phosphate (NADPH) (-0.32 V).

5.3 The metabolic transformation of xenobiotics and endogenous thiols

The metabolic processes which enable the elimination of foreign compounds (xenobiotics) from living systems can be divided into two phases. The first phase (phase I) activates the xenobiotic by revealing or introducing a reactive functional group. The majority of phase I reactions are oxidative; however, reduction and hydrolysis reactions also occur. Phase II processes conjugate an endogenous moiety to reactive xenobiotics enhancing their water solubility. The overall process usually results in a compound more hydrophilic than its precursor and hence easily excreted in bile or urine.

In phase I metabolism, enzymatic oxygenation of thiols affords sulfinic acids via reactive sulfenic species. Sulfenic acids can react with excess thiols to produce disulfides. Exogenous and endogenous disulfides can either be reduced back to thiols or converted to the sulfenic or sulfinic acids via intermediate thiosulfenic and thiosulfinic esters (Figure 5.1). However, it is not known how many xenobiotic thiols are metabolized by these reaction sequences, first proposed by Ziegler (1984). The main route of metabolism of the drugs captopril and penicillamine involves oxidation to disulfides and mixed disulfides. Mixed disulfides are formed by reaction with endogenous thiols, such as glutathione, cysteine and the free cysteinyl thiols of proteins (although drug–protein disulfide conjugates are relatively short-lived). The reactivity of the thiol group is considerably influenced by its empty d-orbitals which allow nucleophilic attack. This is responsible for the characteristic thiol–disulfide exchanges observed in their metabolism. These redox reactions are facilitated by the fact that the outer electrons of sulfur are far removed from the nucleus. The thiol group of captopril and penicillamine is the only functionality which is susceptible to transformation.

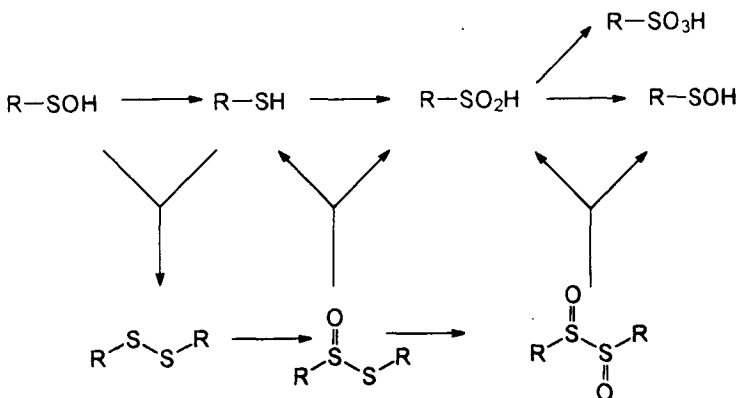


Figure 5.1 Metabolic options for thiols

The majority of phase II conjugation reactions involve the replacement of a proton by the conjugating agent, forming ethers, secondary and tertiary amines and esters from hydroxyl groups, primary and secondary amines and carboxylic acid functionalities, respectively. Sulfur compounds occur in a wide range of valency states and covalent bonding situations. However, only the thiol and dithioic acid ($-CSSH$) have suitably labile protons for substitution by a conjugating agent. Thiols are rarely found in xenobiotics and are not commonly produced through metabolism. When thiols do occur their availability for conjugation is limited by thiol-disulfide exchanges which give dimers or mixed disulfides with endogenous thiols. Certain similarities can be observed between the metabolic reactivity of thiols and hydroxyls in that both species can conjugate with glucuronic acid (in mammals) and glucosides (in molluscs). Only thiols, however, form sulfates and undergo acetylation. The metabolic transformation of thiols is summarized in Table 5.2.

5.3.1 Glutathione conjugation

Generally conjugation occurs as a result of nucleophilic attack by a xenobiotic on the conjugating species. However, the thiolic conjugating agent glutathione (L- γ -glutamyl-L-cysteinylglycine) is an exception to this principle. In this case it is the conjugating species, glutathione, which is nucleophilic; the tripeptide undergoes bond-forming reactions with xenobiotic compounds through the cysteinyl sulfhydryl group. These reactions can involve 1,4-Michael addition across a suitably activated carbon-carbon double bond, such as those found in α,β -unsaturated ketones and allylic esters, addition or substitution at halogenated ethenes, or nucleophilic displacement reactions of epoxides, halo- and nitro-alkanes and alkyl sulfonates. Many of the reactions of glutathione with electrophiles are enzyme-catalysed by GSTs (for a review see Ketterer and Christodoulides 1994).

Further metabolism of glutathione conjugates is often considered as the third phase of xenobiotic metabolism. Detoxification was thought to be the only

Table 5.2 Biological transformations of thiols

Phase	Type of transformation	Product	Formula
I	Oxidation (S-oxygenation)	Sulfenic acid	$R-SOH$
I	Oxidation (S-oxygenation)	Sulfinic acid	$R-SO_2H$
I	Oxidation (S-oxygenation)	Sulfonic acid	$R-SO_3H$
I	Oxidation (disulfide)	Symmetrical disulfide	$R-SS-R$
I	Oxidation (disulfide)	Mixed disulfide	$R'-SS-R''$
II	Conjugation (S-acetylation)	S-Thioacetyl-	$R-S-COCH_3$
II	Conjugation (S-methylation)	S-Methylthioether	$R-S-CH_3$
II	Conjugation (S-glucuronidation)	S-Glucuronide	$R-S-C_6H_9O_6$
II	Conjugation (S-glucosidation)	S-Glucoside	$R-S-C_6H_{11}O_5$
II	Conjugation (S-sulfation)	S-Sulfate	$R-S-SO_3^-$
III	Rearrangement	Thioacyl halide	$R-CSX$
III	Elimination	Thioketene	$R-C=S$
III	Substitution	Episulfonium ion	$R-[S(CH_2)_2]^+$

physiological role for conjugation reactions for many years. This view was supported by experimental evidence which showed that many conjugates are more hydrophilic than the original xenobiotic and therefore easily excreted, and also fail to possess the biological activity of the original compound (Gibson and Skett 1994).

5.3.1.1 The mercapturic acid pathway

Glutathione conjugates undergo further metabolism by various pathways to yield highly polar and water-soluble metabolites; these metabolites can then be easily excreted in the bile or urine. The glutamyl and glycyl residues are sequentially removed by the action of the serine hydrolase, γ -glutamyltransferase, and cysteinylglycine dipeptidase or aminopeptidase M. Subsequent metabolism of the resultant cysteine conjugate usually results in the formation and excretion of a mercapturic acid (the N-acetyl cysteine conjugate), and this route of metabolism has been traditionally thought of as one of detoxification (Chasseaud 1976) (Figure 5.2).

Anomalies began to appear in the literature which indicated that toxications involving conjugations can also occur (Cummings and Prough 1983, Dekant *et al.* 1994). The toxic effects of such conjugates are due to activation or binding to a macromolecular receptor at the site at which toxicity is mediated. The generation of reactive thiols from certain cysteine conjugates has now been well documented as a toxifying event.

5.3.1.2 Cysteine conjugate toxicity results in the formation of reactive thiols

In 1916, Stockman reported that calves fed on soy-meal which had been extracted with trichloroethylene suffered aplastic anaemia and kidney failure (Stockman 1916). The mercapturic acid, glutathionyl- and cysteinyl conjugates were observed

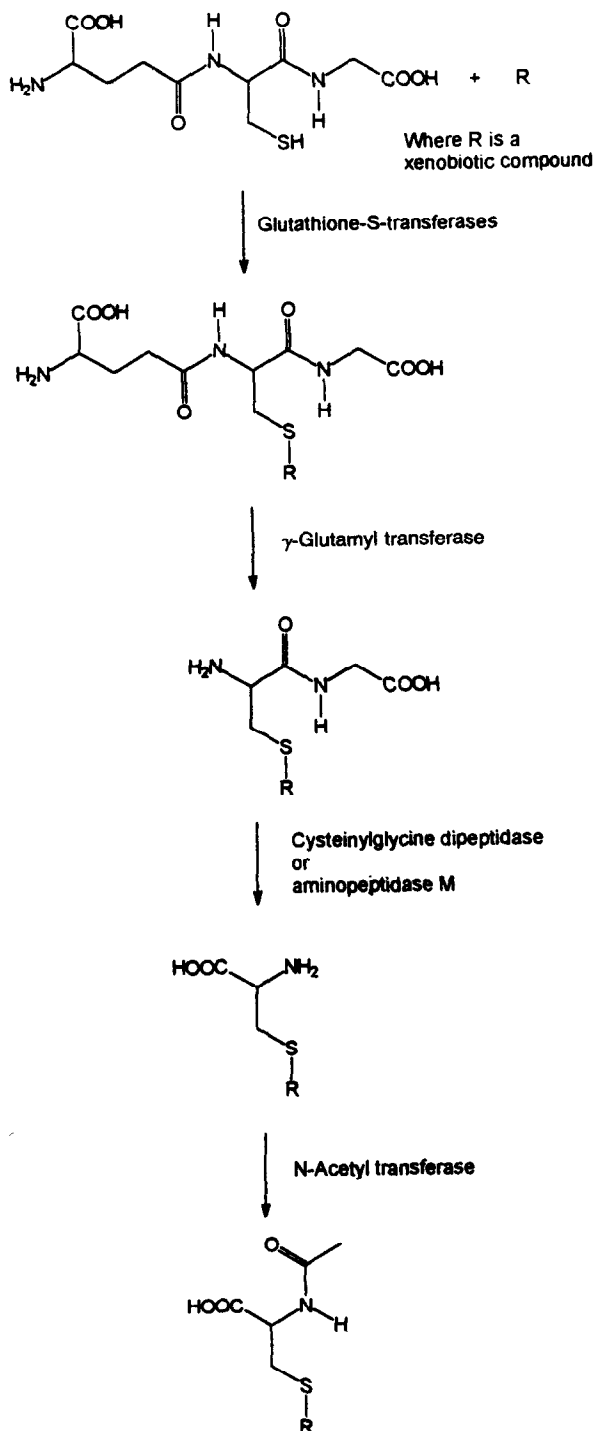


Figure 5.2 The mercapturic acid pathway

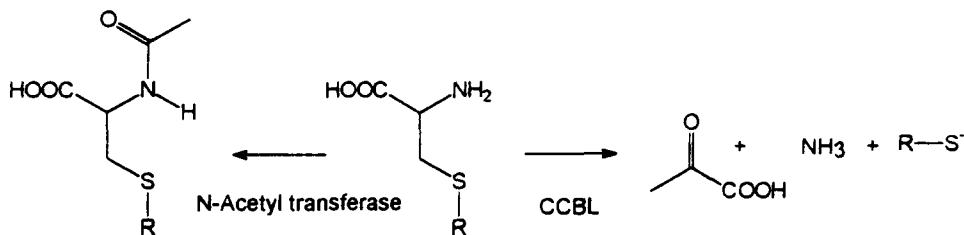


Figure 5.3 Cysteine conjugate metabolism

in the urine of affected animals indicating the normal route of metabolism was intact. A possible mechanism to explain this phenomenon did not emerge until 1957 when McKinney and coworkers postulated that the C—S cleavage of the cysteine conjugate metabolite, S-(1,2-dichlorovinyl)-L-cysteine was involved (McKinney *et al.* 1957, 1959). It is now known that enzymes with cysteine conjugate β -lyase (CCBL) activity are responsible for the generation of equimolar amounts of an unstable thiolate anion, pyruvic acid and ammonia through a β -elimination reaction on the conjugate (Figure 5.3). This thiol yields highly reactive electrophilic species which have been shown to be cytotoxic and mutagenic.

Enzymes with CCBL activity have been isolated from a number of sources including bacteria (Bakke *et al.* 1981, Larsen and Stevens 1985), the *Brassica*, *Cruciferae* and *Allin* families of higher plants (Mazelis 1963, Mazelis *et al.* 1967, Nock and Mazelis 1985), chicken (Bhattacharya and Schultze 1967), rat (Stevens 1985), bovine (Buckberry *et al.* 1993a) and human renal tissue (Buckberry *et al.* 1990, Lash *et al.* 1990), rat (Tateishi *et al.* 1978) and human hepatic tissue (Buckberry *et al.* 1992, Tomisawa *et al.* 1986), and bovine and human lung tissue (Buckberry *et al.* 1993a, 1994). Cysteine conjugate toxicity in human Chang liver cells has also been shown to correlate with CCBL activity in human hepatic tissue (Buckberry *et al.* 1993b). In plant tissue the physiological role of these enzymes is related to the production of characteristic 'thiolic' odours. Physiologically animal CCBL enzymes fulfil two roles: (i) to regulate amino acid metabolism — enzymes exhibiting CCBL activity include glutamine transaminase K (rat and human kidney) (Green and Odum 1985, Lash *et al.* 1990), kynurenine amino transferase (human liver) (Buckberry *et al.* 1992), kynureninase (rat liver) (Stevens 1985), and porcine heart aspartate and alanine aminotransferase (Gaskin *et al.* 1995), CCBL activity has been localized in the cytosolic and mitochondrial regions of mammalian tissues (Buckberry *et al.* 1994, Stevens 1985), and (ii) thiolation of xenobiotics prior to methylation via glutathione conjugation (Parke 1952).

These CCBL enzymes employ pyridoxal phosphate (PLP) as a cofactor in an electrophilic covalent catalytic system. Initial stages of the physiological reaction mechanism which these enzymes catalyse would involve the abstraction of the α -H of the cysteine conjugate to form a highly unstable intermediate carbanion. With PLP the enzymes provide an alternative reaction pathway by formation of a Schiff base between the α -amino group of the substrate amino acid and the PLP-carbonyl group. This stereoelectronically assists proton abstraction by delocalization of the electron pair of the α -C to α -H bond mesomerically with the positively charged pyridinium nitrogen (Figure 5.4). The resultant quinoid intermediate is then protonated to give a ketimine which, with subsequent hydrolysis in the case of

transamination, yields pyridoxamine phosphate and the α -keto acid. This represents a half transamination reaction. The PLP is regenerated using an α -keto acid cosubstrate in a reaction which is the reverse of the above (Braunstein and Shemyakin 1953, Metzler *et al.* 1954). PLP-dependent enzymes are capable of forming a Schiff base with cysteine-conjugated xenobiotics since the active site specifically recognizes the α -amino group of amino acids, but shows broad specificity towards amino acids as a whole, often reacting with groups with broadly related sidechain chemistries. However, following formation of the quinoid intermediate, the highly electronegative characteristics of the S-conjugate side chain cause a β -elimination reaction to take place. This elimination can result in the formation of unstable thiols (Figure 5.4).

5.3.1.3 Different types of thiolic intermediates are formed as a result of β -lysis

Different types of intermediates are formed from S-(1,1-difluoroalkyl)-L-cysteine, S-(1-chloroalkenyl)-L-cysteine and S-(aryl)-L-cysteine derivatives. S-(1,1-difluoroalkyl)-L-cysteine and S-(1-chloroalkenyl)-L-cysteine derivatives generate reactive thiols capable of cytotoxic and mutagenic action. α -Fluoroethanethiols are the products of CCBL-mediated cleavage of S-(2-chloro-1,1,2-trifluoroethyl)-L-cysteine and of S-(1,1,2,2-tetrafluoroethyl)-L-cysteine. Incubation of S-(2-chloro-1,1,2-trifluoroethyl)-L-cysteine with an artificial enzyme system, consisting of PLP and Cu^{2+} ions, or bovine renal CCBL, yields chlorofluoroacetic acid and inorganic fluoride. These products would be expected to be produced as a result of the hydrolysis of the α -fluoroethanethiol following rearrangement to the thione [Figure 5.5 (i)]. The conjugates S-(1,2-dichlorovinyl)-L-cysteine, S-1,2,2-trichlorovinyl-L-cysteine and S-(pentachlorobutadienyl)-L-cysteine yield halovinylthiols which spontaneously rearrange forming thioacyl chlorides or α -thioketenes. Thioacyl chlorides are predominant tautomers of α -chloro substituted enethiols (Mayer and Scheithauer 1976). Alternatively, α -chlorovinyl thiols may eliminate hydrochloric acid to yield thioketenes which are highly reactive towards nitrogen nucleophiles (Adiwidjaja *et al.* 1991) [Figure 5.5 (ii)]. Thioketenes and thioacyl halides are reactive acylating agents and react with amino and hydroxyl groups in proteins, lipids and DNA. N^{ϵ} -(difluorothioacetyl)-L-lysine and N^{ϵ} -(chlorofluorothioacetyl)-L-lysine have been identified as adducts in proteins of subcellular fractions incubated with S-(2-chloro-1,1,2-trifluoroethyl)-L-cysteine and S-(1,2,2)-trichlorovinyl-L-cysteine (Hayden *et al.* 1991a, b, Hayden and Stevens 1990). Difluorothionoacetamide-lipid adducts have also been found in mitochondria (Harris *et al.* 1992). However, these studies employed the use of ^{19}F NMR in adduct detection. This technique will not clearly elucidate the complete adduct structure since the constituent fluorines are too far removed from the original nucleophile atoms to allow full characterization of the target molecules. The authors have attempted to confirm the identity of amino acid adducts formed by CCBL using the artificial PLP-based system in conjunction with electrospray tandem mass spectrometry. However, the lack of active site specificity led to the formation of many side-products and therefore confirmation of the adduct structures was not possible (Buckberry unpublished observations).

Chemically stable thiols are generated as a product of CCBL activity towards aromatic, heterocyclic and alkylaromatic cysteine conjugates (Bakke and

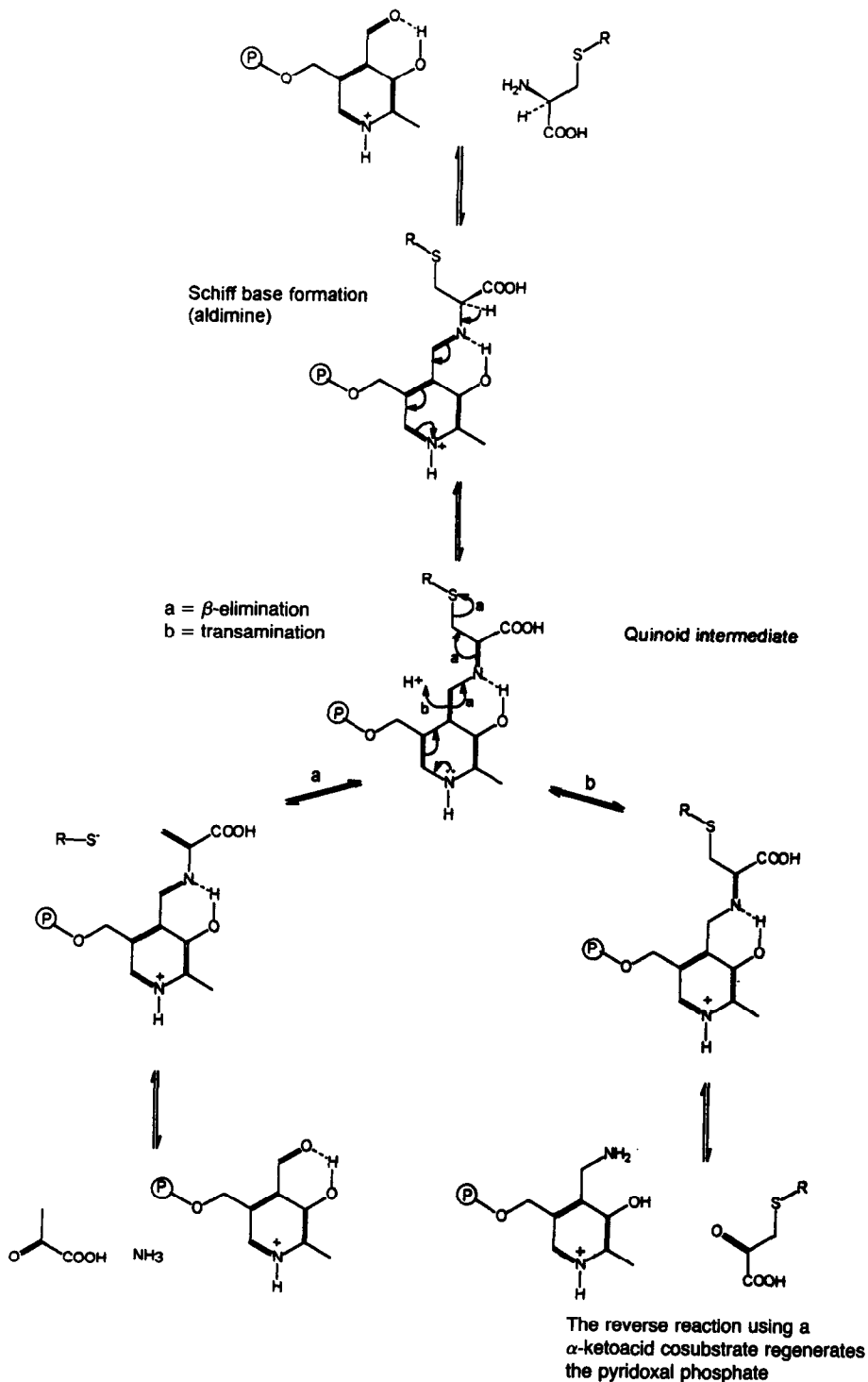
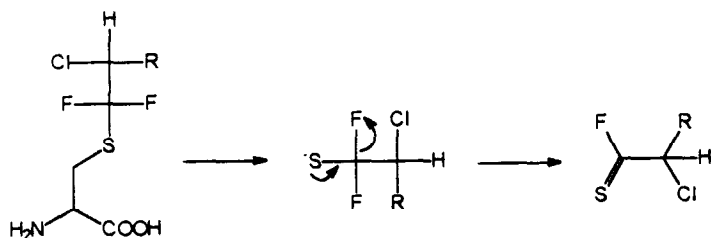
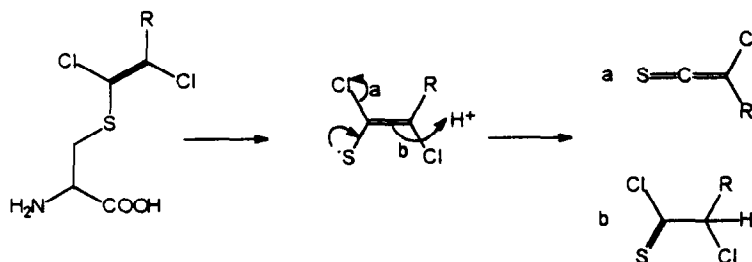


Figure 5.4 Mechanism of β-elimination versus transamination

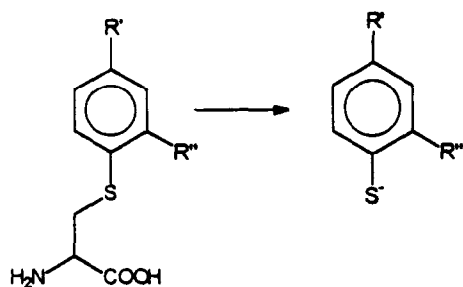
(i) Saturated Aliphatic Conjugates

R = H, S-(2-chloro-1,1-difluoroethyl)-L-cysteine
 R = F, S-(2-chloro-1,1,2-trifluoroethyl)-L-cysteine

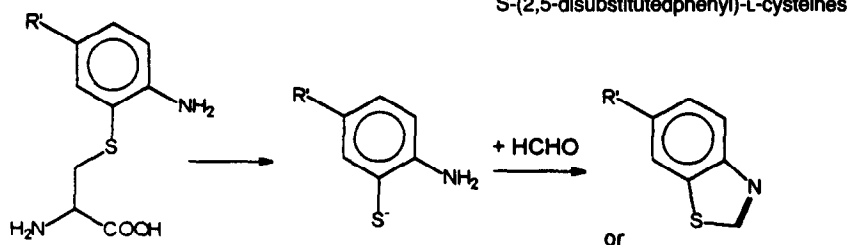
(ii) Unsaturated Aliphatic Conjugates

R = H, S-(1,2-dichlorovinyl)-L-cysteine
 R = Cl, S-(1,2,2-trichlorovinyl)-L-cysteine

a = Thioketene formation
 b = Thioacyl halide formation

(iii) Aromatic Cysteine Conjugates

R' = H, S-(2-substitutedphenyl)-L-cysteines
 R'' = H, S-(4-substitutedphenyl)-L-cysteines
 R', R'' is not H = S-(2,4-substitutedphenyl)-L-cysteines



Adduct formation?

Figure 5.5 Formation of reactive electrophiles from thiolic metabolites

Gustafsson 1984) [Figure 5.5 (iii)]. The toxic potential of these species has not been fully elucidated. It is possible that they are metabolized to sulfenic acids by flavin-dependent monooxygenases, with subsequent formation of mixed disulfides with glutathione and other free thiols (Bakke and Gustafsson 1984). Disulfide formation has been implicated in the toxicity of several thiols, however N-acetyl-S-(pentachlorophenyl)-L-cysteine and S-(2-benzothiazolyl)-L-cysteine (two model substrates for CCBL *in vivo*) failed to induce the renal damage classically associated with CCBL. Following N-deacetylation by aminocyclase, N-acetyl-S-(pentachlorophenyl)-L-cysteine yields pentachlorothiophenol on β -lysis. This may then be either S-methylated to the anisole derivative prior to excretion, or excreted directly into the urine (Renner 1983). The β -lysis product generated from S-(2-benzothiazolyl)-L-cysteine, 2-mercaptobenzothiazole, is not toxic to rat renal proximal tubular cells *in vitro*. This lack of observed toxicity may be due to the inherent stability of the resultant thiols. Stable thiols formed by CCBL *in vivo* may be excreted unchanged or may act as precursors for metabolites such as S-glucuronides, thiomethyl derivatives, sulfones and sulfonates. However, these stable species do not possess the capacity to rearrange to form unstable, reactive thiono species.

Schultze and coworkers first demonstrated that a reactive metabolite formed as a result of C—S lysis of S-(1,2-dichlorovinyl)-L-cysteine causes structural modification of DNA (Bhattacharya and Schultze 1971a, b, 1972, 1973a, b). It is now known that only conjugates capable of forming thioketenes are mutagenic through adduct formation with DNA (Muller *et al.* 1991).

5.3.1.4 Other toxicities related to glutathione conjugation

Glutathione conjugation has been traditionally thought of as a precursor to detoxification. However, the reaction of *vic*-dihaloalkanes with glutathione results in the formation of a highly reactive episulfonium ion which is capable of adduct formation with DNA and other cellular nucleophiles. This was first demonstrated by Rannug *et al.* (1978) who reported that ethylene dichloride was mutagenic to *Salmonella typhimurium* TA 1535 in the presence of glutathione. It was then proposed that glutathione reacted with ethylene dichloride to form a half-mustard (S-(2-chloroethyl)-glutathione) and then an episulfonium ion (Bakke 1986). *Cis*-1,2-dichlorovinylcyclohexane, and *vic*-dihaloalkanes also undergo this transformation (Elfarra *et al.* 1985) (Figure 5.6).

One route of halobenzene metabolism involves conjugation with glutathione via cytochrome P450-catalysed epoxide or benzoquinone formation and this was thought to be an effective mechanism of protection against hepatotoxicity. However, glutathione conjugates of 2-bromohydroquinone are potent nephrotoxins. Activation by CCBL enzymes is unlikely as the primary route of toxicity as the quinone functionality of the metabolite is a more important determinant of toxicity than is the thiol. Multiple renal transport mechanisms which accumulate the glutathione conjugated quinone may be responsible for the toxic effect (den Besten *et al.* 1994, Mertens *et al.* 1991).

The aromatic amines, 4-phenylenediamine (PPD) and 4-aminophenol (PAP), have been linked with glutathione-mediated toxicity via an intermediate thiol; PPD is used extensively as an oxidizable hair dye, in photochemistry and in tyre-vulcanizing processes. The toxic effects of PPD are well recognized in Africa and

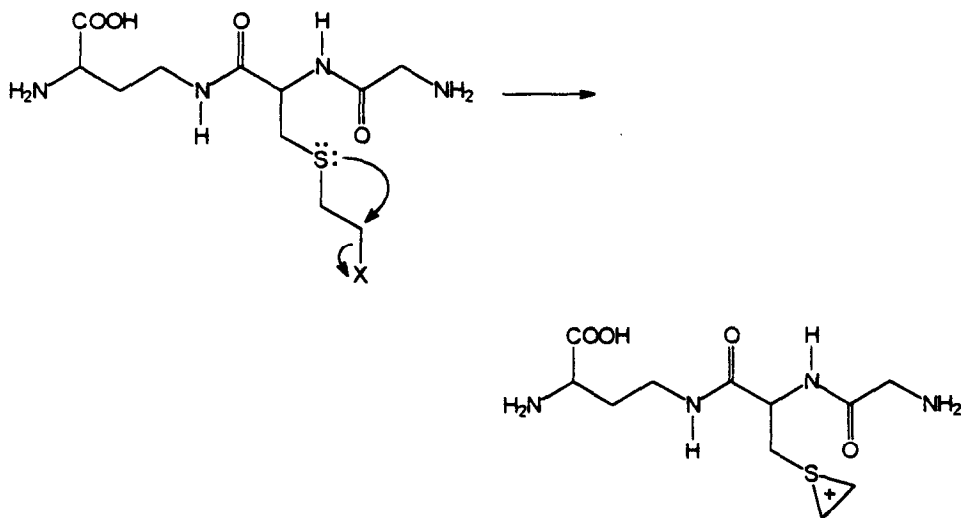


Figure 5.6 The formation of episulfonium ions following glutathione conjugation of *vic*-dihalides

Asia where its availability makes it an attractive poison. PPD causes skin irritation and dermatitis in susceptible individuals. When ingested, PPD causes a severe local mucosal reaction resulting in oedema of the face and neck, followed by rhabdomyolysis and renal failure. PPD is a good hydrogen donor, and in phase I metabolism undergoes a one-electron oxidation providing a cation free radical by cytochrome P450 peroxidase to form a reactive benzoquinonediimine. This diimine is further oxidized to a trimer known as Bandowski's base, a mutagen which also causes anaphylaxis. Recently, Ashraf and coworkers (1994) detected 6-aminobenzothiazole in the urine of a suicide victim and implicated glutathione conjugation in the metabolic route of its formation [Figure 5.5 (iii)]. With hindsight it is possible to predict that the glutathione conjugate of the benzoquinone diimine is subsequently metabolized to the cysteine conjugate and C–S lysed to produce 2,5-diaminothiophenol. This compound could then ring close with formaldehyde to yield the 6-aminobenzothiazole. Both the 6-aminobenzothiazole and the thiolic intermediate are potential nephrotoxins.

PAP is known to cause selective necrosis of the rat renal proximal tubules, but the mechanism of action of this compound is poorly understood. However, PAP produces a dose-dependent decrease in renal glutathione and radiolabelled PAP covalently binds to renal protein (Crowe *et al.* 1979). It has been suggested that nephrotoxicity could result from the formation of phenoxy radicals and the 1,4-benzoquinoneimine (BQI). BQI is highly reactive and the 2- and 3-monosubstituted, 3,5-disubstituted and 2,3,6-trisubstituted thiol adducts of the 4-aminophenol metabolite have been identified. These metabolites can autoxidize faster *in vitro* than can PAP and therefore thioether formation cannot be considered detoxifying in this case. Fowler and Lock demonstrated that 4-amino-3-S-glutathionylphenol exhibits greater nephrotoxicity than does PAP in rats, and that oxidation of PAP is responsible for PAP-induced mitochondrial dysfunction

and tubular cell death in rabbit renal proximal tubules (Fowler *et al.* 1991, Lock *et al.* 1993). As yet there have been no reports of the isolation of the metabolites 6-aminobenzoxazole or 6-hydroxybenzothiazole, which would indicate the involvement of CCBL enzymes in metabolism analogous to that of PPD.

5.3.2 S-methylation

S-methylation of thiols is an important metabolic route in their biotransformation. A wide variety of xenobiotics are metabolized to methylthio-containing metabolites in various species (Stillwell 1981, Tateishi *et al.* 1987). S-methylation can occur via two routes: (i) through the reaction of methionine with electrophilic intermediates of xenobiotics (Kadlubar *et al.* 1976, Scribner *et al.* 1965) and (ii) through conjugation to the nucleophilic tripeptide, glutathione, subsequent metabolism and β -elimination of the cysteine conjugate yielding a thiol which can then undergo methylation (Tateishi 1983). The latter route is the most commonly encountered mechanism of methyl sulfide production. A number of thiol compounds, such as mercaptoacetic acid, 2-mercaptopropanoic acid and methyl mercaptan, are transmethylated by a microsomal enzyme system present in mammalian liver (Bremer and Greenberg 1961). S-adenosylmethionine is the methyl donor in this case and also in the S-methylation of thiopurine and thiopyrimidines (Remy 1963). In microorganisms, S-methylation is catalysed by thiol methyltransferase which is located in the cytoplasm. However, in mammalian tissues two enzymes with differing substrate specificity, subcellular location, and mode of regulation occur. Thiopurine methyltransferase, in common with the equivalent enzyme found in microorganisms, is cytoplasmic and shows substrate specificity for aromatic and heterocyclic compounds; thiol methyltransferase catalyses the S-methylation of aliphatic compounds such as captopril. For a review of the biochemistry of the thiol S-methyltransferases see Wienshilboum (1989).

Despite thiols rarely being observed in xenobiotic compounds, S-methylation of this functionality is important in the metabolism of cysteine. In the initial stages of cysteine biosynthesis, serine and homocysteine condense to form cystathionine, the reaction being catalysed by cystathionine synthase. Cystathionine is then deaminated and cleaved forming cysteine and α -ketobutyrate by cystathionase. Both cystathionine synthase and cystathionase are pyridoxal phosphate-dependent enzymes, as are the cysteine conjugate β -lyases. Since cysteine cannot be stored or excreted, transamination of the α -amino group affords the eventual production of pyruvic acid. The sulfur emerges as either hydrogen sulfide, thiocyanate or sulfate (Cooper 1983, Jakoby and Stevens 1984).

5.3.3 S-glucuronidation

S-glucuronidation was first reported by Parke, in 1952, who isolated thiophenol glucuronide as a metabolite of benzene in the rabbit. The mechanism of thiol introduction to the metabolite remained unknown for a number of years; however it is now thought this is produced via conjugation to glutathione with subsequent C-S lysis of the intermediate cysteine conjugate. The metabolism of ben-

zothiazole-2-sulfonamide (a potential carbonic anhydrase inhibitor) was the first S-glucuronidation to be fully elucidated. In *in vivo* studies in the rat, three metabolites were detected in urine, the benzothiazole-2-thiogluconide (Clapp 1965), the benzothiazole-2-thiol and the benzothiazole-2-mercapturate (Colucci and Buyske 1965). The thiol group is introduced to position 2 via displacement of the sulfonamide by glutathione. The resultant 2-glutathionylbenzothiazole is subsequently converted to the cysteine conjugate which is C–S cleaved to yield the 2-thiol metabolite, or N-acetylated to give the mercapturate (Colucci and Buyske 1965). The thiol is then excreted or converted to the S-glucuronide, a reaction which is catalysed by UDP-glucuronosyl transferase in rat liver microsomal preparations (Illing and Benford 1976). Glucuronyl transferases behave in a similar way towards hydroxyls and sulfhydryls, and both activities have the same subcellular location and pH optima (Dutton and Illing 1972).

5.3.4 Synthetic thiols as chemoprotectants

Cysteinyl esters raise lung, liver and plasma levels of cysteine and other nonprotein sulfhydryls. Hence, protection is afforded against electrophilic attack of short duration such as that experienced on exposure to the gaseous pulmonary oedemagens, perfluoroisobutene, phosgene and sulfur mustards (Lailey *et al.* 1991, Wilde and Upshall 1994). L-Cysteine isopropyl ester has been shown to be particularly effective and can protect against paracetamol toxicity (Butterworth *et al.* 1991). N-acetyl-L-cysteine administration results in increased glutathione levels in bronchioalveolar lavage fluid (Bridgeman *et al.* 1991). Cysteine esters cause much higher levels of cysteine and other nonprotein sulfhydryls in lung tissues due to their greater lipophilicity compared to N-acetyl-L-cysteine (Hobbs and Upshall 1993). Recently, Lailey and Upshall (1994) reported that cysteine, N-acetyl-L-cysteine, cysteine methyl-, dimethyl-, isopropyl-, cyclopentyl-, cyclohexyl- and neopentyl-esters afford lung protection by increasing the levels of protective nucleophiles in the alveolar lining as well as in the lung tissue itself. The level of increase needed for effective protection has not been determined. It should now be noted that L-cysteine and L-cysteine isopropyl ester cause neurotoxicity in rats and mice. This toxicity is both age- and species-dependent and is thought to involve the N-methyl-D-aspartate subtype of the glutamate receptor although its exact role is unclear at this time (Maghsoudloo *et al.* 1994).

The ability of dimercapto chelating agents to protect against heavy metal poisoning was utilized in the Second World War when 2,3-dimercapto-propanol (British anti-Lewisite, BAL) was employed against dichloro-(2-chlorovinyl)-arsine (Lewisite) poisoning (Eagle *et al.* 1946). BAL is poorly water-soluble so was administered topically and as an intramuscular injection, the dosage being limited by its inherent toxicity (Sulzberger *et al.* 1946); as such these factors have limited its effective use. More recently, water-soluble analogues of BAL, such as 2,3-dimercaptopropanesulfonic acid (DMPS) and meso-2,3-dimercaptosuccinic acid, have been used to treat heavy metal poisoning (Aphoshian 1983). These compounds are more effective and less toxic than BAL and can be administered both orally and parenterally (Inns and Rice 1993). More recently, it has been reported that DMPS treatment was effective in a case of severe mercuric chloride poisoning without side-effects (Toet *et al.* 1994).

5.4 The role of thiols in redox processes

In addition to being important in defence against electrophilic species, thiols are involved in many redox processes. They act to protect against free radical damage and in the maintenance of cellular redox state through thiol–disulfide exchange. This section will consider both of these factors.

5.4.1 Thiol–disulfide exchange

Thiols, in particular glutathione, are important in the maintenance of the redox state of cells; a function shared by various reductants such as NADPH, ascorbate and α -tocopherol. Thus the $[GSH]/[GSSG]$ ratio is a useful indicator of cellular redox regulation. Control of this ratio is mediated by glutathione reductase and a number of enzyme systems which catalyse thiol–disulfide exchange. Amongst these are protein disulfide isomerase, thioredoxin reductase and the thioltransferase, glutaredoxin reductase. These thiol–protein oxidoreductases all contain a characteristic Cys-X-Y-Cys moiety in the active site. The thiolic groups of both cysteinyl residues appear to be important in disulfide reduction and thiol oxidation. Whilst the known three-dimensional structures of the functional domain of this protein superfamily are all closely related, forming a hairpin turn between a β -strand and an α -helix near the protein surface, they have quite markedly different redox potentials ranging from a strongly reducing -0.270 V for thioredoxin and -0.230 V for glutaredoxin to -0.110 V for protein disulfide isomerase. These redox potentials are quite different from those of the excised functional domains, and thus can be seen to be dependent on the folding of the backbone in the full protein (Siedler *et al.* 1993). These differences in redox potentials can be seen as arising from the free energy change for disulfide bridge formation which is -13.4 kJ mol $^{-1}$ for thioredoxin and 3.8 kJ mol $^{-1}$ for glutaredoxin (Sandberg *et al.* 1991). Since the enthalpy change on formation of the disulfide bridge will vary little, it is interesting to speculate that these variations in free energy are due to entropy considerations and thus act as a marker of the mobility of cysteinyl sidechains in the reduced form of the proteins.

Although being structurally related, these thiol–disulfide oxidoreductases do have differing functions, resulting from the redox potentials outlined above, in addition to the structural requirements of the active site. For example, it has been suggested that the thioltransferase glutaredoxin acts as a glutathionyl mixed disulfide oxidoreductase, specifically reducing disulfides of the type GSSR (Gravina and Mieyal 1993). Although it has been suggested that thioltransferase can utilize other thiol reductants (Terada 1994), Gravina and Mieyal (1993) argue that this occurs as a result of nonenzymatically catalysed thiol–disulfide exchange processes. They observed that thioltransferase had no effect on the rate of the glutathione-dependent dethiolation of the haemoglobin–cysteaminyl disulfide. In contrast, dethiolation of the haemoglobin–glutathionyl disulfide was shown to be enzymatically catalysed. Where thioltransferase gives apparent overall reduction of nonglutathione-containing disulfides, RSSR', this process is likely to result from the nonenzymatic thiol–disulfide exchange with glutathione giving the required glutathionyl mixed disulfide (Figure 5.7).

This proposed mechanism (Gravina and Mieyal 1993) indicates that involvement

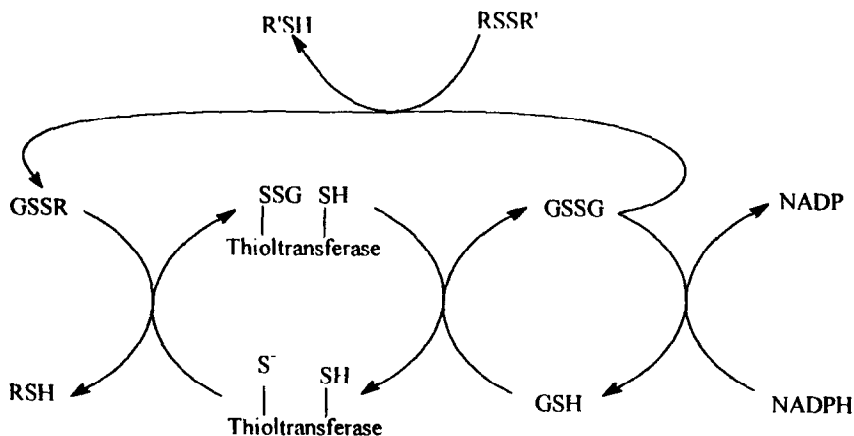


Figure 5.7 The reduction of disulfides mediated by thiol transferase

of the second thiolic functionality in the active site is not necessary for disulfide reduction and thereby explains the activity of mutant thioltransferases without this second thiol group (Yang and Wells 1991). The overall reductive process is thus mediated by glutathione which is regenerated by glutathione reductase. The importance of the physiological role of thioltransferase is clear from the number of enzymes that are oxidatively inactivated by disulfide formation; these include pyruvate kinase, phosphofructokinase and GSTs. Glutathione is the major intracellular nonprotein thiol and so the majority of the mixed disulfides formed on oxidative stress are glutathione-containing. By contrast, thioredoxin, whose active site is exposed on a relatively featureless surface, appears to be a nonspecific oxidoreductase.

The formation of disulfides can be seen not as a consequence of oxidative stress, but rather as a protective mechanism giving easily reversed adduct formation (Del Corso and Mura 1994). Whilst reduced cellular thiols are available, the formation of mixed disulfides acts as a buffer, protecting against irreversible oxidation (Coan *et al.* 1992). The regeneration of the protein following the mechanisms outlined above is energetically favourable as compared to proteolytic degradation and re-synthesis of an irreversibly oxidized protein.

Given the similarity in cellular response to oxidative stress and to heat shock, and the clear involvement of thiolic species as moderators of oxidative stress, Huang and coworkers investigated the involvement of thiol reducing reagents in the heat shock response (Huang *et al.* 1994). They showed that thiols, especially dithiothreitol, inhibited the heat-induced synthesis of heat shock proteins. Given that oxidized thiols had no effect on heat shock response, they concluded that the thiols blocked an early step in the heat shock signal transduction pathway utilizing a redox mechanism.

5.4.2 Radical depletion

Thiols are well known for their ability to protect against ionizing radiation (van den Broeke and Beyersbergen van Heneguowen 1993). Damage from such

radiation results from the formation of reactive oxygen species, including singlet molecular oxygen, hydroxyl, superoxide and other free radicals. These highly reactive species can cause oxidative damage at membranes through lipid peroxidation, and also at proteins and nucleic acids; a process that can result in strand breakage.

The basis of thiol-mediated radiation protection arises through two important properties. (i) The ability of thiols to act as a proton donor source. The S—H bond is relatively weak (typically 349 kJ mol^{-1}) compared to the C—H bond (415 kJ mol^{-1}) and the O—H bond (465 kJ mol^{-1}). Homolytic cleavage of the S—H bond yields the relatively unreactive thiyl radical (reaction 1), which is correspondingly poor at hydrogen abstraction and readily combine to give disulfides (reaction 2).



(ii) Sulfur is relatively electron-rich, having two lone pairs, and has a low electronegativity. These factors mean that electron donation with the concomitant formation of a radical cation proceeds with relative ease.

A recent study into the protective properties of several thiols (Figure 5.8) against UV radiation damage concluded that they were effective in protecting against free radical species following both mechanisms outlined above. N-acetyl cysteine, mercaptopropionylglycine and captopril were found to be especially effective (van den Broeke and Beyersbergen van Heneguowen 1993). A similar study demonstrated that N-acetyl cysteine also affords protection against endogenous thiol oxidation following *t*-butyl hydroperoxide-induced oxidative stress in erythrocytes (Udupi and Rice-Evans 1993). Such protection can arise from elevation of glutathione levels (Cotgreave *et al.* 1991) as well as through intrinsic activity of the N-acetyl cysteine (Aruoma *et al.* 1989). One potential outcome of oxidative stress is lipid peroxidation. Normal erythrocytes do not readily undergo lipid peroxidation by hydroperoxides, however sickle erythrocytes are subject to lipid peroxidation under these conditions. N-acetyl cysteine was seen to protect against lipid peroxidation of sickle erythrocytes at 1 mM concentration. In contrast, mercaptopropionyl glycine amplified oxidative stress in this study, perhaps as a result of an interaction with haemoglobin, producing an intermediate that promotes oxidation (Udupi and Rice-Evans 1993).

5.4.3 Interaction with DNA damaging agents: protection and activation

Thiols are recognized as radioprotectors (Clayman and Copeland 1982) and as playing an important role in protection against mutagenesis and carcinogenesis (Jernström *et al.* 1993). As discussed above, these protective actions are elicited through thiol nucleophilicity and ease of thiol oxidation. In many cases anticancer agents act through DNA damage. In this case thiol protection can lead to drug resistance, a problem that can be overcome through the use of thiol-activated drugs.

Many antineoplastic agents contain a quinone nucleus which is prone to one- and two-electron enzymatic bioreduction by flavoenzymes such as NADPH-

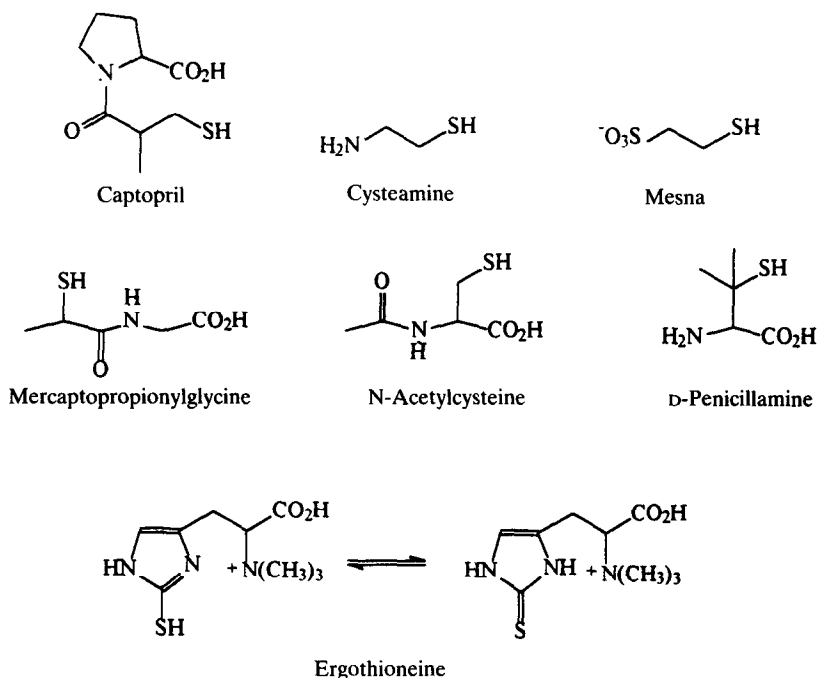


Figure 5.8 Thiols assessed for their ability to protect against UV radiation

cytochrome P450 reductase and DT-diaphorase. The one- and two-electron reduction products of these enzymes, semi- and hydroquinones, respectively, are capable of undergoing autoxidation and can generate reactive oxygen species (reaction 3).



Thiols such as glutathione protect against these reactive species by forming less reactive thiyl radicals and disulfide radical anions according to reactions 4, 5 and 6. It is of note that reaction 5 produces a shift from oxidizing to reducing radicals (Guilvi and Cadenas 1994).



In addition to protecting DNA from radical damage, thiols can also act as repair agents (Becker *et al.* 1994). Mechanistically, this can proceed as outlined above following reactions 7–10.



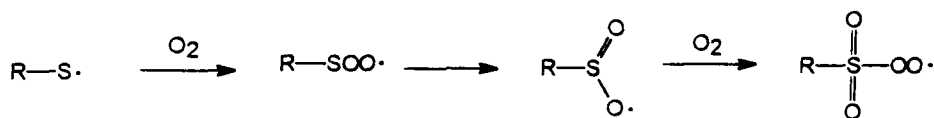


Figure 5.9 The production of thiol peroxy radicals

However, competing with protection through the reactions above, is the production of thiol peroxy radicals (Figure 5.9) which are capable of hydrogen abstraction thus leading to DNA inactivation. Furthermore, reactions of this type suppress formation of the protective $\text{RSSR}^{\cdot-}$ radical.

In their comparative study of these processes using cysteamine and glutathione, Becker and coworkers note that whilst both thiols afford the expected radioprotection, the situation with glutathione is somewhat complicated by the ease of abstraction of the cysteinyl α -proton (Becker *et al.* 1994). This reaction yields the carbon-centred radical, $\cdot\text{GSH}$, perhaps accounting for the lower efficiency of GSH in radioprotection.

Glutathione plays an additional detoxifying role where cytotoxicity is also elicited through macromolecule alkylation (Cramer *et al.* 1988). This mode of detoxification operates through provision of an alternative nucleophilic target for potentially DNA-damaging electrophilic species. The higher nucleophilicity of the thiol group compared to that of the weakly nucleophilic exocyclic amino groups of adenine and guanine aids this process. Whilst many xenobiotics are not initially electrophilic, many otherwise unreactive agents are metabolically activated through the action of mixed function oxidase (MFO)-mediated epoxidation. This mode of activation, whose physiological role is to allow conjugation prior to excretion, provides an electrophilic species with DNA-alkylating capability. For instance, the well-known tumorigenic properties of polyaromatic hydrocarbons and aflatoxin B₁ arise through MFO epoxidation. Glutathione can act, in conjunction with GST, to intercept the electrophilic epoxide prior to DNA alkylation (Croy *et al.* 1978) (Figure 5.10).

Where drug action depends on causing DNA damage, as is the case for many anticancer agents, thiol-mediated protection can lead to resistance. For instance, glutathione is found to inactivate the semiquinone- and hydroquinone-activated forms of mitomycin C, leading to a decrease in DNA adduct formation. By contrast, the disulfide-containing mitomycin C analogue, KW-2149 (Figure 5.11), is activated by cellular thiols and is not dependent on the DT-diaphorase or cytochrome P450 reductase reduction outlined above. Activation by glutathione and cysteine occurs at concentrations as low as 0.1 mM and is distinct at the physiologically relevant concentration of 1 mM. Whilst a detailed mechanism for the thiol-mediated activation of KW-2149 has not been elucidated, it is clear that reduction of the disulfide function by cellular thiols presents a thiolate anion which may be responsible for the nonenzymatic activation of the quinone (Lee *et al.* 1994) (Figure 5.11).

Calicheamicin γ_1^{I} provides a further example of thiol activation. Calicheamicin γ_1^{I} is a trisulfide prodrug which is reduced by thiols to a thiolate anion, which in turn undergoes a series of cyclisations yielding a diradical intermediate (Figure 5.12). The diradical species abstracts protons from DNA and the resulting DNA radicals are oxidatively 'fixed' giving DNA cleavage (Chatterjee *et al.* 1993).

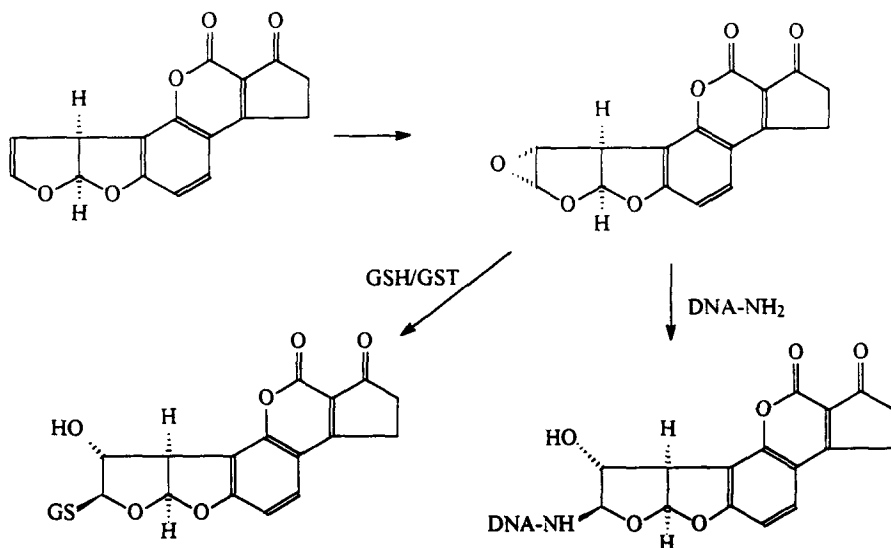


Figure 5.10 The competing pathways of DNA alkylation and glutathione alkylation by aflatoxin B₁ epoxide

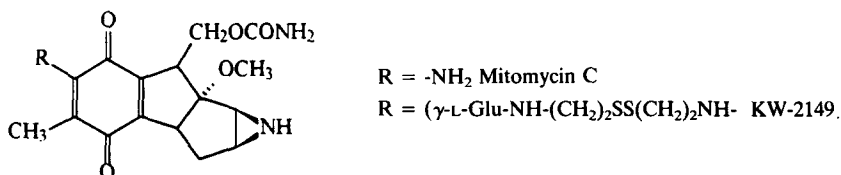


Figure 5.11 The structures of mitomycin C and KW-2149

5.5 Thiol-mediated DNA damage

In addition to providing a defence against DNA damage, the cleavage of DNA in the presence of Cu(II) and thiols has been reported (John and Douglas 1989) and the mechanism recently evaluated (John and Douglas 1993). It was observed that a Cu(II)/thiol system mediated DNA single-strand breaks. Although these occurred at all possible sites within a test DNA fragment, a pattern of preferred sites was elucidated. Two important features were noted: (i) the cleavage pattern was largely independent of the thiol used, i.e. was characteristic of the DNA fragment, with preferential cleavage at cystosine within AC sequences; and (ii) out of a range of metal ions tested, only copper elicited thiol-induced DNA cleavage. These results suggested that DNA cleavage, being thiol-independent, was likely to arise through a reactive oxygen species such as the hydroxyl radical since hydroxyl radical generation by Cu(II) is not unknown (Chiou 1983). It is interesting to note, however, that Fe(II)-generated hydroxyl radicals are 'sequence-neutral', bearing no sequence specificity. The reasons for target selectivity with Cu(II) and thiols are not, as yet, fully clear, but may be due to transfer of a copper ion from the thiol to DNA (Pruetz 1994).

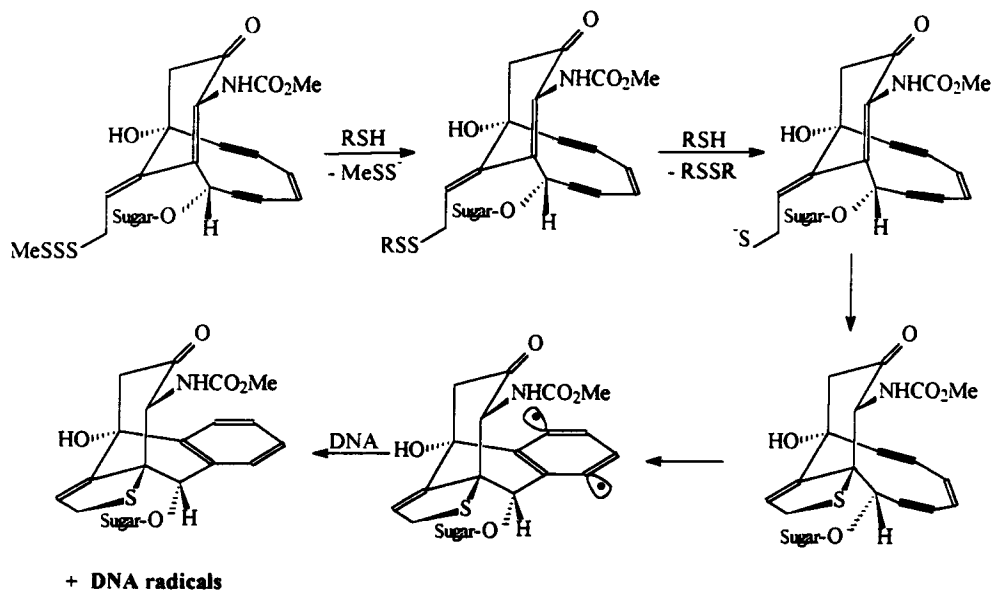


Figure 5.12 Activation of calicheamicin γ_1^I by thiols: formation of a DNA damaging diradical

5.6 Metal binding by thiols

The ability of thiols to bind metals is an important feature of various pharmaceutical agents, and this section represents an illustration of thiolic metal binding by the drugs penicillamine and captopril.

5.6.1 Penicillamine

D-Penicillamine is used in the treatment of several disorders, such as severe active rheumatoid arthritis, Wilson's disease, lead poisoning, cystinuria, primary biliary cirrhosis and active chronic hepatitis (Coleman and Park 1989). Both enantiomers of penicillamine have similar therapeutic properties, however L-penicillamine is not used clinically because it is a potent agonist of vitamin B₆, leading to pyridoxal phosphate deficiency (Kuchinskas and du Vigneaud 1957). Its use in the treatment of Wilson's disease (caused by copper accumulation) and heavy metal poisoning (Walshe 1956, 1981) arises from the ability of penicillamine to act as a bidentate ligand for metal ions. The complexes formed may be monomeric, dimeric or oligomeric (Muijsers *et al.* 1981) and may be excreted, as is the case with copper and lead, or result in the redistribution of the metal between tissues (Lyle 1981). It is possible that metal complexes are responsible for some of the therapeutic effects of penicillamine. For instance, the superoxide dismutase activity of the copper complex (Lengfelder *et al.* 1979) may be responsible for some of the observed anti-inflammatory effects, and its ability to inhibit T cell proliferation may be the reason for immunosuppression by penicillamine (Lipsky 1984). The

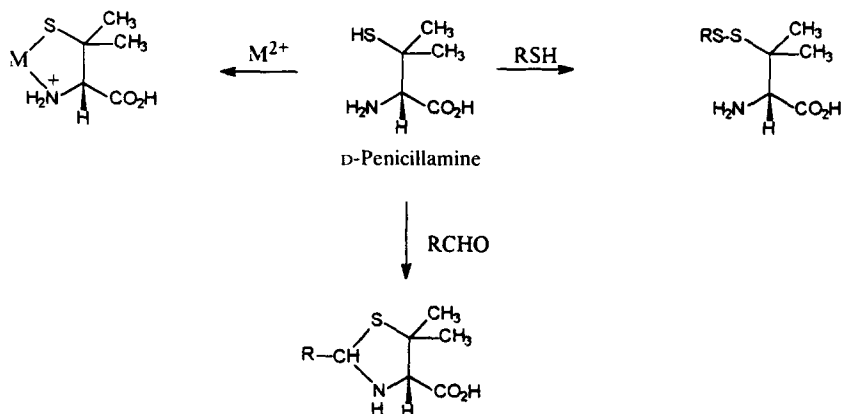


Figure 5.13 Important processes in penicillamine activity

role of penicillamine in other diseases is not as clear, although several possible modes of action have been recognized. These include the formation of unsymmetrical disulfides with protein thiols (Coleman *et al.* 1988) and formation of thiazolidine rings with the aldehyde functionality of pyridoxal phosphate (Kuchinskas and du Vigneaud 1957) and collagen (Deshmukh and Nimni 1969; Siegel 1977) (Figure 5.13).

5.6.2 Captopril

The angiotensin-converting enzyme (ACE) inhibitor captopril (D-3-mercapto-2-methylpropanoylproline) is a further example of a pharmaceutically active thiol whose mode of action is dependent on the metal binding capability of the thiol group (Cushman *et al.* 1977, 1978). It is used for the treatment of hypertension and congestive heart failure and competitively inhibits ACE. Its binding is stabilized through chelation of the active site Zn^{2+} ion, as well as through satisfaction of other hydrogen-bonding and van der Waals interactions. Captopril is metabolized following the expected pathways of disulfide formation with proteins and nonprotein thiols, such as glutathione, and S-methylation (Drummer and Jarrott 1986). Formation of mixed disulfides of captopril with plasma proteins is extensive (Wong *et al.* 1981). This pool of symmetrical and mixed disulfides can be viewed as a reservoir of the drug which can be converted to the free thiol form reductively or by thiol-disulfide exchange either before or after uptake into the cell (Migdalof *et al.* 1984).

5.7 Coenzymes containing a thiolic functionality

Thiol esters play an essential role in carboxylic acid chemistry in living systems. The thiol ester can serve as a carboxylic derivative that is more reactive towards nucleophilic attack than is the corresponding oxygen ester. Thiol esters can also serve to hold temporarily a carboxylic acid unit attached to an enzyme. The cysteinyl thiol group of a peptide can form a covalent link as a thiol ester.

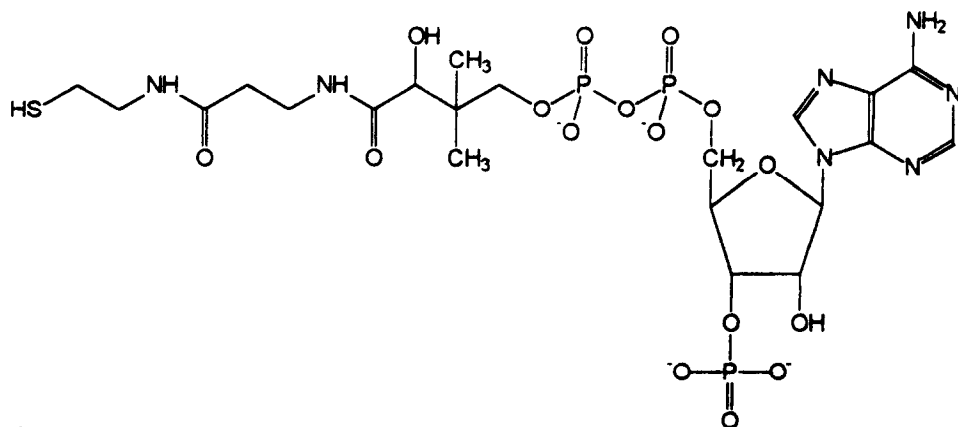


Figure 5.14 The structure of coenzyme A

Coenzyme A (CoA) is the universal carrier of acyl groups and as such is a central molecule to respiratory metabolism (Figure 5.14). The terminal sulfydryl group in CoA is the reactive site, acyl groups being linked to CoA by a thiol ester bond resulting in the formation of acyl-CoA compounds. The acetyl group is the acyl unit most often associated with the coenzyme. The ΔG° for acetyl-CoA has a large negative value, hence its hydrolysis is more favourable than that of an oxygen ester because the double bond character of the C—O bond does not extend significantly to the C—S bond. As a consequence of this, acetyl-CoA has a high acetyl group transfer potential. Acetyl-CoA is involved in fatty acid oxidation and synthesis, pyruvate oxidation and biological acetylations. It can be formed as a result of the enzymatic oxidation of pyruvate or fatty acids, or from free acetate in a reaction catalysed by acetyl-CoA synthetase. The combustion of the acetyl group by the citric acid cycle and oxidative phosphorylation to produce carbon dioxide and water results in the production of ATP and other high-energy compounds, and is an essential component of respiratory metabolism. Fatty acid breakdown takes place in the mitochondria and the intermediates are bound to the thiol group of CoA. Fatty acid chains are constructed by the addition of two-carbon units derived from acetyl-CoA and acetate units are activated by formation of malonyl-CoA at the expense of ATP.

5.8 Summary

Chemistry: Thiols can be compared to alcohols, with which they are direct analogues. Both thiols and alcohols are acidic, nucleophilic, subject to eliminations, capable of complexing metal ions and undergoing redox processes. Being directly below oxygen in the periodic table, sulfur is larger, can expand its valency beyond two and its electrons are more easily polarized. Thus the sulfydryl group is more acidic, a better nucleophile, tends to undergo eliminations more readily, complexes softer cations strongly and is subject to a different range of redox processes. The major thiol metabolites reflect this chemistry.

Metabolism: The metabolic processes which enable the elimination of foreign compounds (xenobiotics) from living systems can be divided into phase I

(activation), Phase II (conjugation) and phase III (conjugate metabolism). Phase I metabolism of thiols is principally oxidative and results in the formation of sulfur acids and disulfides. In phase II reactions the endogenous thiol glutathione can act as a conjugating agent for electrophilic xenobiotics, while nucleophilic xenobiotic thiols are conjugated to methyl and glucuronyl groups. Many S-methylations occur following thiolation via CCBL-mediated cleavage of a cysteine conjugate, the resultant thiols being subsequently methylated. Cysteine conjugate metabolism, which represents the phase III metabolism of glutathione conjugates, can also result in the generation of reactive thiols which, through rearrangement, can be mutagenic and/or cytotoxic.

Redox and DNA damage: Thiols can undergo a plethora of redox processes. Amongst the most important of these are the thiol-disulfide couple and the formation of thiyl radicals. The thiol-disulfide couple is central to maintenance of the cellular redox state. It can also be seen as a protective mechanism against oxidative stress and can be harnessed in drug activation. The ease of thiyl radical formation provides a protective mechanism against free radical-induced DNA damage. However, in the presence of copper, thiols can generate DNA-damaging hydroxyl radicals.

Metal binding: The ability of thiols to bind metals is an important feature of various pharmaceutical agents such as penicillamine, which is used in the treatment of several disorders such as severe active rheumatoid arthritis, Wilson's disease, lead poisoning, cystinuria, primary biliary cirrhosis and active chronic hepatitis, and captopril which is an angiotensin-converting enzyme (ACE) inhibitor.

Coenzymes: Coenzyme A (CoA) is the universal carrier of acyl groups and as such is a central molecule to respiratory metabolism. The terminal sulfydryl group in CoA is the reactive site. The acetyl group is the acyl unit most often associated with the coenzyme.

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5.9 References

- ADIWIDJAJA, G., KIRCH, C., PEDERSEN, F., SCHAUMANN, E., SCHMERSE, G. C. & SENNING, A., 1991, Generation and trapping of dichlorothioketene; *Chemische Berichte*, **124**, 1485-1487.
- APHOSHIAN, H. V., 1983, DMSA and DMPS-water soluble antidotes for heavy metal poisoning, *Annual Review of Pharmacology and Toxicology*, **23**, 193-215.
- ARUOMA, O. I., HALLIWELL, B., HOEY, B. M. & BUTLER, J., 1989, The antioxidant action of N-acetylcysteine: its reaction with hydrogen peroxide, *Free Radical Biology and Medicine*, **6**, 593-597.
- ASHRAF, W., DAWLING, S. & FARROW, L. J., 1994, Systemic paraphenylenediamine

- poisoning: a case report and review, *Human and Experimental Toxicology*, **13**, 167–170.
- BAKKE, J. E., 1986, Catabolism of glutathione conjugates, in Paulson, G. D., Caldwell, J., Hutson, D. H. & Menn, J. J. (Eds), *Xenobiotic Conjugation Chemistry*, ACS Symposium Series 299, pp. 301–321, Washington: American Chemical Society.
- BAKKE, J. E. & GUSTAFSSON, J.-A., 1984, Mercapturic acid pathway metabolites of xenobiotics: generation of potentially toxic metabolites during enterohepatic circulation, *Trends in Pharmacological Sciences*, **December**, 517–521.
- BAKKE, J. E., LARSEN, J. L., ASCHBACHER, P. W., RAFTER, J. J., GUSTAFSSON, J. A., & GUSTAFSSON, B. E., 1981, Role of gut microflora in metabolism of glutathione conjugates of xenobiotics, in Rosen, J. D., Magee, P. S. & Casida, J. E. (Eds), *Sulphur in Pesticide Action and Metabolism*, ACS Symposium Series 158, pp. 165–178, Washington: American Chemical Society.
- BECKER, D., SUMMERFIELD, S., GILLICH, S. & SEVILLA, M. D., 1994, Influence of oxygen on the repair of direct radiation damage to DNA by thiols in model systems, *International Journal of Radiation Biology*, **65**, 537–548.
- DEN BESTEN, C., BROUWER, A., RIETJENS, I. M. C. M. & VAN BLADEREN, P. J., 1994, Biotransformation and toxicity of halogenated benzenes, *Human and Experimental Toxicology*, **13**, 866–875.
- BHATTACHARYA, R. K. & SCHULTZE, M. O., 1967, Enzymes from bovine and turkey kidneys which cleave S-(1,2-dichlorovinyl)-L-cysteine, *Comparative Biochemistry and Physiology*, **22**, 723–735.
- 1971a, Properties of DNA isolated from tissues of calves treated with S-(1,2-dichlorovinyl)-L-cysteine. I. Chemical and physical properties, *Archives of Biochemistry and Biophysics*, **145**, 565–574.
- 1971b, Properties of DNA isolated from tissues of calves treated with S-(1,2-dichlorovinyl)-L-cysteine. II. Primer template activity for bacterial DNA polymerases, *Archives of Biochemistry and Biophysics*, **145**, 575–582.
- 1972, Properties of DNA treated with S-(1,2-dichlorovinyl)-L-cysteine and a lyase, *Archives of Biochemistry and Biophysics*, **153**, 105–115.
- 1973a, Modification of polynucleotides by a fragment produced by the cleavage of S-(1,2-dichlorovinyl)-L-cysteine, *Biochemical and Biophysical Research Communications*, **53**, 172–181.
- 1973b, Hybridisation of DNA modified from S-(1,2-dichlorovinyl)-L-cysteine, *Biochemical and Biophysical Research Communications*, **54**, 538–543.
- BRAUNSTEIN, A. E. & SHEMAKIN, M. M., 1953, A theory of amino acid metabolic processes catalysed by pyridoxal phosphate dependent enzymes, *Biokhimiya*, **18**, 393–411.
- BREMER, J. & GREENBERG, D. M., 1961, Enzymic methylation of foreign sulfhydryl compounds, *Biochimica et Biophysica Acta*, **46**, 217–224.
- BRIDGEMAN, M. M. E., MARSDEN, M., MACNEE, W., FLENLEY, D. C. & RYLE, A. P., 1991, Cysteine and glutathione concentrations in plasma and bronchoalveolar lavage fluid after treatment with N-acetyl-L-cysteine, *Thorax*, **46**, 39–42.
- VAN DEN BROEKE, L. T. & BEYERSBERGEN VAN HENEGUOWEN, G. M. J., 1993, Thiols as potential UV radiation protectors: an *in vitro* study, *Journal of Photochemistry and Photobiology B: Biology*, **17**, 279–286.
- BUCKBERRY, L. D., BLAGBROUGH, I. S., BYCROFT, B. W. & SHAW, P. N., 1990, Human renal C-S lyases: two cytosolic enzymes, *Toxicology Letters*, **53**, 253–255.
- 1992, Kynurenine aminotransferase activity in human liver: identity with human hepatic C-S lyase activity and a physiological role for this enzyme, *Toxicology Letters*, **60**, 241–246.
- 1993a, Bovine pulmonary, hepatic and renal tissues: models for the study of mammalian C-S lyase enzymes, *ATLA*, **21**, 360–370.

- 1993b, Cysteine conjugate toxicity in a human cell line: correlation with C-S lyase activity in human hepatic tissue, *Human and Experimental Toxicology*, **12**, 329-335.
- 1994, Human renal, hepatic, and pulmonary C-S lyases: possible catalysts for the biosynthesis of toxic thiols in phase 3 metabolism, *Pharmaceutical Science Communications*, **4**, 107-116.
- BUTTERWORTH, M., UPSHALL, D. G., SMITH, L. L., & COHEN, G. C., 1991, Cysteine isopropyl ester protects against paracetamol-induced toxicity, *Biochemical Pharmacology*, **43**, 483-488.
- CHASSEAUD, L. F., 1976, Conjugation with glutathione and mercapturic acid excretion, in Arias, I. M. & Jakoby, W. B. (Eds), *Glutathione Metabolism and Function*, pp. 77-114, New York: Raven Press.
- CHATTERJEE, M., CRAMER, K. D. & TOWNSEND, C. A., 1993, Kinetic nature of thiol activation in DNA cleavage by Calicheamicin, *Journal of the American Chemical Society*, **115**, 3374-3375.
- CHIOU, S.-H., 1983, DNA- and protein-scission activities of ascorbate in the presence of copper ion and a copper-protein complex, *Journal of Biochemistry*, **94**, 1259-1267.
- CLAPP, J. W., 1965, A new metabolic pathway for a sulfonamide group, *Journal of Biological Chemistry*, **223**, 207-214.
- CLAYMAN, D. L. & COPELAND, E. S., 1982, Radioprotective agents, in Kirk, R. E. & Othmer, D. F., (Eds), *Encyclopedia of Chemical Toxicology*, pp. 801-832, New York: John Wiley.
- COAN, C., JI, J.-Y., HIDEG, K. & MEHLHORN, R. J., 1992, Protein sulfhydryls are protected from irreversible oxidation by conversion to mixed disulfides, *Archives of Biochemistry and Biophysics*, **295**, 369-378.
- COLEMAN, J. W. & PARK, B. K., 1989, Antirheumatics: penicillamine, in Damani, L. A. (Ed.), *Sulfur-Containing Drugs and Related Organic Compounds*, Vol. 3B, pp. 47-67, Chichester: Ellis Horwood.
- COLEMAN, J. W., FOSTER, A. L., YEUNG, J. H. K. & PARK, B. K., 1988, Drug-protein conjugates. XV. A study of the disposition of D-penicillamine in the rat and its relationship to immunogenicity, *Biochemical Pharmacology*, **37**, 737-742.
- COLUCCI, D. F. & BUYSKE, D. A., 1965, The transformation of a sulfonamide to a mercaptan and to mercapturic acid and glucuronide conjugates, *Biochemical Pharmacology*, **14**, 457-466.
- COOPER, A. J. L., 1983, Biochemistry of the sulfur containing amino acids, *Annual Review of Biochemistry*, **52**, 187-222.
- COTGREAVE, I., MOLDEUS, P. & SCHUPPE, I., 1991, The metabolism of N-acetylcysteine by human endothelial cells, *Biochemical Pharmacology*, **42**, 13-16.
- CRAMER, R. A., ZAKHER, J. & KIM, G., 1988, Role of the glutathione redox cycle in acquired and *de novo* multidrug resistance, *Science*, **241**, 694-697.
- CROWE, C. A., YOUNG, A. C., CALDER, I. C., HAM, K. N. & TANGE, J. D., 1979, The nephrotoxicity of 4-aminophenol. I: The effect on microsomal cytochromes, glutathione and covalent binding in kidney and liver, *Chemico-Biological Interactions*, **27**, 235-243.
- CROY, R. G., ESSIGMAN, J. M., REINHOLD, V. N. & WOGAN, G. N., 1978, Identification of the principal aflatoxin B₁-DNA adduct formed *in vivo* in rat liver, *Proceedings of the National Academy of Sciences, USA*, **74**, 1745-1749.
- CUMMINGS, S. W. & PROUGH, R. A., 1983, Metabolic formation of toxic metabolites, in Caldwell, J. & Jakoby, W. B. (Eds), *Biological Basis of Detoxification*, pp. 1-30, New York: Academic Press.
- CUSHMAN, D. W., CHEUNG, H. S., SABO, E. F. & ONDETTI, M. A., 1977, Design of potent competitive inhibitors of angiotensin-converting enzyme. Carboxyalkanoyl and mercaptoalkanoyl amino acids, *Biochemistry*, **16**, 5484-5491.
- 1978, Design of new antihypertensive drugs: potent and specific inhibitors of angiotensin-

- converting enzyme, *Progress in Cardiovascular Diseases*, **21**, 176–182.
- DEKANT, W., VAMVAKAS, S. & ANDERS, M. W., 1994, Formation and fate of nephrotoxic glutathione S-conjugates: cysteine conjugate β -lyase pathway, in Anders, M. W. & Dekant, W. (Eds), *Advances in Pharmacology, Conjugation-Dependent Carcinogenicity and Toxicity of Foreign Compounds*, Vol. 27, pp. 115–163, London: Academic Press.
- DEL CORSO, A. & MURA, U., 1994, Thiol dependent oxidation of enzymes: the last chance against oxidative stress, *International Journal of Biochemistry*, **26**, 745–750.
- DESHMUKH, K. & NIMNI, M. E., 1969, A defect in the intramolecular and intermolecular cross-linking of collagen caused by penicillamine. II. Functional groups involved in the interaction process, *Journal of Biological Chemistry*, **244**, 1787–1795.
- DRUMMER, O. H. & JARROTT, B., 1986, The disposition and metabolism of captopril, *Medical Research Reviews*, **6**, 75–97.
- DUTTON, G. J. & ILLING, H. P. A., 1972, Mechanism of the biosynthesis of thio-beta-D-glucuronides and thio-beta-D-glucoside, *Biochemical Journal*, **129**, 5439–5550.
- EAGLE, H., MAGNUSON, H. J. & FEISCHMAN, R., 1946, Clinical uses of 2,3-dimercaptopropanol (BAL) I. The systemic treatment of experimental arsenic poisoning (mapharsen, lewisite, phenyl arsenoxide) with BAL, *Journal of Clinical Investigation*, **25**, 451–466.
- ELFARRA, A. A., BAGGS, R. B. & ANDERS, M. W., 1985, Structure–nephrotoxicity relationships of S-(2-chloroethyl)-DL-cysteine and analogues — role for an episulfonium ion, *Journal of Pharmaceutical and Experimental Therapeutics*, **233**, 512–516.
- FOWLER, L. M., MOORE, R. B., FOSTER, J. R. & LOCK, E. A., 1991, Nephrotoxicity of 4-aminophenol glutathione conjugates, *Human and Experimental Toxicology*, **10**, 451–459.
- FRIEDMAN, M., 1973, *The Chemistry and Biochemistry of the Sulfhydryl Group in Amino Acids, Peptides and Proteins*, Braunschweig: Pergamon.
- GANDOLFI, A. J., NAGLE, R. B., SOLTIS, J. J. & PLESCIA, F. H., 1981, Nephrotoxicity of halogenated vinyl cysteine compounds, *Research Communications in Chemical Pathology and Pharmacology*, **33**, 249–261.
- GASKIN, P. J., ADCOCK, H. J., BUCKBERRY, L. D., TEESDALE-SPITTLE, P. H. & SHAW, P. N., 1995, The C–S lysis of L-cysteine conjugates by aspartate and alanine aminotransferase enzymes, *Human and Experimental Toxicology*, **14**, 422–427.
- GIBSON, G. G. & SKETT, P., 1994, *Introduction to Drug Metabolism*, London: Chapman & Hall.
- GRAVINA, S. A. & MIEYAL, J. J., 1993, Thioltransferase is a specific glutathionyl mixed disulfide oxidoreductase, *Biochemistry*, **32**, 3368–3376.
- GREEN, T. & ODUM, J., 1985, Structure/activity studies of the nephrotoxic and mutagenic action of cysteine conjugates of chloro- and fluoroalkenes, *Chemico-Biological Interactions*, **54**, 15–31.
- GUILVI, C. & CADENAS, E., 1994, One- and two-electron reduction of 2-methyl-1,4-naphthoquinone bioreductive alkylating agents: kinetic studies, free radical production, thiol oxidation and DNA-strand-break formation, *Biochemical Journal*, **301**, 21–30.
- HARRIS, J. W., DEKANT, W. & ANDERS, M. W., 1992, *In vivo* detection and characterisation of protein adducts resulting from bioactivation of haloethene cysteine conjugates by ^{19}F NMR: chlorotrifluoroethene and tetrafluoroethene, *Chemical Research in Toxicology*, **5**, 34–41.
- HAYDEN, P. J. & STEVENS, J. L., 1990, Cysteine conjugate toxicity, metabolism, and binding to macromolecules in isolated rat kidney mitochondria, *Molecular Pharmacology*, **37**, 468–476.
- HAYDEN, P. J., ICHIMURA, T., MCCANN, D. J., POHL, L. R. & STEVENS, J. L., 1991a, Detection of cysteine conjugate metabolite adduct formation with specific mitochondrial

- proteins using antibodies raised against haloethane metabolite adduct, *Journal of Biological Chemistry*, **266**, 18415-18418.
- HAYDEN, P. J., YANG, Y., WARD, A. J. I., DULIK, D. M., McCANN, D. J. & STEVENS, J. L., 1991b, Formation of difluorothionoacetyl-protein adducts by S-(1,1,2,2-tetrafluoroethyl)-L-cysteine metabolites: nucleophilic catalysis of stable adduct formation by histidine and tyrosine, *Biochemistry*, **30**, 5935-5943.
- HOBBS, M. & UPSHALL, D. G., 1993, Structure activity relationships of cysteine esters and their effects on thiol levels in rat lung *in vitro*, *Biochemical Pharmacology*, **45**, 1605-1612.
- HUANG, L. E., ZHANG, H., BAE, S. W. & LIU, A. Y.-C., 1994, Thiol reducing reagents inhibit the heat shock response, *Journal of Biological Chemistry*, **48**, 30718-30725.
- ILLING, H. P. A. & BENFORD, D. J., 1976, Observations on the accessibility of acceptor substrates to the active centre of UDP-glucuronosyltransferase, *Biochimica et Biophysica Acta*, **429**, 768-779.
- INNS, R. H. & RICE, P., 1993, Efficacy of dimercapto chelating agents for the treatment of poisoning by percutaneously applied dichloro(2-chlorovinyl)arsine in rabbits, *Human and Experimental Toxicology*, **12**, 241-246.
- JAKOBY, W. B. & STEVENS, J. L., 1984, Cysteine conjugate β -lyase and the thiomethyl shunt, *Biochemical Society Transactions*, **12**, 33-35.
- JERNSTRÖM, B., MORGENSTERN, R. & MOLDEUS, P., 1993, Protective role of glutathione, thiols and analogues in mutagenesis and carcinogenesis, *Basic Life Sciences*, **61**, 137-147.
- JOHN, D. C. A. & DOUGLAS, K. T., 1989, Apparent sequence preference in cleavage of linear B-DNA by the Cu(II):thiol system, *Biochemical and Biophysical Research Communications*, **165**, 1235-1242.
- 1993, Sequence-dependent reactivity of linear DNA to chemical cleavage by Cu(II): thiol combinations including cysteine or glutathione, *Biochemical Journal*, **289**, 463-468.
- KADLUBAR, F. F., MILLER, J. A. & MILLER, E. C., 1976, Hepatic metabolism of N-hydroxy-N-methyl-4-aminoazobenzene and other N-hydroxy arylamines to reactive sulfuric acid esters, *Cancer Research*, **36**, 2350-2359.
- KETTERER, B. & CHRISTODOULIDES, L. G., 1994, Enzymology of cytosolic glutathione-S-transferases, in Anders, M. W. & Dekant, W. (Eds), *Advances in Pharmacology, Conjugation-Dependent Carcinogenicity and Toxicity of Foreign Compounds*, Vol. 27, pp. 37-70, London: Academic Press.
- KUCHINSKAS, E. J. & DU VIGNEAUD, V., 1957, An increased vitamin B₆ requirement in the rat on a diet containing L-penicillamine, *Archives of Biochemistry and Biophysics*, **68**, 69-75.
- LAILEY, A. F. & UPSHALL, D. G., 1994, Thiol levels in rat bronchio-alveolar lavage fluid after administration of cysteine esters, *Human and Experimental Toxicology*, **13**, 776-780.
- LAILEY, A. F., HILL, L., LAWSTON, I. W., STANTON, D. & UPSHALL, D. G., 1991, Protection by cysteine esters against chemically induced pulmonary oedema, *Biochemical Pharmacology*, **42**, S47-S54.
- LARSEN, G. L. & STEVENS, J. L., 1985, Cysteine conjugate β -lyase in the gastrointestinal bacterium *Eubacterium limosum*, *Molecular Pharmacology*, **15**, 199-209.
- LASH, L. H., NELSON, R. M., VAN DYKE, R. A. & ANDERS, M. W., 1990, Purification and characterisation of human kidney cytosolic cysteine conjugate β -lyase activity, *Drug Metabolism and Disposition*, **18**, 50-54.
- LEE, J.-H., NAITO, M. & TSURUO, T., 1994, Non enzymatic reductive alkylolation of 7-N-{2-[[2-(γ -L-glutamylamino)ethyl]dithio]ethyl}} mitomycin C by thiol molecules: a novel mitomycin C derivative effective on mitomycin C-resistant tumor cells, *Cancer Research*, **54**, 2398-2403.
- LENGFELDER, E., FUCHS, C., YOUNES, M. & WESNER, U., 1979, Functional aspects

- of the superoxide dismutase action of Cu-penicillamine, *Biochimica et Biophysica Acta*, **567**, 492–502.
- LIPSKY, P. E., 1984, Immunosuppression by D-penicillamine *in vitro*. Inhibition of human T cell lymphocyte proliferation by copper or ceroplasmin-dependent generation of hydrogen peroxide and protection by monocytes, *Journal of Clinical Investigation*, **73**, 53–65.
- LOCK, E. A., CROSS, T. J. & SCHNELLMANN, R. G., 1993, Studies on the mechanism of 4-aminophenol-induced toxicity to renal proximal tubules, *Human and Experimental Toxicology*, **12**, 383–388.
- LYLE, W. H., 1981, Penicillamine and metal poisoning, *Journal of Rheumatology Supplement*, **7**, 96–99.
- LYTTLE, M. H., HOCKER, M. D., HUI, H. C., CALDWELL, C. G., AARON, D. T., ENGQVIST-GOLDSTEIN, Å., FLATGUARD, J. E. & BAUER, K. E., 1994, Isozyme-specific glutathione-S-transferase inhibitors: design and synthesis, *Journal of Medicinal Chemistry*, **37**, 189–194.
- MAGHSOUDLOO, M., UPSHALL, D. G. & COHEN, G. M., 1994, Neurotoxicity of L-cysteine and L-cysteine isopropyl ester is species and age dependent, *Human and Experimental Toxicology*, **13**, 204–209.
- MAYER, R. & SCHEITHAUER, S., 1976, Thiocarbonsaure-halogenide, in Mueller, E. (Ed.), *Methoden der Chemie*, pp. 615–627, Germany: Houben-Wey.
- MAZELIS, M., 1963, Demonstration and characterisation of cysteine sulfoxide lyase in the *Cruciferae*, *Phytochemistry*, **2**, 15–22.
- MAZELIS, M., BEIMER, N. & CREVELING, R. K., 1967, Cleavage of L-cysteine by soluble enzyme preparations from *Brassica* species, *Archives of Biochemistry and Biophysics*, **120**, 371–378.
- McKINNEY, L. L., WEAKLEY, F. B., ELDRIDGE, A. C., CAMPBELL, R. E., COWAN, J. C. & BIESTER, H. E., 1957, S-(1,2-Dichlorovinyl)-L-cysteine: an agent causing fatal aplastic anaemia in calves, *Journal of the American Chemical Society*, **79**, 3932–3933.
- McKINNEY, L. L., PICKEN, J. C., WEAKLEY, F. B., ELDRIDGE, A. C., CAMPBELL, R. E., COWAN, J. C. & BIESTER, H. E., 1959, Possible toxic factor of trichloroethylene extracted soybean oil meal, *Journal of the American Chemical Society*, **81**, 909–915.
- MERTENS, J. J. W. M., TEMMINK, J. H. M., VAN BLADEREN, P. J., JONES, T. W., LO, H.-H., LAU, S. S. & MONKS, T. J., 1991, Inhibition of γ -glutamyl transpeptidase potentiates the nephrotoxicity of glutathione conjugated chlorohydroquinones, *Toxicology and Applied Pharmacology*, **110**, 45–60.
- METZLER, D. E., IKAWA, M. & SNELL, E. E., 1954, A general mechanism for vitamin B6 catalysed reactions, *Journal of the American Chemical Society*, **76**, 648–653.
- MIGDALOF, B. H., ANTONACCIO, M. J., MCKINSTRY, D. N., SINGHVI, S. M., LAN, S.-J., EGLI, P. & KRIPALANI, K. J., 1984, Captopril: pharmacology, metabolism and disposition, *Drug Metabolism Reviews*, **15**, 841–869.
- MUIJSERS, A. O., VAN DER STADT, R. J., HENRICHS, A. M., BAAS, J. & VAN DER KORST, J. K., 1981, D-Penicillamine and copper in rheumatoid arthritis, in Maini, R. N. & Berry, H., (Eds), *Modulation of Autoimmunity and Disease: The Penicillamine Experience*, pp. 150–158, London: Praeger Westport Publications.
- MULLER, D. A., URBAN, G. & DEKANT, W., 1991, Sulfur containing proreac-tive intermediates hydrolysis and mutagenicity of halovinyl 2-nitrophenyl disulfides, *Chemico-Biological Interactions*, **77**, 159–172.
- NOCK, L. P. & MAZELIS, M., 1985, Some new observations on the properties of garlic allinase, *Plant Physiology*, **77**(Suppl.), 115.
- PARKE, D. V., 1952, The metabolism of aromatic compounds, PhD Thesis, University of London.

- PARKE, D. V. & WILLIAMS, R. T., 1951, Studies in detoxification 38: the metabolism of benzene, *Biochemical Journal*, **48**, 624–629.
- PATAI, S. (Ed.) 1974, *The Chemistry of the Thiol Group, Parts 1 and 2*, London: Wiley-Interscience.
- PRUETZ, W. A., 1994, Interaction between glutathione and Cu(II) in the vicinity of nucleic acids, *Biochemical Journal*, **302**, 373–382.
- RANNUG, U., SUNDVALL, A. & RAMEL, C., 1978, The mutagenic effect of 1,2-dichloroethane on *Salmonella typhimurium*. I. Activation through conjugation with glutathione *in vitro*, *Chemico-Biological Interactions*, **20**, 1–16.
- REMY, C. N., 1963, Metabolism of thiopyrimidines and thiopurines. S-Methylation with S-adenosylmethionine transmethylase and catabolism in mammalian tissues, *Journal of Biological Chemistry*, **238**, 1078–1084.
- RENNER, G., 1983, Formation of pentachlorothioanisole from N-acetyl-S-(pentachlorophenyl)-cysteine in blood and liver of rats *in vivo*, *Life Science*, **33**, 1427–1431.
- SANDBERG, V. A., KREN, B., FUCHS, J. A. & WOODWARD, C., 1991, *Escherichia coli* glutaredoxin: cloning and overexpression; thermodynamic stability of the oxidised forms and report of an N-terminal extended species, *Biochemistry*, **30**, 5475–5484.
- SCRIBNER, J. D., MILLER, J. A. & MILLER, E. C., 1965, 3-Methylmercapto-N-methyl-4-aminoazobenzene; an alkaline-degradation product of a labile protein-bound dye in the livers of rats fed N,N-dimethyl-4-aminoazobenzene, *Biochemical and Biophysical Research Communication*, **20**, 560–565.
- SIEDLER, F., RUDOLPH-BÖHNER, S., DOI, M., MUSIOL, H.-J. & MORODER, L., 1993, Redox potentials of active site bis(cysteiny) fragments of thiol-protein oxidoreductases, *Biochemistry*, **32**, 7488–7495.
- SIEGEL, R. C., 1977, Effect of D-penicillamine on collagen cross-linking *in vitro*, *Journal of Biological Chemistry*, **252**, 254–259.
- STEVENS, J. L., 1985, Isolation and characterisation of a rat liver enzyme with both cysteine conjugate β -lyase and kynureninase activity, *Journal of Biological Chemistry*, **260**, 7945–7950.
- STILLWELL, W. G., 1981, A new pathway of drug metabolism, *Trends in Pharmacological Sciences*, **2**, 250–252.
- STOCKMAN, S., 1916, Cases of poisoning in cattle by feeding on meal from soya bean after extraction of the oil, *Journal of Comparative Pathology and Therapeutics*, **29**, 95–107.
- SULZBERGER, M. B., BAER, R. L. & KANOF, A., 1946, Clinical uses of 2,3-dimercaptopropanol (BAL). III. Studies on the toxicity of BAL on percutaneous and parenteral administration, *Journal of Clinical Investigation*, **25**, 474–479.
- TATEISHI, M., 1983, Methylthiolated metabolites, *Drug Metabolism Reviews*, **14**, 1207–1234.
- TATEISHI, M., SUZUKI, S. & SHIMIZU, H., 1978, Cysteine conjugate β -lyase activity in rat liver, *Journal of Biological Chemistry*, **253**, 8854–8859.
- TATEISHI, M., TOMISAWA, H., FUKAZAWA, H. & ICHIHARA, S., 1987, Metabolism of N-acetyl-L-cysteine conjugate and related enzymes, *Yakugaku Zasshi*, **107**, 1–16.
- TERADA, T., 1994, Thioltransferase can utilise cysteamine as same as glutathione as a reductant during the restoration of cystamine treated glucose 6-phosphate dehydrogenase activity, *Biochemistry and Molecular Biology International*, **34**, 723–727.
- TOET, A. E., VAN DIJK, A., SABELKOU, T. J. F. & MEULENBELT, J., 1994, Mercury kinetics in a case of severe poisoning treated with dimercapto-1-propane sulfonate (DMPS), *Human and Experimental Toxicology*, **13**, 11–16.
- TOMISAWA, H., ICHIHARA, S., FUKAZAWA, H., ICHIMOTO, N., TATEISHI, M. & YAMAMOTO, I., 1986, Purification and characterisation of human hepatic cysteine conjugate β -lyase, *Biochemical Journal*, **235**, 569–575.

- UDUPI, V. & RICE-EVANS, C., 1993, Thiol compounds as protective agents in erythrocytes under oxidative stress, *Free Radical Research Communications*, **16**, 315–323.
- WALSHE, J. M., 1956, Penicillamine, as new oral therapy for Wilson's disease, *American Journal of Medicine*, **21**, 487–495.
- 1981, The discovery and therapeutic use of D-penicillamine, *Journal of Rheumatology Supplement*, **7**, 3–8.
- WIENSHILBOUM, R., 1989, Thiol S-methyltransferases, I: Biochemistry, in Damani, L. A. (Ed.), *Sulfur-Containing Drugs and Related Organic Compounds*, Vol. 2A, pp. 121–142, Chichester: Ellis Horwood.
- WILDE, P. E. & UPSHALL, D. G., 1994, Cysteine esters protect cultured rodent lung slices from sulfur mustard, *Human and Experimental Toxicology*, **13**, 743–748.
- WONG, K. K., LAN, S. J. & MIGDALOF, B. H., 1981, *In vitro* biotransformations of [¹⁴C]captopril in the blood of rats, dogs and humans, *Biochemical Pharmacology*, **30**, 2643–2650.
- YANG, Y. F. & WELLS, W. W., 1991, Catalytic mechanism of thiol transferase, *Journal of Biological Chemistry*, **266**, 2766–2771.
- ZIEGLER, D. M., 1984, Metabolic oxygenation of organic nitrogen and sulfur compounds, in Mitchell, J. R. & Horning, M. G. (Eds), *Drug Metabolism and Drug Toxicity*, pp. 33–53, New York: Raven Press.

Sulfur–Sulfur Compounds

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6.1 Introduction

Sulfur–sulfur bonds are the backbone of biochemistry. Often, they have a structural role, involved in the maintenance of protein shape and function, as in keratin (Ogawa 1989). Disulfide groups confer unique properties as they can hold the whole molecule in a fixed conformation. However, this relatively rigid frame exists in a state of dynamic equilibrium, where formation of mixed disulfides with small molecules such as cysteine or glutathione (γ -glutamyl-cysteinyl-glycine) will break the intramolecular bonding and disrupt the protein structure. The dual requirement for strong bonds which are readily made and equally readily disassembled leads to continuous formation and re-formation of sulfur–sulfur bonds, and is essential for biochemical function (Kim 1992). Proteins may also act in transmission of molecular signalling. In this latter case, the disulfide bond,

-S-S-, engages in a series of interconversions, breaking and remaking the sulfur bonds to form heterodimers with other molecules often via reduction to thiols (-SH). The loosely-held electrons in the outermost orbitals of the sulfur atoms confer a certain 'flexibility' to the electronic configuration, increasing the possibility of reactions with other sulfur-containing molecules, so that protein-protein and ligand-receptor links can be made rapidly.

6.2 Sulfur-sulfur bonds in protein

6.2.1 Protein folding and three-dimensional structure

Protein stability is quantitatively described by the standard Gibbs energy change (ΔG_D°), involved in unfolding the unique three-dimensional structure to randomly-coiled polypeptide chains. As naturally-occurring disulfide links stabilize proteins against chemical and physical degradation, the presence of these crosslinks is critical for protein function. The formation of -S-S- bridges from -SH groups occurs during the protein-folding process in the endoplasmic reticulum (ER) of eukaryotic cells and the periplasm of prokaryotes. The 'protein-folding problem' has been discussed in some detail (Betz 1993, Houk *et al.* 1987); there are still large discrepancies between the theoretical models and the experimental results and it is clear that stabilizing factors in native and denatured protein states have to be taken into consideration. Nevertheless, rearrangements of disulfide bonds in proteins are known to be catalysed by other proteins which act as thiol-disulfide oxidoreductases. Protein disulfide isomerase (PDI) is found in eukaryotes and dsbA (from the mutant disulfide bond formation A-type) in prokaryotes (Noiva 1994). These proteins are soluble and their active sites have sequences which are similar to that of the redox protein thioredoxin (Nelson and Creighton 1994). Both PDI and dsbA themselves contain -S-S- bonds and are believed to facilitate protein folding by forming heterodimer intermediates with cysteine residues on the polypeptide chains (Freedman *et al.* 1988, Goldenberg 1992). The formation and isomerization of -S-S- links then controls folding of the nascent protein into the required tertiary configuration. PDI has been shown to have chaperone-like activity, preventing aggregation of polypeptide chains and ensuring productive folding (Creighton 1988, Freedman *et al.* 1988). A 1:1 PDI:protein ratio is known to be required, giving evidence for formation of a PDI-protein complex (Puig and Gilbert 1994). Thioredoxin has similar oxido-reductive properties, also interconverting -S-S- and -SH groups, but as it does not bind to peptides it cannot catalyse the folding process (Wang and Tsou 1993). Studies on the folding of antibodies have shown that in the presence of PDI, the formation of the correct disulfide bonds is possible at higher oxidative conditions than for the spontaneous reaction (Lilie *et al.* 1994). Both PDI and dsbA, in addition to hsp 150 (heat shock protein 150) (Simonen *et al.* 1994), their equivalent in yeast (*Saccharomyces cerevisiae*) are required for protein translocation to the ER, the formation of the appropriate disulfide bonds and also for secretion. Although formation of disulfide bonds between cysteine residues on the isomerases and polypeptides is a common mechanism, this does not in itself explain what makes the process so dynamic. Studies on dsbA have shown that it has one accessible cysteine (Cys 30) and a

second cysteine which, although it is only two amino acid residues away (Cys 33), is buried in the interior of the molecule. These two cysteines can form disulfide bonds internally which (because of steric effects) are one thousand times more reactive than a normal -S-S- bond (Nelson and Creighton 1994). The mixed disulfide between either cysteine or glutathione is 10-fold more reactive still (Zapun *et al.* 1994), and this lability must be the driving force behind the disulfide interchanges and the rearrangements of protein conformation. Recently, a PDI-type protein has been localized to the plasma membrane of β -lymphocytes; it may possibly also be involved in regulation of the thiol/disulfide status of the cell membrane proteins (Kroning *et al.* 1994).

6.2.2 Stability of structural proteins

Protein disulfide formation in the lens of the eye destroys its function and leads to opacities and cataracts (Giblin 1980). This has been investigated in human lens crystallins which, as the name suggests, are normally translucent (Miesbauer *et al.* 1994); disease states such as diabetes which tend to alter the thiol redox status by decreasing the level of glutathione (via a reduction in NADPH) can therefore be associated with cataract formation. Dehydration-induced injury to biological systems and materials similarly reflects the oxidation of thiols to disulfides (Levitt 1978). The interconversions of -SH/-S-S- groups in muscle proteins under the influences of processing and long-term storage ('hanging') have been much studied. Generally, the formation of thiols rather than disulfides leads to a product which is more readily eaten and digested, but decomposition is more rapid at high free -SH content (Hofmann and Hamm 1978). Cruciferin, the main storage protein in rapeseed (*Brassica napus* L.), is a legumin-like globulin which is hexameric, composed of α and β polypeptides which are linked to disulfides. Similar -S-S-bridges occur in the cruciferins from radishes and *Arabidopsis thaliana*, and the two polypeptide classes represent biochemical markers for two different Cruciferae globulin gene families (Inquellow *et al.* 1993).

The formation of -S-S- bonds is also important in controlling the aggregation of peptide monomers into multimers and their subsequent secretion out of the cell, as has been shown for the assembly of fibronectin into the extracellular matrix (Mosher and McKeown-Longo 1985) and the assembly and secretion of human fibrinogen (Zhang and Redman 1994). Disulfide bridging also regulates and stabilizes the structures of the immunoglobulins and hence partially determines their specificity and function. This was described for the constant light-chain fragment of Ig (Hamaguchi 1982) and since then has been found to be a general feature. Haptoglobins (Hp), which bind to haemoglobin (Hb), have structures similar to the immunoglobulins. Typically, they form heterodimers with two Hb-binding units (β -chains) and two other units (α -chains) linked by disulfide bonds. However, the exact extent and location of disulfide bridging varies both intraspecies (the forms found in the dog, bear and cat lack disulfide bonds between α -chains) and interspecies (some genotypes in man and ruminants have a highly polymeric molecule with extra disulfide links between α -chains) (Morimatsu *et al.* 1993). Thiol-disulfide exchange is also important in the blood-clotting process and the role of the dissociable complex of thrombin and thrombospondin has been discussed in detail (Detwiler *et al.* 1987).

Many proteins have conserved sequence motifs with structurally distinct domains, often comprising short disulfide-stabilized sequences which are present in the extracellular portions of membrane proteins and in secreted proteins. Examples include complement C9/LDL receptor domains, immunoglobulin repeats and epidermal growth factor (EGF)-like repeats. These domains seem to be required for ligand binding and combine with themselves or with other types of domains to construct complex mosaic proteins. SRCR (scavenger receptor cysteine-rich) domains have been recognized in many proteins which are now grouped into a superfamily containing examples from mammalian, amphibian and invertebrate species. All these proteins are cysteine-rich and have up to 11 SRCR domains per chain. The mammalian SRCR-domain-containing proteins are generally expressed on the surfaces of immune system cells such as macrophages, T-cells and B-cells. They seem to be analogous to the cysteine-containing protein domains found in the immunoglobulin superfamily, suggesting that these disulfide bridges are essential for interactions involving recognition and host defence (Resnick *et al.* 1994). Increasing the intramolecular disulfide bonding, as would be expected, improves the thermal stability of a protein. Enhancement of this type has been introduced by molecular engineering techniques into proteins which are used for industrial purposes so that they are less readily degraded in commercial use (Şowdhamini and Balaram 1993, Wetzel 1987).

The determination of the positions of disulfide bonds in proteins is now usually carried out using fast atom bombardment mass spectrometry (FAB-MS) (Smith and Zhou 1990). This has shown that thiol groups which are located deep inside the protein structure tend not to form disulfide bonds in the native protein (Goto 1988), although they may do so if the protein unfolds. If this happens, the original configuration is unlikely to be regained even under reducing conditions unless a PDI-type enzyme is present. FAB-MS has been widely used to update the knowledge about protein disulfide bonds which has been collected over decades (Inoue 1989, Mannervik and Larson 1981, Straub 1968); it has the advantage of requiring only microgram amounts of materials and of giving the molecular ion, so that high precision can be obtained.

6.2.3 Regulation of enzyme activity

Any enzyme containing an accessible thiol group at or near the active site can potentially be modulated by reaction with low-molecular weight compounds containing thiols or disulfide links. Formation of mixed protein-thiol complexes or intramolecular disulfides can increase or decrease activity, since the presence of disulfide dimers can radically alter both the shape of the molecule and its potential for interaction with other molecules, as has been shown for aequorin bioluminescence (Nagano and Tsuji 1990). The oxidation of protein thiols by disulfides and formation of heteroprotein disulfides has been referred to as 'thiolation'. This process is capable of conferring the specificity required for biological control mechanisms, as signals can be transmitted by changes in the thiol/disulfide ratio (Gilbert 1990). This has been described in detail for oxygenic photosynthetic systems where, in the light, electrons from chlorophyll are transferred to thioredoxin via ferredoxin. The reduced thioredoxin then modulates

the activities of key enzymes. To date, covalent modification of enzymes may be carried out by the following equations:



In reaction sequence (2) RSH is usually glutathione (GSH) (γ -glutamyl-cysteinyl-glycine); the concentrations of this compound can be altered by both endogenous and exogenous oxidizing systems so that the total thiol status is continuously fluctuating. The requirement for functional thiol groups to maintain membrane integrity means that any compound which can oxidize GSH *in vivo* will lead to increased membrane fragility. This is clearly seen in ‘favism’ [so named because susceptible individuals are affected after eating beans (*Vicia fava*)]. In this condition, a reduced level of glucose-6-phosphate dehydrogenase activity leads to low concentrations of NADPH (the product of the enzyme reaction) and hence to decreased GSH levels. Compounds which oxidize GSH (such as antimalarial drugs or vicine and divicine which are found in beans) will therefore overload an already fragile system causing damage to membranes such as those of the red blood cell. As these cells have no repair capacity, this gives rise to haemolytic anaemia.

Thiol redox status is a major determinant of tissue damage. Disulfides such as cystamine (which interconverts with cysteamine *in vivo*) are radioprotective and inhibit utilization of glucose by various tissues, presumably by formation of mixed disulfides between enzyme and thiol. Toxicity from paraquat poisoning is known to occur via generation of hydrogen peroxide, formed by cyclic reduction with NADPH and re-oxidation by oxygen. Normally, hydrogen peroxide oxidizes glutathione (GSH) to give the disulfide, GSSG. However, when NADP/NADPH or GSH/GSSG ratios were determined in lung tissues poisoned with paraquat, no changes were found, though there was good correlation between levels of paraquat in the lung, toxicity and the formation of protein-mixed disulfides (Ecobichon 1991).

6.2.4 Thiol/disulfide exchange

Thiol/disulfide exchange has recently been indirectly implicated in the numerous reactions controlled by nitric oxide (NO). This is synthesized from arginine and oxygen by NO-synthase (NOS) enzymes, citrulline being the other product. NO appears to be involved in regulating neurotransmission in the central nervous system (CNS) and in ischaemic brain damage (Butler *et al.* 1995). It also acts (Olesen *et al.* 1994) as a peripheral nerve transmitter controlling smooth muscle and blood flow and pressure (gastrointestinal tract, bladder sphincter, penile erection). Further roles include its release in shock states caused by endotoxins and sepsis and in inflammatory disease involving cytokine action. This wide range of functions is carried out by a small molecule which, like oxygen, is a weak free radical; as such, NO interacts with free thiol groups in cysteine, glutathione and proteins to form nitroso–thiol complexes (Culotta and Koshland 1992).

Guanylate cyclase enzymes, which synthesize cyclic GMP (cGMP) are widely distributed, particularly being found in platelets, smooth muscle cells and the CNS.

These enzymes are controlled by the thiol/disulfide ratio; the bovine liver enzyme is inactivated by GSSG while GSH blocks activation by NO. There is some evidence to suggest that NO reacts with thiol groups on the enzyme itself, causing conformational changes which lead to increased formation of cGMP. The presence of regulatory thiol groups will therefore modulate the activity of guanylate cyclase by combining with NO before it can react with the enzyme. It seems possible that the role of NO in neuronal long-term potentiation and synaptic depression is due to its capacity to increase production of cGMP; activation of a cGMP-dependent protein kinase then subsequent protein phosphorylation has been suggested as a mechanism.

The damaging effects of NO appeared to be due to its reaction with superoxide (O_2^-) to form the peroxynitrite anion ($ONOO^-$). Although under physiological conditions superoxide is kept at a very low level, ~ 10 pM, by the action of superoxide dismutase (SOD), this reaction is about threefold slower than the reaction with NO. Peroxynitrite, once formed, has a half-life of ~ 1 sec, nitrating tyrosine residues and directly oxidizing sulfhydryl groups to give disulfides. This reaction in proteins may underlie the modifications of low-density lipoprotein (LDL) which culminate in the accumulations of lipid in atherosclerotic lesions. As might be expected, thiols are protective, and in their presence tissues such as platelets convert peroxynitrite to S-nitroso thiols in a detoxification pathway.

The actual concentration of tissue disulfides available to take part in biochemical reactions must include both compounds like GSSG and also accessible disulfide groups in proteins. Many workers have tried to obtain some estimates of the potential ranges. Decades of work, however, have led to few conclusions. Reliable methods for the estimation of GSH and GSSG do now exist, primarily HPLC based, but the real problems lie in the potential for artefact formation (Pheifer and Briggs 1995). Thiols are readily oxidized in neutral or alkaline solution and this is catalysed by cations such as Fe^{2+} . However, they can also be oxidized by peroxides in acid solutions and these are easily produced in biological systems where most oxygenated tissues contain endogenous peroxides. Haemoproteins, especially when oxygenated, can catalyse peroxide formation and these radicals are also produced at a low level as byproducts of the oxidative phosphorylation pathway. Measurement of GSSG in cells containing functional mitochondria is therefore difficult. Most methods employ immediate homogenization of tissue in strong acid or snap-freezing before processing. It seems probable that the ratio of GSH to GSSG in rat liver is $\sim 300:1$ and, if so, thiol/disulfide modulation of enzymes *in vivo* may only be important in conditions of oxidative stress, while S-thiolation of proteins may be both selective and specific. However, this presupposes that there is rapid equilibration between the components of the thiol/disulfide redox pair. This may well not be the case; many investigators in this field have come to the conclusion that there are subcellular compartments or glutathione 'pools' that slowly come into equilibrium over a matter of days rather than minutes.

The factors controlling the interconversions of thiols and disulfides are not fully understood. Enzymatically, glutathione reductase will reduce a disulfide bond and as the enzyme itself contains a thiol group, it may participate in a feedback loop (Carlberg and Mannervik 1982). Chemically, disulfides can be reduced by thioredoxin, glutathione, dithiothreitol and sometimes by cysteine but *in vivo* reduction can also take place via the superoxide anion which converts $-S-S-$ to $-SH$ (Peterson *et al.* 1994). The Cu/Zn-linked SOD enzymes which convert

superoxide to hydrogen peroxide themselves contain a disulfide bond so that they too may be modulated by the presence of free thiol groups. GSH can be oxidized to GSSG by H_2O_2 , this reaction being catalysed by glutathione peroxidase which again has a free -SH group near the active site. Transamination of cysteine leads to formation of mercaptopyruvate which will react with available thiols to give pyruvate and persulfides (RSSH). These readily transhydrogenate with GSH to form H_2S and GSSG so that this series of interconversions comes full circle. The flavin-containing microsomal monooxygenases can also oxidize sulfur compounds. The conversion of cysteamine to cystamine goes via this route; this enzyme system has been studied in depth for xenobiotic substrates but its role in endogenous metabolism is not clearly understood. As has been described, peroxynitrite can oxidize thiols while NO itself has the potential to interact with zinc-sulfur-sulfur clusters in proteins such as metallothionein, releasing the metal and removing the activity (Maret 1994). In the long term, better understanding of control mechanisms for the -SH/-S-S- ratio must have clinical relevance. Studies on microtubule proteins show that antimetabolic drugs appear to exert their effects by altering the -SH/-S-S- ratio, indirectly confirming that cell behaviour is regulated (at least partially) by this means and suggesting that control of disulfide bonding could also be important in anticancer therapy (Stearns 1988). Improved drug design for disease states where thiol groups are involved, such as trypanosomiasis (Ondarza 1989) and hyperlipoproteinaemia (Yang *et al.* 1994), could also result from increased knowledge about factors involved in *in vivo* redox potential.

Thiol-disulfide exchange seems to be particularly important in carbohydrate metabolism; the starch-binding domain of glucoamylases contains a disulfide bridge which is conserved in proteins from fungal, yeast and bacterial sources (Coutinho and Reilly 1994). Glycogen phosphorylase phosphatase is rapidly inactivated by disulfides while glycogen synthetase, which has four accessible sulfhydryl groups, is inactivated by oxidized glutathione and can be reactivated with dithiothreitol (Ziegler 1985). Phosphofructokinase is similarly reversibly inhibited by GSSG and other disulfides. Conversely, glucose-6-phosphate dehydrogenase and fructose 1,6-bisphosphatase appear to be activated by disulfides especially GSSG, although hexokinase is usually inhibited, as is glyceraldehyde-3-phosphate dehydrogenase (Vaidyanathan *et al.* 1993). A pineal enzyme, serotonin N-acetyl transferase, is diurnal, with maximum activity at night, correlating with synthesis of melatonin (N-acetyl-5-methoxytryptamine). It is inhibited by a variety of disulfides (including insulin) and this inactivation is reversed by thiols (Klein and Namboodin 1981). As many neuropeptides contain a thiol/disulfide group, they may be able to exert subtle control in this system. Similar regulation can occur in plants; the activity of maize NADP-malic-enzyme is controlled by the thiol redox ratio (Drincovich and Andrea 1994) so that this seems to be a general mechanism for very different life forms.

Calpain is a member of the cysteine proteases, with a cysteine residue in the catalytic domain. It is selective for a group of cellular proteins, including cytoskeletal proteins (spectrin), membrane receptors, G-proteins and protein kinase C, all of which have thiol groups at or near the active site. High levels of sulfhydryls, for instance cysteine itself, will form a disulfide bridge with the -SH group on calpain and hence inhibit its action (Wang and Yuen 1994). In general, disulfide exchange is the mechanism by which thiol inhibitors of trypsin-like proteinases exert their effects (Steven 1981).

Thiol/disulfide exchange with GSSG or peptides containing available -S-S- groups similarly activates collagenases, via a decrease in molecular weight which is also seen on activation by limited proteolysis. This suggests that, like many extracellular enzymes, collagenase is secreted as a proenzyme which only exerts its hydrolysis effects under very specific conditions. However, this mechanism seems to be physiologically relevant, as it comes at the end of a cascade of reactions during phagocytosis. In this process, leukocytes generate hydrogen peroxide, and this converts GSH (catalysed by myeloperoxidase and glutathione peroxidase) to GSSG which in turn activates the latent collagenase. As the collagenase activation is readily reversible, this mechanism permits 'bursts' of cellular destruction when required; generally, once collagen is formed the turnover is slow, apart from specialized cases like the resorbing uterus or the tail of a tadpole.

6.2.5 Nucleic acid insertion

Interprotein formation of -S-S- bonds can be seen to occur when alphaviruses penetrate the host cells. Alphavirus membrane glycoproteins are organized in an icosahedral lattice, the stability of this space-enclosing structure being dependent on -S-S- bridges which are protected from the environment by the glycoprotein configuration. At neutral pH, the virus glycoprotein interacts with a host cell receptor, giving conformational changes which expose the previously-protected -S-S- bridges. These are then reduced by thiol exchange (at a more acid pH) and link to thiol residues in the host cell proteins, forming rigid intercellular bonds and allowing cell fusion to take place (Brown and Edwards 1992). Very similar processes appear to take place for HIV-1, HIV-2 and SIV viruses, all of which have envelope glycoproteins shielding disulfide structures (Gregory *et al.* 1991, Hoie 1991) and may be a general mechanism for penetration and infection with viruses of this type. The Sindbis E1 virus is known to have a series of rearrangements of metastable disulfide bonds during its maturation in the cell, while E1-E1 disulfide bonds maintain the structural integrity of the host-derived envelope (Mulvey and Brown 1994). This is also true for guinea-pig cytomegalovirus and Newcastle Disease virus (Britt and Harrison 1994, McGinnes and Morrison 1994). As this reduction of critical disulfides in viral envelope glycoproteins is the initial event which triggers entry of viral RNA/DNA and cell infection, control of thiol status could have a therapeutic role (Ryser *et al.* 1994). Patients with HIV infection and *Pneumocystis carinii* pneumonia (PCP) have been found to have high levels of GSSG and low levels of glutathione itself (Adams *et al.* 1993), although it is not clear whether this is the cause or the result of the infection. Despite these findings, thiol-related therapy has not received much attention, although D-penicillamine (β,β -dimethyl cysteine) which has a free -SH group has been used to treat HIV-1 infection.

It is interesting that the same mechanism used by viruses to penetrate cell membranes and insert their nucleic acid content is also found in a different context. The eggs of sea urchins are surrounded by a glycoprotein coat which is known to contain -S-S- bridges (Keller and Vacquier 1994). These afford sites for sperm recognition and bonding; fertilization can therefore be blocked by dithiothreitol or any agent which disrupts disulfide bridging (Ohlendorf *et al.* 1994). Other studies have shown the same mechanism in a mammalian system. The surface

sperm-recognition glycoprotein on the zona pellucida of mouse ova is ZP₃, which again contains disulfide bridges. The corresponding surface protein on mouse sperm, Sp56, is a homomultimer ($M_r = 110\,000$) where the monomers are held together with internal -S-S- bonds. Fusion of sperm with egg is thought to take place after both glycoproteins meet in a docking action after which Sp56 forms disulfide links with ZP₃ producing intermolecular bonds (Cheng *et al.* 1994). As in the case of viruses, once the two cells are joined together by -S-S- bridging, the transfer of nucleic acids provides molecular information to redirect the functioning of the acceptor cell. Spermiogenesis itself involves disulfide links, which stabilize sperm chromatin by forming intra- and intermolecular bridges between cysteine residues on protamines. This seems to be a general mechanism in mammals, although the corresponding protein in cuttle fish has a zinc-finger motif (Martin-Ponthieu *et al.* 1994).

6.2.6 Hormones, receptors and signalling

Less is known about the interactions of glycoprotein hormones or growth factors with their receptors. Thyroxine-stimulating hormone (TSH) luteinizing hormone (LH), follicle-stimulating hormone (FSH) and choriogonadotrophin all contain disulfide bonds which are protected by the glycoprotein structure and are essential to the hormone action (Giudice and Pierce 1978, Pierce 1981). This is also true of placental and pituitary glycoprotein hormones, and the prothoracotrophic hormone of the silkworm (*Bombyx mori*) (Ishibashi *et al.* 1994). Such information as is available suggests a mode of action which again involves glycoprotein recognition by hormone and receptor, then intermolecular disulfide bonding. Disulfide linkages in the hormone prolactin are essential for its secretion and its storage in secretory granules (Jacobs and Lorenson 1985), probably because the electrochemical proton gradient induces -SH/-S-S- interchange in transport proteins at both sides of the membrane. The exact position of disulfide bonds is critical for hormone action; the activity of oxytocin and vasopressin analogues has been shown to be dependent on the precise location of these intramolecular bridges (Jost 1987). This is hardly surprising as rearrangements could lead to drastic changes in molecular configuration and presentation. As might be expected, every signalling agent with a disulfide bond seems to have a receptor which contains the same group (Barger *et al.* 1992), and there is often evidence that disulfide exchanges are vital for cell signalling even in nonmammalian systems, for instance the growth hormone receptors from the striped bass (*Morone saxatilis*) also contain disulfide links (Gray and Tsai 1994). Human platelet-derived growth factor and interferon α A both owe their structures and biological activity to disulfide bridges (Haniu *et al.* 1994, Wetzel *et al.* 1983) and, conversely, the human interferon α and β receptor is a disulfide-linked dimer involved in direct signal transduction (Novick *et al.* 1994). Rhodopsin and its GMP-coupled receptors have been extensively studied; the -S-S- bond is highly conserved in the protein and required for its function, probably because it appears to be a key component in determining the stability of the metarhodopsin II structure and its coupling to transduction (Davidson *et al.* 1994). The experimental evidence suggests that many cell-surface receptors which are coupled to their effector systems via G-proteins possess intramolecular disulfide bridges; it will be remembered that guanylcyclase itself

is thiol-modulated. Studies on the β -adrenergic receptors have revealed that disulfide cleavage by thiol compounds activates the receptor in the same way as does agonist binding (Malbon *et al.* 1987). Other receptor systems also involve disulfide links. The insulin receptor is a member of the tyrosine kinase family of transmembrane signalling proteins and is particularly involved in metabolic regulation. It functions as a dimer, with disulfide bonds; when activated by the ligand, these links are broken, phosphorylation of another protein takes place and this initiates the signalling effect (Lee and Pilch 1994). Thiol-disulfide exchange is integral to proteins that function as voltage-gated channels and receptor-channel complexes and seems to be a general mechanism (Zuazaga *et al.* 1994). The combination of disulfide bridges in both ligand and receptor can also be shown for apolipoprotein β -100 (Yang *et al.* 1994) where the lipoprotein complexes are known to be held together by -S-S- bonds (Berg 1994, Blanco-Vaca and Pownall 1993). The coordinated expression of Apo E and the Apo E/Apo β receptors appears to regulate the transport of cholesterol and phospholipids during the early and intermediate phases of the re-innervation process in the CNS. When dendritic remodelling and synaptogenesis take place, the neurones repress the synthesis of cholesterol via the Apo E receptor pathway. Apo E itself is vital for the functioning of the cholinergic system, probably because phospholipid homeostasis is essential for neuronal function. The $\epsilon 4$ allele is associated with Alzheimer's Disease (AD) and represents an important risk factor. Human Apo E is coded for on chromosome 19; the substitution of arginine for cysteine residues at sites 112 and 158 in the protein sequence of 299 amino acids gives rise to the $\epsilon 4$ allele. As many as 80% of AD subjects have at least one copy of $\epsilon 4$ although the frequency in age-matched controls was <5% (Mullen and Crawford 1993). It has been shown that plasma cysteine levels are raised in AD (Heafield *et al.* 1990) so it is possible that one of the factors in the disease is modification of -S-S- bonding in the Apo E receptor; this could be disrupted by elevated plasma thiol levels if the intramolecular bonds were broken.

Disulfide bonds are common to a range of peptides with growth factor properties and/or neurological activity (Dixon *et al.* 1987). In some cases, although the functions are very different, there is a high degree of structural similarity between peptides. For instance, a series is known where they all contain three disulfide bonds which maintain a 'clover leaf' configuration and appear to be essential for activity. These are breast-cancer associated peptide (pS2), pancreatic spasmolytic polypeptide (PSP) and frog-skin peptides (spasmolysins, B8). Insulin-like growth factor (IGF-1), which stimulates cell growth and division, also has three disulfide bonds holding the peptide in a rigid configuration but this is different from the 'clover-leaf' structure seen for the pS2-type peptide (Thim 1989). It has been suggested as therapy for motor neurone disease (MND) on the basis of the improvement seen in the histochemical parameters of skeletal muscle from the 'wobbler' mouse after its administration. This genetic mutant has muscle atrophy and denervation and has been suggested as an animal model for the human condition although only ~5% of MND patients have a familial pattern with a high input from hereditary factors (Hantai *et al.* 1994). As might be expected, the IGF-binding protein (IGFBP) also contains disulfide bonds, as do other components of the CNS. Both the NMDA subtype of the glutamate receptor and the dopamine uptake system have this structure. In consequence, cysteine, with a free thiol group, is potentially excitotoxic; high cysteine levels interact with the -S-S-

structure in the glutamate receptor and stimulate continuous signalling. This in turn allows calcium ions to enter the cell and activate a sequence of hydrolytic enzymes which finally leads to cell death. This disruption of the delicate balance of neurological function may explain the damaging effect of thiols on the brain. The major degenerative diseases such as Parkinson's and Alzheimer's Disease seem to be associated with reduced levels of glutathione, and oxidant stress has been suggested as a factor in the aetiology of these diseases. It seems probable that the concentrations of thiols in the CNS are critical, both insufficiency and excess leading to cell toxicity or dysfunction.

6.3 Sulfur–sulfur bonds in toxins

Disulfide bridges between Cys-X-X-X-Cys and Cys-X-Cys are a feature of many bioactive peptides and toxins (Kobayashi and Tamaoki 1990); an α -helical motif is formed which gives specific interactions with receptor proteins. Many spider toxins which exert their mode of action by specific binding to tissues or receptors contain disulfides, huwen toxin-1 from the spider *Selenocosmia huwena* (Zhang and Liang 1993) being a good example. When these compounds reach the cell surface, the disulfide links are reductively cleaved and the resulting thiols then activate the receptor by interaction with its target disulfides. If this process is interrupted, toxicity does not occur. Thioredoxin, which is a powerful reducing protein and itself contains catalytically active disulfides, can block the action of snake venoms such as α - or β -bungarotoxin, scorpion and bee venoms and diphtheria toxin (Lozano *et al.* 1994). Presumably, the toxins are reductively cleaved and inactivated by thioredoxin in preference to interacting with receptors in the cell surface and thioredoxin therapy has therefore been proposed as a therapeutic measure. Many venoms are also neurotoxins. Mamba venoms contain several small proteins which are highly specific for muscarinic receptors. Five different types (M_1 – M_5) are expressed in human brain; they are intrinsic membrane receptor proteins which are coupled with G-proteins. They are all 64–66-residue peptides with molecular mass of 7–7.5 kDa, and have similar sequences, containing four disulfide bonds between cysteine residues which are conserved. These are again found in the family of 'three-fingered' snake toxins, which presumably all fold in a similar way. This includes cardiotoxins, fasciculin from the mamba snake (*Dendro aspis*) which is a specific noncompetitive inhibitor of acetyl choline esterase, and calciseptine, also a dendrotoxin, which blocks Ca^{2+} channels (Jerusalinsky and Harvey 1994). Sequence information and three-dimensional structures should shed light on the patterns of recognition between toxins and receptors; however, by analogy with other examples, one can assume that this will involve disulfide bonding between the two molecules.

6.4 Sulfur–sulfur bonds in small molecules

6.4.1 General properties

Probably because of their potential for interconversion with the -SH group, disulfides are highly active biologically. Generally, toxicity seems to be due to

redox cycling producing free radical damage or to inhibition of enzyme function by formation of mixed disulfides with a thiol group at the active site (Munday 1989). Methods for their analysis in biological fluids are often not entirely satisfactory; direct chemical reduction with sodium borohydride, sodium amalgam or phosphines has been used (Jocelyn 1987). Electrochemical detection with specialized electrodes, often coupled with HPLC, is another option (Kadin 1987, Shoup 1987). Currently, estimation of compounds such as glutathione disulfide (GSSG) is best achieved by reduction (dithiothreitol) then derivatization and HPLC separation of the products, using fluorescence detection (Pheifer and Briggs 1995).

Some drugs or prodrugs function precisely because they can interact with biological thiols to produce disulfides. D-Penicillamine ($\beta\beta$ -dimethyl cysteine) is frequently used for treatment of rheumatoid arthritis (RA) although the mode of action is not clearly understood. It is known that cysteine levels in serum of RA patients are often elevated, leading to formation of the inflammatory cysteinyl leukotrienes (Waring and Emery 1992). As D-penicillamine forms a mixed disulfide with cysteine which is more readily excreted than the homodimer of either compound, it may act by reducing cysteine concentrations and therefore reducing the potential for inflammation and autoimmune responses.

Disulfides can act to inhibit cell proliferation and have been used, especially selenodithiols, as anticancer agents (Sundaram and Milner 1993). In one series of experiments the rate of cell division in culture HT29 colon adenocarcinoma cells was reduced to zero. It seems possible that they inhibit protein formation in eukaryotic cells by direct binding to elongation factor EF-2 (Parrish *et al.* 1992).

The biological activity of disulfides has been extensively studied. Generally, they are bacteriostatic and fungistatic, probably because they are inhibitors of protein synthesis. Although toxicity is rarely seen in man, domestic and farm animals can suffer severe anaemia if they consume large amounts of onions. The toxic principle is dipropyl disulfide which, as in the case of favism, removes the glutathione needed for maintenance of the integrity of the red blood cell. A similar condition is seen in cattle consuming kale which is rich in S-methyl cysteine sulfoxide ('kale anaemia factor'). This is a precursor of dimethyl disulfide which also acts as a haemolytic agent. This reaction does not appear to take place in man, possibly because the S-methyl cysteine sulfoxide is acetylated or degraded to inorganic sulfate (R. H. Waring unpublished data).

6.4.2 Organoleptic properties

One of the most characteristic properties of sulfur compounds is their smell. Generally, this is less strong as the sulfur atom becomes more oxidized, so that thiols have a stronger odour than do disulfides, and sulfates have little if any discernible scent although their volatility is also lower. If perception of odour depends on the interaction of thiols or disulfides with similar groups on the receptor surface, followed by configurational changes and signal transmission, then this is not unexpected. Sulfur compounds are the major sources of odour and flavour of many foodstuffs, particularly the *Allium* and *Brassica* families of vegetables and of the rare and expensive truffles. Disulfides are probably best

known for their contribution to the flavour of onions, but they are also biologically active as they block human platelet aggregation (Kawakishi and Morimitsu 1994). This effect is due to inhibition of the prostaglandin endoperoxide synthase step in the platelet arachidonic acid cascade although the activity of human 5'-lipoxygenase, which is involved in the synthesis of leukotrienes from arachidonic acid in leukocytes, is also reduced. These two effects may explain the well-known antithrombotic properties of onions and related species. Most sulfur compounds are thermally unstable and readily rearrange or degrade, making identification or isolation more difficult; this lability at high temperatures is a recurrent theme in papers on the sulfur-containing constituents of vegetables. When vegetables are cut or crushed, the enzyme cysteine sulfoxide- β -lyase (CS-lyase) is released and this converts S-alkenyl-L-cysteine sulfoxides to thiols and disulfides. These volatiles can be analysed by trapping on specific adsorbents (Tenax GC) then solvent or thermal desorption and dynamic headspace GC (Pino 1992) or HPLC (Ziegler and Sticher 1989).

Both methods have been used to study disulfides and related compounds. *Caucas* (*Allium victorialis*), one of the garlic species, contains 1-propenyl disulfides, and also vinyl dithiins, which were identified by NMR and mass spectrometry (Nishimura *et al.* 1988). Welsh onions and scallions have been reported as containing more than 40 volatile sulfur components, the principal constituents being 1-[alk(en)yl thio] alk(en)yl disulfides and alkyl tetra- or penta-thio-alkanes and alkenes (Kuo and Ho 1992a). Dried caucas powders contained disulfides and trisulfides, in addition to the antithrombotic vinyl dithiins, but these components all decrease on storage (Wijaya *et al.* 1991). The characteristic odour of garlic has largely been ascribed to diallyl disulfide, although propyl allyl disulfide, methyl allyl trisulfide and diallyl trisulfide also contribute (Sheen *et al.* 1992). Cooking garlic with oil (frying as opposed to baking) favours formation of vinyl dithiins over di- and trisulfides (Yu *et al.* 1993). The levels of allyl tri- and disulfides decreased on prolonged heating, so that losses of garlic flavour in cooking may be significant (Yu and Wu 1988). (Those recipes which describe roast chicken stuffed with garlic cloves presumably rely on this fact to achieve edible results.) Despite the presence of many sulfur-containing compounds in garlic oils, the main components in exhaled air after ingestion of garlic are 2-propene-1-thiol and diallyl disulfide; it is probable that most volatile garlic constituents are degraded by enzymes in the digestive system (Laakso *et al.* 1989). Some individuals are garlic-sensitive, and this effect can be reproduced in guinea pigs. The main allergenic component appears to be diallyl disulfide, although garlic-sensitive patients also had positive reactions towards allicin (diallyl sulfinate), allyl propyl disulfide and allyl mercaptan (Papageorgiou *et al.* 1983). Allyl propyl disulfide and dipropyl disulfide have been found as volatiles from pulverized onion, and detected in exhaust air from an onion-processing plant (Tsuji *et al.* 1991). Other aroma compounds identified in onions include methyl propyl disulfide and propenyl disulfides, in addition to dipropyl trisulfide and methyl propyl trisulfide (Kallio and Salorinne 1990) and propenyl-trisulfides (Sass-Kiss *et al.* 1989). Some of these compounds have biocidal activity; dialkyl disulfides and trisulfides were shown to be toxic when they were tested against *Bruchidius atrolineatus*, a pest of stored cow peas. Dimethyl and diethyl trisulfides gave the highest mortality of adults, eggs and larvae at 0.013 and 0.25 mg l⁻¹, respectively, being more effective than carbon tetrachloride fumigation but less than by using dichlorvos (Nammour *et al.* 1989). Small amounts of polysulfides have also been

detected in onions; diallyl tetra- and pentasulfides (Misharina and Golovnya 1993) and 1,3,5-trithianes have been reported, particularly after steam distillation of onion oils (Farkas *et al.* 1992). Other plants have been found to contain polysulfur constituents; resin from *Ferula* species (Min *et al.* 1987), seeds from the neem tree (*Azadirachta indica*, Balandrin *et al.* 1988) and fruits of *Durio zibenthinus*, the durian, all contain polysulfides. The rind of durian fruit is notorious for smelling of 'drains' although the creamy caramel-flavoured fruit is much appreciated by connoisseurs. Because of the odour, durians are not allowed to be carried on public transport in Singapore. The predominant volatiles are H₂S, ethyl hydrosulfide and diethyl polysulfides, although other components have been reported (Moser *et al.* 1980). Polysulfides have been described as constituents of the aroma of fresh cooked ground beef; the higher the degree of heating the greater the concentrations of polysulfides (MacLeod and Ames 1986). The same effect has been noted for onion. Polysulfides have also been found among the volatile metabolic products from the decomposition of poultry carcasses. The appearance of H₂S and CH₃SH on the sixth day of storage, due to pseudomonad-mediated degradation of sulfur-containing amino acids from meat proteins, signalled incipient spoilage. Two days later, eleven sulfur-containing components were detected by headspace analysis with gas-liquid chromatography (GLC) (Viehweg *et al.* 1989a). These workers have concluded that the specific metabolic capabilities of the spoilage flora mainly determine the 'off' odours; sulfur compounds, identified by GC/MS were produced almost exclusively by pseudomonads and *Alteromonas putrefaciens* and gave the characteristic odour of rotten meat (Viehweg *et al.* 1989b). Oligosulfides were again responsible for the putrid odours associated with the inflorescence of the voodoo lily (*Sauromatum guttatum*) (Borg-Karlson *et al.* 1994). The smell attracts pollinators such as scarab beetles and dung flies, while the emission of volatiles is intensified by raised temperatures produced in the brown-purple top of the flower. This increase in heat is probably due to uncoupling of the oxidative phosphorylation pathway by salicylic acid. The heat and smell are thought to stimulate the activity and mating behaviour of the insects and therefore enhance pollination. The stink morel (*Phallus impudicus*) produces a similar smell and attracts flies belonging to the same genera as those which pollinate the voodoo lily. These insects disperse the spores of the mushroom, which also becomes warmer than ambient temperature so that the sulfur-attractants are volatilized. Other sulfur components can accompany the major constituents. The voodoo lily was found to produce dimethyl di-, tri- and tetrasulfides, while the di- and trisulfides were identified from the mushroom, with a variety of other odorous volatiles. The smell is presumably intended to attract flies of the family Sarcophagaceae which feed or lay their eggs in rotten meat and onions, both of which release similar constituents (Shankaranarayana *et al.* 1974). Tests showed that flies of the genera *Calliphora*, *Lucilia* and *Sarcophaga* were attracted to the scent of dimethyldisulfide so these odour cues seem to be an example of chemically convergent evolution as a strategy in dispersal of pollen or spores. Sulfur-containing volatiles appear to act as attractants and pheromones in many species, particularly those which feed on plants of the *Allium* and *Brassica* families. Odour discrimination is carried out by olfactory sensory organs on the antenna of the insect and when a sulfur volatile is present, a 'profile' of action potentials is released. Both the cabbage root fly and the onion fly can respond to concentrations of sulfur volatiles of less than one part in a million and they locate the food source by flying up the concentration gradient. Volatiles containing the propyldithio

grouping (methyl propyl disulfide, propenyl propyl disulfide, dipropyl disulfide) act as attractants to the onion fly and also induce the deposition of eggs. These compounds are all breakdown products of alkylcysteine sulfoxides. These are released in crushed plant tissue so that those plants which are diseased or damaged are more likely to be attacked (Cole 1983). As *Allium* and *Brassica* species are grown for their sulfur-containing flavour constituents, any decrease in odorous volatiles would probably lead to a decreased commercial acceptability of these vegetables. There may however be scope for plant breeding programmes to alter the ratios of sulfur-containing constituents.

Although the odour of disulfides is usually thought of as being repugnant, in extreme dilution it can be attractive to mammals. Dimethyl disulfide (DMDS) mimics the effect of milk on behaviour in neonatal rats and also promotes postnatal attachment to the maternal nipples. The pleasure which babies obviously derive from the act of 'sucking' may be partly due to opioid release, and in agreement with this hypothesis the DMDS signal is blocked by naloxone administration suggesting kappa opioid involvement in the response (Smotherman and Robinson 1992). Some vestiges of this early attraction may explain why addition of DMDS improves the voluntary food intake of sheep on a vegetable diet high in allyl cyanide (Duncan and Milne 1993).

Along with other sulfur constituents, DMDS can be found in beers and is a component of the famous 'Burton nose', the name for a 'sulfury' smell found in beer made from Burton on Trent water. This is rich in calcium sulfate which is reduced by yeasts to give thiols and disulfides. DMDS, dimethyl trisulfide and diethyl disulfide have all been found, with methane-, ethane- and *t*-butyl thiols (Hough *et al.* 1982a). Some Canadian beers also have sulfur flavour components. These have been identified as dimethyl polysulfides and trisulfides, derived primarily from the malting process (Williams and Gracey 1982a). The interactions of sulfur dioxide or other sulfur-containing agents with wort and beer constituents were a major source of polysulfides, and levels of these increased if packaged beer was stored for prolonged periods at room temperature or for a few days at 48°C (Williams and Gracey 1982b). Higher polysulfide levels were associated with a marked reduction in consumer satisfaction with the product. The essential oil of hops only contains traces of sulfur-containing compounds but these have low detection thresholds. Analysis by GLC using a flame photometric detector showed that DMDS (flavour threshold 7.5 ppb) and dimethyl trisulfide (0.1 ppb) were present, with 2,3,5-trithiahexane and dimethyltetrasulfide (0.2 ppb) (Hough *et al.* 1982a). Thiophenes and cyclic polysulfides have also been reported (Elvidge *et al.* 1982). Most of these compounds arise when hops in the field are treated with elemental sulfur to control mildew or are bleached with sulfur dioxide during roasting in the oast-house. Higher temperatures increase the formation of sulfur volatiles and these may markedly affect the flavour of the beer, giving 'cooked-vegetable', 'onion-like', 'rubbery' sulfur aromas with low thresholds and low customer acceptability (Hough *et al.* 1982b).

6.4.3 Di- and polysulfides in plants and bacteria

Trisulfides are also biologically active. Selenotrisulfides can be detected when cells are incubated with [⁷⁵Se]selenite although they may be formed nonenzymically

(Frenkel *et al.* 1991). In other systems selenotrisulfides are inhibitors of RNA polymerase II purified from wheat germ where they inhibit the formation of the enzyme–DNA binary complex; in *E. coli* RNA polymerase they also inhibit elongation (Frenkel and Falvey 1989). Similar inhibitory effects on protein synthesis have led to the use of thiuran disulfides and trisulfides as components of antifouling agents on polythene fish nets; pentasulfides and polysulfides are also active in these preparations (Akashi *et al.* 1989, Kadota and Tsurumi 1992) and their functions have been reviewed (Wood 1982). Both disulfides and polysulfides are active against cattle ticks, ~5 mg of the allyl derivatives being effective per animal (Ferrando 1986).

Like other sulfur compounds, polysulfides are strong antioxidants inhibiting lipid peroxidation in rat liver microsomes (Horie *et al.* 1992). They are readily formed when cysteine and glutathione are heated at pH 7.5 in an aqueous medium for 1 h at 180°C, and have been reported as smelling of roasted sesame seeds (Zhang and Ho 1991). They can be determined in human blood by alkylation with penta-fluorobenzylbromide then partial desulfuration with potassium cyanide to the disulfide. The lower limit of detection was $0.005 \mu\text{mol l}^{-1}$ and this method has been used forensically (Kage *et al.* 1991). Generally, polysulfides display biocidal activity; for example, they are active against brown fruit mites and pear leaf blister mites (Ol'Khovskaya-Burkova *et al.* 1967). Benzoyl polysulfides have been suggested for therapeutic treatment of liver disorders as they were effective in improving abnormal enzyme levels and blood coagulation time after hepatic damage induced by D-galactosamine in rats (Ogata *et al.* 1989). Relatively little information is available on polysulfide toxicity, although one death from polysulfide poisoning is known to have occurred when a child was given an enema containing a mixture of polysulfides by mistake; this was 100 times stronger than naturally sulfurated spa water (Jaulmes *et al.* 1969). In mice, the LD_{50} for a disulfide was 37 mg kg^{-1} , and for a sulfide 20 mg kg^{-1} , suggesting that polysulfide toxicity is not usually of major importance. When a mixture of CaS_4 and CaS_5 was given orally to rats, polysulfides were found in blood, lung, liver and kidney, at levels of $0.196 \mu\text{mol ml}^{-1}$, $0.018 \mu\text{mol g}^{-1}$, $0.051 \mu\text{mol g}^{-1}$ and $0.013 \mu\text{mol g}^{-1}$, respectively, with undetectable levels in other tissues. As might be expected, sulfides (as opposed to polysulfides) were widely distributed in all organs (Kage *et al.* 1993).

Cyclic di- and polysulfides are also known to exist. Probably the best-known examples of the former are lipoic acid and asparagusic acid, which is responsible for the smell which may occur in human urine after ingestion of asparagus. Production of this odour appears to be due to a dominant gene which controls asparagusic acid metabolism; ~40% of a Caucasian population produce odorous urine after eating asparagus, with ~6% being 'super-excretors'. Analysis of the volatiles showed that the main components were methane thiol, dimethyl sulfide, dimethyl disulfide and dimethyl sulfoxide and these could be detected as early as 1 h after eating the asparagus and up to 36 h later (Waring *et al.* 1987a, b). Asparagusic acid itself is biologically active, controlling fungal growth in the plant and repelling insect attack (Iriuchijima 1977). It is known to be readily reduced to the ring-opened dithiol (dihydroasparagusic acid). Cyclic disulfides such as thioctic acid have been used in combination with BAL (1,3-dithio propane) to treat heifers poisoned with sodium arsenite (Hatch *et al.* 1978). As thiosulfates were not effective, the therapeutic effect is presumably due to the potential presence of thiol groups.

Asparagusic acid and its derivatives possess growth inhibitory properties on a range of plant seedlings including lettuces and are active at concentrations of 6×10^{-7} M, comparable with abscisic acid. Six-membered rings containing the -S-S- bond have also been found in asparagus, as has a cyclic trisulfide (1,2,3-trithione-5-carboxylic acid). Although asparagusic acid and lipoic acid both contain a 1,2-dithiolane ring, their biosynthesis appears to be unrelated and their biochemical functions are quite different. Lipoic acid is best known as one of the minor constituents of the B-vitamin complex. Like most disulfides, it can be readily reduced to the dithiol and this interconversion is important in the metabolic sequences involved in the action of pyruvate dehydrogenase.

Polysulfides and cyclic polysulfides are synthesized by the sulfur-metabolizing hyperthermophilic bacteria such as *Archea Thermococcus tadjuricus* and *T. acidaminovorans*; most are derivatives of trithiolanes, tetrathianes and penta-thiepanes and are derived from amino acids (Ritzau *et al.* 1993). Similarly, cyclic polysulfides can be found in seeds of *Parkia speciosa* Hassk, derived from djenkolic acid and dichrostachinic acid as amino acid precursors (Susilo and Gmelin 1982). As in the case of asparagusic acid, these sulfur compounds seem to confer protection from fungal attack; they showed activity against *Candida albicans* and also against Gram-positive and -negative bacteria (Gmelin *et al.* 1981, Mitsudera *et al.* 1990). The red alga *Chondria californica* is known to synthesize a range of cyclic polysulfides, including a 12-membered heterocycle containing eight S-atoms, and these have antibiotic activity (Wratten and Faulkner 1976). A major problem in using polysulfides as pharmaceutical agents is that it is not easy to get reproducible preparations. Generally they are extracted from natural products and the yield, both qualitatively and quantitatively, depends on the other components in the mixture. It is obvious, however, that the biological activity of sulfides underlies the use of, for instance, garlic in folk medicine. Polysulfides occurring as chains or eight-membered rings of sulfur atoms are found in many sulfur springs and the employment of sulfur water in treating hepatic and arthritic disease has a long history; the hot springs at Bath have been used therapeutically since Roman times and are rich in sulfate with some dissolved sulfur and polysulfides. Sulphate can be formed *in vivo* by oxidation of cysteine or other sulfur-containing precursors, and the concentration of this anion is known to be reduced in plasma from patients with liver or arthritic problems (Bradley *et al.* 1994, Davis *et al.* 1994). This could affect sulfation of bile acids and biocomponents such that inadequate supplies could easily precipitate clinical dysfunction. Sulphate absorption across the gut is relatively slow, while absorption of sulfides and polysulfides appears to be rapid; drinking sulfur-containing water may therefore be therapeutically useful if oxidation to sulfate can occur *in vivo*. These absorbed sulfides and polysulfides are thought to be substrates for the hepatic microsomal flavin monooxygenase (FMO) systems which catalyse oxidation of sulfides to sulfoxides and initiate their oxidative degradation to sulfate itself (Ziegler 1988).

The lachrymatory S-alkyl or alkenyl cysteine sulfoxides which are precursors of di- and trisulfides in the *Allium* species also give rise to more oxidized derivatives. The enzyme, alliinase, catalyses breakdown of alliin (S-ak(en)yl cysteine sulfoxides) when the plant tissue is bruised or crushed to disrupt the cells. The resulting products are alk(en)yl sulfinates (allicins, R-S-S(O)-R¹) and pyruvic acid. These products are formed stoichiometrically (Lawson and Hughes 1992). The thiosulfinates readily dissociate to give disulfides (R-S-S-R¹) and thiosulfonates

(R-S(O)-S(O)-R¹), the disulfides often disproportionating to give the sulfides and trisulfides which have already been discussed. As thiosulfinates readily react with thiol groups, they have wide-ranging biological effects. Generally, they have antibacterial, antimicrobial and antifungal properties and can have quite powerful actions. Di(prop-2-enyl) thiosulfinate is as effective as metronidazole in controlling *Entamoeba histolytica*, a parasite which causes amoebic dysentery. The same compound strongly inhibits the aggregation of platelets *in vitro* and reduces liver cholesterol and lipids (Wagner *et al.* 1990). It was also effective as a hypoglycaemic agent in cases of mild diabetes. However, as it acted as a mild allergen in some individuals and could cause diarrhoea if taken in large amounts, there are obvious problems for its routine therapeutic use. Some thiosulfinates are active as antitumour agents and may be responsible for the inhibition of tumour promotion which has been reported for oils from onion and garlic. It is not clear whether thiosulfinates exert some of their activity by dissociation to disulfides; they are very unstable at room temperature or in aqueous solution and are best stored in cold alcoholic solutions (4°C) or as solids at -20°C. Alkenyl thiosulfinates and dialkenyl thiosulfonates have been identified in volatile fractions from Welsh scallions (Kuo and Ho 1992a, b). These compounds also inhibit 5-lipoxygenase and cyclooxygenase *in vitro* and probably *in vivo* (Kawakishi and Morimotsu 1994) and have been shown to act as anti-asthmatic agents (Wagner *et al.* 1990). Isolation and characterization was only achieved after solvent extraction at ambient temperatures and analysis by GC-MS; as they are unstable to heat they are not present in distilled oils.

6.4.4 Thiosulfates and thiosulfites

Thiosulfates and thiosulfites contain sulfur atoms in a higher oxidation state; like the other sulfur-sulfur bond compounds discussed in this chapter, they too have biological activity and can act as sulfur sources for various life forms. This topic has been extensively reviewed (Soerbo 1989). Duckweed fronds will utilize thiosulfate (Fraser 1974), while *Thiomicrospira denitrificans*, an anaerobic microorganism, can oxidize thiosulfate and reduce nitrate (Timmer-Ten Hoor 1975). Similarly, cysteine-deficient *Actinomyces olivaceus* mutants reduced thiosulfates using them as cysteine precursors (Dekhtyarenko *et al.* 1975), while *Thiobacillus* strains oxidized thiosulfate and polythionates, forming sulfates in aerobic conditions (Kelly and Tuovinen 1975). Many microorganisms can form thiosulfate from other sulfur-containing compounds. *Desulfovibrio vulgaris* will convert sulfite to thiosulfate (Suh and Akagi 1969), while *Chromatium vinosum* cultures, using sulfide as a source, produced thiosulfate and elemental sulfur (S₆, S₇ and S₈ rings) (Steudel *et al.* 1990). This oxidation of sulfide can be carried out by turbellarians, showing that invertebrates can also use this detoxification pathway (Powell *et al.* 1980). In turn, thiosulfate is used as a source by *Mathanosarcina* in anaerobic cultivation to manufacture vitamin B₁₂ (Nagai and Nishio, 1987). *Thiobacillus* species will also utilize thiosulfates as an energy source (Martinez Garcia *et al.* 1980), especially in acidic media; this may be a factor in corrosion of stainless steels (Dhawale 1993). Thiosulfates appear to have low *in vivo* toxicity; their ingestion by sheep increased the haemoglobin level and leukocyte and erythrocyte counts, with an increase in organic and total sulfur content in blood (Korelov 1973), suggesting that some conversion to inorganic sulfate had occurred. Cyanide ions

react with thiosulfate, under the influence of the enzyme rhodanese, to form the less damaging thiocyanate and sulfite anions (Smith 1991). Intravenous injection of sodium thiosulfate is now a preferred method for treating acute cyanide poisoning. Low levels of thiosulfates occur endogenously so that this provides some protection from chronic low-level toxicity due to cyanogenic glycosides in the diet. These compounds are fairly widely distributed, being found in many plants including cassava, almonds and barley. Short-chain alkyl thiosulfates, especially the amyl, can protect against dinitro-*o*-cresol poisoning (Luk'yanchuk *et al.* 1985); however the higher homologues, such as nonyl and decyl salts, were found to be ineffective. In contrast, *n*-decylaminoethanethiosulfuric acid has been successfully used to protect *Macacca mulatta* (Hamilton *et al.* 1968) and rhesus monkeys (Sharp *et al.* 1967) from ionizing radiation damage from X-rays. A similar effect was found in rodents using 2-aminoethyl and propyl thiosulfuric acids. These appeared to decrease endogenous radiosensitizers in tissues and increase endogenous radioprotective agents (Bogatyrev *et al.* 1973). The exact mechanisms were unclear, although thiosulfates can act as metal chelating agents (Foye and Hu 1979) and also react with cysteine to form disulfides and oxygenated polysulfide derivatives (Scarf *et al.* 1977) which might act as free-radical traps.

Thiosulfates have a range of reported chemical and biological activities; they can inhibit corrosion of carbon steel by aqueous nitrates (as in fertilizer solution) 280-fold more effectively than can controls with no inhibitor (Sansing and Novotny 1976) and will remove traces of hypochlorite from tap water (Karigome *et al.* 1994, Levy 1992). Alkylthiosulfates have also been shown to be active against Ehrlich ascites carcinoma, SN-36 leukemia and sarcoma 180 in mice (Chae *et al.* 1972); the mechanisms are not clear, but probably involve interactions with thiol groupings. As thiosulfates react with free sulfhydryl compounds, they inhibit the production of the 'cooked flavour' of heated milk, formed by heat-denaturation of β -lactoglobulin (Ferretti 1973). Thiosulfates alter the respiration rate in plant tissue, possibly through altering the -SH/-S-S- ratios in the photosynthetic pathways. They can be used to accelerate crop maturation in soybeans (Nakama 1981) or to act as desiccants and defoliants in lupins (Shutov 1982), thus improving the quality of the seed yield. Potassium thiosulfate has been used to stimulate the ripening of mandarin oranges (Nakama 1980, Young 1972) presumably again by interacting with respiration in the growing plant. Thiosulfates are also used as components in preparations to preserve cut carnation flowers (Takahashi *et al.* 1991); this capacity to extend the life of cut blooms is now used commercially on a large scale. The properties of thiosulfates have led to further practical applications as they can be utilized to prolong the shelf-life of vegetables that have been disinfected with per-acids (French and Mearns 1991). This latter use involves removal of damaging free-radical species which cause tissue browning, analogous to the discolouration seen in cut fruit and vegetables after the action of peroxidases. Thiosulfates will in fact directly inhibit the peroxidase-induced discolouration of potato and sweet potato (Ishida *et al.* 1985) and can be used to improve the yield of protein and starch from these vegetables. The action of papain can similarly be blocked by thiosulfates which act on thiols at or near the active site of the enzyme (Patarasakulchai and Southwell-Keely 1984). Conversely, thiosulfates activated glutamic dehydrogenase in pea seedlings (Pahlich *et al.* 1972) and β -galactosidase (Herrmann *et al.* 1991), presumably again by interacting with thiols as inhibition could be achieved by using sulfides.

These reactions are further examples of the Protean nature of the disulfide bond, which can readily be converted to a thiol, but in the process re-forms the -S-S- link with a sulfur atom in another molecule. Although the sources of the individual sulfur atoms may vary, disulfide bonds play a major role in linking structure with function in biology. These interconversions are found in all life forms and have probably changed little throughout evolutionary history.

6.5 Summary

The unique flexibility of structure and function which is conferred by sulfur-sulfur bonds is a basis for protein structure and cell signalling. The activity of many biomolecules depends on the -SH/-S-S- ratio, so that directly or indirectly, the redox state of the organism determines regulation of metabolic processes; this mechanism of control is common to all life forms.

6.6 References

- ADAMS, J. D., JARESKO, G. S., LOUIE, S. G., KLAIDMAN, L. K., KENNEDY, D., SHARMA, O. & BOYLEN, C. T., 1993, *Pneumocystis carinii* pneumonia in HIV-infected patients: effects of the diseases on glutathione and glutathione disulfide, *Journal of Medicine*, **24**, 337-352.
- AKASHI, H., INOUE, T. & KITORA, Y., 1989, Synergistic antifouling agents containing N-phenylmaleimides, polysulfides and/or thiuram disulfides for fish nets, *Japan Kokai Tokyo Koho, Japanese Patent* JP 01090105 cited in CA **111**, 189610y.
- BALANDRIN, M. F., LEE, S. M. & KLOCKE, J. A., 1988, Biologically active volatile organosulfur compounds from seeds of the neem tree (*Azadirachta indica*), *Journal of Agricultural and Food Chemistry*, **36**, 1048-1054.
- BARGER, S. W., WOŁCHOK, S. R. & VAN ELDIK, L. J., 1992, Disulfide-linked S100 β dimers and signal transduction, *Biochimica et Biophysica Acta*, **160**, 105-112.
- BERG, K., 1994, Lipoprotein: an overview, *Chemistry and Physics of Lipids*, **67-68**, 9-16.
- BETZ, S. F., 1993, Disulfide bonds and the stability of globular proteins, *Protein Sciences*, **2**, 1551-1558.
- BLANCO-VACA, F. & POWNALL, H. J., 1993, Disulfide linked dimers of apolipoprotein D in urine, *Electrophoresis*, **14**, 1086-1087.
- BOGATYREV, G. P., GONCHARENKO, E. W., CHIRKOV, Y. Y. & KUDRYASHOV, Y. B., 1973, Radioprotective activity and possible mechanisms of the action of some potential radioprotectors, *Biologicheskde Nauhi*, **16**, 50-53 cited in CA **79**(9), 11626j.
- BORG-KARLSON, A.-K., ENGLUND, F. O. & UNELIUS, C. R., 1994, Dimethyl oligosulfides, major volatiles released from *Sauromatum guttatum* and *Phallus impudicus*, *Phytochemistry*, **35**, 321-323.
- BRADLEY, H., GOUGH, A., SOKHI, R. S., HASSALL, A., WARING, R. H. & EMERY, P., 1994, Sulphate metabolism is abnormal in patients with rheumatoid arthritis, confirmation by *in vivo* biochemical findings, *Journal of Rheumatology*, **21**, 1192-1196.
- BRITT, W. J. & HARRISON, C., 1994, Identification of an abundant disulfide-linked complex of glycoproteins in the envelope of guinea pig cytomegalovirus, *Virology*, **201**, 294-302.

- BROWN, D. T. & EDWARDS, J., 1992, Structural changes in α -viruses accompanying this process of membrane penetration, *Seminars in Virology*, **3**, 519–527.
- BUTLER, A. R., FLITNEY, F. W. & WILLIAMS, D. L. H., 1995, NO, nitrosonium ions, nitroxide ions, nitrosothiols and iron-nitrosyls in biology: a chemist's perspectives, *Trends in Pharmacological Sciences*, **16**, 18–22.
- CARLBERG, I. & MANNERVIK, B., 1982, Reaction of 2,4,6-trinitrobenzenesulfonate with the active site of glutathione reductase, *Developmental Biochemistry*, **21** (Flavins, Flavoproteins), 53–56.
- CHAE, D. K., CHUNG, W. K. & LEE, N. B., 1972, Synthesis and antineoplastic activity of alkyl thiosulfates and diaryl disulfides, *Yakhak Hoeji*, **16**, 149–154 cited in CA **80**, 116081h.
- CHENG, A., LE, T., PALACIOS, M., BOOKBINDER, L. H., WASSARMAN, P. M., SUZUKI, F. & BLEIL, J. D., 1994, Sperm-egg recognition in the mouse, *Journal of Cell Biology*, **125**, 867–878.
- COLE, R. A., 1983, Sulfur-containing compounds in vegetables as insect attractants, in Mitchell, S. C. & Waring, R. H. (Eds), *Sulfur in Xenobiotics*, Proceedings of Symposium, pp. 41–55, Birmingham: University of Birmingham.
- COUTINHO, P. M. & REILLY, P. J., 1994, Structure–function relationships in the catalytic and starch-binding domains of glucoamylase, *Protein Engineering*, **7**, 393–400.
- CREIGHTON, T. E., 1988, Towards a better understanding of protein folding pathways, *Proceedings of the National Academy of Sciences, USA*, **85**, 5082–5086.
- CULOTTA, E. & KOSHLAND, D. E., 1992, NO News is good news, *Science*, **258**, 1862–1865.
- DAVIDSON, F. F., LOEWEN, P. C. & KHORANA, H. G., 1994, Structure and function in rhodopsin, *Proceedings of the National Academy of Sciences, USA*, **91**, 4029–4033.
- DAVIS, M. H., KLOVRZA, L., WARING, R. H. & ELIAS, E., 1994, Plasma cysteine and sulphate levels in patients with cirrhosis of the liver, *Clinical Sciences*, **87**, 357–362.
- DEKHTYARENKO, T. D., BOIN, L. F. & MATSELYUKH, A., 1975, Use of inorganic precursors of cysteine synthesis by cysteine-deficient mutants of *Actinomyces olivaceus*, *Mikrobiologika (Kiev)*, **37**, 320–324 cited in CA **83**(17), 144244a.
- DETWILER, T. C., TURK, J. L. & BROWNE, P. C., 1987, Thiol-sulphate exchange by thrombospondin, *Seminars in Thrombosis and Hemostasis*, **13**, 276–280.
- DHAWALE, S. W., 1993, Thiosulfate: an interesting sulfur oxo anion that is useful in both medicine and industry, *Journal of Chemical Education*, **70**, 12–14.
- DIXON, J. E., YAZDANPARAST, R., SMITH, D. & ANDREWS, P. C., 1987, Identification of post-translational modifications in neuropeptides, in Walsh, K. A. (Ed.), *Methodology in Protein Sequencing Analysis*, Proceedings of the International Conference 6th Meeting (1986), pp. 493–503, Clifton, NJ: Humana Press.
- DRINCOVICH, M. F. & ANDREO, C. S., 1994, Redox regulation of maize NADP-malic enzyme by thiol-disulfide interchange: effect of reduced thioredoxin on activity, *Biochimica et Biophysica Acta*, **1206**, 10–16.
- DUNCAN, A. J. & MILNE, J. A., 1993, Effects of oral administration of Brassica secondary metabolites on the voluntary food intake and metabolism of sheep, *British Journal of Nutrition*, **70**, 631–645.
- ECOBICHON, D. J., 1991, Toxic effects of pesticides, in Amder, M. O., Doull, J. & Klaassen, C. D. (Eds), *Casarett and Doull's Toxicology: The Basic Science of Poisons*, pp. 565–622, New York: Pergamon.
- ELVIDGE, J. A., JONES, S. P. & PEPPARD, T. L., 1982, Chemistry of hop constituents, *Journal of the Chemical Society, Perkin Transactions*, **1**, 1089–1094.
- FARKAS, P., HRADSKY, P. & KOVAC, M., 1992, Novel flavour components identified in the steam distillate of onion, *Zeitschrift fur Lebensmittel Untersuchung und Forschung*, **195**, 459–462.

- FERRANDO, J. A. S., 1986, Allylic sulfides as tickicides, *Belgian Patent*, 903756 cited in CA 105(25), 218898g.
- FERRETTI, A., 1973, Inhibition of cooked flavor in heated milk by use of additives, *Journal of Agricultural and Food Chemistry*, 21, 939-942.
- FOYE, W. O. & HU, J. M., 1979, Metal-binding abilities of radioprotective aminoalkyl disulfides and thiosulfates, *Journal of Pharmacological Sciences*, 68, 202-205.
- FRASER, G. G., 1974, Sulfur nutrition in duckweed *Spirodela oligorrhiza Mauri Ora*, 2, 147-156 cited in CA 85(19), 139824v.
- FREEDMAN, R. B., BULLEID, N. J., HAWKINS, H. C. & PAVER, J. L., 1988, Role of protein disulfide-isomerase in the expression of native proteins, *Biochemical Society Symposium* (1988), 55, 167-192.
- FRENCH, M. S. & MEARN, S. R., 1991, Prevention of discoloration or wilting of vegetables disinfected with per acids, *British Patent*, GB 90-12790 cited in CA 116(13), 127380t.
- FRENKEL, G. D. & FALVEY, D., 1989, Selenotrisulfide inhibits initiation by RNA polymerase II but not elongation, *Journal of Inorganic Biochemistry*, 35, 179-189.
- FRENKEL, G. D., FALVEY, D. & MACVICAR, C., 1991, Products of the reaction of selenite with intracellular sulfhydryl compounds, *Biological Trace Elements Research*, 30, 9-18.
- GIBLIN, F. J., 1980, Disulfide formation in high molecular weight protein of X-ray induced cataract, *Developmental Biochemistry*, 9, 95-99.
- GILBERT, H. F., 1990, Molecular and cellular aspects of thiol-disulfide exchange, *Advances in Enzymologically Related Areas of Molecular Biology*, 63, 69-172.
- GIUDICE, L. C. & PIERCE, J. G., 1978, Glycoprotein hormones, in McKearn, K. W. (Ed.), *Structure and Functions of Gonadotropins*, pp. 81-110, New York: Plenum.
- GMELIN, R., SUSILIO, R. & FENWICK, G. R., 1981, Cyclic polysulfides from *Parkia speciosa*, *Phytochemistry*, 20, 2521-2523.
- GOLDENBERG, D. P., 1992, Native and non-native intermediates in the BPTI folding pathway, *Trends in Biochemical Sciences*, 17, 257-261.
- GOTO, Y., 1988, Folding process of proteins - unfolding and refolding of the immunoglobulin fragments, *Tanpakushitsu Kakusan Koso*, 33(6), 1083-1097 cited in CA 108(25), 219973b.
- GRAY, E. S. & TSAI, R. W., 1994, Characterisation of striped bass growth hormone receptors by disulfide-bond reduction and cross-linking studies, *Journal of Experimental Zoology*, 268, 428-435.
- GREGORY, T., HOIE, J., WATANABE, C. & SPELLMAN, M., 1991, Structure and function in recombinant HIV-1 gp 120 and speculation about the disulfide bonding in the gp 120 homologs of HIV-2 and SIV, *Advances in Experimental Medicine and Biology*, 303, 1-14.
- HAMAGUCHI, K., 1982, The role of disulfide bridges in globular proteins, *Tanpakushitsu Kakusan Koso*, 27, 1616-1625 cited in CA 97, 160685L.
- HAMILTON, H. E., MELVILLE, G. S. & STORK, E. J., 1968, Radioprotection of primates with 2-(1-decylamino) ethane thiosulfuric acid in dimethyl sulfoxide, *US Clearing-house Federal Sciences Technical and Information AD-691409*, United States Governmental Research and Development Reports, 1969, 69, 39 cited in CA 72(9), 39566a.
- HANIU, M., HSIEH, P., ROHDE, M. F. & KENNEY, W. C., 1994, Characterisation of disulfide linkages in platelet-derived growth factor, *Archives of Biochemistry and Biophysics*, 310, 433-437.
- HANTAI, D., AKAABOUN, M., LAGARD, C., MURAWSKY, M., HOUEYOU, L., FESTOFF, B. W., VAUGHT, J. L., RIEGER, F. & BLANDET, B., 1994, Beneficial effects of IGF-1 on wobbler mouse motor neurone disease, *Abstracts of the Proceedings of the 5th International Symposium ALS/MND*, Noordwijk, Holland, International Alliance ALS/MND Association, p. 140.

- HATCH, R. C., CLARK, J. D. & JAIN, A. V., 1978, Use of thiols and thiosulfate for treatment of experimentally induced acute arsenite toxicosis in cattle, *American Journal of Veterinary Research*, **39**, 1411–1414.
- HEAFIELD, M. T. E., FEARN, S., STEVENTON, G. B., WARING, R. H. & WILLIAMS, A. C., 1990, Plasma cysteine and sulphate levels in motor neurone, Parkinson's and Alzheimer's Disease, *Neuroscience Letters*, **110**, 216–222.
- HERRMANN, R., GUDER, H. J. & JUNIUS-COMER, M., 1991, Use of azide, cyanate, thiosulfate and rhodamide salts for enhancement of enzymic reactivity of β -galactosidase, *German Patent*, DE 90-4007836 cited in CA **115**(5), 45157r.
- HOFMANN, K. & HAMM, R., 1978, Sulfhydryl and disulfide groups in meats, *Advances in Food Research*, **24**, 1–11.
- HOIE, J. A., 1991, Hypothetical assignment of intrachain disulfide bonds for HIV-2 and SIV envelope glycoproteins, *AIDS Research and Human Retroviruses*, **7**, 495–499.
- HORIE, T., AWAZU, S., ITAKURA, Y. & FUWA, T., 1992, Identified diallyl polysulfides from an aged garlic extract which protects the membranes from lipid peroxidation, *Planta Medica*, **58**, 468–469.
- HOUGH, J. S., BRIGGS, D. E., STEVENS, R. & YOUNG, T. W., 1982a, *Malting and Brewing Science*, Vol. II 2nd Edn, pp. 806–808, London and New York: Chapman & Hall.
- 1982b, *Malting and Brewing Science*, Vol. II, 2nd Edn, pp. 448–451, London and New York: Chapman & Hall.
- HOUK, J., SINGH, R. & WHITESIDES, G. M., 1987, Measurement of thiol-disulfide interchange reactions and thiol pK_a values, *Methods in Enzymology*, **143**, 129–140.
- INOUE, M., 1989, Glutathione: dynamic aspects of protein mixed disulfide formation, *Coenzymes and Cofactors*, **3** (Glutathione, Chemistry, Biochemistry and Medical Aspects, Part B), 613–644.
- INQUELLO, V., RAYMOND, J. & AZANZA, J. L., 1993, Disulfide interchange reactions in IIS globulin subunits of Cruciferae seeds, Relationships to gene families, *European Journal of Biochemistry*, **217**, 891–895.
- IRIUCHIJIMA, S., 1977, Malformin and asparagusic acids as sulfur-containing compounds having biological activities towards plants, *Yuki Gosei Kagaku Kyokaiishi*, **35**, 394–395 cited in CA **87**(9), 65267h.
- ISHIBASHI, J., KATAOKA, H., ISOGAI, A., KAWAKAMI, A., SAEGUSA, H., YAGI, Y., MIZOGUCHI, A., ISHIZAKI, H. & SUZUKI, A., 1994, Assignment of disulfide bond location in PTTH of the silkworm *Bombyx mori*: a homodimeric peptide, *Biochemistry*, **33**, 5912–5919.
- ISHIDA, M., HAGA, R., ODAWARA, H., TAKAHASHI, S. & EBARA, K., 1985, *Japanese Patent* 84-38580 cited in CA **103**(23), 195219d.
- JACOBS, L. S. & LORENSON, M. Y., 1985, Extractability of rat pituitary prolactin, *FIDIA Research in Series*, **1**, 455–461.
- JAULMES, P., HAMELLE, G. & BEALATON, I., 1969, Polysulfide intoxication, *Medicine Legale et Dommage Corporel*, **2**, 174–177 cited in CA **72**(5), 20150p.
- JERUSALINSKY, D. & HARVEY, A. L., 1994, Toxins from mamba venoms: small proteins with selectivities for different subtypes of muscarinic acetylcholine receptors, *Trends in Pharmacological Sciences*, **15**, 424–430.
- JOCELYN, P. C., 1987, Chemical reduction of disulfides, *Methods in Enzymology*, **143**, 246–256.
- JOST, K., 1987, Important structural modifications. III. Modifications in the disulfide bridge, in Jost, K., Lebl, M. & Brtnik, F. (Eds), *CRC Handbook, Neurohypophyseal Hormone Analogs*, Vol. 1, pp. 144–188, Boca Raton, FL: CRC Press.
- KADIN, H., 1987, Electrochemical reduction of disulfides, *Methods in Enzymology*, **143**, 257–264.

- KADOTA, O. & TSURUMI, K., 1992, Controlled-release antifouling compositions for fishnets and ropes, *Japan Kokai Tokkyo Koho*, JP 04225904 cited in CA **118**(11), 96279b.
- KAGE, S., NAGATA, T. & KUDO, K., 1991, Determination of polysulfides in blood by gc and gc/ms, *Journal of Chromatography*, **564**, 163–169.
- KAGE, S., NAGATA, T., KIMURA, K., KUDO, K., IMAMURA, T. & URAKAWA, N., 1993, Forensic toxicological analysis of polysulfides in biological materials, *Hochudoku* **11**, 134–135 cited in CA **119**(13), 133005c.
- KALLIO, H. & SALORINNE, L., 1990, Comparison of onion varieties by headspace gc/ms, *Journal of Agricultural and Food Chemistry*, **38**, 1560–1564.
- KARIGOME, A., HAYASHI, R. & HASHIZUME, Y., 1994, Tap water treatment for enzymic reaction, *Japanese Patent JP*, 92-187694 cited in CA **120**(19), 242708a.
- KAWAKISHI, S. & MORIMITSU, Y., 1994, Sulfur chemistry of onions and inhibitory factors of the arachidonic acid cascade, *ACS Symposium Series*, **546** (Food Phytochemicals for Cancer Prevention 1), 120–127.
- KELLER, S. H. & VACQUIER, V. D., 1994, The isolation of acrosome-reaction inducing glycoproteins from sea urchin egg jelly, *Developmental Biology*, **162**, 304–312.
- KELLY, D. P. & TUOVINEN, O. H., 1975, Metabolism of inorganic sulfur compounds by *Thiobacillus ferrooxidans*, *Plant Soil*, **43**, 77–93.
- KIM, Y. H., 1992, Biochemistry of disulfides, in Oae, S. & Okuyama, T. (Eds), *Organic Sulfur Chemistry: Biochemical Aspects*, pp. 137–194, Boca Raton, FL: CRC Press.
- KLEIN, D. C. & NAMBOODIN, M. A. A., 1981, Inactivation of pineal N-acetyltransferase by disulfide exchange, in Usdin, E., Weiner, N. & Youdin, M. B. H. (Eds), *Function and Regulation of Monoamine Enzymes: Basic Clinical Aspects*, pp. 711–722, London: Macmillan.
- KOBAYASHI, Y. & TAMAOKI, H., 1990, A new α -helical motif in bioactive peptides, *Seikagaku*, **62**, 15002–15006 cited in CA **114**(13), 17009y.
- KORELOV, V. T., 1973, Morphological and biochemical composition of sheep blood during feeding of some sulfur preparations, *Trudy Vsesyuznyi Nauchno-Issledovatel'skii Instituta Ovisevodi Kozovodshi*, **34**, 200–203 cited in CA **84**(17), 120126b.
- KRONING, H., KAHNE, T., ITTENSON, A., FRANKE, A., & ANSORGE, S., 1994, Thiol-protein disulfide-oxidoreductase (PD1): a new plasma membrane constituent of mature human β -lymphocytes, *Scandinavian Journal of Immunology*, **39**, 346–350.
- KUO, M. C. & HO, C. T., 1992a, Volatile constituents of the distilled oils of Welsh onions (*Allium fistulosum*) and scallions (*Allium fistulosum* L. var. *caespitosum*), *Journal of Agricultural and Food Chemistry*, **40**, 111–117.
- 1992b, Volatile constituents of the solvent extracts of Welsh onions and scallions, *Journal of Agricultural and Food Chemistry*, **40**, 1906–1910.
- LAAKSO, I., SEPPANEN-LAAKSO, T., HILTUNEN, R., MUELLER, B., JANSEN, H. & KNOBLOCH, K., 1989, Volatile garlic odour components: gas phases and adsorbed exhaled air analysed by head-space gc/ms, *Planta Medica*, **53**, 257–261.
- LAWSON, L. D. & HUGHES, B. G., 1992, Characterisation of the formation of allicin and other thiosulfates from garlic, *Planta Medica*, **58**, 345–350.
- LEE, J. & PILCH, P. F., 1994, The insulin receptor: structure, function and signalling, *American Journal of Physiology*, **266**, C319–334.
- LEVITT, J., 1978, Role of sulfhydryl and disulfide groups in damage to biological systems at low water activities, in Crowe, J. H. & Clegg, J. S. (Eds), *Dry Biological Systems*, pp. 243–256, New York: Academic Press.
- LEVY, E., 1992, Removal of chlorine from beverages with thiosulphates, *US Patents* 90-606307 cited in CA **116**(21), 213344r.
- LILIE, H., McLAUGHLIN, S., FREEDMAN, R., BUCHNER, J., 1994, Influence of protein disulfide isomerase on antibody folding *in vitro*, *Journal of Biological Chemistry*, **269**, 14290–14296.

- LOZANO, R. M., YEE, B. C. & BUCHANAN, B. B., 1994, Thioredoxin-linked reductive inactivation of venom neurotoxins, *Archives of Biochemistry and Biophysics*, **309**, 356–362.
- LUK'YANCHUK, V. D., MIRYAN, N. I. & LINK, A. I., 1985, Comparative evaluation of the detoxifying effect of sodium thiosulfate and its alkyl derivatives on dinitro-orthocresol poisoning, *Farmakologia Zhurnal (Kiev)*, **1**, 75–76 cited in CA **102**(21), 180455h.
- MACLEOD, G. & AMES, J. M., 1986, The effect of heat on beef aroma: comparisons of chemical composition and sensory properties, *Flavour and Fragrance Journal*, **1**, 91–104.
- MALBON, C. C., GEORGE, S. T. & MOXHAM, C. P., 1987, Intramolecular disulfide bridges: avenues to receptor activation?, *Trends in Biochemical Sciences*, **12**, 172–175.
- MANNERVIK, B. & LARSON, K., 1981, Mixed (unsymmetric) disulfides, coenzyme A-glutathione disulfide as an example, *Methods in Enzymology*, **77**, 420–424.
- MARET, W., 1994, Oxidative metal release from metallothionein via zinc/thiol disulfide interchange, *Proceedings of the National Academy of Sciences, USA*, **91**, 237–241.
- MARTIN-PONTHIEU, A., WOUTERS-TYROU, D., PUDLO, B., BUISINE, E. & SAUTIERE, P., 1994, Isolation and characterisation of a small putative zinc finger protein from cuttle fish epididymal sperm cells, *European Journal of Biochemistry*, **220**, 463–468.
- MARTINEZ GARCIA, J. P., MARTINEZ, G. P., ALCAIDE, M. E., HERNANDEZ, H. P. & HERNANDEZ, G. P., 1980, Effect of anionic detergents on aciduric species of *Thiobacillus* using oxidative sodium thiosulphate, *Agrochimica*, **24**(2–3), 127–136 cited in CA **94**(11), 77525y.
- MCGINNES, L. W. & MORRISON, T. G., 1994, The role of the individual cysteine residues in the formation of the mature antigenic HN protein of Newcastle disease virus, *Virology*, **200**, 470–483.
- MIESBAUER, L. R., ZHOU, X., YANG, Z., SUN, Y., SMITH, D. L. & SMITH, J. B., 1994, Post-translational modifications of water-soluble human lens crystallins from young adults, *Journal of Biological Chemistry*, **269**, 12494–12502.
- MIN, Z., MAI, Q., MIZUNO, M., TANAKA, T. & INUMA, M., 1987, Polysulfanes in the volatile oils of *Ferula* species, *Planta Medica*, **53**, 300–302.
- MISHARINA, T. A. & GOLOVNYA, R. V., 1993, Changes in the composition of volatiles in ripe and unripe onions in storage, freezing and cooking, *Prikladnaia Biokhimiya Microbiologiya*, **29**, 911–921 cited in CA **120**(11), 132679j.
- MITSUDERA, H., KAMIKADO, T., UNEME, H., MANABE, Y., 1990, Synthesis and biological activity of 5-alkylthio-1,3-dithianes, *Agricultural and Biological Chemistry*, **54**, 1723–1730.
- MORIMATSU, M., NAKAGAWA, N., MOMINOKI, K., SYUTO, B., NAIKI, M. & SAITO, M., 1993, Comparative study on the molecular properties of mammalian haptoglobins, *Kachiku Seikayaku Kenkyukaiho*, **30**(1), 23–30 cited in CA **119**, 91859b.
- MOSER, R., DUEVEL, D. & GREVE, R., 1980, Volatile constituents and fatty acid composition of lipids in *Durio zibethinus*, *Phytochemistry*, **19**, 79–81.
- MOSHER, D. F. & MCKEOWN-LONGO, P. J., 1985, Assembly of fibronectin-containing extracellular matrix: a glimpse of the machinery, *Biopolymers*, **24**, 199–210.
- MULLEN, M. & CRAWFORD, F., 1993, Molecular and genetic advances in Alzheimer's Disease, *Trends in Neurological Sciences*, **16**, 398–409.
- MULVEY, M. & BROWN, D. T., 1994, Formation and re-arrangement of disulfide bonds during maturation of the Sindbis virus E1 glycoprotein, *Journal of Virology*, **68**, 805–812.
- MUNDAY, R., 1989, Toxicity of thiols and disulfides, involvement of free-radical species, *Free Radicals in Biology and Medicine*, **7**, 659–673.

- NAGAI, S. & NISHIO, N., 1989, Vitamin B₁₂ and its manufacture with Methanosarcina, *Japanese Patent*, 62257394 cited in CA 108(2), 185271d.
- NAGANO, K. & TSUJI, F. I., 1990, Dimeric interaction of calcium-binding photoprotein aequorin, in Rivier, J. E. & Marshall, G. R. (Eds), *Peptides: Chemistry, Structure and Biology*, Proceedings of the American Peptide Symposium, 11th Meeting (1988), pp. 508–509, London: ESCON Science.
- NAKAMA, K., 1980, Agent for accelerating the ripening of agricultural products, *Japanese Patent*, 79-47276 cited in CA 94(1), 1074e.
- 1981, Agent and method for accelerating the maturation of field and garden crops, *Japanese Patent*, 79-47376 cited in CA 96(5), 29952e.
- NAMMOUR, D., AUGER, L. & HUIGNARD, J., 1989, Insecticidal effect of sulfur compounds (disulfides and trisulfides) in *Bruchidius atrolineatus*, *Insect Science and Its Application*, 10, 49–53.
- NELSON, J. W. & CREIGHTON, T. E., 1994, Reactivity and ionisation of the active site cysteine residues of DsbA, a protein required for disulfide bond formation *in vivo*, *Biochemistry*, 33, 5974–5983.
- NISHIMURA, H., WIJAYA, C. H. & MIZUTANI, J., 1988, Volatile flavor components and antithrombotic agents: vinyl dithiols from *Allium victorialis*, *Journal of Agricultural and Food Chemistry*, 36, 563–566.
- NOIVA, R., 1994, Enzymic catalysis of disulfide formation, *Protein Expression and Purification*, 5, 1–13.
- NOVICK, D., COHEN, B. & RUBINSTEIN, M., 1994, The human interferon α/β receptor: characterisation and molecular cloning, *Cell*, 77, 391–400.
- OGATA, Y., IKEDA, M. & NAGAGAWA, J., 1989, Benzyl polysulfides for prophylactic and therapeutic treatment of liver disorders, *Japanese Patent* 01180821 cited in CA 112(9), 70028v.
- OGAWA, H., 1989, Significance of sulfhydryl group and disulfide bond in keratinisation, *Nippikyo Japan* 12, 14–20 cited in CA 112, 195600g.
- OHLENDIECK, K., PARTIN, J. S. & LENNARZ, W. J., 1994, The subunit composition of the biologically active sperm receptor in egg plasma membranes from *S. purpuratus*, *Journal of Cell Biology*, 125, 817–824.
- OLESON, J., THOMSEN, L. L. & IVERSEN, H., 1994, Nitric oxide is a key molecule in migraine and other vascular headaches, *Trends in Pharmacological Sciences*, 15, 149–153.
- OL'KHOVSKAYA-BURKOVA, A. K., SUSHA, U. L. & LOPATIN, L. V., 1967, Results of testing ammonium polysulfides against fruit and pear mites, *Sadovodstvo (Kiev)*, 6, 97–106 cited in CA 72(17), 89236p.
- ONDARZA, R. N., 1989, Enzyme regulation by biological disulfides, *Bioscience Reports*, 9, 593–604.
- PAHLICH, E., JAEGER, H. J. & STEUBING, L., 1972, Effect of sulfur dioxide on the activities of glutamate dehydrogenase and glutamine synthesis from pea seedlings, *Angewante Botanische*, 46, 183–197.
- PAPAGEORGIOU, C., CORBET, J. P., MENEZES-BRANDAO, F., PECEGUEIRO, M. & BENEZRA, C., 1983, Allergic contact dermatitis to garlic (*Allium Sativum*, L.), *Archives of Dermatological Research*, 275, 229–234.
- PARRISH, W. B., WILLETT, D. N., KRALICK, F. A. & ORR, G. N., 1992, Method for the stimulation of cell growth and the inhibition of cell proliferation by the utilisation of selenodithiols such as selenodiglutathione, *US Patent* 89-443608 cited in CA 117(15), 143443s.
- PATARASAKULCHAI, N. & SOUTHWELL-KEELY, P. T., 1984, Se-arylselenenylthiosulfates and S-arylsulfenylthiosulfates as thiol-blocking reagents, *Biochemical Journal*, 221, 780–797.
- PETERSON, D. A., ARCHER, S. L. & WEIR, E. M., 1994, Superoxide reduction of a

- disulfide: a model of intracellular redox modulation, *Biochemical and Biophysical Research Communications*, **200**, 1586–1591.
- PHEIFER, J. H. & BRIGGS, D. E., 1995, The estimation of thiols and disulfides in barley, *Journal of the Institute of Brewing*, **101**, 5–10.
- PIERCE, J. G., 1981, Problems in the determination of disulfide bonds in the glycoprotein hormones of the pituitary and placenta, *Developmental Biochemistry* (Chemical Synthesis and Sequencing of Peptides and Proteins), 221–229.
- PINO, J. A., 1992, Headspace sampling methods for the volatile constituents of garlic, *Journal of Science of Food and Agriculture*, **59**, 131–133.
- POWELL, E. N., CRENSHAW, M. A. & RIEGER, R. M., 1980, Adaptations to sulfide in sulfide-system meiofauna, *Marine Ecology: Progress Series*, **2**, 169–177.
- PUIG, A. & GILBERT, H. F., 1994, Protein disulfide isomerase exhibits chaperone and anti-chaperone activity in the oxidative refolding of lysozyme, *Journal of Biological Chemistry*, **269**, 7764–7771.
- RESNICK, D., PEARSON, A. & KRIEGER, M., 1994, The SRCR superfamily: a family reminiscent of the Ig superfamily, *Trends in Biochemical Sciences*, **19**, 508–510.
- RITZAU, M., KELLER, M., WESSELS, P., STETTER, K. O. & ZEECK, A., 1993, New cyclic polysulfides from hyperthermophilic archaea of the genus *Thermococcus*, *Liebigs Annalen der Chemie*, **8**, 871–876.
- RYSER, H. J., LEVY, E. M., MANDEL, R. & DISCIULLO, G. J., 1994, Inhibition of human immunodeficiency virus infection by agents that interfere with thiol–disulfide interchange upon virus–receptor interactions, *Proceedings of the National Academy of Sciences, USA*, **91**, 4559–4563.
- SANSING, J. E. & NOVOTNY, J., 1976, Inhibition of corrosive action of zinc-containing fertiliser solutions, *US Patent* 75-570102 cited in CA **84**(13), 88679w.
- SASS-KISS, A., PETRO-TURZA, M., SZARFOLDI-SZALMA, I., PINO, J. & ROSADNO, A., 1989, Investigations on the volatile oil of Mako anions, *Nahrung*, **33**, 413–421 cited in CA **112**(5), 34579s.
- SCARF, A., COLE, E. R. & SOUTHWELL-KEELY, P. T., 1977, Comparative reactivities of alkyl selenosulfates and alkyl thiosulfates with cysteine, *Phosphorus Sulfur*, **3**, 285–289.
- SHANKARANARAYANA, M. L., RAGHAVEN, B., ABRAHAM, K. O. & NATARAJAN, C. P., 1974, Sulfur volatiles, *CRC Critical Reviews in Food Technology*, **29**, 395–413.
- SHARP, J. C., KELLY, D. D. & BRADY, J. V., 1967, Radioattenuating effects of *n*-decylaminoethane thiosulfuric acid in the rhesus monkey, in Vagtborg, H. (Ed.), *Use of Non-Human Primates in Drug Evaluation Symposium*, pp. 338–346, Austin, TX: University of Texas Press, cited in CA **72**(5), 18952h.
- SHEEN, L. Y., LIN, S. Y. & TSAI, S. J., 1992, Odor assessments for volatile compounds of garlic and ginger essential oils by sniffing gas chromatography, *Zhongguo Nongye Huaxue Huizhi*, **30**, 14–24 cited in CA **117**(13), 130135t.
- SHOUP, R. E., 1987, Minireview: RSH/RSSR applications using the dual mercury/gold amperometric detector, *Current Separations*, **8**, 38–43.
- SHUTOV, G., 1982, Defoliation and desiccation agents, *Belorussian Patent* DE 3048526 cited in CA **97**(9), 67832u.
- SIMONEN, M., JAMSA, E. & MAKAROW, M., 1994, The role of the carrier protein and disulfide formation in the folding of β -lactamase fusion proteins in the endoplasmic reticulum of yeast, *Journal of Biological Chemistry*, **269**, 13887–13892.
- SMITH, D. L. & ZHOU, Z., 1990, Strategies for locating disulfide bonds in proteins, *Methods in Enzymology*, **193** (Mass Spectrometry), 374–389.
- SMITH, R. P., 1991, Toxic responses of the blood, in Amdur, M. A., Doull, J. & Klaassen, C. D. (Eds), *Casarett and Doull's Toxicology* 4th Edn, pp. 257–281, Oxford: Pergamon.
- SMOTHERMAN, W. P. & ROBINSON, S. R., 1992, Dimethyl disulfide mimics the effects

- of milk on fetal behaviour and responsiveness to cutaneous stimuli, *Physiology and Behaviour*, **52**, 761–765.
- SOERBO, B., 1989, Metabolism of thiosulfate and thiosulfate esters, *Coenzymes and Cofactors*, **3** (Glutathione, Chemistry, Biochemistry and Medical Aspects, Part B), 411–439.
- SOWDHAMINI, R. & BALARAM, P., 1993, Protein structure and stability, in Gupta, M. W. (Ed.), *Thermostable Enzymes*, pp. 2–21, New Delhi: Narosa.
- STEARNS, M. E., 1988, Importance of the thiol/disulfide status of microtubule proteins for anti-mitotic drug action, *Pharmacology and Therapeutics*, **37**, 25–36.
- STEUDEL, R., HOLDT, G., VISSCHER, P. T. & VAN GEMERDEN, H., 1990, Search for polythionates in cultures of *Chromatium vinosum* after sulfide incubation, *Archives of Microbiology*, **153**, 432–437.
- STEVENS, F. S., 1981, Thiol inhibitors of proteases – the reversible disulfide exchange in the regulation of trypsin-like enzymes, in Turk, J. & Vitale, L. (Eds), *Proteinases, Their Inhibitors: Structure, Function and Applied Aspects*, pp. 305–316, Ljubljana: Mladinska Krujiga.
- STRAUB, F. B., 1968, Role of disulfide bridges in some pancreatic enzymes, in Fric, P. (Ed.), *Progress in Pancreatology*, pp. 71–79, Prague: Czechoslovakia Medical Press.
- SUH, B. & AKAGI, J. M., 1969, Formation of thiosulfate from sulfite by *Desulfovibrio vulgaris*, *Journal of Bacteriology*, **99**, 210–215.
- SUNDARAM, S. G. & MILNER, J. A., 1993, Impact of organosulfur compounds in garlic on canine mammary tumor cells in culture, *Cancer Letters*, **74**, 85–90.
- SUSILO, R. & GMELIN, R., 1982, Precursors of cyclic polysulfides in seeds of *Parkia speciosa* Hassk, *Zeitschrift fur Naturforschung*, **37C**, 584–586.
- TAKAHASHI, T., TAMURA, Y. & KANDU, O., 1991, An agent for preservation of cut flowers containing phosphate as major ingredient, *Japanese Patent* 89-301885 cited in CA **115**(15), 155289t.
- THIM, L., 1989, A new family of growth-factor-like peptides, *FEBS Letters*, **250**, 85–90.
- TIMMER-TEN HOOR, A., 1975, A new type of thiosulphate oxidising, nitrate reducing microorganism: *Thiomicrospira denitrificans* species nova, *Netherlands Journal of Sea Research*, **9**, 344–350 cited in CA **84**(23), 161550d.
- TSUJI, M., FUJIMORI, K., NAKUNO, T. & OKUMO, T., 1991, Identification of volatile sulfur compounds in plants, *Hyogo-Kenritsu*, **23**, 106–108 cited in CA **117**(25), 250230s.
- VAIDYANATHAN, V. V., SASTRY, P. S. & RAMASARMA, T., 1993, Regulation of the activity of glyceraldehyde-3-phosphate dehydrogenase by glutathione and H₂O₂, *Molecular and Cellular Biochemistry*, **129**, 57–65.
- VIEHWEG, S. H., SCHMIDT, R. E. & SCHMIDT-LORENZ, W., 1989a, Microbial spoilage of refrigerated fresh broilers, *Lebensmittel-Wissenschaft Technologie*, **22**, 346–355.
- 1989b, Microbial spoilage of refrigerated fresh broilers, *Lebensmittel-Wissenschaft Technologie*, **22**, 356–367.
- WAGNER, H., DORSCH, W., BAYER, T., BREU, W. & WILLER, F., 1990, Antiasthmatic effects of onions: inhibition of 5-lipoxygenase and cyclooxygenase *in vitro* by thiosulfates and cepaenes, *Prostaglandins, Leukotrienes and Essential Fatty Acids*, **39**, 59–62.
- WANG, C. C. & TSOU, C. L., 1993, Protein disulfide isomerase is both an enzyme and a chaperone, *FASEB Journal*, **7**, 1515–1517.
- WANG, K. K. W. & YUEN, P. W., Calpain inhibition: an overview of its therapeutic potential, *Trends in Pharmacological Sciences*, **15**, 412–419.
- WARING, R. H. & EMERY, P., 1992, Genetic factors predicting persistent disease: the role of defective enzyme systems, *Baillieres Clinical Rheumatology*, **6**, 277–350.
- WARING, R. H., FENWICK, G. R. & MITCHELL, S. C., 1987a, The chemical nature of the urinary odour following asparagus ingestion, *Xenobiotica*, **17**, 235–244.

- WARING, R. H., LAND, D. G., MITCHELL, S. C. & THORPE, W. V., 1987b, Odorous urine following asparagus ingestion in man, *Experientia*, **43**, 382–383.
- WETZEL, R., 1987, Harnessing disulfide bonds using protein engineering, *Trends in Biochemical Sciences*, **12**, 478–482.
- WETZEL, R., JOHNSTON, P. D. & CZARNIECKI, C. W., 1983, Roles of the disulfide bonds in a human α interferon, in De Maeyer, E. (Ed.), *Biology of Interferon Systems*, Proceedings of the 2nd International TNO Meeting, pp. 101–112, Amsterdam: Elsevier.
- WIJAYA, C. H., NISHIMURA, H., TANAKA, T. & MIZUTARNI, J., 1991, Influence of drying methods on volatile sulfur constituents of caucas, *Journal of Food Sciences*, **56**, 72–75.
- WILLIAMS, R. S. & GRACEY, D. E. F., 1982a, Beyond dimethyl sulfide: the significance to flavor of thioesters and polysulfides in Canadian beers, *Journal of the American Society of Brewing Chemists*, **40**, 68–71.
- 1982b, Factors influencing the levels of polysulfides in beer, *Journal of the American Society of Brewing Chemists*, **40**, 71–74.
- WOOD, J. L., 1982, Biochemical functions of persulfides, *Advances in Experimental Medicine and Biology*, **148**, 327–342.
- WRATTEN, S. J. & FAULKNER, D. J., 1976, Cyclic polysulfides from the red alga *Chondria californica*, *Journal of Organic Chemistry*, **41**, 2465–2467.
- YANG, C., GU, Z. W., YANG, M. & GOTTO, A. M., 1994, Primary structure of Apo β -100, *Chemistry and Physics of Lipids*, **67–68**, 99–104.
- YOUNG, D. C., 1972, Solutions of ammonium nitrate, sulfate and chloride containing a thiosulfate as defoliants and desiccants, *US Patent* 63-421688 cited in CA **77**(25), 160997y.
- YU, T. H. & WU C. M., 1988, Effects of heating on garlic essential oils, *Shih P'in K'o Hsueh (Taipei)*, **15**, 385–393 cited in CA **111**(15), 132766z.
- YU, T. H., WU, C. M. & HO, C. T., 1993, Volatile components of deep-oil fried, micro-wave heated and oven-baked garlic slices, *Journal of Agricultural and Food Chemistry*, **41**, 800–805.
- ZAPUN, A., COOPER, L. & CREIGHTON, T. E., 1994, Replacement of the active-site cysteine residues of DsbA, a protein required for disulfide bond formation *in vivo*. *Biochemistry*, **33**, 1907–1914.
- ZHANG, D. & LIANG, S., 1993, Assignment of the three disulfide bridges of huwentoxin-1, a neurotoxin from the spider *Selenocosmia huwena*, *Journal of Protein Chemistry*, **12**, 735–740.
- ZHANG, J. Z. & REDMAN, C. M., 1994, Role of interchain disulfide bonds on the assembly and secretion of human fibrinogen, *Journal of Biological Chemistry*, **269**, 652–658.
- ZHANG, Y. & HO, C. T., 1991, Comparison of the volatile compounds formed from the thermal reaction of glucose with cysteine and glutathione, *Journal of Agricultural and Food Chemistry*, **39**, 760–763.
- ZIEGLER, D. M., 1985, Role of reversible oxidation–reduction of enzyme thiols–disulfides in metabolic regulation, *Annual Reviews of Biochemistry*, **54**, 305–329.
- 1988, Functional groups activated via flavin-containing monooxygenases, in Miners, J. D. (Ed.), *Microsomes and Drug Oxidation*, Proceedings of the International Symposium 7th Meeting (1987), pp. 297–304, London: Taylor & Francis.
- ZIEGLER, S. J. & STICHER, O., 1989, HPLC of S-alk(en)yl-L-cysteine derivatives in garlic including quantitative determination of (+)-S-allylcysteine sulfoxide (alliin), *Planta Medica*, **55**, 372–378.
- ZUAZAGA, Z., STEINACKER, A. & DEL CASTILLO, J., 1994, The role of sulfhydryl and disulfide groups of membrane proteins in electrical conduction and chemical transmission, *Puerto Rico Health Sciences Journal*, **3**, 125–139.

Sulfur–Selenium and Sulfur–Phosphorus Compounds

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1.1 Introduction

This short chapter describes two associations of sulfur that have been exploited within the biological field and which have not been covered elsewhere in this book, namely the inorganic sulfur–selenium combinations and the organic sulfur–phosphorus compounds. It is true that sulfur can form a vast array of inorganic compounds with many elements, as has been admirably covered in the chapter on sulfur chemistry, and details of inorganic toxicities (e.g. the airborne toxins, sulfur dioxide and hydrogen sulfide) have been mentioned in other chapters and can also be gleaned from excellent comprehensive works on toxicology (Ellenhorn and Barceloux 1988, Hayes 1982, Kurt 1983, Lilis 1983, Rom and Barkman 1983, Sörbo 1972).

1.2 Sulfur–selenium compounds

Sulfur may combine with selenium to give a range of eutectic mixtures, possessing general formulae of $\text{Se}_n\text{S}_{8-n}$, but disproportionating into disordered structures with selenium and sulfur placed statistically over the atomic sites and several different combinations within the same crystal lattice. Twelve-membered molecules, in addition to six- and seven-membered ring sizes, have also been proposed (Steudel and Laitinen 1982).

Selenium sulfide ('selenium disulfide'; SeS_2) has found a major use in the

medical field in the treatment of skin disorders. These disorders include dandruff (Matson 1956), psoriasis (Borglund and Enhamre 1987), seborrhoeic dermatitis (Grosshans and Bressiux 1988), scalp ringworm (tinea capitis) (Shelley *et al.* 1987) and several other dermatoses (e.g. transient acantholytic dermatosis – Grove's disease) and papillomatoses (e.g. congruent and reticulated papillomatosis of Gourgerot and Carteaud). This group of disorders is characterized mainly by inflammation of the skin (dermatitis) or hair follicles (folliculitis), an overaction of the sebaceous glands, and varying degrees of exfoliation of the epidermis (pityriasis). Fungal infections are thought to play a major role in the majority of conditions for which selenium sulfide has been found to be effective, but an overall causative factor still remains elusive (Mitchell *et al.* 1993).

Selenium sulfide, both in aqueous suspension and formulated as a shampoo, has been shown to possess antimicrobial activity against several species belonging to different genera of fungi and bacteria (Butterfield *et al.* 1987, McGinley and Leyden 1982). Studies have suggested that its action is fungicidal in nature and not fungistatic, and it has been proposed that its mechanism of action may be the interference with normal fungal sulfur metabolism. Selenium sulfide-catalysed irreversible polymerization of free thiol groups within the fungal cell walls has been proposed, thereby preventing expansion, halting cell division and leading to eventual cell lysis following gross functional disruption.

Inhibitory effects upon epidermopoiesis (cytostatic effect) have also been postulated, and interference with enzyme systems and cell turnover by selenium sulfide ions has been advocated as a mechanism of action. Investigations with selenium trisulfide have indicated that during nucleic acid transcription the compound can associate with free RNA polymerase II, forming an inactive enzyme that is then unable to interact with the template DNA to form the required enzyme–DNA binary transcription complex. Thus, selenium trisulfide interferes at the initiation stage and subsequently has the effect of decreasing the number of transcription complexes which are active during elongation, with a resulting decrease in the overall rate of RNA synthesis (Frenkel and Falvey 1989). Such interactions could be possible for other selenium sulfur compounds possessing different stoichiometry. The selenium present in selenium sulfide may also act as an intramolecular catalyst, replacing part of the sulfur in ring molecules and forming networks for the transference of electrons, thereby making the sulfur a more effective therapeutic agent. This subject has been reviewed recently (Mitchell *et al.* 1993).

7.3 Sulfur–phosphorus compounds

Sarin (isopropyl methylphosphonofluoridate) together with soman (pinacolyl methylphosphonofluoridate) and tabun (ethyl N-dimethylphosphoramido-cyanidate) (Saunders 1957) are alkoxy organophosphorus compounds which possess a phosphorus–oxygen double bond ($P=O$). They are extremely potent cholinesterase inhibitors developed for use as military nerve gases which have, on occasion, been released upon human populations. Although not strictly speaking gases, they can be dispersed readily as aerosols to devastating effect, being rapidly fatal in high doses; even lower doses may cause prolonged incapacity due to ocular problems.

Inhibition of cholinesterase by organophosphorus compounds appears to be a two-stage process. Simplistically, the enzyme combines reversibly with the inhibitor to produce an enzyme-inhibitor complex and then a stable covalent linkage forms between the enzyme protein and the phosphorus. Cholinesterase activity disappears until the enzyme-phosphorus link is hydrolysed, which may be a very slow reaction, leading to effectively irreversible inhibition. The stability of the phosphorylated enzyme is also enhanced through 'aging' which occurs via transfer of an alkyl group from the bound phosphate ester to the enzyme (Aldridge 1976, Hobbiger 1976, Johnson 1987). Such compounds may not be specific inhibitors of cholinesterases but also inhibit a wide group of carboxylic esterases (e.g. trypsin, chymotrypsin, lipases).

Developed along identical and intermeshed lines of research were the less homicidal phosphorothionate insecticides which have become favoured replacements for the more persistent organochlorine pesticides, and which still retain important use for crop protection and for the control of vector-borne diseases such as malaria (Brooks 1989, DeMatteis 1989). Amongst these compounds, parathion and malathion are probably the best-known examples.

These particular compounds contain a thiono sulfur function attached to the phosphorus atom ($P=S$) and as such are relatively stable and safe. Toxicity ensues from the oxidative conversion of the proinsecticide to the corresponding phosphate which possesses the anticholinesterase activity. This desulfuration reaction has been shown to be enzyme catalysed (Diggle and Gage 1951) and that the enzyme involved was microsomal in location and the reaction had the characteristics of a monooxygenase (Davison 1955, Murphy and DuBois 1957, Nakatsugawa and Dahm 1965, 1967), in particular cytochrome P450 (Kamatani *et al.* 1976, Neal 1976, Norman *et al.* 1973). The recent application of ^{31}P NMR has also implicated a monooxygenase system in the oxidative desulfuration of thiophosphonic amino acid analogues during their incubation with rabbit liver microsomes in the presence of NADPH (Shumyantseva *et al.* 1995).

The mechanism of the critical step in this metabolic activation of phosphorothionates has been investigated over many years and is outlined below for parathion. The reaction of the thiono sulfur of parathion with an activated form of oxygen (enzyme generated) produces an S-oxide intermediate which may exist as a variety of resonating structures. This S-oxide may then either immediately react internally to form a three-membered phosphaoxathiirane ring which disrupts to lose sulfur and produce the neurotoxic paraoxon ($P=O$) derivative (Herriott 1971, Ptashne *et al.* 1971); or alternatively nucleophilic attack by water on the S-oxide moiety may first liberate *p*-nitrophenol, and then subsequent internal phosphaoxathiirane ring formation yields diethyl phosphate or diethyl phosphorothionate dependent upon whether sulfur or oxygen is excluded (Knaak *et al.* 1962, Ptashne *et al.* 1971) (Figure 7.1). This mechanism involving a phosphaoxathiirane ring is analogous to the proposed formation of an oxathiirane ring during the conversion of thiocarbonyl S-oxides to carbonyl compounds (Snyder 1974).

Both insects and humans are capable of converting phosphorothionates to their toxic oxon derivatives but most mammalian species possess more effective hydrolytic enzymes than do insects, thereby inactivating and escaping toxicity at low doses. This use of the proinsecticidal phosphorothionate ($P=S$) instead of the corresponding immediately active phosphate ($P=O$) provides an opportunity for

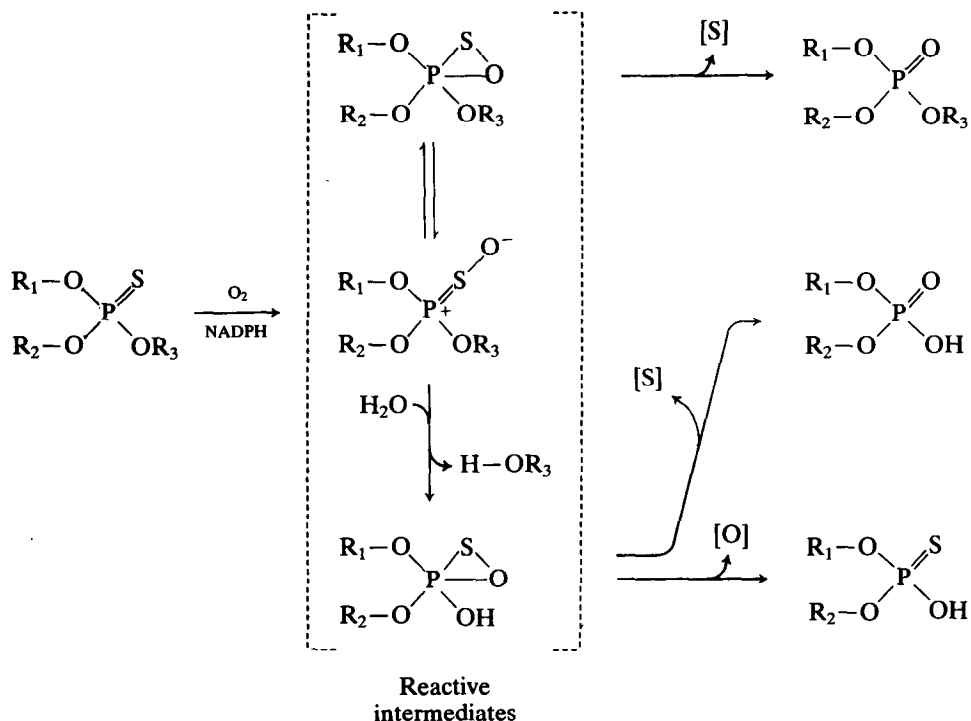


Figure 7.1 Postulated intermediates in the oxidative desulfuration and oxidative hydrolytic breakdown of a phosphorothionate

the organism to metabolize the compound by routes leading to deactivation rather than toxicity (Brooks 1989). Nevertheless, these compounds are still to be considered dangerous.

As desulfuration is an activating pathway, whereas hydrolytic breakdown affords protection, the balance between these two pathways dictates toxicity. This balance presumably depends upon the chemical structure of the phosphorothionate and the relative ease with which sulfur and/or the aryl group are lost from the intermediate reactive oxygen complex (Ptashne and Neal 1972). It has been suggested (DeMatteis 1989) that desulfuration may take place preferentially within the active centre of the catalytic cytochrome leading to deleterious suicidal inactivation (DeMatteis 1974, DeMatteis and Seawright 1973, Hunter and Neal 1975), whereas the reactive S-oxide may diffuse away and, being then and only then allowed to come into contact with water, undergo hydrolytic breakdown. Evidence for the former activity has been provided using parathion (and carbon disulfide) where cyanide treatment released as thiocyanate the sulfur bound to microsomes, suggesting that during desulfuration a considerable proportion of the reactive sulfur binds to cysteine residues in the apoprotein of cytochrome P450 to form hydrodisulfides (DeMatteis 1989) as well as with other amino acids (Neal 1980).

7.4 References

ALDRIDGE, W. N., 1976, Survey of major points of interest about reactions of cholinesterases, *Croatia Chemica Acta*, **47**, 225-233.

- BORGLUND, E., & ENHAMRE, A., 1987, Treatment of psoriasis with topical selenium sulphide, *British Journal of Dermatology*, **117**, 665–666.
- BROOKS, G. T., 1989, Agricultural importance of sulphur xenobiotics, in Damani, L. A. (Ed.), *Sulphur-Containing Drugs and Related Organic Compounds*, Vol. 1A, pp. 64–70, Chichester: Ellis Horwood.
- BUTTERFIELD, W., ROBERTS, M. M. & DAVE, V. K., 1987, Sensitivities of *Pityrosporum* sp. to selected commercial shampoos, *British Journal of Dermatology*, **116**, 233–235.
- DAVISON, A. N., 1955, The conversion of Schradan (OMPA) and parathion into inhibitors of cholinesterase by mammalian liver, *Biochemical Journal*, **61**, 203–209.
- DEMATTEIS, F., 1974, Covalent binding of sulphur to microsomes and loss of cytochrome P-450 during the oxidative desulphuration of several chemicals, *Molecular Pharmacology*, **10**, 849–854.
- 1989, Phosphorothionates, in Damani, L. A. (Ed.), *Sulphur-Containing Drugs and Related Organic Compounds*, Vol. 1B, pp. 9–33, Chichester: Ellis Horwood.
- DEMATTEIS, F. & SEAWRIGHT, A. A. S., 1973, Oxidative metabolism of carbon disulphide by the rat. Effect of treatments which modify the liver toxicity of carbon disulphide, *Chemical Biological Interactions*, **7**, 375–388.
- DIGGLE, W. M. & GAGE, J. C., 1951, Cholinesterase inhibition by parathion *in vivo*, *Nature*, **168**, 998.
- ELLENHORN, M. J. & BARCELOUX, D. G., 1988, *Medical Toxicology: Diagnosis and Treatment of Human Poisoning*, pp. 818, 836, 874, New York: Elsevier.
- FRENKEL, G. D. & FALVEY, D., 1989, Selenotrisulfide inhibits initiation by RNA polymerase II but not elongation, *Journal of Inorganic Biochemistry*, **35**, 179–189.
- GROSSHANS, E. & BRESSIEUX, A., 1988, L'Eczéma séborrhéique (la pityrospore), *Annals of Dermatology and Venereology*, **115**, 79–86.
- HAYES, W. J., 1982, *Pesticides Studied in Man*, Baltimore: Williams & Wilkins.
- HERRIOTT, A. W., 1971, Peroxy acid oxidation of phosphinothioates, a reversal of stereochemistry, *Journal of the American Chemical Society*, **93**, 3304–3305.
- HOBBIGER, F., 1976, Pharmacology of anticholinesterase drugs, in Zaimis, E. (Ed.), *Neuromuscular Junction: Handbuch der Experimentellen Pharmakologie*, Vol. 42, pp. 487–451, Berlin: Springer.
- HUNTER, A. L. & NEAL, R. A., 1975, Inhibition of hepatic mixed-function oxidase activity *in vitro* and *in vivo* by various thiono-sulfur-containing compounds, *Biochemical Pharmacology*, **24**, 2199–2205.
- JOHNSON, M. K., 1987, Organophosphate-induced delayed neuropathy: anomalous data lead to advances in understanding, in DeMatteis, F. & Lock, E. A. (Eds), *Selectivity and Molecular Mechanisms of Toxicity*, pp. 27–58, New York: Macmillan.
- KAMATAKI, T., LEE LIN, M. C. M., BELCHER, D. H., & NEAL, R. A., 1976, Studies of the metabolism of parathion with an apparently homogenous preparation of rabbit liver cytochrome P-450, *Drug Metabolism and Disposition*, **4**, 180–189.
- KNAAK, J. B., STAHMANN, M. A. & CASIDA, J. E., 1962, Peroxidase and ethylenediaminetetraacetic acid – ferrous iron – catalysed oxidation and hydrolysis of parathion, *Journal of Agricultural and Food Chemistry*, **10**, 154–158.
- KURT, T. L., 1983, Chemical asphyxiants, in Rom, W. N. (Ed.), *Environmental and Occupational Medicine*, pp. 289–300, Boston: Little, Brown and Company.
- LILIS, R., 1983, Carbon disulfide, in Rom, W. N. (Ed.), *Environmental and Occupational Medicine*, pp. 627–631, Boston: Little, Brown and Company.
- MATSON, E. J., 1956, Selenium sulfide as an antidandruff agent, *Journal of the Society of Cosmetic Chemists*, **7**, 459–466.
- MCGINLEY, K. J. & LEYDEN, J. J., 1982, Antifungal activity of dermatological shampoos, *Archives of Dermatological Research*, **272**, 339–342.

- MITCHELL, S. C., NICKSON, R. M. & WARING, R. H., 1993, The biological activity of selenium sulfide, *Sulfur Reports*, **13**, 279–292.
- MURPHY, S. D. & DUBOIS, K. P., 1957, Enzymatic conversion of dimethoxy ester of benzotriazine dithiophosphoric acid to an anticholinesterase agent, *Journal of Pharmacology and Therapeutics*, **119**, 572–583.
- NAKATSUGAWA, T. & DAHM, P. A., 1965, Parathion activation enzymes in the fat body microsomes of the American cockroach, *Journal of Economic Entomology*, **58**, 500–509.
- 1967, Microsomal metabolism of parathion, *Biochemical Pharmacology*, **16**, 25–38.
- NEAL, R. A., 1976, Studies on the enzymatic mechanism of metabolism of diethyl 4-nitrophenyl phosphorothionate (parathion) by rat liver microsomes, *Biochemical Journal*, **105**, 289–297.
- 1980, Microsomal metabolism of thiono-sulfur compounds: mechanisms and toxicological significance, *Reviews in Biochemistry and Toxicology*, **2**, 131–171.
- NORMAN, B. J., VAUGHN, W. K. & NEAL, R. A., 1973, Studies on the mechanism of metabolism of diethyl *p*-nitrophenyl phosphorothionate (parathion) by rabbit liver microsomes, *Biochemical Pharmacology*, **22**, 1091–1101.
- PTASHNE, K. A. & NEAL, R. A., 1972, Reaction of parathion and malathion with peroxytrifluoroacetic acid, a model system for mixed function oxidase, *Biochemistry*, **11**, 3224–3228.
- PTASHNE, K. A., WOLCOTT, R. M. & NEAL, R. A., 1971, Oxygen-18 studies on the chemical mechanisms of the mixed function oxidase catalysed desulfuration and dearylation reactions of parathion, *Journal of Pharmacology and Experimental Therapeutics*, **179**, 380–395.
- ROM, W. N. & BARKMAN, H., 1983, Respiratory irritants, in Rom, W. N. (Ed.), *Environmental and Occupational Medicine*, pp. 273–283, Boston: Little, Brown & Company.
- SAUNDERS, B. C., 1957, *Some Aspects of the Chemistry and Toxic Action of Organic Compounds Containing Phosphorus and Fluorine*, pp. 91–94, Cambridge: Cambridge University Press.
- SHELLEY, W. B., SHELLEY, E. D. & BURMEISTER, V., 1987, The infected hairs of tinea capitis due to *Microsporum canis*: demonstration of uniqueness of the hair cuticle by scanning electron microscopy, *Journal of the American Academy of Dermatology*, **16**, 354–361.
- SHUMYANTSEVA, V. V., MESHKOV, S. V., IVANOV, YU. D., ALEXANDROVA, O. V., UVANOV, V. YU. & ARCHAKOV, A. I., 1995, Interaction of organophosphorus analogues of amino acids with P450, *Xenobiotica*, **25**, 219–227.
- SNYDER, J. P., 1974, Oxathiiranes. Differential orbital correlation effects in the electrocyclic formation of sulfur-containing three-membered rings, *Journal of the American Chemical Society*, **96**, 5005–5007.
- SÖRBO, B., 1972, The pharmacology and toxicology of inorganic sulfur compounds, in Senning, A. (Ed.), *Sulfur in Organic and Inorganic Chemistry*, Vol. 2, pp. 143–169, New York: Marcel Dekker.
- STEUDEL, R. & LAITINEN, R., 1982, Cyclic selenium sulfides, *Topics in Current Chemistry*, **102**, 177–197.

Charged Sulfur Compounds

P. KESTELL

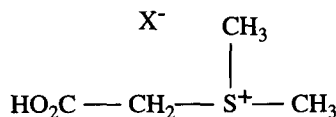
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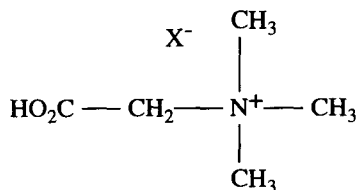
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8.1 Introduction

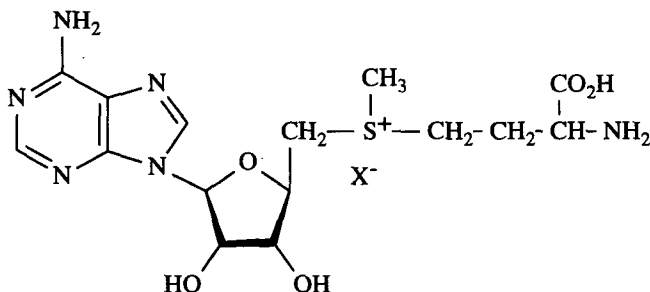
Apart from a few exceptions, sulfonium compounds and their related salts are by far the most important charged sulfur compounds encountered in biology. In addition, the analogy of their structures to those of many quaternary ammonium compounds contributed to the scientific rationale of their development. The impetus most likely originated from the studies by Brown and Fraser (1866, 1867) relating the chemical constitution and physiological action of substances in which the 'salts of the ammonium bases derived from strychnia, brucia, thebaia, codeia, morphia and nicotia were used to demonstrate certain principles'. These substances had some of the most striking pharmacological and toxicological actions of known quaternary ammonium compounds. The results raised the question of



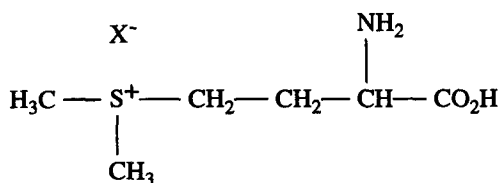
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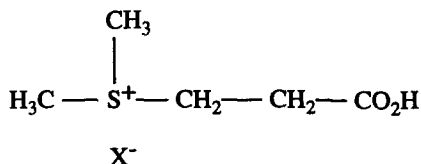
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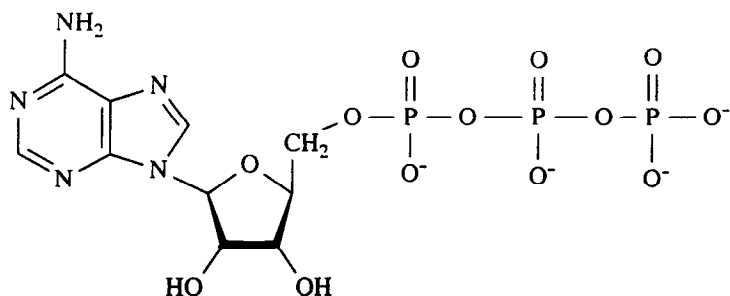


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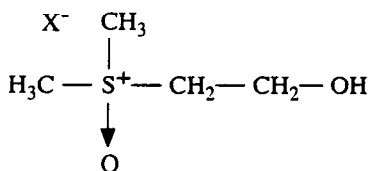


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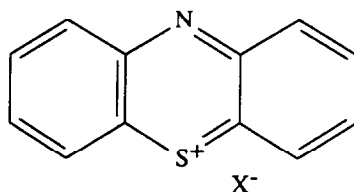
whether analogues of these chemicals might possess similar biological properties. The original sulfonium compounds, prepared by Brown and Letts (1878) and by Letts (1878), had relatively simple chemical structures based on carboxymethyl-dimethylsulfonium salts (1), making them structurally analogous to the naturally occurring quaternary ammonium compound found in plants, betaine (2). In order to describe this new class of compounds the investigators named them thetines, a term that reflected the nature and relationship of these substances to betaine. A quote from the original publication puts this succinctly: 'We have given the substance the name thetine to recall its relation to betaine and the fact that it contains sulfur'. Since then, the number of known sulfonium compounds has dramatically increased. A small number of these have been found to occur in living organisms and include S-(5'-deoxyadenosyl-5')-L-methionine (S-adenosyl-L-methionine, AdoMet, 3), S-methyl-L-methionine (MeMet, 4) and dimethyl- β -propiothetin (5). The discovery of the biological role of AdoMet, particularly in enzymatic group transfer reactions such as transmethylation which were



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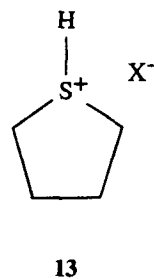
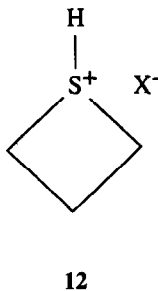
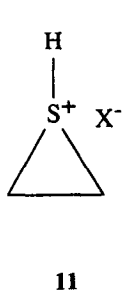
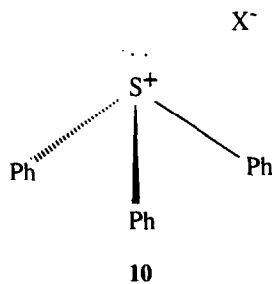
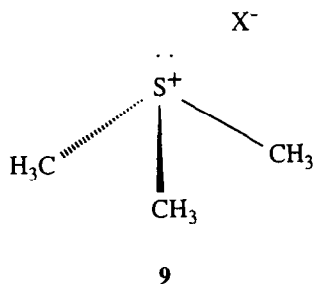


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discovered during the middle half of this century, has led to an overwhelming amount of published material which reflects the status and unique nature of this molecule in biochemistry. Indeed, as AdoMet is involved in so many of the cellular metabolic reactions, its function and status in cellular terms has been compared to that of adenosine triphosphate (ATP, 6).

Examples of other charged sulfur compounds having any biological significance are very rare. There have been reports of a compound corresponding to a (2-hydroxyethyl)dimethyl-sulfoxonium salt (7) occurring in the marine bryozoan, *Alcyonidium gelatinosium*, which is responsible for a form of contact dermatitis encountered by North Sea fishermen (Carle and Christophersen 1982, Carle *et al.* 1982). Also reference has been made to the tricyclic phenazothionium ion (8) as an intermediate in the metabolic oxidation of phenothiazine in mammals (Mitchell 1982).

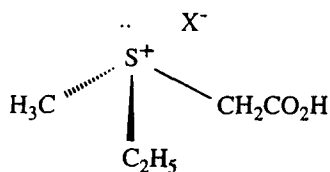
An immense amount of knowledge has been acquired over the years, as documented by du Vigneaud (1952), Young and Maw (1958), Challenger (1959), Schlenk (1965), Shaperio and Schlenk (1965), Salvatore *et al.* (1977), Maw (1981) and Crooks (1989) in their respective books and reviews. In view of their excellent work, this chapter will only outline the well-established processes and highlight the more recent and additional features of these compounds. The various mechanisms by which sulfonium compounds interact with biological systems, and their primary biological role as key biochemicals necessary to sustain essential cellular functions, will be discussed. It is hoped that this will emphasize the current knowledge about the mechanisms by which these molecules interact with living systems, and underline their uses as biochemical and pharmacological probes. Topics will be presented in terms of their chemical, biological, pharmacological and toxicological properties.



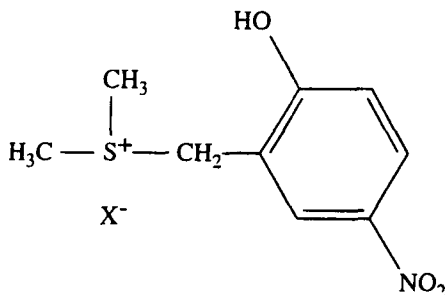
8.2 Sulfonium compounds: specific chemical group characteristics

The parent compound of this group is the simple sulfonium ion H_3S^+ which is structurally analogous to the hydronium ion H_3O^+ . Substitution of all the hydrogen atoms with organic groups such as methyl or phenyl results in the formation of their respective sulfonium derivatives: trimethylsulfonium (9) and triphenylsulfonium (10) ions. As mentioned previously, the sulfur atom is formally trivalent in all these compounds and bears a positive charge. The electronic arrangement of the valence shell of the positively charged sulfur atom is best considered as having five electrons which are distributed in three bonds and one lone pair of electrons. The steric conformation is such that most simple sulfonium compounds are pyramidal, but substitution of more bulky groups as in 10 bends the conformation as a consequence of mutual avoidance of these groups (Simonetta and Gavezzotti 1981). Sulfonium compounds can also exist in cyclic structures, the simplest being the three-membered ring associated with the thiiranium or episulfonium ion (11). Other similar cyclic ring systems which have been identified and studied include the four-membered thiathetium ion (12) and the five-membered thiolanium ion (13).

Optical isomers of sulfonium ions are possible if three different groups are substituted around the sulfonium pole. The first successful resolution of the sulfonium compound into enantiomeric forms was achieved by Pope and Peachy (1900) who resolved ethylmethylthetin (14) via its camphorsulfonate salts. Since then a large number of optically pure sulfonium salts have been prepared, either by resolution or by stereospecific synthesis (Andersen 1981). From a biological perspective this is an important feature and was recognized as such by Cantoni (1951a, b, 1952, 1953) during the course of numerous studies leading to the initial discovery of AdoMet and its pivotal role in many biochemical reactions. In theory



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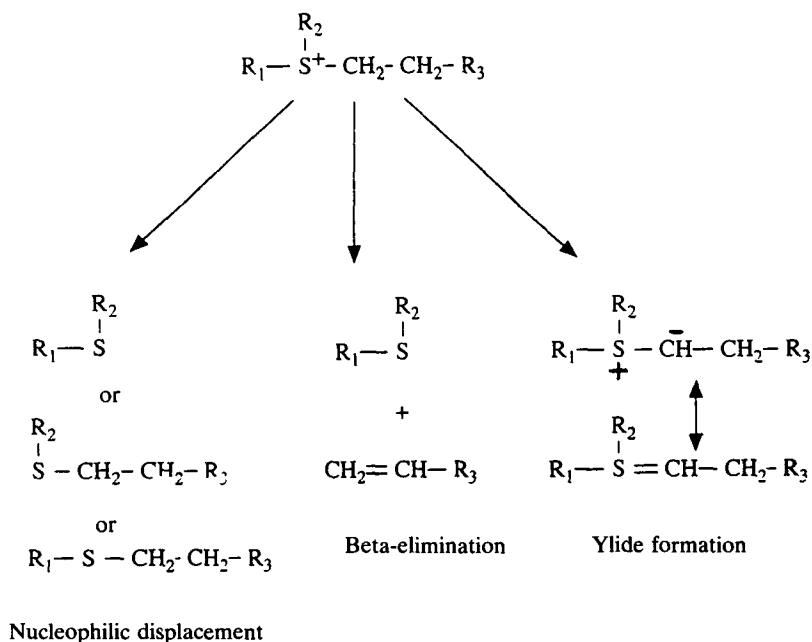


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at least four diastereomers are possible as a consequence of the two chiral centres, one at the sulfonium pole and the other at the α -carbon atom of the amino acid moiety. Initial studies by de la Haba *et al.* (1959) employed the designations (+) and (−) based on polarimetry and showed that enzymatically produced AdoMet has a (−) configuration. It was assumed that the same configuration existed in the naturally occurring isomer. Later, Zappia *et al.* (1969) and Borchardt and Wu (1976) confirmed these findings and showed that the (−) isomer of AdoMet was the only one which was indeed utilized by biological systems. The absolute configuration of the sulfonium chiral centre of naturally occurring AdoMet has been determined by Cornforth *et al.* (1977) by correlation with the absolute configuration of diastereometric S-carboxymethyl-(S)-methionine salts. It was deduced that the stereochemistry was 5'-[[(3S)-3-amino-carboxypropyl)methyl-(S)-sulfonio]-5'-deoxyadenosine and thereby confers the S configuration at the sulfonium pole. Studies by Wu *et al.* (1983) and by Hoffman (1986) with this compound have shown that the S configuration of the sulfur atom can undergo racemization which, like many stereo isomers of sulfonium salts, is subject to chiral instability at the sulfonium pole. The stereomutation or configurational change at the sulfur atom can occur by mechanisms involving pyramidal inversion or reversible dissociation into a carbonium ion and neutral sulfide molecule via an S_N1 mechanism or via S_N2 attack at the α -carbon followed by re-formation of the salt (Andersen 1981).

The chemical reactions and properties of sulfonium salts are well understood and have been studied in detail since the first reports describing their syntheses were made during the later half of the nineteenth century. Consequently there have been many publications covering the various aspects, with the most notable being those reference books and reviews by du Vigneaud (1952), Challenger (1959), Schlenk (1965), Stirling (1977) and Stirling and Patai (1981). Some of the more relevant reactions applicable to the biological field are summarized in Scheme 1. Probably the most important of these are the nucleophilic displacement reactions in which there is C-S⁺ bond fission and the β -elimination reactions which yield an olefin and a thioether. In addition, α -deprotonization of sulfonium salts with suitable reagents results in the formation of sulfonium ylides which are relatively stable compounds due to the ability of the sulfur atom to stabilize the adjacent carbanion (Knipe 1981).

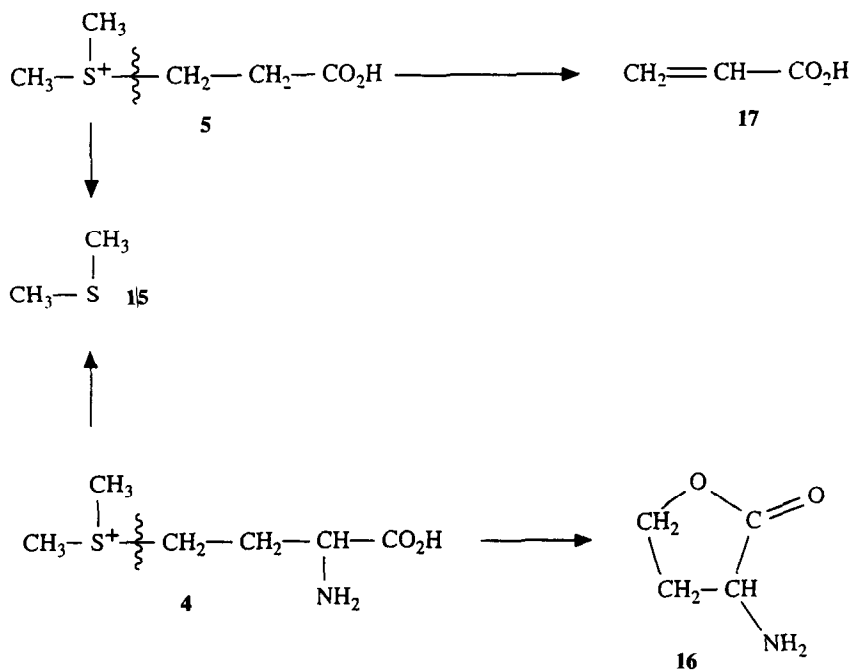
Many sulfonium salts are chemically unstable in solution, particularly when pH values exceed 7. In such environments, complex mixtures of decomposition products can arise, presumably as the result of the sulfonium salt undergoing one



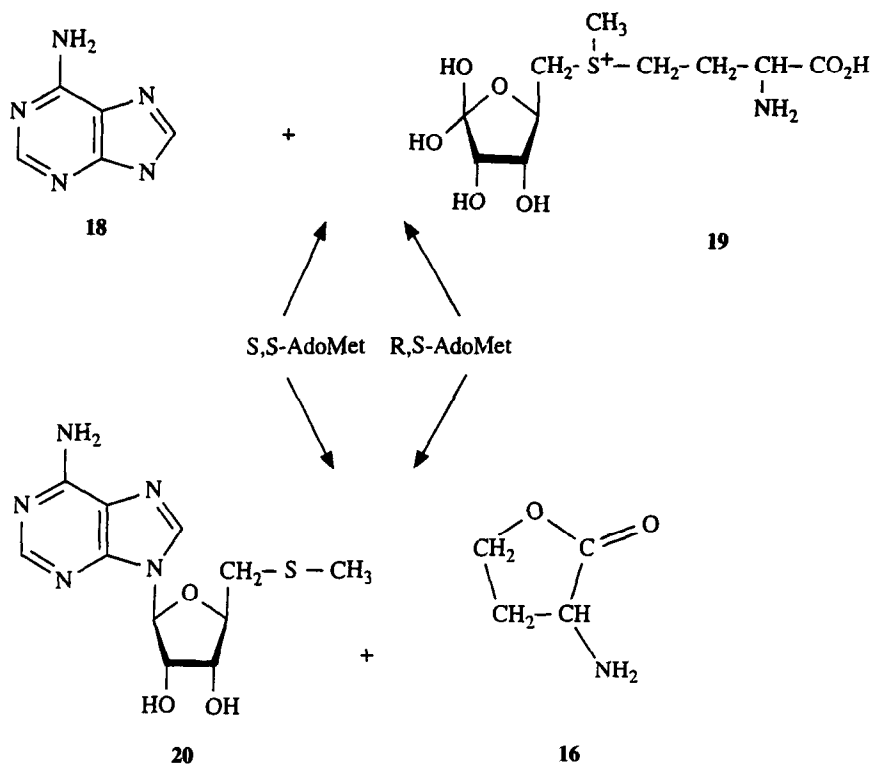
Scheme 1

or more of the reactions outlined in Scheme 1. The solution chemistry of the naturally occurring compounds, AdoMet, MeMet and dimethyl- β -propiothetin, has been investigated and the principal reactions are reviewed by Schlenk (1965), Maw (1981) and Crooks (1989). Under alkaline conditions, both MeMet and dimethyl- β -propiothetin decompose to give dimethylsulfide (15), and the products that arise from the remaining portion of the molecule are α -aminobutyrolactone (homoserine lactone) (16) and acrylic acid (17), respectively (Scheme 2). It has been postulated that the presence of α -aminobutyrolactone amongst the decomposition products of MeMet results from an intramolecular group participation mechanism involving the carboxyl moiety. Under similar conditions, the N-glycosidic bond of AdoMet is cleaved with adenine (18) and S-pentosylmethionine (19) being formed (see Scheme 3). AdoMet is somewhat less stable than either of the other naturally occurring sulfonium compounds since it can undergo racemization in solution by previously mentioned mechanisms and is susceptible to degradation even under slightly acidic conditions to give 5'-deoxy-5'-methylthioadenine (MTA, 20) and α -aminobutyrolactone, as shown in Scheme 3. Both Wu *et al.* (1983) and Hoffman (1986) recognized the biological and biochemical implications of the chemical and chiral instability of AdoMet and the problems associated with studying this compound under stable environments. Likewise, it has also posed problems when quantifying AdoMet in biological samples. Procedures have to be employed that minimize the degradation of the molecule during analysis.

The chemical reactivity and properties of the sulfonium group have been exploited for various purposes in protein and enzyme biochemistry. Of particular use has been the ability of some sulfonium compounds to react or inactivate certain specific moieties within proteins or enzymes. One such reagent, dimethyl

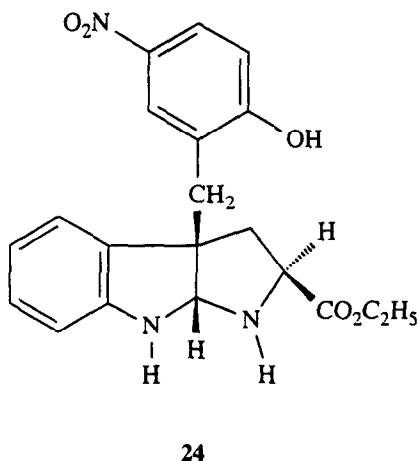
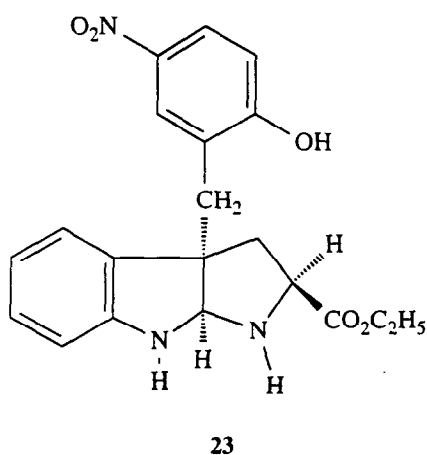
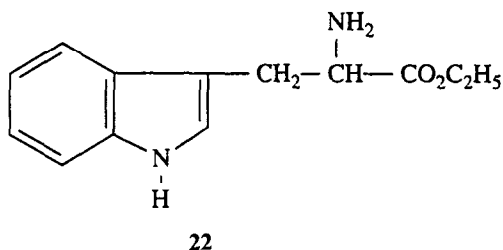


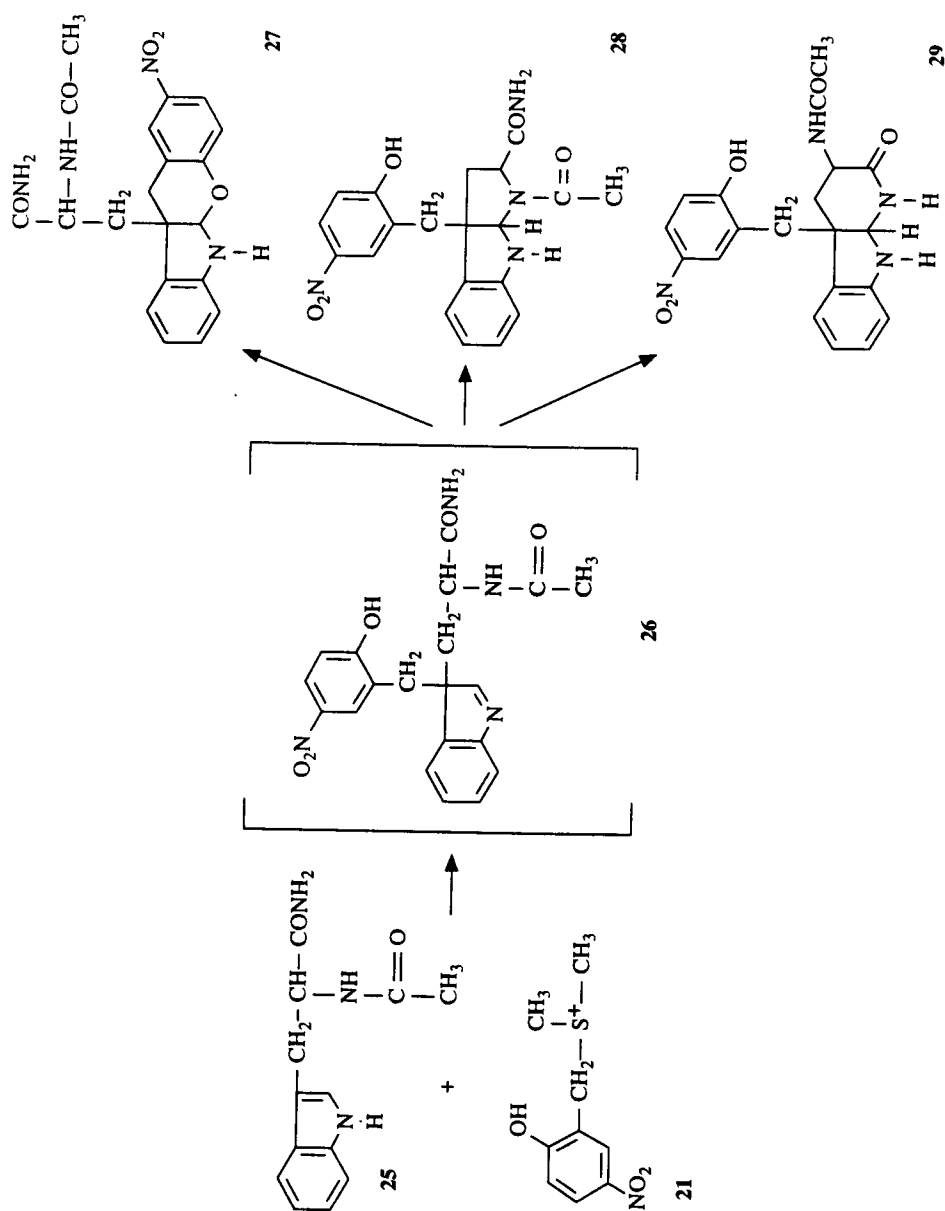
Scheme 2



Scheme 3

(2-hydroxy-5-nitrobenzyl)sulfonium bromide (**21**), was discovered by Horton and Tucker (1970) to react selectively with tryptophanyl residues in model proteins, thereby providing a means by which the role of this moiety could be studied in relation to its functional activity. The technique of chemically modifying proteins by dimethyl (2-hydroxy-5-nitrobenzyl)sulfonium bromide has proved useful in many instances, and together with other investigational techniques such as circular dichroism and solvent perturbation difference spectroscopy have revealed the complex nature of the structure–functional relationships of many enzymes. Those enzyme systems which have been studied in this way include human plasminogen (Hochschwender and Laursen 1981, Llinas *et al.* 1983), human thrombin (Villanueva 1981), human antithrombin (Blackburn and Sibley 1980, Karp *et al.* 1984, Scully *et al.* 1991), rabbit muscle creatine kinase (Zhou and Tsou 1985), rabbit skeletal muscle myosin subfragment (S-1) (Peyser 1990, Werber *et al.* 1987), bovine pancreatic carboxypeptidase A (Liu Wu and Horton 1979) yeast lactate dehydrogenase (Mayer *et al.* 1982) and yeast glyceraldehyde-3-phosphate dehydrogenase (Heilman and Pfeleiderer 1975). The chemistry associated with the reaction between dimethyl (2-hydroxy-5-nitrobenzyl)sulfonium bromide and the tryptophan residues of proteins is somewhat complex. Therefore in order to gain some insight into the reaction most investigations have used model compounds to mimic the tryptophan moiety in the peptide backbone. Tucker *et al.* (1971) examined the reaction of (2-hydroxy-5-nitrobenzyl)sulfonium bromide with L-tryptophan ethyl ester (**22**) in aqueous solutions at pH 4.7 and found that two diastereomeric monosubstitution products (**23**, **24**) were formed by alkylation of the reagent at position 3 of the indole nucleus followed by cyclization through the



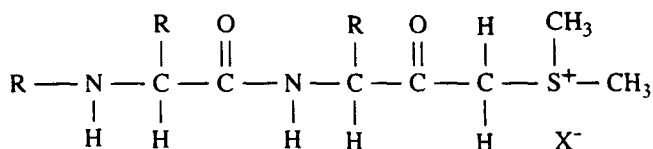
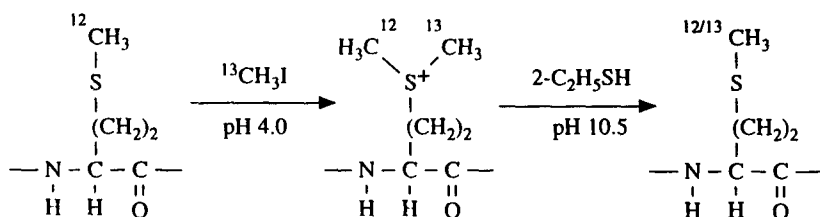


Scheme 4

nucleophilic α -amino group. A similar mechanism has been postulated to account for the compounds produced when (2-hydroxy-5-nitrobenzyl)sulfonium bromide is reacted with N-acetyl-L-tryptophan amide (**25**) using the same conditions as reported for the ethyl ester of tryptophan (Heinrich *et al.* 1973). The reaction, outlined in Scheme 4, assumes that alkylation leads to the formation of the intermediate (**26**) which then undergoes cyclization by a number of possible routes leading to the formation of the observed products (**27**, **28**, **29**). The major route appeared to be that giving rise to the 2-piperidone derivative (**29**).

Chemical modification of the methionine residues in proteins and enzymes via conversion to their sulfonium moieties has also been considered as a means of investigating the role of this amino acid in the structure–function relationships of glucagon (Rothgeb *et al.* 1977), α -chymotrypsin (Glover *et al.* 1976) and sperm whale myoglobin (Jones *et al.* 1975, 1976). In addition, methionine sulfonium moieties have been found to be useful intermediates in the ^{13}C enrichment of the methionine methyl groups at the active site of α -chymotrypsin (Matta *et al.* 1980, 1981) and in specific sequences of the transmembrane sialoglycoprotein, glycophorin A (Hardy and Dill 1982), and were prepared by a reaction scheme outlined in Scheme 5. The structural characteristics of the ^{13}C methyl methionine labelled proteins were then studied by ^{13}C NMR spectroscopy.

Affinity labelling of specific amino acid residues using appropriate group-specific reagents at the active sites of target enzymes has also proved to be an invaluable technique for elucidating key aspects of structure, function and reaction mechanisms of proteases. Among the reagents examined in recent years have been peptidyl sulfonium salts of general chemical structure given by **30**. They have been evaluated as affinity labelling reagents in the following enzymes: a variety of cysteine and serine proteases such as papain and cathepsin B (Pliura *et al.* 1992a, b, Shaw 1988, Zumbunn *et al.* 1988), together with clostripain (Rauber *et al.* 1988) and cancer procoagulant (Falanga 1989), plus the proinsulin-processing endopeptidases (Rhodes *et al.* 1989). Also peptidyl sulfonium salts have been found to be

**30****Scheme 5**

specific and potent inactivators of transglutaminases (Pliura *et al.* 1992a, b). The studies demonstrated the application of these compounds as useful affinity-labelling reagents and also discovered that peptidyl sulfonium salts have attractive features which favour them over other reagents. For example, they are less reactive towards other biological nucleophiles such as thiols and therefore there are fewer side-reactions, and they display a much narrower range of antiproteolytic activity. The mechanism of action was originally thought to involve the transfer of a methyl group from the sulfur atom to an amino acid residue at the active site but evidence suggests that a more likely process is that of the peptidyl moiety being transferred instead. It has also been postulated that the ylide of the peptidyl sulfonium salt may be the active form by which affinity labelling occurs. The ylide seems to be the most prevalent form at physiological pH as demonstrated with Cbz-Phe-AlaCH₂S⁺(CH₃)₂ which at pH 7 was found to be 50% (Shaw 1988).

8.3 Sulfonium compounds: biological interactions

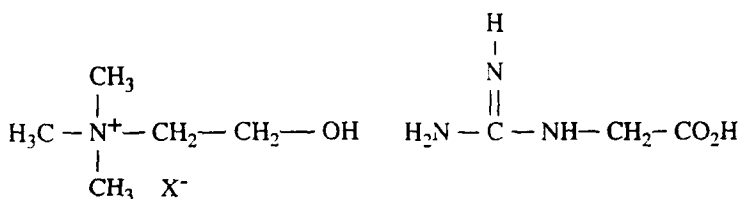
8.3.1 Physiological and metabolic roles

8.3.1.1 S-adenosyl-L-methionine

AdoMet and its metabolites are ubiquitous in the plant and animal kingdoms (Crooks 1989, Maw 1981). Accurate quantitation of these compounds has been made possible with recent advances in chromatographic and radioenzymatic techniques.

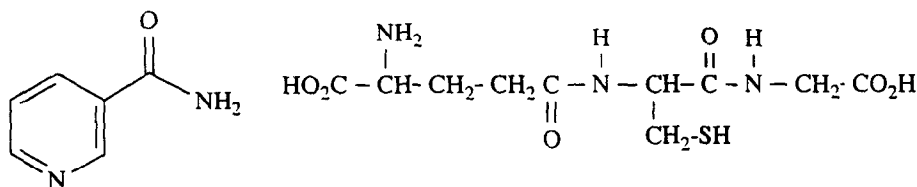
The discovery of AdoMet in biology and its pivotal role in many biochemical processes was the result of many years of intensive research which began in the early part of this century. The development of the concept of transmethylation as a biochemical reaction arose from studies on the metabolism of Met in growing rats where it was shown that this amino acid could not be replaced in the diet by either homocysteine or homocystine unless certain other quaternary ammonium compounds were present such as choline (31) or betaine. The results implied that the function of the S-methyl group in Met was in some way connected with nutritional health via the metabolic fate of this moiety. Subsequent investigations by Borsook and Dubnoff (1940, 1947), Perlzweig *et al.* (1943) and Cantoni (1951a, b) into the methylation of guanidino acetic acid (32) and nicotinamide (33) substantiated the requirement not only for Met to be involved in such reactions, but also for ATP, which appears to act in a different capacity to that of a phosphorylating agent. Additionally, it was found that ATP could convert Met to a biochemically active species by an enzymic process which occurred prior to the transfer of the methyl group of the amino acid. The compound referred to as the 'active Met' was eventually isolated by Cantoni (1952, 1953) who, after investigating its properties, concluded that it was a sulfonium compound containing an S-adenosyl moiety with a chemical structure similar to that given for 3.

AdoMet has diverse and strategic roles in many cellular processes. It is recognized that the primary function of this molecule is to serve as the methyl donor in numerous biological methylation reactions by a metabolic pathway collectively termed transmethylation. Additionally, AdoMet is also involved with the biosynthesis of essential nutrients such as glutathione (GSH, 34) and the



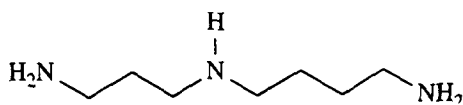
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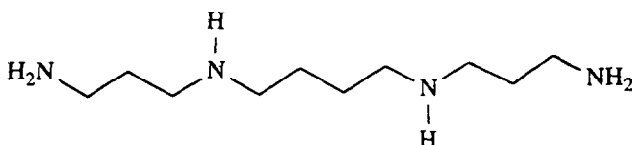


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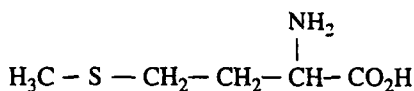
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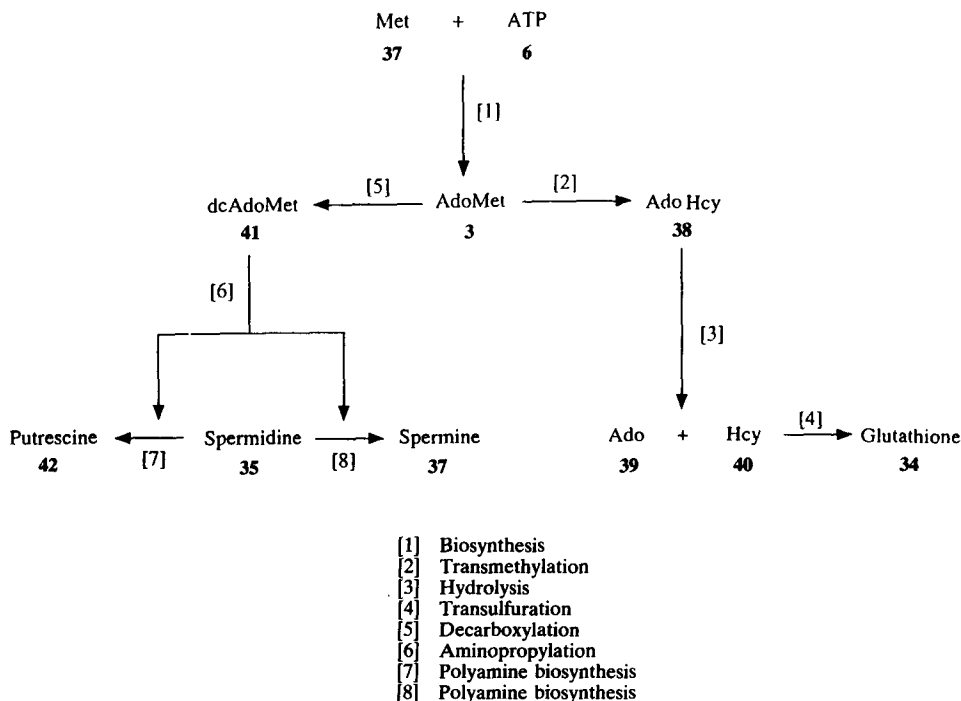


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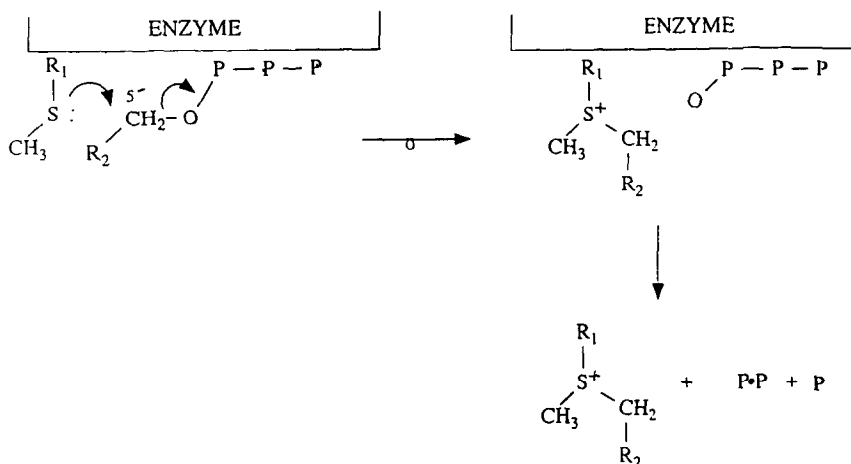
polyamines, spermidine (35) and spermine (36), which are produced via the transulfuration and aminopropylation pathways, respectively (Salvatore *et al.* 1977, Shapiro and Schlenk 1965, Usdin *et al.* 1979, 1982, du Vigneaud 1952). A summary of the biosynthetic and metabolic routes involving AdoMet is given in Scheme 6. The enzymes associated with these biochemical reactions have increasingly become of interest as chemotherapeutic targets, and the effort made to characterize their physical and chemical properties has in turn led to the design of selective inhibitors. The enzyme which catalyses the biosynthesis of AdoMet from L-methionine (Met, 37) and ATP is ATP: L-methionine S-adenosyltransferase (MAT). During the



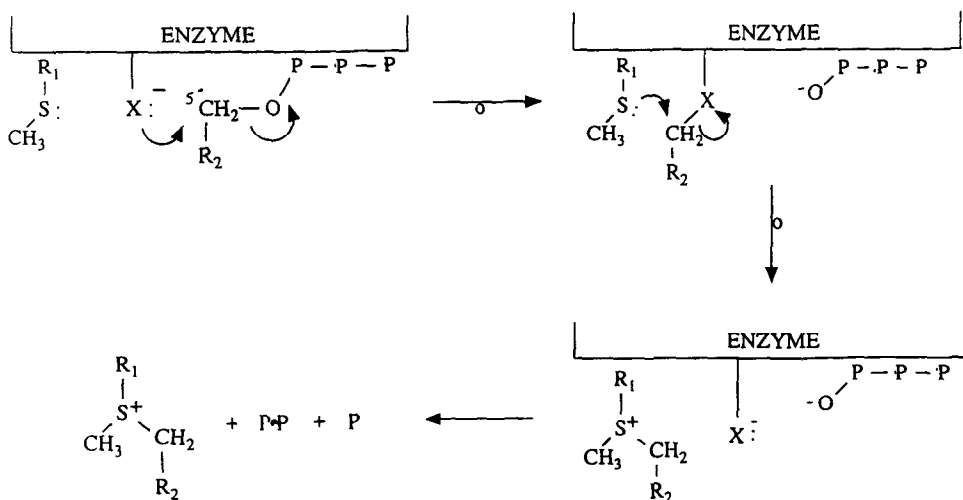
Scheme 6

reaction, the 5'-deoxyadenosyl moiety of ATP is transferred to the methionine sulfur atom leaving the terminal phosphate to be released as inorganic phosphate and the remaining phosphates as pyrophosphate (Parry and Minta 1982). It is thought to proceed by one of two possible mechanisms, as shown in Scheme 7. The first of these postulates that ATP and methionine bind to the enzyme in such a way that a single displacement reaction takes place at C-5' of ATP to give AdoMet and enzyme-bound tripolyphosphate (Mechanism A). The second mechanism (Mechanism B) proceeds by a nucleophilic displacement of the tripolyphosphate moiety from the C-5' of ATP by a functional group present at the active site of the enzyme. Following this, a second displacement of C-5' by the sulfur atom of methionine would result in the transfer of the adenosyl group to this moiety. Of the two mechanisms, the single displacement of Mechanism A appears to be the more favourable, as attempts to detect the adenosyl-enzyme intermediate have failed. Further evidence to support this was provided by Parry and Minta (1982) whilst elucidating the stereochemistry of AdoMet formed by yeast MAT. The mechanism confirms the unusual role played by ATP in the biosynthesis of AdoMet which is completely dephosphorylated. This contrasts with its fate during the majority of biochemical reactions in which ATP participates, whereby ATP is only partially dephosphorylated to adenosine diphosphate (ADP). The MATs are widely distributed in animal tissues, with the highest concentrations being found in the liver. MATs can exist in multiple forms which differ in each tissue from which they are prepared.

Mechanism A

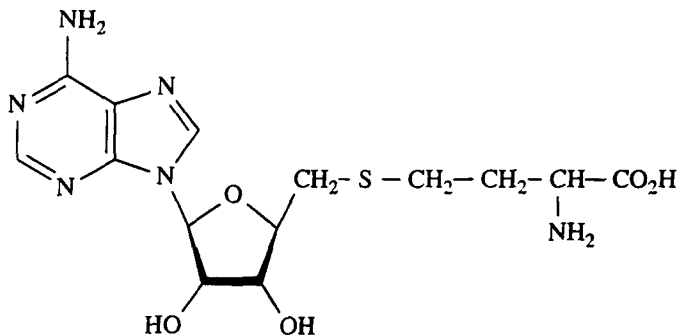


Mechanism B



Scheme 7

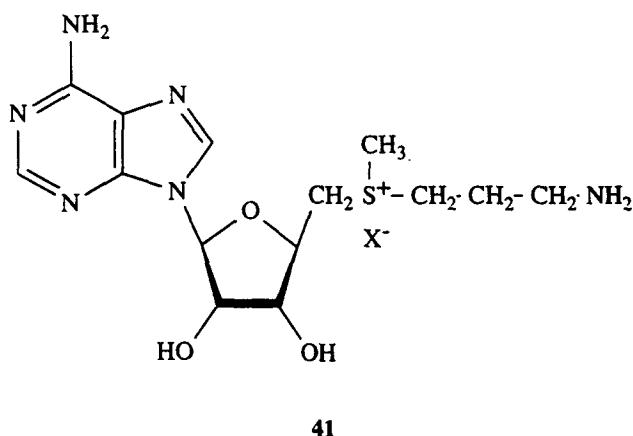
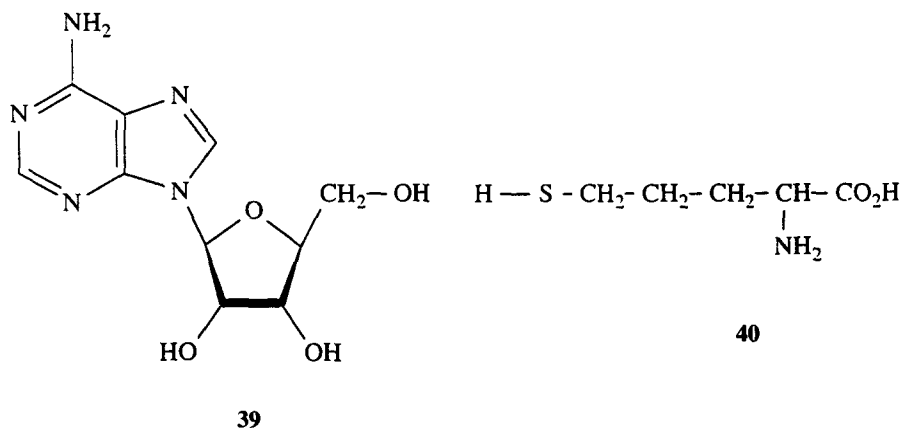
Once formed, AdoMet is rapidly metabolized to S-adenosylhomocysteine (AdoHcy, **38**) through its involvement with one of the many transmethylation reactions that are catalysed by an appropriate methyltransferase. The latter is usually highly specific with regard to the methyl acceptor substrate. Low molecular weight substrates for AdoMet-dependent transferases include amino acids, hormonal catecholamines, purine bases, neurotransmitter amines, sterols and terpenes, carboxylic acids and sugars, thus highlighting the ability of AdoMet to donate its methyl group to a large number of compounds of diverse molecular



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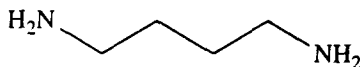
structure and to a variety of atoms contained within that structure (oxygen, nitrogen, sulfur or carbon). Many of the aforementioned compounds are important physiologically and AdoMet therefore regulates their activation or inactivation to varying degrees. In addition, AdoMet-mediated methylation can also occur with macromolecules such as polysaccharides, phospholipids, proteins and nucleic acids. Until recently it was impossible to assign exact physiological functions to many of the macromolecule methylations. However, it has become apparent that AdoMet-dependent methylations play a role in posttranslational modification of proteins, in posttranscriptional modification of nucleic acids and in regulating membrane functions through modification of membrane phospholipids. The role of methylation of eukaryotic DNA has not been established, but a relation to growth, differentiation and carcinogenesis has been suggested. Carboxylation of proteins seems to play a role in processes such as chemotaxis and secretion. Thus, in many respects, AdoMet-mediated methylation of physiological and cellular constituents is comparable with phosphorylation due to widespread involvement with biological regulation (Borchardt, 1980, Crooks 1989, Maw 1981, Ueland 1982). A number of reaction mechanisms have been postulated by which the methyl group is transferred from AdoMet to the acceptor molecule, but all seem to be open to much speculation and criticism. However, of these, the most favoured are the random Bi-Bi and ping-pong mechanisms which have been reviewed by Crooks (1989).

In any discussion regarding the transmethylation pathway, reference should be made to the properties of the resulting product AdoHcy and its interaction with biological methylations. The formation of this molecule from AdoMet was first demonstrated by Cantoni and Scarano (1953), and later shown by Gibson *et al.* (1961) to be a competitive inhibitor of AdoMet-dependent transmethylation during studies investigating the enzymatic synthesis of phosphatidylcholine in rat liver. This observation was later confirmed by others who also demonstrated that AdoHcy was a potent competitive inhibitor of almost all the known AdoMet-dependent methyltransferases (Ueland 1982). The inhibitor constants for AdoHcy are often equal to or lower than the K_m values for AdoMet, indicating that the catalytic sites of most transmethylases have nearly the same or higher affinity for AdoHcy than for AdoMet (Crooks 1989, Ueland 1982). Thus AdoHcy plays a major regulatory role in controlling AdoMet-mediated cellular methylations and so any factors which govern AdoHcy production or destruction in cells must also influence



the degree of methylated species. To this end, the enzyme AdoHcy hydrolase, which is widely distributed in animal tissues and in yeasts, and cleaves AdoHcy to adenosine (Ado, 39) and L-homocysteine (Hcy, 40), also plays a complementary role in regulating cellular methylation (Borchardt *et al.* 1976, Crooks 1989, Salvatore *et al.* 1977, Ueland 1982, Usdin *et al.* 1979, 1982).

AdoMet also undergoes metabolism by decarboxylation, resulting in the formation of the corresponding decarboxylated derivative, S-adenosyl-(5')-3-methylthiopropylamine (dcAdoMet, 41). The enzyme responsible for the catalytic decarboxylation of AdoMet, AdoMet decarboxylase, appears to have a covalently bound pyruvate prosthetic group which is unlike those of many amino acid decarboxylases. The enzyme is synthesized as a precursor with an $M_r \sim 37\,000$ and is then converted to the $M_r = 32\,000$ enzyme subunit in a reaction which generates the pyruvate group at the active site (Pegg 1988). Upon formation, dcAdoMet is rapidly utilized in polyamine biosyntheses as an aminopropyl group donor as illustrated in Scheme 6. Thus, biosynthesis of spermidine or spermine involves the transference of this group to the amino group of either one of the acceptor substrates, putrescine (42) or spermidine. The reactions are catalysed by appropriate aminopropyltransferases, which for spermine is spermine synthase and in the case of spermidine is spermidine synthase. The side-product of both



reactions is MTA which appears to act as a feedback inhibitor of these enzymes in much the same way as AdoHcy interacts with AdoMet-dependent methyltransferases. In addition, dcAdoMet possesses some weak methyl donor activity and can act as a substrate for yeast homocysteine-S-methyltransferase (Crooks 1989, Maw 1981).

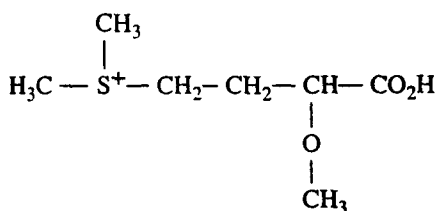
A complete understanding of the physiological and cellular roles of polyamines is far from established, and further aspects of AdoMet and dcAdoMet interactions associated with polyamine biosynthesis and metabolism may yet be discovered. What has been substantiated is that the polyamines are necessary to maintain cell growth and are essential for maintaining many cellular processes of which various aspects have been included in reviews by Pegg and McCann (1982), Tabor and Tabor (1984), Pegg (1986) and Porter and Sufrin (1986). There is also growing evidence to suggest that abnormal polyamine metabolism is associated with the development of tumours, and this in turn has led to monitoring of these compounds in biological fluids in cancer patients as a means of screening for neoplastic disease. However, despite intensive efforts in many laboratories the general utility of this concept has so far not been fully substantiated. In another approach to establishing polyamines as biological markers of malignant growth, the activity of associated enzymes in polyamine metabolism have been scrutinized (Pegg 1988).

8.3.1.2 *S*-methyl-L-methionine

In contrast to AdoMet, the physiological roles and metabolic functions of other naturally occurring sulfonium compounds have not been so rigorously investigated. In the case of MeMet there is evidence to suggest that this compound plays a significant role in both the biosynthesis of Met in plants whereby it acts as a methyl donor, and as a putative source of ethylene during the ripening of flowers and fruits. It was originally discovered in asparagus tips and cabbage leaves and further studies confirmed the ubiquitous nature of MeMet in the plant kingdom. It has been detected in a wide variety of vegetables (Khuchua and Stasyak 1975, Kovacheva 1974, McNicol 1986, Wong and Carson 1966), fruit (Wong and Carson 1966) and green tea (Kiribuchi and Yamanishi 1963). Although MeMet is a compound found exclusively in plants, its detection in cow's milk has been attributed to the transference of the compound from the animal's diet (Keenan and Lindsay 1968). Biosynthesis of MeMet has been demonstrated in a number of plant species which requires the presence of both Met and AdoMet in order for the methylation reaction to proceed via catalysis with a AdoMet:Met methyltransferase (Green and Davis 1960, Karr *et al.* 1967). The products of the reaction are MeMet and AdoHcy. Original work by Shapiro (1956) and Shapiro and Yphantis (1959) with microorganisms established that there is an enzymic process by which the methyl group of MeMet could be transferred specifically to Hcy, resulting in the formation of two molecules of methionine. Subsequent studies by Sato *et al.* (1958), Abrahamson and Shapiro (1965) and Dodd and Cossins (1969) showed that the same reaction occurred in plants and seeds.

8.3.1.3 Dimethyl- β -propiothetin

Although synthesized by Carrara (1893) during the late nineteenth century, it was not established that dimethyl- β -propiothetin was a naturally occurring compound until many decades later. It was the first sulfonium compound to be identified in a living organism and was isolated by Challenger and Simpson (1948), who re-investigated the earlier observations made by Hass (1935) regarding the evolution of dimethyl sulfide from the marine algae, *Polysiphonia fastigiata* and *P. nigrescens*, when it was exposed to air. They successfully extracted the dimethyl sulfide precursor from *P. fastigiata* and conclusively identified it as dimethyl- β -propiothetin. This compound has been found to be a widely distributed substance not only in marine algae and other marine organisms such as plankton, molluscs, crustacea and fish (Blunden and Gordon 1986, Blunden *et al.* 1992, Crooks 1989, Maw 1981, Sciuto *et al.* 1989, White 1982), but also in plants (Rhodes and Hanson 1993). A related tertiary sulfonium compound has been reported in marine algae which has been identified as (-)-(S)-4-dimethylsulfonio-2-methoxybutyrate (43)

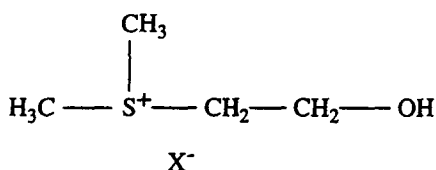
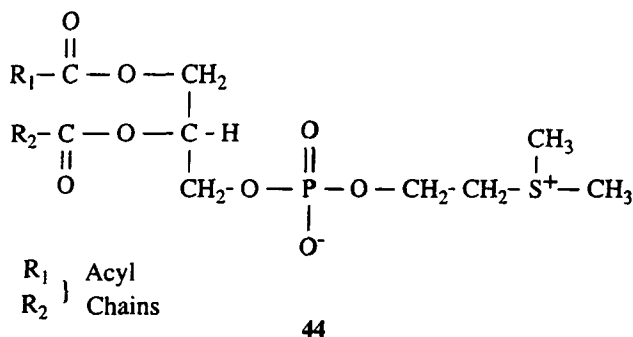


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(Sciuto *et al.* 1982). Both compounds are present at very high concentrations, and it is postulated that they have an important role in overcoming salt or water stresses in these organisms, especially those plants associated with arid or salty environments (Blunden and Gordon 1986).

8.3.1.4 Phosphatidyl sulfocholine (44)

The presence of a novel sulfolipid and sulfonium analogue of phosphatidyl choline, phosphatidyl sulfocholine, has been reported in the following marine diatoms: *Nitzschia alba*, *Cylindrotheca fusiformis*, *Phaeodactylum tricornutum* and *Navicula pelliculosa* (Anderson *et al.* 1976, 1978, Bisseret *et al.* 1984). Its role in these organisms remains somewhat unclear but studies using the nonphotosynthetic marine diatom, *Nitzschia alba*, suggest that it replaces the function of the ubiquitous phospholipid, phosphatidyl choline, in cell membranes. In this diatom, the major phospholipids have been identified and found to be based on sulfur rather than the nitrogenous bases such as phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine encountered in other living organisms. Thus, the absence of nitrogen-containing phospholipids suggests that the commonly occurring pathways for biosynthesis in plants of nitrogenous phospholipids are not functional in *Nitzschia alba* and that these are replaced by more novel biosynthetic routes involving sulfur-containing compounds, possibly derived from



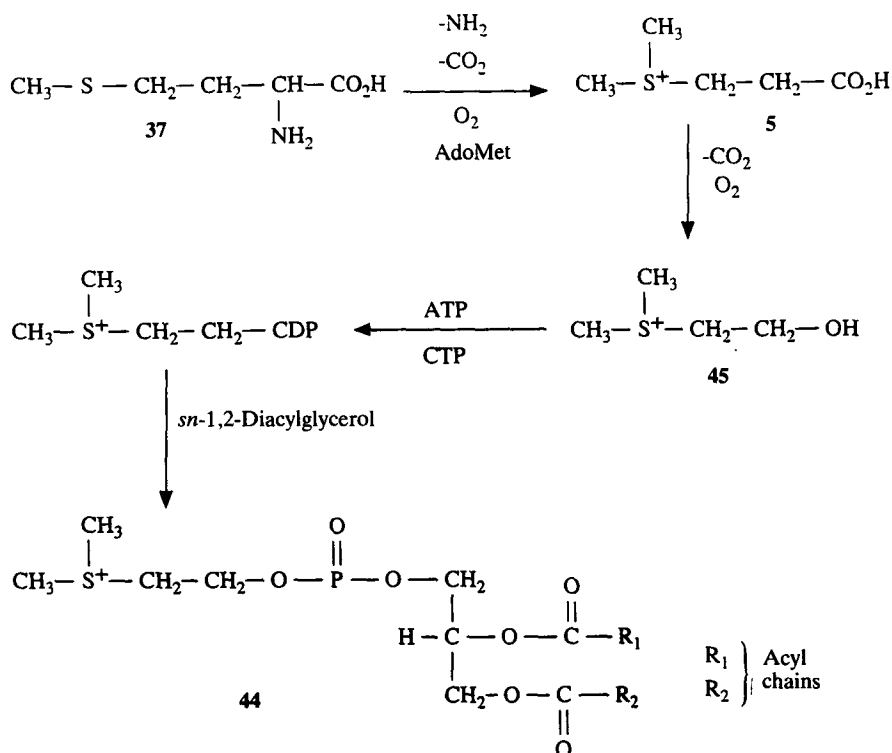
cysteine or methionine. Studies by Anderson *et al.* (1979) indicate that the sulfocholine moiety most probably arises from Met via dimethyl- β -propiothetin which is present in many marine organisms (see Scheme 8). This would involve deamination of methionine, followed by oxidative decarboxylation and methylation to produce dimethyl- β -propiothetin. Oxidative decarboxylation of the latter would then give sulfocholine (45). Thus, the most likely route leading to the biosynthesis of phosphatidyl sulfocholine from sulfocholine would be those analogous pathways associated with the biosynthesis of phosphatidyl choline from choline involving ATP, cytidine triphosphate (CTP) and *sn*-1,2-diacyl glycerol, as shown in Scheme 8.

8.3.2 Pharmacological and therapeutic effects

Sulfonium compounds exhibit a wide variety of pharmacological effects, some of which have been exploited for therapeutic purposes in treating a number of human diseases. Many of the wide and diverse pharmacological effects exhibited by sulfonium compounds are reported in the following examples.

8.3.2.1 Naturally occurring compounds

AdoMet interacts with the central nervous system (CNS) in a profound manner, affecting the serotonergic, dopaminergic and β -adrenergic systems. This is reflected by the elevated levels of 5-hydroxytryptamine and 5-hydroxyindole acetic acid in some regions of rat brain following its administration, and by alterations in the brain membrane fluidity in aging rats (Stramentinoli 1986). It has been proposed that modification of CNS catecholamine and indolamine metabolism might form the biochemical basis of depression. In view of this and because of



Scheme 8

its CNS effects in rodents, AdoMet has undergone extensive clinical evaluation as an antidepressant agent (Chawla *et al.* 1990). In general, AdoMet has been proven to be at least as effective in treating depression as the standard regimens of tricyclic antidepressants such as imipramine, amitriptyline or clomipramine, and has exhibited fewer side-effects. Hence the clinical use of AdoMet as an antidepressant agent is increasingly being considered. Also AdoMet has been reported by Chawla *et al.* (1990) to have therapeutic benefits when used clinically as an essential nutrient in treating malnourished patients either alone or with dietary supplements. Replenishment therapy using AdoMet has been extended to patients whose levels have been depleted by liver disease. The clinical efficacy of AdoMet in the treatment of cholestasis resulting from hepatic diseases, pregnancy or administration of oestrogen-containing oral contraceptives has been demonstrated (Almasio *et al.* 1990). Several cooperative mechanisms appear to underlie the anticholestatic action of AdoMet, the most important being the restoration of normal hepatocyte membrane fluidity and Na^+ , K^+ ATPase activity, through a reversal of the reduction in phospholipid methylation produced by hepatotoxic agents. In addition, AdoMet may act by overcoming the effects of an impaired MAT enzyme system and by promoting trans-sulfuration pathway reactions which consequently improve the detoxifying capacity of this metabolic system (Mato *et al.* 1990, Pisi and Marchensini 1990). Concomitant with these studies have been detailed investigations into the *in vivo* disposition, metabolism

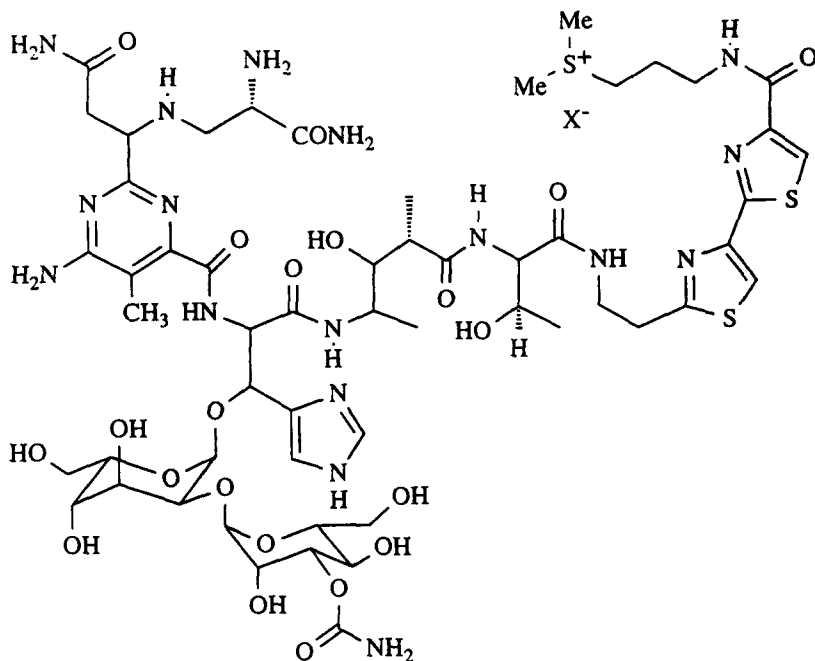
and excretion of AdoMet in rat, dog and man after intravenous or oral administration. Much of this has been summarized in reviews by Stramentinoli (1986, 1987) and Kaye *et al.* (1990). Although well absorbed orally, AdoMet has a reduced bioavailability which is due to a significant first-pass effect in the liver. AdoMet is not decomposed in gastric juices since studies show that it is stable within this environment. Dose-dependent peak plasma levels are achieved within 3–6 h of oral administration and plasma levels approach baseline after 24 h. The volumes of distribution measured in most of these studies have been found to be quite small. An important feature of the distribution of AdoMet is its ability to cross the blood–brain barrier. The metabolism of exogenous AdoMet appears to follow the known pathways of endogenous AdoMet metabolism, and preliminary data suggests that the process is largely unaffected in patients with chronic liver disease. In addition to these well-studied pharmacological effects, AdoMet has also been reported to have anti-inflammatory and analgesic activity (Stramentinoli 1986, 1987).

MeMet is another naturally occurring sulfonium compound which has undergone pharmacological evaluation with the purpose of determining whether it might be of therapeutic use (Maw 1981). This compound was originally established as having anti-ulcer properties when extracts of cabbage leaves containing MeMet were shown to prevent the development of experimentally induced gastric ulcers in guinea pigs. The active factor in the extract was named vitamin U, i.e. MeMet. Much effort has subsequently been made to demonstrate the same action in other species including man (Salim, 1987, Strehler 1955, Tanaka *et al.* 1969, Yoshinaka and Nakamura 1981). MeMet has attracted considerable interest in Japan and Russia on account of this and other medicinal properties. These include anti-inflammatory activity, stimulation of skin regeneration and the healing of wounds, and the ability of MeMet to lower blood cholesterol levels. Due to the structural similarities, dimethyl- β -propiothetin has also undergone evaluation as an anti-ulcer agent in rats. Of the two naturally occurring molecules, dimethyl- β -propiothetin was found to be more effective as an anti-ulcer agent than was MeMet (Ishida *et al.* 1990, Nakajima 1991).

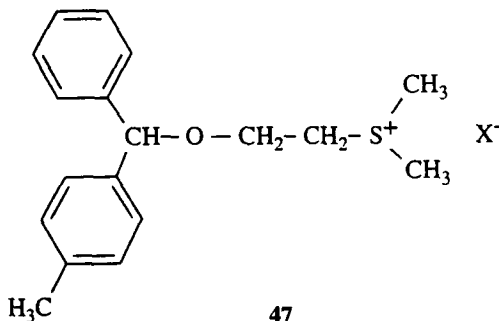
A brief reference should be made to bleomycin A₂ (46) which has a sulfonium group contained within a complex molecular structure. It is the major component of the antitumour drug, bleomycin, which is an important clinical agent used to treat Hodgkin's disease, non-Hodgkin's disease, testicular cancer, and head and neck cancer. It is also used in combination with vinblastine and *cis*-dichlorodiammineplatinum, a standard regimen which produces high cure rates in patients with germinal neoplasms of the testis. However, the relationship between the pharmacological and therapeutic properties of bleomycin A₂ and the sulfonium moiety appear to be unconnected (Chabner 1990, Twentyman 1984).

8.3.2.2 Analogues of pharmaceutical compounds

Some sulfonium analogues of Benadryl have been found to exhibit weak antihistamine activity (Exner *et al.* 1953, Protiva and Exner 1951). Of the many compounds evaluated in these studies, compound (47) proved to be five times as effective as the parent compound, Benadryl. A sulfonium analogue of theophylline, the iodide salt of 7-(β -methylthioethyl)-theophylline (48), has been shown to have the same but weaker pharmacological properties as theophylline (Maj *et*



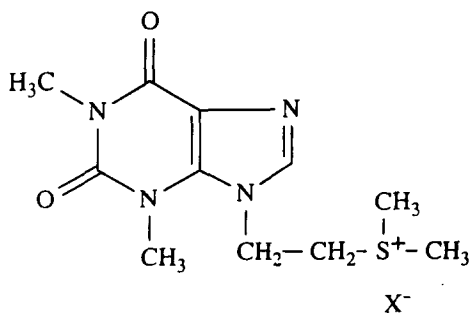
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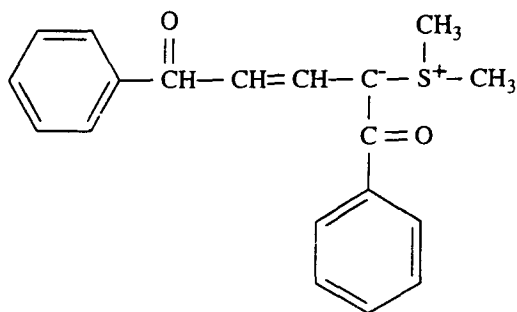
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al. 1962) whilst sulfonium derivatives of 1-aryl-4-oxo-4-aryl-2-butenylides (**49**) are known to possess antimicrobial activity. Additionally, sulfonium analogues of platelet activating factor (PAF) have recently been synthesized and evaluated in terms of both their pharmacological and physiological activities (Kates *et al.* 1991). Of most interest was the finding that the sulfonium analogue of PAF (**50**) and the 2-benzyl sulfonium analogue (**51**) displayed a high degree of cytotoxicity towards HL60 cells whilst they exhibited weak hypotensive and platelet aggregating activity.

There have been reports of sulfonium compounds exhibiting *in vitro* cytotoxicity and *in vivo* antitumour properties, the most notable being those involving tri(2-chloroethyl)sulfonium or alkyl-di(2-chloroethyl)sulfonium halides, which have highly reactive alkylating functional groups. They were found to have a pronounced inhibitory effect on the development of the Ehrlich carcinoma in mice and upon the



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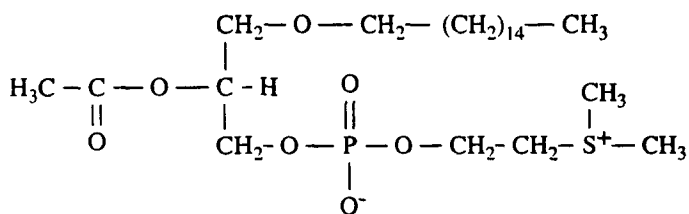
Yoshida ascites sarcoma of rats (Luettringhaus *et al.* 1959, Luettringhaus and Machatzke 1963, Markitantova 1964). In addition, a number of substituted phenacyl sulfonium bromides synthesized by Rutter (1951) exhibited *in vivo* antitumour activity when administered to sarcoma 37 tumour-bearing mice.

A review by Tada (1986) has outlined the structure–activity relationships and drug design of dimethylsulfonium compounds as biological response modifiers and antitumour agents. More recent studies show that a new dimethylsulfonium compound, Suplatast Tosilate (52), designed to treat allergy disorders probably acts in a similar fashion to a biological response modifier in that it can suppress IgE syntheses in both mice and humans following administration (Matsuura *et al.* 1992, Yanagihara *et al.* 1993a, b).

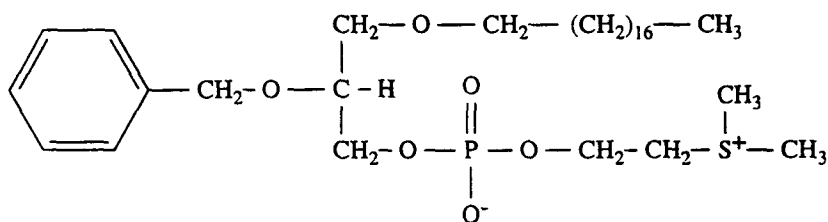
8.3.2.3 Sulfur analogues of amines and quaternary ammonium compounds

A number of pharmacological studies have employed synthetic sulfonium compounds as probes for determining the structure–activity relationships of pharmacologically active nitrogen analogues. Indeed they have proved useful in the course of studies establishing the biochemical and physiological roles of many endogenous amines and quaternary ammonium salts due to their similarities in chemical structure. In general, sulfonium analogues have been found to exhibit the same effects as their nitrogen-containing counterparts but to a lesser degree. Thus, the sulfonium analogue of choline, sulfocholine, can be substituted for choline in phospholipid biosynthesis (Anderson and Bilan 1981, Bjerve and Bremer 1969, Maw and du Vigneaud 1948), and serve as a substrate for choline acetyltransferase to afford acetylsulfocholine (53) (Frankenberg 1973). Both processes were found to be less efficient than if choline was employed. The pharmacological properties of acetylsulfocholine resembles acetylcholine in many ways by the effects elicited on cat's blood pressure, rabbit's intestine and frog's heart and rectal muscle (Ing *et al.* 1952). The effects are somewhat diminished in comparison to acetylcholine.

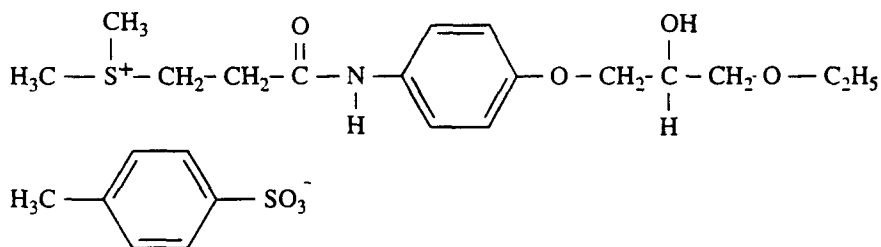
Sulfonium analogues have been used to explore the structure–activity relationships of the endogenous neurotransmitter dopamine (54). One compound in particular, [2-(3,4-dihydroxyphenyl)ethyl]dimethylsulfonium iodide (55), was



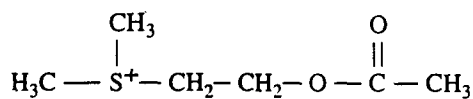
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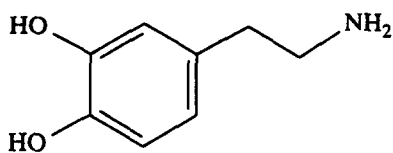
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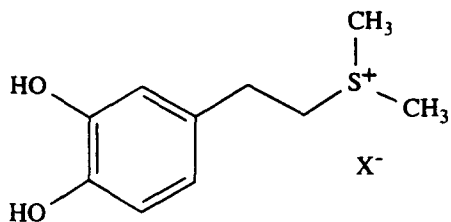
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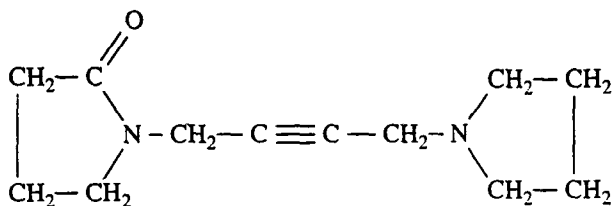
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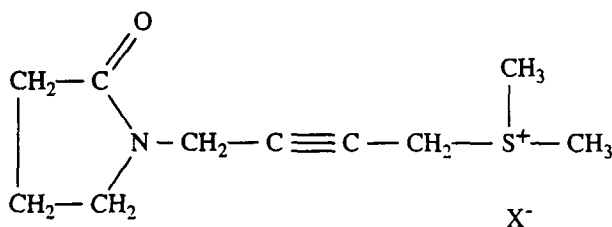
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shown to mimic dopamine by its ability to inhibit not only the high-affinity binding of radiolabelled dopamine to crude preparations of rat striatal membranes, but also the depolarization-evoked release of radiolabelled acetylcholine from mouse striatal slices (Anderson *et al.* 1981, Turowski *et al.* 1984). Additionally, a circling behaviour was observed when this compound was injected directly into the striatum of rats. Despite the lack of potency, the properties of **55** were interesting enough to warrant the synthesis of other sulfonium analogues of dopamine to be included in subsequent studies. Hamada *et al.* (1984) synthesized analogues of **55** and dopamine to determine whether similar structural modifications in **55** would produce similar pharmacological effects in dopamine if it was modified in the same way. Also Chang *et al.* (1987) used analogues of **55** to investigate whether a charged or uncharged chemical species would be best suited to interact with the dopamine receptor. A similar approach has been used to study the interactions of ammonium, sulfonium and sulfide analogues of metoclopramide with the dopamine D₂ receptor (Harrold *et al.* 1994).

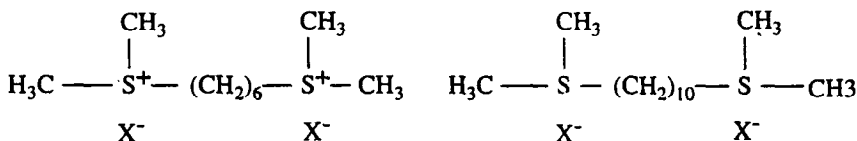
Other sulfonium analogues of pharmacologically active quaternary ammonium salts include those related to the neuromuscular chemical transmitters and blocking agents associated with the autonomic and somatic motor nervous systems. Again, the pharmacological properties of these analogues are in many ways similar to those of the quaternary ammonium salts, but they are less potent (Della Bella 1963). Their inclusion in studies investigating structure-activity relationships has enabled the determination of the conformational and configurational requirements of the receptors involved at the neuromuscular junction and the basic structural requirements necessary for the transmitter to illicit activity. One important factor which governs the manner in which these compounds interact with the receptors is the size of the central atom in the onium moiety. It was recognized as such from the early studies which characterized and compared the pharmacological and toxicological properties of quaternary onium salts which included many tri- or tetra-alkyl substituted ammonium, sulfonium, phosphonium, stibonium and arsonium compounds. These onium salts displayed a 'curare'-like action upon the motor nerve endings of skeletal muscle in addition to 'muscarinic' or 'nicotinic' actions as measured by their blood pressure effects in anaesthetized cats (Hunt and Renshaw 1925, Ing and Wright 1933). Similar findings were obtained during pharmacological investigations of sulfonium analogues of the naturally occurring betaine and its corresponding esters (Hunt and Renshaw 1932). Generally, it was found that the order in which the methyl onium ions displayed potency was as follows: Me₄N > Me₃S > Me₄P > Me₄As, and that the ethyl homologues were inactive. It was deduced that the decrease in activity was a consequence of replacing the central nitrogen atom with sulfur, phosphorus and arsenic, respectively, and that the overall size of the cation must in some way be of importance to activity. Similar conclusions were drawn by Ing *et al.* (1952) to explain the activity of some onium analogues of acetylcholine. It was suggested that a molecule with a large head, such as sulfonium or arsonium, would be less able to fit the receptor group normally holding the quaternary ammonium group in acetylcholine and thereby reduce the interaction necessary for the pharmacological effect. Further evidence to implicate the pivotal role of the central atom in receptor interactions also arose during more recent studies concerning the synthesis and pharmacological evaluation of sulfonium analogues of the muscarinic agents, dioloxane (2-methyl-4-trimethylammoniummethyl-1,3-dioloxane iodide) (Elferink



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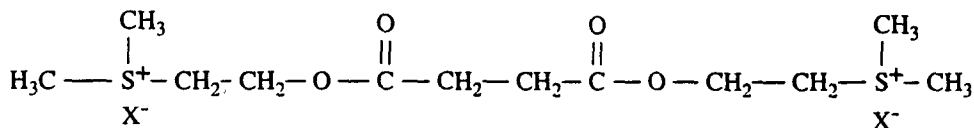


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and Saleminck 1975) and oxotremorine (56) (Ringdahl 1988). In the later study, the muscarinic properties of compound 57 were most surprising as its potency was far greater than that of oxotremorine.

The sulfonium analogues of polymethylenebis(trialkylammonium) salts and succinylcholine have been synthesized and evaluated as neuromuscular blocking agents. These include the salts of hexamethylene-1,6-bis(dimethylsulfonium) (58) (Della Bella 1955), decamethylene-1,10-(dimethylsulfonium) (59) (Walker 1950) and succinylbis-(sulfocholine) (60) (Della Bella *et al.* 1956) and like their nitrogen analogues were found to possess the ability to block neuromuscular transmission. From their studies of ganglion-blocking agents, Barlow and Vane (1956) suggested that in order to make an effective comparison of potency and toxicity between tertiary ammonium and sulfonium salts, the number of methyl or ethyl groups associated with the onium moiety in each analogue must be the same. Therefore,

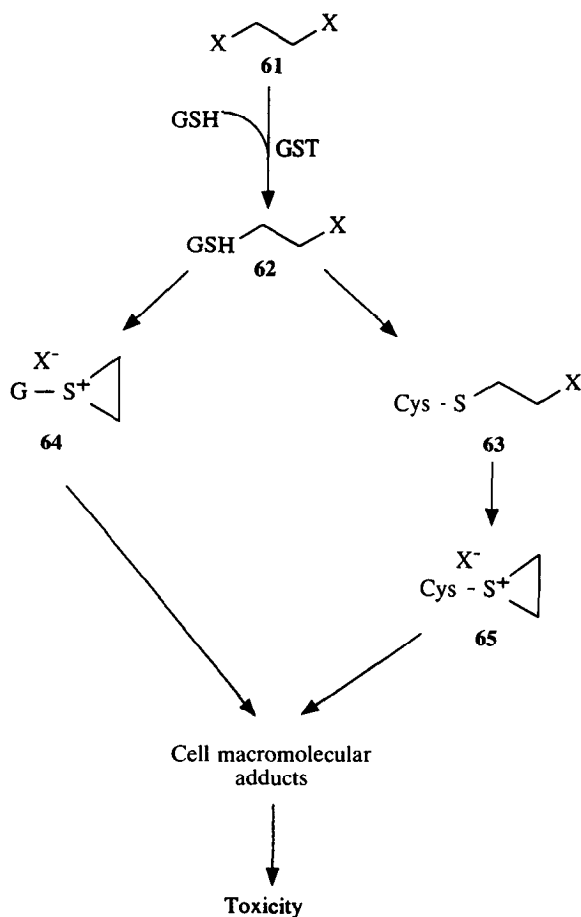
with this in mind, further studies by Della Bella *et al.* (1959) and Lewis *et al.* (1963) identified the critical factor which determined the nature of the pharmacological effect, in addition to the potency of this class of compounds, as being the number of methylene groups present in the chain.

8.4 Sulfonium compounds: toxicological interactions

8.4.1 Formation of sulfonium compounds during toxic events

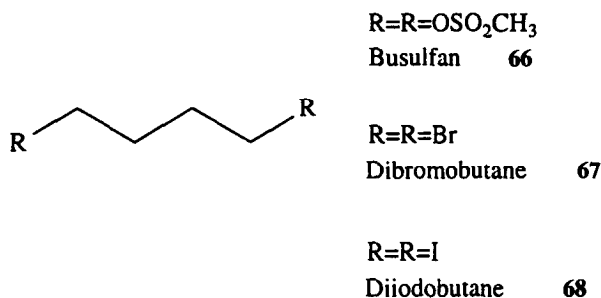
The formation of sulfonium intermediates and their interaction with cellular processes have featured in a number of toxicological mechanisms. It has been proposed that thiiranium or episulfonium ions are the ultimate toxic species associated with the toxicity of 1,2-dihaloethanes (**61**) (Crooks 1989, Dekant and Vamvakas 1993, Van Bladeren *et al.* 1987), a group of compounds once commonly used as soil fumigants, gasoline additives, solvents and synthetic intermediates. Toxicological studies show that these compounds are potent mutagens and carcinogenic substances in animals producing liver, lung, stomach, mammary adrenal, skin and kidney tumours (Olson *et al.* 1972, Rannug *et al.* 1978, Storer and Conolly 1983, Weisburger 1977, Wong *et al.* 1982). It is envisaged that 1,2-dihaloethanes are initially metabolized via a glutathione S-transferase (GST)-catalysed displacement reaction to yield the corresponding S-(2-haloethyl)glutathione conjugates (**62**), as shown in Scheme 9. These compounds then undergo further biotransformation to the cysteine conjugates (**63**) by metabolic pathways associated with the formation of mercapturic acids. Both the glutathione or cysteine conjugates may be considered as biosynthetic sulfur half-mustards which have strong alkylating and electrophilic properties attributable to neighbouring group interactions in nucleophilic displacement reactions (Dohn and Casida 1987, Jean and Reed 1989). Thus the highly reactive episulfonium ions (**64**, **65**) are formed through the internal displacement of the second halogen atom by the sulfur atom. It is postulated that these intermediate species react with biological macromolecules forming covalent adducts which in turn give rise to toxicity (Rannug *et al.* 1978, Van Bladeren *et al.* 1980, Webb *et al.* 1985). The concept of episulfonium ions as ultimate intermediates in the metabolism of vicinal dihaloethanes is supported by structure-activity relationships on the mutagenicity of several derivatives on N-acetyl-S-(2-haloalkyl)-L-cysteine (Van Bladeren *et al.* 1979), by the stereochemistry of the DNA adducts (Peterson *et al.* 1988) and by chemical studies of the transient species using model compounds (Dohn and Casida 1987, Henkel and Amato 1988). Similar mechanisms involving the formation of reactive episulfonium ion intermediates have been proposed to account for the metabolism and toxicity of other dihaloalkanes, such as 1,2-dichloropropane (Bartels and Timchalk 1990) and 1,2-dibromo-3-chloropropane (Pearson *et al.* 1990).

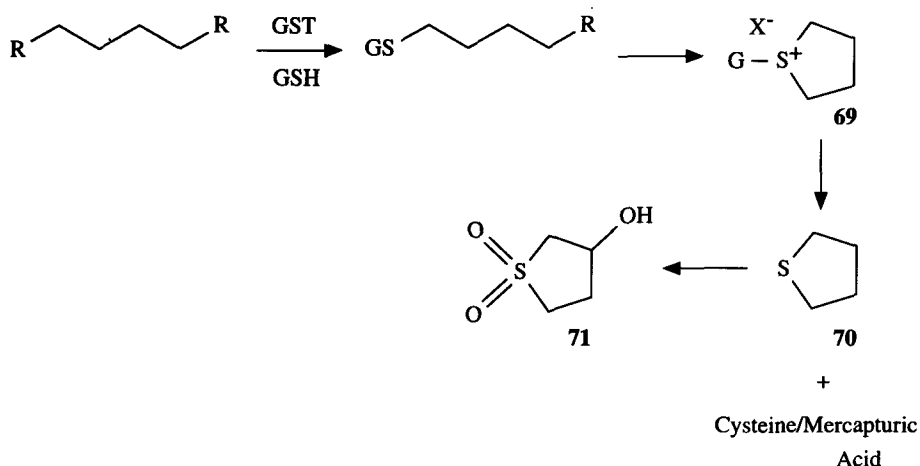
Cyclic sulfonium ion intermediates have also been encountered during investigations into the metabolic fate of 1,4-disubstituted butanes which arise from mechanisms similar to those described for the 1,2-dihaloethanes. Examples are found in the metabolism of the bifunctional alkylating and chemotherapeutic agent, Busulfan, or 1,4-butanediol dimethanesulfonate (**66**) (Hassan and Ehrsson 1987a, b, Roberts and Warwick 1959, 1961a, b) and of the mutagens 1,4-dibromo-



Scheme 9

(67) and 1,4-diiodobutanes (68) (Marchand and Abdel-Monem 1985, Marchand *et al.* 1988, Onkenhout *et al.* 1986). These compounds are principally biotransformed via conjugation with glutathione, forming an unstable conjugate which rapidly cyclises to a five-membered thiolanium ion glutathione conjugate (69) as shown in Scheme 10. The sulfonium intermediate undergoes further metabolism to the cysteine and mercapturic acid derivatives, which are unstable at physiological pH. These yield tetrahydrothiophene (70) which in turn is rapidly





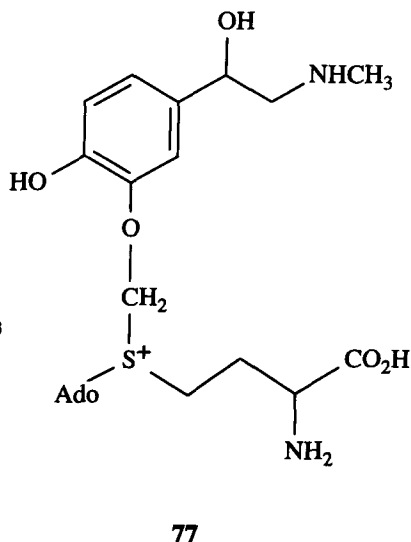
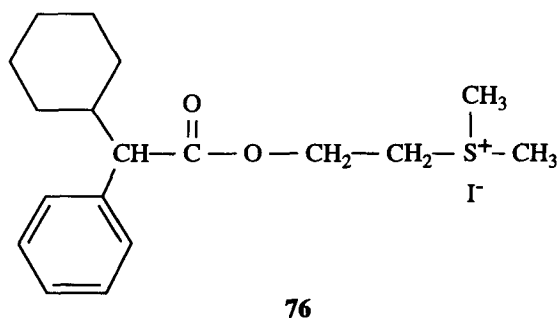
Scheme 10

metabolized by oxidation to 3-hydroxysulfolane (71). It has not been established whether the cyclic sulfonium ion intermediates have any significant role in the toxicity of the parent compounds. In comparison to the episulfonium ion, the thiolanium ion is chemically a more stable compound and therefore unlikely to react with cellular macromolecules.

Bioactivation and toxicity via S-adenosylated derivatives has also been proposed (see Scheme 11). The hepatocarcinogenicity of ethionine (72) and vinthionine (73) is thought to be due to the resulting S-adenosylethionine and S-adenosylvinthionine having the capability of mimicking AdoMet and enzymatically transferring ethyl or vinyl groups to macromolecules such as DNA. However, in the case of vinthionine, S-adenosylvinthionine (74) would be expected to be much more reactive towards cellular nucleophiles than would either vinthionine or S-adenosylethionine (75) because of the inductive and electron-sharing stabilization of the carbanion intermediate formed during nucleophilic addition at the β -carbon atom of the vinyl group (Crooks 1989, Leopold *et al.* 1982).

8.4.2 Inhibition of enzymes

A number of sulfonium salts have been found to inhibit specific cellular enzymes and prevent them from carrying out their normal physiological functions. These compounds should be considered as potentially toxic and treated with due care. Triphenylsulfonium chloride has been found to inhibit oxidative phosphorylation and adenosine triphosphatase activity in addition to the electron-transfer system in the NAD-cytochrome b region of the respiratory chain – enzyme systems which are associated with the mitochondria of cells (Barrett and Selwyn 1976). Several alkylsulfonium and alkyldisulfonium salts, such as decamethylenebis(dimethylsulfonium) and *n*-octadecyldimethylsulfonium bromides, are potent inhibitors of phospholipase C (Young *et al.* 1991, 1992). This



enzyme catalyses the hydrolysis of the glycerophosphate ester bond in phospholipids and features prominently in mechanisms associated with inflammation. Sulfonium compounds of similar structure can also inhibit cholinesterase, an enzyme which deactivates the pharmacological actions of acetylcholine. Thus bis and polyonium molecules which contain sulfonium moieties, and monoalkylsulfonium compounds having less complicated chemical structures, such as trimethylsulfonium iodide, display anticholinesterase activity in addition to neuromuscular blocking activity (Cohen *et al.* 1985, Kirpekar *et al.* 1962). Other compounds which have anticholinesterase activity include 2-(phenylcyclohexylacetoxy)ethyltrimethylsulfonium iodide (**76**) or Thiospasmin, a sulfonium compound possessing strong spasmolytic or muscle relaxing properties (Protiva and Exner 1953).

Chemically designed enzyme inhibitors can be useful as biochemical tools as a means to provide information about the general properties of the enzyme, together with more specific details such as that regarding the active site. These compounds also have chemotherapeutic potential if the inhibitor can selectively inactivate the target enzyme without causing any disruption to other cellular enzymes and related cellular processes. In this context, the majority of the reported studies involving sulfonium compounds have been related to the inactivation of AdoMet-dependent enzymes involving simple analogues of AdoMet (Borchardt *et al.* 1976, Gnegy and Lotspeich 1976), as well as more complex derivatives such as **77**, which can be viewed as transition-state analogues since the methyl donor and acceptor are combined in a single structure (Anderson *et al.* 1981, Benghiat and Crooks 1983, Tang *et al.* 1981). These compounds have been studied as competitive inhibitors. Additionally, the properties of sulfonium analogues of cationic intermediates have been reported (Croteau *et al.* 1986), and the ability of peptidyl sulfonium salts to inhibit proteases has been described in a previous section.

8.4.3 Detoxification

Detoxification mechanisms involving sulfonium compounds are relatively few in number. However, from recent studies it has been suggested that in addition to the previously mentioned roles, AdoMet also has the ability to become involved in the detoxification of xenobiotics if they are metabolized via AdoMet-dependent methyltransferase reactions. Ansher and Jakoby (1986) and Crooks *et al.* (1988) have identified nonspecific AdoMet-dependent N-methyltransferases in rabbit liver which can metabolize a wide range of amines, and Mozier *et al.* (1988) and Mozier and Hoffman (1990) have detected the presence of an AdoMet-dependent thioether transferase in mice which can metabolize several alkyl sulfides including 2-chloroethyl ethyl sulfide, an analogue of the highly toxic sulfur mustard, bis(2-chloroethyl)sulfide.

8.5 References

- ABRAHAMSON, L. & SHAPIRO, S. K., 1965, The biosynthesis of methionine: partial purification and properties of homocysteine methyltransferase of jack bean meal, *Archives of Biochemistry and Biophysics*, **109**, 376–382.
- ALMASIO, P., BORTOLINI, M., PAGLIARO, L. & CORTOLTI, M., 1990, Role of S-adenosyl-L-methionine in the treatment of intrahepatic cholestasis, *Drugs*, **40**, 111–123.
- ANDERSEN, K. K., 1981, Stereochemistry and chiroptical properties of the sulphonium group, in Stirling, C. J. M. & Patai, S. (Eds), *The Chemistry of the Sulphonium Group*, Part 1, pp. 229–266, Chichester: Wiley.
- ANDERSON, G. L., BUSSOLOTTI, D. L. & COWARD, J. K., 1981, Synthesis and evaluation of some multisubstrate adducts as inhibitors of catechol O-methyltransferase, *Journal of Medicinal Chemistry*, **24**, 1271–1277.
- ANDERSON, K. & BILAN, P., 1981, Replacement of mouse LM fibroblast choline by a sulonium analog. Effects on membrane properties as determined by virus probes, *Biochimica et Biophysica Acta*, **640**, 91–99.
- ANDERSON, K., KURUVILLA, A., URETSKY, N. & MILLAR, D. D., 1981, Synthesis and pharmacological evaluation of sulfonium analogues of dopamine: nonclassical dopamine agonists, *Journal of Medicinal Chemistry*, **24**, 683–687.
- ANDERSON, R., KATES, M. & VOLCANI, B. E., 1976, Sulphonium analogue of lechithin in diatoms, *Nature*, **263**, 51–53.
- 1978, Identification of the sulfolipids in the nonphotosynthetic diatom, *Nitzschia alba*, *Biochimica et Biophysica Acta*, **528**, 89–106.
- 1979, Studies on the biosynthesis of sulfolipids in the diatom, *Nitzschia alba*, *Biochimica et Biophysica Acta*, **573**, 557–561.
- ANSHER, S. S. & JAKOBY, W. B., 1986, Amine-N-methyltransferases from rabbit liver, *Journal of Biological Chemistry*, **267**, 3996–4001.
- BARLOW, R. B. & VANE, J. R., 1956, The ganglion-blocking properties of hexamethylene bisdialkylsulphonium salts, *British Journal of Pharmacology*, **11**, 198–201.
- BARRETT, R. H. & SELWYN, M. J., 1976, Effects of triphenylsulphonium ions on mitochondria: inhibition of adenosine triphosphatase activity, *Biochemical Journal*, **156**, 315–322.
- BARTELS, M. J. & TIMCHALK, C., 1990, 1,2-Dichloropropane: investigation of the mechanism of mercapturic acid formation in the rat, *Xenobiotica*, **20**, 1035–1042.
- BENGHIAT, E. & CROOKS, P. A., 1983, Multisubstrate adducts as potential

- inhibitors of S-adenosylmethionine dependent methylases: inhibition of indole N-methyltransferase by (5'-deoxyadenosyl)[3-(3-indolyl)prop-1-y]methylsulfonium and (5'-deoxyadenosyl)[4-(3-indolyl)but-1-y]methylsulfonium salts, *Journal of Medicinal Chemistry*, **26**, 1470-1477.
- BISSERET, P., ITO, S., TREMBLAY, P. A., VOLCANI, B. E., DESSERT, D. & KATES, M., 1984, Occurrence of phosphatidylsulfocholine, the sulfonium analog of phosphatidylcholine in some diatoms and algae, *Biochimica et Biophysica Acta*, **796**, 320-327.
- BJERVE, K. S. & BREMER, J., 1969, Sulfocholine (dimethylhydroxyethylsulfonium chloride) and choline metabolism in the rat, *Biochimica et Biophysica Acta*, **176**, 570-583.
- BLACKBURN, M. N. & SIBLEY, C. C., 1980, The heparin binding site of antithrombin III. Evidence for a critical tryptophan residue, *Journal of Biological Chemistry*, **255**, 824-826.
- BLUNDEN, G. & GORDEN, S. M., 1986, Betaines and their sulphonio analogues in marine algae, *Progress in Phycological Research*, **4**, 39-80.
- BLUNDEN, G., SMITH, B. E., IRONS, M. W., YANG, M. H., ROCH, O. G. & PATEL, A. V., 1992, Betaines and tertiary sulfonium compounds from 62 species of marine algae, *Biochemical Systematics and Ecology*, **20**, 373-388.
- BORCHARDT, R. T., 1980, S-Adenosyl-L-methionine dependent macromolecule methyltransferases: potential targets for the design of chemotherapeutic agents, *Journal of Medicinal Chemistry*, **23**, 347-357.
- BORCHARDT, R. T. & WU, Y. S., 1976, Potential inhibitors of S-adenosylmethionine-dependent methyltransferases. 5. Role of the asymmetric sulfonium pole in the enzymatic binding of S-adenosyl-methionine, *Journal of Medicinal Chemistry*, **19**, 1099-1103.
- BORCHARDT, R. T., WU, Y. S., HUBER, J. A. & WYCPALEK, A. F., 1976, Potential inhibitors of S-adenosylmethionine-dependent methyltransferases. 6. Structural modifications of S-adenosyl-methionine, *Journal of Medicinal Chemistry*, **19**, 1104-1110.
- BORCHARDT, R. T., CREVELING, C. R. & UELAND, P. M., 1986, *Biological Methylation and Drug Design*, New Jersey: Humana Press.
- BORSOOK H. & DUBNOFF, J. W., 1940, The formation of creatine from glycocyamine in the liver, *Journal of Biological Chemistry*, **132**, 559-574.
- BORSOOK, H. & DUBNOFF, J. W., 1947, On the role of oxidation in the methylation of guanidoacetic acid, *Journal of Biological Chemistry*, **171**, 363-375.
- BROWN, A. C. & FRASER, T. R., 1866-1867, On the connection between chemical constitution and physiological action of the salts of the ammonium bases derived from strychnia, brucia, thebaia, codeia, morphia, and nicotia, *Journal of Anatomy and Physiology* **2**, 224-242.
- 1867-1869, V - On the connection between chemical constitution and physiological action. Part I - On the physiological action of the salts of ammonium bases, derived from strychnia, brucia, thebaia, codeia, morphia, and nocotea, *Transactions of the Royal Society of Edinburgh*, **25**, 151-203.
- BROWN, C. & LETTS, E. A., 1878, On dimethyl-thetine and its derivatives, *Transactions of the Royal Society of Edinburgh*, **28**, 571-582.
- CANTONI, G. L., 1951a, Activation of methionine for transmethylation, *Journal of Biological Chemistry*, **189**, 745-754.
- 1951b, Methylation of nicotinamide with a soluble enzyme system from rat liver, *Journal of Biological Chemistry*, **189**, 203-216.
- 1952, The nature of the active methyl donor formed enzymatically from L-methionine and adenosinetriphosphate, *Journal of the American Chemical Society*, **74**, 2942-2943.
- 1953, S-Adenosylmethionine; a new intermediate formed enzymatically from L-methionine and adenosinetriphosphate, *Journal of Biological Chemistry*, **204**, 403-416.

- CANTONI, G. L. & SCARANO, E., 1953, The formation of S-adenosylhomocysteine in enzymatic transmethylation reactions, *Journal of the American Chemical Society*, **76**, 4744.
- CARLE, J. S. & CHRISTOPHERSEN, C., 1982, Dogger bank itch. 4. An eczema-causing sulfoxonium ion from the marine animal, *Alcyonidium gelatinosum* [Bryozoa], *Toxicon*, **20**, 307–310.
- CARLE, J. S., THYBO, H. & CHRISTOPHERSEN, C., 1982, Dogger bank itch. 3. Isolation, structure determination and synthesis of a hapten, *Contact Dermatitis*, **8**, 43–47.
- CARRARA, G., 1893, Sopra alcune tetine isomere, *Gazzetta Chimica Italia*, **23**, 493–509.
- CHABNER, B. A., 1990, Bleomycin, in Chabner, B. A. & Collins, J. M. (Eds), *Cancer Chemotherapy-Principles and Practise*, pp. 341–355, Philadelphia: J.B. Lippincott.
- CHALLENGER, F., 1959, *Aspects of the Organic Chemistry of Sulphur*, London: Butterworths.
- CHALLENGER, F. & SIMPSON, M. I., 1948, Studies on biological methylation. Part XII. A precursor of the dimethyl sulphide evolved by *Polysiphonia fastigiata*. Dimethyl 2-carboxyethylsulphonium hydroxide and its salts, *Journal of the Chemical Society*, 1591–1597.
- CHANG, Y.-A., ARES, J., ANDERSON, K., SABOL, B., WALLACE, A., FAROQUI, T., URETSKY, N. & MILLAR, D. D., 1987, Dopamine agonists: effects of charged and uncharged analogues of dopamine, *Journal of Medicinal Chemistry*, **30**, 214–218.
- CHAWLA, R. K., BONKOVSKY, H. L. & GALAMBOS, J. T., 1990, Biochemistry and pharmacology of S-adenosyl-L-methionine and rationale for its use in liver disease, *Drugs*, **40**, 98–110.
- COHEN, S. G., CHISHTI, S. B., ELKIND, J. L., REESE, H. & COHEN, J. B., 1985, Effects of charge, volume and surface binding of inhibitor and substrate moieties to acetylcholinesterase, *Journal of Medicinal Chemistry*, **28**, 1309–1313.
- CORNFORTH, J. W., REICHARD, S. A., TALAY, P., CARRELL, H. L. & GLUSKER, J. P., 1977, Determination of the absolute configuration at the sulfonium centre of S-adenosylmethionine. Correlation with the absolute configuration of the diastereomeric S-carboxymethyl-(S)-methionine salts, *Journal of the American Chemical Society*, **99**, 7292–7300.
- CROOKS, P. A., 1989, Sulphonium salts, in Damani, L. A., (Ed.), *Sulphur-Containing Drugs and Related Organic Compounds: Chemistry, Biochemistry and Toxicology*, Vol. 1, Part B, pp. 155–180, Chichester: Ellis Horwood.
- CROOKS, P. A., GODIN, C. S., DAMANI, L. A., ANSHER, S. S. & JAKOBY, W. B., 1988, Formation of quaternary amines by N-methylation of azaheterocycles with homogenous amine N-methyltransferase, *Biochemical Pharmacology*, **37**, 1673–1677.
- CROTEAU, R., WHEELER, C. J., AKSELA, R. & OEHLISCHLAGER, A. C., 1986, Inhibition of monoterpene cyclases by sulfonium analogs of presumptive carbocationic intermediates of the cyclization reaction, *Journal of Biological Chemistry*, **261**, 7257–7263.
- DEKANT, W. & VAMVAKAS, S., 1993, Glutathione-dependent bioactivation of xenobiotics, *Xenobiotica*, **23**, 873–887.
- DELLA BELLA, D., 1955, Über ein gangleinlähmendes Sulphoniumderivat: Das hexamethylen-1,6-bis-dimethylsulphonium, *Naunyn-Schmiedebergs Archiv für Experimentelle Pathologie Pharmakologie*, **226**, 335–339.
- 1963, Pharmacological properties of derivatives containing a sulfonium function replacing a quaternary ammonium function, *Archivio Italiano di Scienze Farmacologiche*, **13**, 70–82.
- DELLA BELLA, D., VILLANI, R. & ZUANAZZI, G. F., 1956, Über die curareähnliche Wirkung eines Disulfoniumabkömmlings, des Succinyl(bis)sulfacholins, *Naunyn-Schmiedebergs Archiv für Experimentelle Pathologie Pharmakologie*, **229**, 536–543.

- DELLA BELLA, D., CALIARI, W. & ROGNONI, F., 1959, On the relationship between the structure and pharmacological properties of certain derivatives of polymethylene-bis-dimethylsulfonium, *Archivio Italiano di Scienze Farmacologiche*, **9**, 549-555.
- DODD, W. & COSSINS, E. A., 1969, Metabolism of S-adenosylmethionine in germinating pea seeds: turnover and possible relationships between recycling of sulfur and transmethylation reactions, *Archives of Biochemistry and Biophysics*, **133**, 216-223.
- DOHN, D. R. & CASIDA, J. E., 1987, Thiiranium ion intermediates in the formation and reactions of S-(2-haloethyl)-L-cysteines, *Bioorganic Chemistry*, **15**, 115-124.
- ELFERINK, J. G. R. & SALEMINK, C. A., 1975, Relation between structure and muscarinic activity. III. Synthesis and cholinergic activity of some -onium compounds related to 2-methyl-4-trimethylammoniummethyl-1,3-dioxolane iodide, *Arzneimittel Forschung*, **25**, 1858-1860.
- EXNER, O., BOROVICKA, M. & PROTIVA, M., 1953, Antihistamine substances, XXIII. Sulfonium analogues of antihistaminics of the monoamine and diamine series, *Collection of Czechoslovak Chemical Communications*, **18**, 270-274.
- FALANGA, A., SHAW, E., DONATI, M. B., CONSONNI, R., BARBUI, T., & GORDON, S., 1989, Inhibition of cancer procoagulant by peptidyl sulfonium salts; *Thrombosis Research*, **54**, 389-398.
- FRANKENBERG, L., HEIMBÜRGER, NILSSON, C. & SÖBO, B., 1973, Biochemical and pharmacological studies on the sulfonium analogues of choline and acetylcholine, *European Journal of Pharmacology*, **23**, 37-46.
- GIBSON, K. D., WILSON, J. D. & UDENFRIEND, S., 1961, The enzymatic conversion of phospholipid ethanolamine to phospholipid choline in rat liver, *Journal of Biological Chemistry*, **236**, 673-679.
- GLOVER, G. I., MARIANO, P. S. & PETERSON, J. R., 1976, Reinvestigation of the phenyl bromide modifications of α -chymotrypsin, *Biochemistry*, **15**, 3754-3760.
- GNEGÝ, M. E. & LOTSPEICH, F. J., 1976, Inhibitors of RNA methyltransferases. S-Adenosylsulfonium salts, *Journal of Medicinal Chemistry*, **19**, 1191-1195.
- GREEN, R. C. & DAVIS, N. B., 1960, Biosynthesis of S-methylmethionine in the jack bean, *Biochimica et Biophysica Acta*, **43**, 360-362.
- DE LA HABA, G., JAMIESSON, G. A., MUDD, S. H. & RICHARDS, H. H., 1959, S-Adenosylmethionine: the relation of configuration at the sulfonium center to enzymatic reactivity, *Journal of the American Chemical Society*, **81**, 3975-3980.
- HAMADA, A., CHANG, Y. A., URETSKY, N. & MILLAR, D. D., 1984, Dopaminergic agonists: comparative actions of amine and sulfonium analogues of dopamine, *Journal of Medicinal Chemistry*, **27**, 675-680.
- HARDY, R. E. & DILL, K., 1982, Magnetic resonance study of glycophorin A-containing ¹³C-enriched methionines, *Federation of European Biochemical Society Letters*, **143**, 327-331.
- HARROLD, M. W., SRIBURI, A., MATSUMOTO, K., MILLAR, D. D., FAROQUI, T. & URETSKY, N., 1994, The interaction of ammonium, sulfonium and sulfide analogues of metoclopramide with the dopamine D₂ receptor, *Journal of Medicinal Chemistry*, **36**, 3166-3170.
- HASS, P., 1935, The liberation of methyl sulphide by seaweed, *Biochemical Journal*, **29**, 1297.
- HASSAN, M. & EHRSSON, H., 1987a, Metabolism of ¹⁴C-busulfan in isolated perfused rat liver, *European Journal of Drug Metabolism and Pharmacokinetics*, **12**, 71-76.
- 1987b, Urinary metabolites of busulfan in the rat, *Drug Metabolism and Disposition*, **15**, 399-402.
- HEILMANN, H. D. & PFLEIDERER, G., 1975, On the role of tryptophan residues in the mechanism of action of glyceraldehyde-3-phosphate dehydrogenase as tested by specific modification, *Biochimica et Biophysica Acta*, **384**, 331-341.
- HEINRICH, C. P., ADAM, S. & ARNOLD, W., 1973, The reaction of dimethyl(2-hydroxy-

- 5-nitrobenzyl)sulfonium bromide with N-acetyl-L-tryptophan amide, *Federation of European Biochemical Society Letters*, **33**, 181–183.
- HENKEL, J. G. & AMATO, G. S., 1988, Methyl mercapturate episulfonium ion: a model reactive metabolite of dihaloethanes, *Journal of Medicinal Chemistry*, **31**, 1279–1282.
- HOCHSCHWENDER, S. M. & LAURSEN, R. A., 1981, The lysine binding sites of human plasminogen. Evidence for a critical tryptophan in the binding site of kringle 4, *Journal of Biological Chemistry*, **256**, 11172–11176.
- HOFFMAN, J. L., 1986, Chromatographic analysis of the chiral and covalent instability of S-adenosyl-L-methionine, *Biochemistry*, **25**, 4444–4449.
- HORTON, H. R. & TUCKER, W. P., 1970, Dimethyl(2-hydroxy-5-nitrobenzyl)sulfonium salts. Water-soluble environmentally sensitive protein reagents, *Journal of Biological Chemistry*, **245**, 3397–3401.
- HUNT, R. & RENSHAW, R. R., 1925, On some effects of arsonium, stibonium, phosphonium, and sulfonium compounds on the autonomic nervous system, *Journal of Pharmacology and Experimental Therapeutics*, **25**, 315–355.
- 1932, Sulfonium compounds and the autonomic nervous system, *Journal of Pharmacology and Experimental Therapeutics*, **44**, 63–79.
- ING, H. R. & WRIGHT, W. M., 1933, Further studies on the pharmacological properties of onium salts, *Proceedings of the Royal Society of London B*, **114**, 48–63.
- ING, H. R., KORDIC, P. & TUDOR WILLIAMS, D. P. H., 1952, Studies on the structure–action relationships of the choline group, *British Journal of Pharmacology*, **7**, 103–116.
- ISHIDA, Y., OGIHARA, Y. & OKABE, S., 1990, Effects of a crude extract of a marine dinoflagellate, containing dimethyl- β -propiothetin, on HCl-ethanol-induced gastric lesions and gastric secretion in rats, *Japanese Journal of Pharmacology*, **54**, 333–338.
- JEAN, P. A. & REED, D. J., 1989, *In vitro* dipeptide, nucleoside and glutathione alkylation by S-(2-chloroethyl)glutathione and S-(2-chloroethyl)-L-cysteine, *Chemical Research in Toxicology*, **2**, 455–460.
- JONES, W. C., ROTHGEB, T. M. & GURD, F. R. N., 1975, Specific enrichment with ^{13}C of the methionine methyl groups of sperm whale myoglobin, *Journal of the American Chemical Society*, **97**, 3875–3877.
- 1976, Nuclear magnetic resonance studies of sperm whale myoglobin specifically enriched with ^{13}C in the methionine groups, *Journal of Biological Chemistry*, **251**, 7452–7460.
- KARP, G. I., MARCUM, J. A. & ROSENBERG, R. D., 1984, The role of tryptophan residues in heparin–antithrombin interactions, *Archives of Biochemistry and Biophysics*, **233**, 712–720.
- KARR, D., TWETO, J. & ALBERSHEIM, P., 1967, S-Adenosylmethionine: methionine methyl transferase from wheat germ, *Archives of Biochemistry and Biophysics*, **121**, 732–738.
- KATES, M., ADAMS, G. A., BLANK, M. L. & SNYDER, F., 1991, Chemical synthesis and physiological activity of sulfonium analogues of platelet activating factor, *Lipids*, **26**, 1095–1101.
- KAYE, G. L., BLAKE, J. C. & BURROUGHS, A. K., 1990, Metabolism of exogenous S-adenosyl-L-methionine in patients with liver disease, *Drugs*, **40**, 124–128.
- KEENAN, T. W. & LINDSAY, R. C., 1968, Evidence for a dimethyl sulfide precursor in milk, *Journal of Dairy Science*, **51**, 112–114.
- KHUCHUA, G. N. & STASYAK, A. B., 1975, Method of electrophoretic determination of S-methylmethionine, *Prikladnaia Biokhimiia i Mikrobiologiia*, **11**, 914–921.
- KIRIBUCHI, T. & YAMANISHI, T., 1963, Studies on the flavor of green tea. Part IV. Dimethylsulfide and its precursor, *Agriculture and Biological Chemistry*, **27**, 56–59.

- KIRPEKAR, S. M., LEWIS, J. J. & MUIR, T. C., 1962, Anti-cholinesterase activity in bis- and polyonium neuromuscular blocking agents, *Biochemical Pharmacology*, **11**, 937–942.
- KNIPE, A. C., 1981, Reactivity of sulfonium salts, in Stirling, C. J. M. & Patai, S., (Eds), *The Chemistry of the Sulphonium Group*, Part 1, pp. 313–385, Chichester: John Wiley.
- KOVACHEVA, E. G., 1974, Method for measuring S-methylmethionine in natural products, *Prikladnaia Biokhimiia i Mikrobiologiya*, **10**, 129–135.
- LEOPOLD, W. R., MILLAR, J. A. & MILLAR, E. C., 1982, Comparison of some carcinogenic, mutagenic and biochemical properties of S-vinylhomocysteine and ethionine, *Cancer Research*, **42**, 4364–4374.
- LETTIS, E. A., 1878, Action of iodoacetic and bromoacetic ethyl ether on sulphide of methyl, *Transactions of the Royal Society of Edinburgh*, **28**, 618–624.
- LEWIS, J. J., MARTIN-SMITH, M. & MUIR, T. C., 1963, Observations upon roles of ether, oxygen, alkyl group size, number of onium centres and interonium distance upon neuromuscular block in bis- and polyonium compounds, *British Journal of Pharmacology*, **20**, 307–318.
- LIU-WU, L. N. Y. & HORTON, R., 1979, Structure of 2-hydroxy-5-nitrobenzylated carboxypeptidase A, *Biochimica et Biophysica Acta*, **577**, 22–33.
- LLINAS, M., DE MARCO, A., HOCHSCHWENDER, S. M. & LAURSEN, R. A., 1983, A ¹H-NMR study of isolated domains for human plasminogen. Structural homology between kringles 1 and 4, *European Journal of Biochemistry*, **135**, 379–391.
- LÜTTRINGHAUS, A. & MACHATZKE, H., 1963, Cytostatisch wirkende Sulfoniumsalze. II: Sulfoniumsalze mit einem und zwei-2-halogenäthylresten, *Arzneimittel Forschung*, **13**, 366–370.
- LÜTTRINGHAUS, A., KIMMIG, J., MACHATZKE, H. & JÄNNER, M., 1959, Cytostatisch wirkende Sulfoniumsalze. Tri-(2-chloräthyl)- und di(2-chloräthyl)-vinyl-sulfoniumsalze, *Arzneimittel Forschung*, **9**, 748–753.
- MAJ, J., SOWINSKA, H. & SYPNIEWSKA, M., 1962, Pharmacological properties of sulfur derivatives of theophylline, *Archivum Immunologiae Therapiae Experimentalis*, **10**, 125–131.
- MARCHAND, D. H. & ABDEL-MONEM, M. M., 1985, Glutathione S-transferases catalyzed conjugation of 1,4-disubstituted butanes with glutathione *in vitro*, *Biochemical and Biophysical Research Communications*, **128**, 360–367.
- MARCHAND, D. H., REMMEL, R. P. & ABDEL-MONEM, M. M., 1988, Biliary excretion glutathione conjugate of busulfan and 1,4-diiodobutane in the rat, *Drug Metabolism and Disposition*, **16**, 85–92.
- MARKITANTOVA, A. E., 1964, Animal experimental study of the anticancer effect of 2-hydroxy- and 2-chloroethyl sulfonium salts, *Chemical Abstracts*, **60**, 4653e.
- MATO, J. M., CORRALES, F., MARTIN-DUCE, A., ORTIZ, P., PAJARES, M. A. & CABRERO, C., 1990, Mechanisms and consequences of the impaired trans-sulfuration pathway in liver disease: Part 1. Biochemical implications, *Drugs*, **40**, 58–64.
- MATSUURA, N., MORI, H., NAGAI, H. & KODA, A., 1992, Effects of suplastat tosilate (IPD-1151T) on antibody formation in mice, *Folia Pharmacologica Japonica*, **100**, 485–493.
- MATTA, M. S., LANDIS, M. E., PATRICK, T. B., HENDERSON, P. A., RUSSO, M. W. & THOMAS, R. L., 1980, ¹³C-Enriched S-methyl probe at the active site of an enzyme: [S-[¹³C]methylmethionine-192]-α-chymotrypsin (MSMC), *Journal of the American Chemical Society*, **102**, 7152–7154.
- MATTA, M. S., HENDERSON, P. A. & PATRICK, T. B., 1981, Preparation and ¹³C NMR characterisation of [[ε-¹³C]methionine-192]-α-chymotrypsin, *Journal of Biological Chemistry*, **256**, 4172–4174.
- MAW, G. A., 1981, The biochemistry of sulphonium salts, in Stirling, C. J. M. & Patai,

- S. (Eds), *The Chemistry of the Sulphonium Group*, Part 2, pp. 703–770. Chichester: John Wiley.
- MAW, G. A. & DU VIGNEAUD, V., 1948, Investigations of the biological behaviour of the sulfur analogue of choline, *Journal of Biological Chemistry*, **176**, 1029–1036.
- MAYR, U., HENSEL, R., DEPARADE, M., PAULY, H. E., PFLEIDERER, G. & TROMMER, W. E., 1982, Structure–function relationship in the allosteric L-lactate dehydrogenases from *Lactobacillus casei* and *Lactobacillus curvatus*, *European Journal of Biochemistry*, **126**, 549–558.
- MCNICOL, P. K., 1986, Analysis of S-methylmethionine and S-adenosylmethionine in plant tissue by a dansylation, dual-isotope method, *Analytical Biochemistry*, **158**, 93–97.
- MITCHELL, S. C., 1982, Mammalian metabolism of orally administered phenothiazine, *Drug Metabolism Reviews*, **13**, 319–343.
- MOZIER, N. M. & HOFFMAN, J. L., 1990, Biosynthesis and urinary excretion of methyl sulfonium derivatives of the sulfur mustard analog, 2-chloroethyl ethyl sulfide, and other thioethers, *FASEB Journal*, **4**, 3329–3333.
- MOZIER, N. M., MCCONNELL, K. P. & HOFFMAN, J. L., 1988, S-Adenosyl-L-methionine: thioether S-methyltransferase, a new enzyme in sulfur and selenium metabolism, *Journal of Biological Chemistry*, **263**, 4527–4531.
- NAKAJIMA, K., 1991, Dimethyl- β -propiothetin, new potent resistive-agent against stress-induced gastric ulcers in rats, *Journal of Nutritional Science and Vitaminology*, **37**, 229–238.
- OLSEN, W. A., HABERMAN, R. T., WEISBURGER, E. K., WARD, J. M. & WEISBURGER, J. H., 1972, Induction of stomach cancer in rats and mice by halogenated aliphatic fumigants, *Journal of the National Cancer Institute*, **51**, 1993–1995.
- ONKENHOUT, W., VAN LOON, W. M. G. M., BUIJS, W., VAN DER GEN, A. & VERMEULEN, N. P. E., 1986, Biotransformation and quantitative determination of sulfur-containing metabolites of 1,4-dibromobutane in the rat, *Drug Metabolism and Disposition*, **14**, 608–612.
- PARRY, R. J. & MINTA, A., 1982, Studies of enzyme stereochemistry. Elucidation of the stereochemistry of S-adenosylmethionine formation by yeast methionine adenosyltransferase, *Journal of the American Chemical Society*, **104**, 871–872.
- PEARSON, P. G., SODERLUND, E. J., DYBING, E. & NELSON, S. D., 1990, Metabolic activation of 1,2-dibromo-3-chloropropane: evidence for the formation of reactive episulfonium ion intermediates, *Biochemistry*, **29**, 4971–4981.
- PEGG, A. E., 1986, Recent advances in the biochemistry of polyamines in eukaryotes, *Biochemical Journal*, **234**, 249–262.
- 1988, Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy, *Cancer Research*, **48**, 759–774.
- PEGG, A. E. & MCCANN, P. P., 1982, Polyamine metabolism and function, *American Journal of Physiology*, **243**, C212–221.
- PERLZWEIG, W. A., BERNHEIM, M. L. C. & BERNHEIM, F., 1943, The methylation of nicotinamide by rat liver *in vitro*, *Journal of Biological Chemistry*, **150**, 401–406.
- PETERSON, L. A., HARRIS, T. M. & GUENGERICH, F. P., 1988, Evidence for an episulfonium ion intermediate in the formation of S-[2-(N7-guanyl)ethyl]glutathione in DNA, *Journal of the American Chemical Society*, **110**, 3284–3291.
- PEYSER, Y. M., MUHLRAD, A. & WERBER, M. M., 1990, Tryptophan-130 is the most reactive tryptophan residue in rabbit skeletal myosin subfragment-1, *FEBS Letters*, **259**, 346–348.
- PISI, E. & MARCHESINI, G., 1990, Mechanisms and consequences of the impaired sulfuration pathway in liver disease: Part II – clinical consequences and potential for pharmacological intervention in cirrhosis, *Drugs*, **40**, 65–72.
- PLIURA, D. H., BONAVENTURA, B. J., SMITH, R. A., COLES, P. J. & KRANTZ, A., 1992a, Comparative behaviour of calpain and cathepsin B toward peptidyl

- acyloxymethyl ketones sulphonium methyl ketones and other potential inhibitors of cysteine proteinases, *Biochemical Journal*, **288**, 759–762.
- PLIURA, D. H., BONAVENTURA, B. J., PAULS, H. W., KILLACKEY, J. F. & KRANTZ, A., 1992b, Irreversible inhibition of transglutaminases by sulfonium methylketones: optimization of specificity and potency with omega-aminoacyl spacers, *Journal of Enzyme Inhibition*, **6**, 181–194.
- POPE, W. J. & PEACHEY, S. J., 1900, Asymmetric optically active sulphur compounds. *d*-Methylethylthetin platinichloride, *Journal of the Chemical Society*, **77**, 1072–1075.
- PORTER, C. W. & SUFRIN, J. R., 1986, Interference with polyamine biosynthesis and/or function by analogs of polyamines or methionine as a potential anticancer chemotherapeutic strategy, *Anticancer Research*, **6**, 525–542.
- PROTIVA, M. & EXNER, O., 1951, Antihistamine substances. XXI. Sulfonium analogues of antihistamines of the ether series, *Collection of Czechoslovak Chemical Communications*, **16**, 689–695.
- 1953, Synthetic spasmolytics VI. Sulfonium analogs of the ester type spasmolytes, *Chemicke Listy*, **47**, 213–218.
- RANNUG, U., SUNDALL, A. & RAMEL, C., 1978, The mutagenic effect of dichloroethane on *Salmonella typhimurium*. I. Activation through conjunction with glutathione *in vitro*, *Chemico-Biological Interactions*, **20**, 1–16.
- RAUBER, P., WALKER, B., STONE, S. & SHAW, E., 1988, Synthesis of lysine-containing sulphonium salts and their properties as proteinase inhibitors, *Biochemical Journal*, **250**, 871–876.
- REDDY, R. G., REDDY, R. G. & SARADA, S., 1989, Biological activity of sulphur ylides I. Antimicrobial properties of some new carbonyl stabilized sulphonium ylides, *Biochemistry International*, **19**, 215–218.
- RHODES, C. J., ZUMBRUNN, A., BAILYES, E. M., SHAW, E. & HUTTON, J. C., 1989, The inhibition of proinsulin-processing endopeptidase activities by active-site-directed peptides, *Biochemical Journal*, **258**, 305–308.
- RHODES, D. & HANSON, A. D., 1993, Quaternary ammonium and tertiary sulfonium compounds in higher plants, *Annual Review of Plant Physiology and Plant Molecular Biology*, **44**, 357–384.
- RINGDAHL, B., 1988, Dimethylsulphonium and thiolanium analogues of the muscarinic agent oxotremorine, *Journal of Medicinal Chemistry*, **31**, 164–168.
- ROBERTS, J. J., & WARWICK, G. P., 1959, Metabolism of myleran (1,4-dimethanesulfonyloxybutane), *Nature*, **183**, 1509–1510.
- 1961a, The mode of action of alkylating agents II, *Biochemical Pharmacology*, **6**, 205–216.
- 1961b, The formation of 3-hydroxytetrahydrothiophen-1,1-dioxide from meleran, S- β -L-alanyl tetrahydrothiophenium mesylate, tetrahydrothiophen and tetrahydrothiophen-1,1-dioxide in the rat, rabbit and mouse, *Biochemical Pharmacology*, **6**, 217–227.
- ROTHGEB, T. M., JONES, B., HAYES, D. F. & GURD, R. S., 1977, Methylation of glucagon, characterisation of the sulfonium derivative and regeneration of the native covalent structure, *Biochemistry*, **16**, 5813–5818.
- RUTTER, H. A., 1951, Preparation of some sulfonium salts as possible anticancer agents, *Journal of the American Chemical Society*, 5905.
- SALIM, A. S., 1987, Gastric mucosal cytoprotection in the rat by cysteine, *Journal of Pharmacy and Pharmacology*, **39**, 553–555.
- SALVATORE, F., BORECK, E., ZAPPIA, V., WILLIAMS-ASHMAN, H. G. & SCHLENK, F., 1977, *The Biochemistry of Adenosylmethionine*, New York: Columbia University Press.
- SATO, C. S., BYERRUM, R. U., ALBERSHEIM, P. & BONNER, J., 1958, Metabolism of methionine and pectin esterification in a plant tissue, *Journal of Biological Chemistry*, **233**, 128–131.

- SCHLENK, F., 1965, The chemistry of biological sulfonium compounds, *Fortschritte der Chemie Organischer Naturstoffe*, **23**, 61–112.
- SCIUTO, S., PIATTELLI, M., & CHILLEMI, R., 1982, (–)-(S)-Dimethyl-sulfonio-2-methoxybutyrate from the red algae, *Rytiphloea tinctoria*, *Phytochemistry*, **21**, 227–228.
- SCIUTO, S., CHILLEMI, R., MORRONE, R., PATTI, A. & PIATTELLI, M., 1989, Dragendorff-positive compounds in some Mediterranean red algae, *Biochemical Systematics and Ecology*, **17**, 5–10.
- SCULLY, M. F., SHAH, N., ELLIS, V. & KAKKER, V. V., 1991, Influence of tryptophan modification upon digestion of antithrombin III by elastase, *Thrombosis and Haemostasis*, **65**, 351–354.
- SHAPIRO, S. K., 1956, Biosynthesis of methionine from homocysteine and S-methyl-methionine in bacteria, *Journal of Bacteriology*, **72**, 730–735.
- SHAPIRO, S. K. & SCHLENK, F., 1965, *Transmethylation and Methionine Biosynthesis*, Chicago: University of Chicago Press.
- SHAPIRO, S. K. & YPHANTIS, D. A., 1959, Assay of S-methylmethionine and S-adenosylmethionine homocysteine transmethyases, *Biochimica et Biophysica Acta*, **36**, 241–244.
- SHAW, E., 1988, Peptidyl sulfonium salts, *Journal of Biological Chemistry*, **263**, 2768–2772.
- SIMONETTA, M. & GAVEZZOTTI, A., 1981, General and theoretical aspects, in Stirling, C. J. M. & Patai, S. (Eds), *The Chemistry of the Sulphonium Group*, Part 1, pp. 1–14, Chichester: John Wiley.
- STIRLING, C. J. M., 1977, Sulfonium salts, in Oae, S. (Ed.), *Organic Chemistry of Sulfur*, pp. 473–525, New York: Plenum.
- STIRLING, C. J. M. & PATAI, S., 1981, *The Chemistry of the Sulphonium Group*, Chichester: John Wiley.
- STORER, R. D. & CONOLLY, R. B., 1983, Comparative *in vivo* genotoxicity and acute hepatotoxicity of three 1,2-dihaloethanes, *Carcinogenesis*, **4**, 1491–1494.
- STRAMENTINOLI, G., 1986, AdoMet as a drug: pharmacokinetic and pharmacological aspects, in Borchardt, R. T., Creveling, C. R. & Ueland, P. M. (Eds), *Biological Methylation and Drug Design*, pp. 315–326, New Jersey: Humana Press.
- 1987, Pharmacological aspects of S-adenosylmethionine, *American Journal of Medicine*, **83**(5A), 35–42.
- STREHLER, E., 1955, Gegenwartigen Stand der sogenannte “Vitamin U” – Therapie der Magendarm-ulcera, *Gastroenterologia*, **84**, 119–132.
- TABOR, C. W. & TABOR, H., 1984, Polyamines, *Annual Review of Biochemistry*, **53**, 749–790.
- TADA, Y., 1986, Structure–activity relationships and drug design of anticarcinogenic dimethylsulfonium compounds, *Chemical Abstracts*, **105**, 1n.
- TANAKA, T., OHARA, J., TAKEZOE, K., ANDO, T. & HOKARI, N., 1969, Healing effects of methylmethionine chloride on gastric ulcers and chronic dyspepsia, *New Remedies and Therapies*, **8**, 20–22.
- TANG, K. C., MARIUZZA, R. & COWARD, J. K., 1981, Synthesis and evaluation of some stable multisubstrate adducts as specific inhibitors of spermidine synthase, *Journal of Medicinal Chemistry*, **24**, 1277–1284.
- TUCKER, W. P., WANG, J. & HORTON, R. H., 1971, The reaction of dimethyl(2-hydroxy-5-nitrobenzyl)sulfonium salts and tryptophan ethyl ester, *Archives of Biochemistry and Biophysics*, **144**, 730–733.
- TUROWSKI, B., SZKRYBALO, M., ANDERSON, K., MILLAR, D. & URETSKY, N., 1984, Effect of a sulfonium analog of dopamine on the release of [3H]acetylcholine from mouse striatal slices, *Biochemical Pharmacology*, **33**, 2371–2376.

- TWENTYMAN, P. R., 1984, Bleomycin-mode of action with particular reference to the cell cycle. *Pharmacology and Therapeutics*, **23**, 417-441.
- UELAND, P. M., 1982, Pharmacological and biochemical aspects of S-adenosylhomocysteine and S-adenosylhomocysteine hydrolase, *Pharmacological Reviews*, **34**, 223-253.
- USDIN, E., BORCHARDT, R. T. & CREVELING, C. R., 1979, *Transmethylation*, New York: Elsevier/North Holland.
- 1982, *Biochemistry of S-Adenosylmethionine and Related Compounds*, London: Macmillan.
- VAN BLADEREN, P. J., BREIMER, D. D. & MOHN, G. R., 1979, Stereoselective activation of vicinal dihalogen compounds to mutagens by glutathione conjugation, *Biochemical Pharmacology*, **28**, 2521-2524.
- VAN BLADEREN, P. J., BREIMER, D. D., ROTTEVEEL-SMITS, G. M., JONG, R. A. W., BUIJS, W., GEN, A. & MOHN, G. R., 1980, The role of glutathione conjugation in the mutagenicity of 1,2-dibromoethane, *Biochemical Pharmacology*, **29**, 2975-2982.
- VAN BLADEREN, P. J., BRUGGEMAN, I. M., JONGEN, W. M. F., SCHEFFER, A. G. & TEMMINK, J. H., 1987, The role of conjugating enzymes in toxic metabolite formation, in Benford, D. J., Bridges, J. W. & Gibson, G. G. (Eds), *Drug Metabolism - From Molecules to Man*, pp. 151-170, London: Taylor & Francis.
- DU VIGNEAUD, V., 1952, *A Trail of Research in Sulphur Chemistry and Metabolism and Related Fields*, New York: Cornell University Press.
- VILLANUEVA, G. B., 1981, Conformational differences between high clotting human α -thrombin and nonclotting τ -thrombin, *Biochemistry*, **20**, 6519-6525.
- WALKER, J., 1950, Some new curarising agents, *Journal of the Chemical Society*, 193-197.
- WEBB, W., ELFARRA, A., THOM, R. & ANDERS, M. W., 1985, S-(2-chloroethyl)-DL-cysteine (CEC)-induced cytotoxicity: a role for the episulfonium ion, *Pharmacologist*, **27**, 228.
- WEISBURGER, E. K., 1977, Carcinogenicity studies on halogenated hydrocarbons, *Environmental Health Perspectives*, **21**, 7-16.
- WERBER, M. M., PEYSER, Y. M. & MUHLRAD, A., 1987, Modification of myosin subfragment 1 tryptophans by dimethyl(2-hydroxy-5-nitrobenzyl)sulfonium bromide, *Biochemistry*, **26**, 2903-2909.
- WHITE, R. H., 1982, Analysis of dimethylsulfonium compounds in marine algae, *Journal of Marine Research*, **40**, 529-536.
- WONG, F. F. & CARSON, J. F., 1966, Isolation of S-methylmethionine sulfonium salt from fresh tomatoes, *Journal of Agriculture and Food Chemistry*, **14**, 247-249.
- WONG, L. C., WINSTON, J. M., HONG, C. B. & PLOTNICK, H., 1982, Carcinogenicity and toxicity of 1,2-dibromoethane in the rat, *Toxicology and Applied Pharmacology*, **63**, 155-165.
- WU, S. E., HUSKEY, W. P., BORCHARDT, R. T. & SCHOWEN, R. L., 1983, Chiral instability at sulfur of S-adenosylmethionine, *Biochemistry*, **22**, 2828-2831.
- YANAGIHARA, Y., KINIWA, M., IKIZAWA, K., SHIDA, T., MATSUURA, N. & KODA, A., 1993a, Suppression of IgE production by IPD-1151T (Suplatast Tosilate), a new dimethylsulfonium agent: (2) Regulation of human IgE response, *Japanese Journal of Pharmacology*, **61**, 31-39.
- YANAGIHARA, Y., KINIWA, M., IKIZAWA, K., YAMAYA, H., SHIDA, T., MATSUURA, N. & KODA, H., 1993b, Suppression of IgE production by IPD-1151T (Suplatast Tosilate), a new dimethylsulfonium agent: (1) Regulation of murine IgE response, *Japanese Journal of Pharmacology*, **61**, 23-30.
- YOSHINAKA, Y. & NAKAMURA, M., 1981, Combined effects of methylmethionine sulfonium chloride and antacids on various experimental gastric ulcers in rats, *Pharmacometrics*, **21**, 921-925.

- YOUNG, L. & MAW, G. A., 1958, *The Metabolism of Sulphur Compounds*, London: Methuen.
- YOUNG, P. R., SNYDER, W. R. & MCMAHON, R. F., 1991, Inhibition of *Clostridium perfringens*, phospholipase C hydrolysis of a thiophosphate analog of lysophosphatidylcholine by micelle-bound ammonium and sulfonium cations, *Lipids*, **26**, 957–959.
- 1992, Inhibition of *Clostridium perfringens* phospholipase C by ammonium and sulfonium dications, *Biochimica et Biophysica Acta*, **1121**, 297–300.
- ZAPPIA, V., ZYDEK-CWICK, C. R. & SCHLENK, F., 1969, The specificity of S-adenosyl-L-methionine sulfonium stereoisomers in some enzyme systems, *Biochimica et Biophysica Acta*, **178**, 185–187.
- ZHOU, H. M. & TSOU, C. L., 1985, An essential tryptophan residue for rabbit muscle creatine kinase, *Biochimica et Biophysica Acta*, **830**, 59–63.
- ZUMBRUNN, A., STONE, S. & SHAW, E., 1988, The synthesis and properties of peptidylmethylsulphonium salts with two catatonic residues as potential inhibitors of prohormone processing, *Biochemical Journal*, **256**, 989–994.

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