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THIOUREA AND ITS BIOLOGICAL INTERACTIONS

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Thiourea, the sulfur analogue of urea, has been known for over a century and a quarter during which time it has found a variety of uses, some within the biological field. Most noted of these have been its employment as a plant growth stimulator to break bud dormancy and increase crop yield (1920–40) and more recently as a therapeutic agent to treat thyroid dysfunction (1940–50). These and other biological applications, together with the biotransformation of thiourea and its effect upon living systems are reviewed. Finally, an insight into its possible molecular mechanisms of interaction is provided.

CONTENTS

1. INTRODUCTION

1.1. *Historical*

The experiments of Wöhler in the early part of the 19th century enabled important advances to be made in the chemical sciences.[1,2] His observations that the passage of cyanogen into ammonium hydroxide solution produced a mixture of ammonium cyanide and urea (carbamide) instead of the expected ammonium cyanide and ammonium cyanate, and that urea could be formed from (the missing) ammonium cyanate by the application of heat, impelled chemists to come to terms with two important issues.

Firstly, urea, the chief nitrogenous constituent of urine, had been isolated from this liquid by Rouelle some fifty years earlier (1773)[3,4] although Boerhaave (1732) may have an even earlier claim to this compound.[5,6] Accordingly, it was viewed as an 'organic compound' which could only be produced through the activity of a 'vital force' which resided within living organisms, a concept which in one form or another (eg. pneuma) dated back more than twenty centuries and was still supported at this time by many eminent chemists including Berzelius. The formation of urea by the action of heat on ammonium cyanate, which was produced from purely mineral sources, demonstrated that an organic compound could be made in the laboratory from inorganic substances without the intervention of 'vital force'. [However, this does not logically dispose of the concept of 'vital force'. The carbon which at least one of the reagents must always contain may have acquired the 'vital force' at some previous period in its history when it was part of a living organism].

Secondly, up to the beginning of the 19th century, it was considered axiomatic in chemistry that substances of the same composition must possess the same properties. Although a few anomalies had already emerged, when Liebig (1823)[7,8] reported an elemental analysis of silver fulminate (argent fulminant) which was identical to that known for silver cyanate, most workers simply considered it an error. At that time it was implausible that two compounds (now known to possess isomeric anions) with such obviously different chemical properties could share the same chemical composition. Wöhler's production of urea by the molecular rearrangement of ammonium cyanate, together with Faraday's discovery of a hydrocarbon in oil gas which had the same empirical composition as ethylene (olefiant gas) but which showed a totally different behaviour[9] (now known, however, to be butylene), added further contradictions to the current dogma and helped chemistry to become more receptive to the concept of isomerism.

By analogy with Wöhler's synthesis of urea, many chemists tried, without apparent success, to produce the corresponding sulfur urea by the action of heat on ammonium thiocyanate (sulfocyanate of ammonium). Liebig had found that heating partially decomposed the compound to yield ammonia, hydrogen sulfide (hydrosulfuric acid) and carbon disulfide (bisulfide of carbon) and left a residue (containing thiourea?), which he named melam. This residue split on further heating into ammonia and mellon. Hofmann, during his researches into anilides and polyammonias had synthesized a number of substitution products of sulfur urea in which he clearly recognised the presence of the thione group, but he failed to isolate thiourea itself.[10,11]

It was left to Reynolds, in a paper entitled, 'On the isolation of the missing sulphur urea', to describe the successful synthesis of thiourea.[12] The reaction required a much higher temperature (170 °C versus 100 °C) than the corresponding synthesis of urea and is by no means complete since the reaction is reversible (1).[13]

$$NH_4SCN \rightleftharpoons NH_2{\cdot}CS{\cdot}NH_2 \quad (1)$$

Both the forward and reverse reactions were thought to be monomolecular[14] but later observations suggested that the conversion of ammonium thiocyanate into thiourea is disturbed by side reactions.[15] Thiourea is not the only compound which can be separated from the melt, several workers have isolated crystals melting at 144°C, containing both thiourea and ammonium thiocyanate, and have given the formula $NH_4SCN{\cdot}3SC(NH_2)_2$ or $NH_4SCN{\cdot}4SC(NH_2)_2$ to this eutectic mixture.[16–18] Three years later an almost theoretical yield of thiourea was obtained by Baumann who treated cyanamide with hydrogen sulfide in absolute diethyl ether (2).[19–21]

$$NH_2CN + H_2S \rightarrow HN_2{\cdot}CS{\cdot}NH_2 \quad (2)$$

Modifications of both of these methods have been employed in the commercial production of thiourea.[22–29]

1.2. *Structure*

Considerable discussion has taken place over the molecular structure of thiourea. Two possible opposing structures exist with respect to the carbon-sulfur bonding, the thione-containing structure **I** being called the 'normal' and the thiol-containing structure **II** the 'pseudo' or 'iso' form. Other structural formulae have been proposed. Lecher and coworkers favoured a zwitter-ion **III, IV** for a time but concluded it impossible and abandoned it to return to the 'normal' symmetrical structure **I**.[30–33] Results from ultraviolet studies led Rivier and Borel to reject the 'normal' structure and pronounce a preference for a cyclic form in which the sulfur was attached to the carbon by a single valency and where one of the nitrogen centres was pentavalent **V**.[34] The same structure had been proposed earlier by Werner.[35] By their own admittance, these results were difficult to reconcile with X-ray crystallographic data on thiourea (and salts) which suggested that the molecule possessed a plane of symmetry.[36,37]

Amidst the arguments, a general opinion emerged that thiourea may undergo thione-thiol tautomerism. The thione form has been shown by X-ray crystallography[38,39] and proton location by both electron[40–42] and neutron[43] diffraction studies to be present in the solid state. This appears to be a general rule when thione-thiol tautomerism is possible.[38,39] In the liquid state and in aqueous solution, most of the available evidence also points to the predominance of the thione form in equilibrium with minute amounts of the thiol tautomer. The preference of the thione tautomer is attributed, in part, to the π-electron stabilization, this being especially important in cyclic thiones.[44]

However, under differing circumstances thiourea appears to react chemically as if it had either a thione or thiol structure.[45] Force-field investigations have implied that thiourea contains a hybridized $C(sp^2)=S$ bond,[46] similar to carbonyl groups, although other workers

have stated that the local-wave function of the sulfur atom in thiocarbonyl groups is nearly unhybridized.[47] However, in general, the C=S π-bond is more readily polarisable than the C=O π-bond and sulfur is therefore better able to stabilize a negative charge. Observations made with thiocarbonyl halides have suggested that the C=S group is generally polar, as in structure **VI**.[47,48]

As would be expected, such compounds as thiourea, with their relatively high basicity[49] and large polarizability, will be powerful nucleophiles. Quantitative studies on the nucleophilicity of the thione group have been made extensively with thiourea, which reacts rapidly with alkyl halides, acylating agents and aromatic substrates to form S-substituted derivatives. In reactions towards platinum, thiourea is about 60 times as reactive as the iodide anion and thereby belongs amongst the strongest nucleophiles known.[50-52]

2. BIOLOGICAL APPLICATIONS

2.1. *Plant Growth Stimulator*

Thiourea itself, although no substituted derivatives,[53] possesses the ability to stimulate plant growth.[54] The germination and sprouting of a variety of seeds can be hastened, including those from lettuce, peach, gladiolus and various tree species.[55-59] Treatment with thiourea shortened the resting period of Jerusalem artichoke[60,61] and potato tubers[62] and caused the early development of dormant buds in potato tubers, gladiolus bulblets and various woody plants (lilac, crab apple, azalea).[63-66] In the potato, a vegetable extensively studied, thiourea inhibited the usual single-bud development and permitted the growth of two or more (up to five) accessory buds from a single 'eye'.[63-67] This enabled the number and weight of sprouts formed to increase[68,69] but decreased the overall size of the tubers obtained.[70] Although thiourea has a multitude of measurable effects on plant biochemistry the specific reason(s) for this stimulatory activity remain uncertain. Thiourea has also been used as a herbicide for several grass types, but again, its precise mechanism of action is unknown.[71,72]

2.2. *Use as a Biocide*

Thiourea is toxic to the larvae of many insects including the mosquito (genus *Anopheles*),[73] clothes moths (*Tineola biselliella & Tinea pellionella*), black carpet beetle (*Attagenus piceus*),[74-76] common blowfly (*Phenicia sericata; Lucilia sericata*),[76] common housefly (*Musca domestica*)[77,78] and various flesh flies whose larvae develop in putrefying or living tissues.[75,76] Combined with a copper salt, thiourea has been employed as a dusting powder[79] and also serves as a moth repellent.[74] It has been recommended as a general insecticide,[80] which claims to be destructive to all forms of insect life.[81]

A definite and marked inhibitory action on the development and activity of pathogenic organisms has been purported.[82-84] Deleterious metabolic effects on trypanosomes have been observed following injection into infected mice[85] and its antimycotic effect[86] suppresses fungal growth[87-89] permitting the effective control of stem-end rot and green mould decay of oranges.[90] Thiourea has been shown to possess antibacterial activity,[91] particularly

a slight antitubercular effect[86] with some improvements being observed in rabbits previously infected with the human (*Mycobacterium tuberculosis*) and avian (*Mycobacterium avium*) tubercle bacilli.[92] Toxicity to lactic acid producing bacilli (*Lactobacillus acidophilus*) has also been seen.[93] Claims of an inactivating effect on Venezuelan equine encephalomyelitis virus and the abolition of its antigenic properties have also been made.[94,95]

2.3. *Effects on the Thyroid Gland*

Thioureas have been shown to affect the functioning of the thyroid gland and have been used as drugs to modify thyroid activity.[96] Structure-activity studies indicate that the thiocarbamide group (S=C−N) is essential amongst this class of compounds for the antithyroid properties.

The mechanism of action is believed to be based on its potent inhibition of thyroid peroxidase (tyrosine iodinase), an enzyme which is concentrated in membranes at or near the apical surface of the thyroid cell. This enzyme catalyses the iodination step in the biosynthesis of the thyroid hormones, the 'organification reaction' of iodine.[97,98] With thiourea present, the iodide anion, following its uptake into the cell, is prevented from being converted into iodine and/or a reactive intermediate before or during its attachment to one of the captive tyrosine residues in the thyroglobulin glycoprotein.

This process of iodination seems not to involve molecular iodine. One suggestion is that the iodinium ion (I^+) may be the active species which iodinates the tyrosyl acceptor.[99–101] Others have implicated hypoiodite intermediates (OI^-)[102] and the production of radicals ($I^{\cdot}$).[103] Iodide ions (I^-) can be oxidised sequentially, the removal of the first electron giving the radical ($I^{\cdot}$) and the removal of the second electron, the iodinium ion (I^+). It had also been proposed that the reactive intermediate in this iodination reaction could be sulfenyl iodide.[104,105] Thiourea reacts very rapidly with β-lactoglobulinsulfenyl iodide, liberating iodide and forming an inactive mixed disulfide. However, further studies have since indicated that this sulfenyl iodide was not the active iodinating species.[106] A recent notion is that iodination involves two active sites on thyroid peroxidase, one of which preferentially oxidizes iodide to the active species (probably a radical) and the other converts tyrosine to a tyrosine radical. Iodotyrosine formation is then thought to occur by a reaction between the two radicals, whilst both are still attached to the enzyme, the tyrosine residue remaining part of the thyroglobulin glycoprotein. It has been shown that the sulfur moiety of thiourea becomes bound to the thyroid protein implying that this drug may compete with the active intermediate for an active site essential for the iodination reaction.[107] Subsequent investigations have substantiated this mechanism and shown that antithyroid drugs bind to and inactivate the peroxidase only when the haem of the enzyme is in an oxidised state.[108,109] Thiourea does not interfere directly with the action of the thyroid hormones on cellular metabolism.

Antithyroid drugs are used to prepare patients for thyroidectomy and are also prescribed for prolonged periods in the hope of inducing life-long remission from hyperthyroidism. Initial high doses over an eight to twelve week period usually return the thyroid activity to normal, whereafter the dose is reduced to the minimum necessary to maintain the patient in the euthyroid state. Unfortunately, following the withdrawal of the drug after

a few years of treatment, less than fifty percent of thyrotoxic patients will remain euthyroid for prolonged periods and require no further therapy. The use of thiourea as an antithyroid drug has now been superseded by the substituted thioureas and other antithyroid medications.

2.4. *Metabolic Suppressant*

Attempts have been made to exploit the overall retarding effect of thiourea on thyroid function in two ways.

Thiourea was briefly investigated as a protective agent against low pressure.[110] This was based on the observation that rats exposed to 200 mm Hg (32,000 ft) of pure oxygen gas for two hours had their survival rates increased from 25% to 100% following dietary exposure to thiourea (0.5% v/v) for twelve days. The rationale was that the thiourea suppressed the activity of the thyroid gland and thus decreased the basal metabolic rate, thereby reducing the demand for oxygen. The development of pressurised cabins within aircraft has now averted this problem.

Thiourea retards the metamorphic process in tadpoles; it is suggested that it acts by inhibiting the formation of normal thyroid function.[111] The use of thiourea as a metabolic suppressant has been used to delay the hatching of avian embryos[112,113] but the commercial use of this treatment remains doubtful. Strangely, it has been reported that the treatment of chick eggs at different stages of incubation with thiourea caused increased embryonic development 3 to 10 days beyond normal.[112]

2.5. *Antioxidant*

The thione-thiol nature of thiourea has led to it to being examined both as an antioxidant and a radioprotective agent.

Thiourea has been shown to protect fats from oxidation when combined with skimmed-milk powder and water[114] and the soaking or dipping of fruit slices in thiourea solution (0.1%w/v) prevents their browning on exposure to air.[115] It has been suggested as an antioxidant for Vitamin C.[116–120] The incorporation of thiourea as an antioxidant into ointments containing sulfonamides, tannic acid and peroxides applied to wounds has also been attempted[121] but studies indicated that those preparations containing large amount of thiourea (1–10% v/v) caused unacceptable interference with normal thyroid functioning. The present-day use of thiourea as an antioxidant seems limited to the research laboratory where it finds application as a radical scavenger.[122–124]

Its passing role as a radioprotective agent arose from observations that addition of thiourea to an aqueous solution of carboxypeptidase and D-amino acid oxidase decreased the inactivation of these enzymes by subsequent X-irradiation.[125] Thiourea was also shown to reduce radiation mortality in mice if injected before exposure.[126] In this context this latter effect is a general property; the role of thiols is strictly protective, that is they must be administered before exposure to the radiomimetic agent.

TABLE 1. Acute toxicity (LD_{50}) of thiourea in rat, rabbit and dog.

Species	Dose route	LD_{50} mg/kg body weight	Reference
Rat			
a) domestic			
	oral	2,500	131
	intraperitoneal	1.25 to 640	129
	intraperitoneal	4.0 ± 0.2	130
	intraperitoneal	44 ± 13	130
	intraperitoneal	640 ± 191	130
	subcutaneous	10,000	132
b) wild*			
	intraperitoneal (A)	1,220 ± 230	130
	intraperitoneal	1,340 ± 230	130
	intraperitoneal	1,830 ± 135	129,130
Rabbit			
	oral	10,000	132
	intravenous	10–11,000	128
Dog			
	intravenous	10–11,000	128

*Wild animals were Norway Rats (*Rattus norvegicus*) except (A) which were Alexandrine Rats (*Rattus rattus alexandrinus*). Other rats were tame animals from different institutional colonies, maintained on different diets. Consult reference 130 for a fuller explanation.

Values represent the range observed or the mean ± s.e.

3. TOXICITY

3.1. *Lethality*

Acute and chronic feeding studies to rats and rabbits have indicated that thiourea is a non-toxic substance,[127] being less toxic to dogs and rabbits than urea.[128] Only the thioureas containing the full thioureido grouping linked to a benzene ring exhibit high toxicity (eg. monoarylthioureas).[129] However, several authors have found marked variation in the response of rats from different colonies, or rats maintained on different diets[128–132] (Table 1). The explanation for this is unknown.[130] Additionally, a diet containing 1% (v/v) of thiourea has been shown to be fatal to chicks.[133]

3.2. *Carcinogenesis*

Studies in mice involving the chronic administration of between 0.2 to 2.0% (by weight) thiourea mixed with the diet or drinking water did not appear to show any increase in the incidence of tumours in these animals.[134,135] However, one adenoma (benign neoplasm) of the thyroid has been observed in a castrated animal receiving thiourea and these workers also reported hyperplastic thyroids[136] whilst others have observed non-malignant follicular cystic changes.[134] A single subcutaneous injection of 2.5 g/kg body weight to neonatal mice produced no increase in lung adenomas after six months, unlike the reference compound, urethane, which produced a 100% incidence of tumours.[137] However, a large

intake of thiourea (5 g/kg body weight) in the diet of mice produced a significant increase in intracranial bone tumours (hyperplastic benign osteoma of the skull) which was shown not to be due the antithyroid effect but via a direct action on the pituitary.[138] Thiourea has, conversely, decreased the incidence of mammary tumours in mice, this probably being via a hormonal mechanism as the animals remained anoestrous during the thiourea administration, the effect being more or less equivalent to ovariectomy and ovarian atrophy.[139]

Unlike the mouse, the rat appears to be a more susceptible species. Administration of thiourea (0.25% by weight) to the drinking water of rats resulted in the development of malignant thyroid tumours in some of these animals, with the tumours invading the thyroid veins and metastases being found within other tissues such as the lungs.[140,141] As intimated previously, one has to be aware of the existence of indirect mechanisms leading to thyroid carcinogenesis, the ultimate being a hormonal imbalance of the hypothalamo-pituitary-thyroid system. Induction of thyroid tumours by antithyroid substances may be a result of the suppression of the rate of synthesis of thyroxine, thus leading to a hormonal imbalance.[142–144] Indeed, the possible synergistic effect on tumorigenesis by coadministration of thiourea with other chemicals[145,146] and known tumour initiators[147] suggests that thiourea possesses a significant tumour promoting effect or promoting tendency on the rat thyroid.

An increased incidence of liver tumours has also been observed in chronically fed rats.[145,148] Other workers have observed epidermoid carcinomas (malignant neoplasms) involving the area between the ear duct and the orbit[149] and associated sebaceous glands (Zymbal gland; Meibomian [tarsal] gland in the upper eyelid).[150,151] Hepatomas have also been observed in rainbow trout after feeding on thiourea (1200 ppm) for up to 20 months.[152]

A series of short-term tests for mutagenesis which purport to predict mammalian carcinogenesis, also suggest that thiourea should be regarded as a carcinogen. The chemical has been shown to be weakly, but definitely, genotoxic and mutagenic in cultured mammalian cell lines by causing an increased appearance of 8-azaguanine resistant mutants in hamster cells and eliciting a linear increase in DNA repair replication in rat hepatocytes.[153] Thiourea has also been shown to produce a significant increase in the eye spot frequency in the unstable zeste-white system of the fruit fly (*Drosophila melanogaster*)[154] thereby indicating mutagenic properties.

However, thiourea failed to enhance the incidence of foci deficient in adenosine-5′-triphosphatase (ATPase) either by initiation or by promotion in the rat liver foci bioassay where a mixture of polychlorinated biphenyls was used as promoting agent and diethylnitrosamine was employed as an initiator.[155] Thiourea also decreased DNA strand-break production induced by X-ray exposure and by chemicals which intercalate between the nucleic acid helices. This was not due to an inactivation of the intercalators or to a decrease in their uptake and it was suggested that thiourea may alter the chromatin structure in a fashion which may dissociate intercalator-induced strand-break production from lethality and the mechanism of X-ray break production.[122] The protective action of thiourea against organoplatinum compound-induced mutations in bacteria (*Escherichia coli*) has also been reported and thiourea is thought to react directly with monofunctional platinum-DNA adducts.[156,157]

Despite all these investigations, although regarded as a potential carcinogen, it is not known if thiourea is actually carcinogenic in man. In a report detailing the adverse effects encountered by over five hundred patients undergoing thiourea treatment no specific mention was made of tumours.[158]

3.3. *Adverse Reactions*

Thiourea has been known to produce a decrease in blood pressure via a vagal reflex which originates at cardiac chemoreceptors and results in sinus bradycardia, hypotension and probable peripheral vasodilation (Bezold-Jarisch reflex). Although this was once viewed as a potential therapeutic technique (eg. mephenesin) none of the drugs currently favoured for the treatment of hypertension operate through this mechanism.[159]

The main effect of thiourea on overall metabolism is mediated by its antithyroid action. The depression of the synthesis of the thyroid hormones results in the depression of the basal metabolic rate. This is a measure of the minimum amount of cell activity associated with continuous organic function. Thiourea may also act directly with macromolecules. The pretreatment of rats, previously induced with phenobarbitone or 3-methylcholanthrene (cytochrome P450 enzyme inducers), brought about a significant decrease in the *N*-demethylation of benzphentamine but not in overall cytochromes P450 content.[160] This may have been due to the metabolic desulfuration of thiourea with the released 'reactive sulfur' binding to and inhibiting the monooxygenase.[161] The use of thiols (eg. glutathione) to protect against thiourea toxicity implicates an oxidative mechanism in terms of preventing covalent protein binding in the liver and lungs.[162]

An insight into the adverse reactions which may occur during the treatment of thyrotoxicosis may be gleaned from a case example reported in the literature, where 83 g of thiourea were administered to a patient over a five-week period. The patient initially showed a marked improvement in her condition before developing granulocytopenia and thrombocytopenia which required an immediate blood transfusion to reverse.[163] Such large amounts of drug intake were quite common; 3 g per day in three divided doses for three weeks followed by 1 g once or twice daily for maintenance therapy not being unusual.[164] Although it is difficult to obtain exact figures it appeared that about ten percent of patients treated with thiourea developed adverse reactions, the most common of these being pyrexia and gastrointestinal disturbances (Table 2).[158,163–172] However, it was the production of blood dyscrasias, especially agranulocytosis, which attracted most concern. In addition, hyperplasia of the thyroid may result and, in some instances, it has been intimated that tumours may develop from this hyperplastic condition.[147,173]

Thiourea may also cause injury to the pulmonary microcirculation.[174] The extensive extravasation of high-protein liquid from the lung capillaries into the lung interstitial areas, alveoli, airways and pleura of experimental animals treated with thiourea produces a pathophysiological and clinical picture which closely resembles the respiratory distress syndrome observed in adults.[175,176] In the rat, the site of injury has been shown to be the pulmonary endothelial cells with little damage to other cell types within the alveolar capillary unit. The extent of thiourea binding correlated well with the extent of oedema.[177] The exact mode of action remains unclear, although there is good evidence to suggest that thiourea requires metabolic activation.[178–180] The observation that significant elevations

TABLE 2. Adverse reactions which have been associated with thiourea therapy

Fever
Gastrointestinal disturbances (vomiting)
Cutaneous eruptions (rash, urticaria)
Pain (arthralgia - joint pain, myalgia - muscular pain)
Blood dyscrasias (leukocytopenia, thrombocytopenia)
Halitosis (sweetish breath)
Mucous membrane irritation and inflammation (conjunctivitis)
Nausea
Lymph node enlargement
Oedema

Complaints are listed, as far as possible, in descending order of occurrence. Terms in brackets give other descriptions within these categories reported by some investigators.

Compiled from data in references.[158,163-172]

in plasma histamine levels occurred in rats treated with thiourea suggests that histamine may be implicated; such a rise would result in marked vasoconstriction which would lead to significantly altered fluid dynamics in the pulmonary vasculature.[181,182]

4. METABOLISM

The thiourea molecule does not stay long within the mammalian body. Virtually all (98%) of the radioactivity associated with an injected dose of [^{35}S]-thiourea could be accounted for in 0–48 hour rat urine with practically nothing being found in faeces or expired air.[183] Similar observations had previously been made following oral and parenteral administration of the compound to rabbits.[184] In man, thiourea has been shown to be rapidly absorbed from the intestine and rapidly excreted in the urine; 75% of the dose appearing within 0–10 hours, 96% during the first day[185] and a total recovery (of sulfur) obtained within two days.[186] However, other workers have been unable to account for up to 25% of an administered dose.[187]

Studies involving both [^{35}S]-thiourea and [^{14}C]-thiourea have shown that radioactivity is mainly localized in highly perfused organs (liver, kidney, lung) (rat;[178,180,188] mice;[189,190]), blood cells[178,191] which are freely permeable to thiourea,[192] and the thyroid gland,[193] the concentrations in the latter organ reaching up to thirty times that found in other tissues.[194]

The rate of elimination of radioactive sulfur from the organs was slower than that of radioactive carbon, suggesting that the sulfur is more avidly held within the tissues. Disulfide formation from thiourea[195–197] is known to take place readily in the presence of Cu^{2+} ions, probably involving the initial formation of a thiourea-metal complex prior to oxidation.[198] The process of thiol-disulfide exchange is of paramount importance in biology and in the case of thiourea-mixed disulfide formation (eg. with protein thiols) it is a potentially favourable reaction.[196,199,200] In addition, it has been shown that the enzyme thyroid peroxidase can mediate the translocation (transsulfuration) of the sulfur from thiourea to proteins within thyroid tissue.[201,202] This covalent binding is NADPH-dependent suggesting that metabolic activation is an essential prerequisite for binding.

Initial metabolic studies implied that the compound was eliminated unchanged in the urine[184] but other investigations revealed that a small proportion of the dose (8–12%) had

been oxidised to sulfate.[183,186] It was found that oxidation of thiourea to sulfate occurs in the thyroid gland[194] and thiourea has been shown to be desulfurated to urea by thyroid tissue.[180,201,202] The presence of a substance with a sweetish odour, resembling that of leeks, has also been observed both in the exhalations of dogs and the breath of thyrotoxicosis patients after administration of thiourea treatment.[164,165,172,203] It has been suggested that this compound is probably dimethyl sulfide.[204]

These findings indicate that, although the majority of administered thiourea appears to pass through the body unmetabolised, extensive degradation of at least a small portion must take place. Other work has shown that, unlike the related antithyroid thiouracils, incubation with guinea pig hepatic UDP-glucuronyl transferase does not produce any conjugated metabolites.[205] Studies in non-mammalian systems have shown that thiourea was not converted to dimethyl sulfide by fungal cultures (*Scopulariopsis brevicaulis*).[206] In frozen peaches thiourea was oxidised to thiourea sulfonic acid (trioxide of thiourea) but there was no evidence for the presence of formamidine disulfide, the dioxide of thiourea, related ammonium salts or urea.[207] In potato tubers radioactivity from [^{35}S]-thiourea was incorporated into the amino acid methionine, with only small amounts present in cysteine, cystine and glutathione.[208]

5. BIOLOGICAL EFFECTS

5.1. *Neurological*

Immersion of mud eels (*Amphipnous cuchia* Ham.) in a dilute thiourea solution (0.33 ppm) caused atrophic changes in the neurones but no discernable change in the cell bodies of the hypothalamic nuclei.[209] Histological indications of moderate activation of the neurosecretory hypothalamo-hypophyseal system have also been reported in the toad and the newt following chronic injection of thiourea.[210]

That these may not be direct effects but linked to perturbations in thyroid hormone levels is suggested by examination of the brain of hypothyroid animals which reveals deficient development, particularly of axonal and dendritic networks with severe impairment of myelinization. In addition, cretinism (infantile hypothyroidism) leads to a general stunting of body growth and mental retardation. It is apparent that the thyroid hormones are important determinants of genetically ordained development programmes.

5.2. *Reproductive*

Treatment of the eggs and nymphal stages of the red cotton bug (*Dysdercus similis*) with thiourea led to a high overall mortality, with adverse effects on ecdysis (moulting) and the production of smaller, weaker and morphologically abnormal adults. Examination of the ovaries showed that they contained a large number of immature, pathological oocytes with degenerating follicular epithelium, which was thin with a regular outline in the early stages, but later became multilayered, with pyknotic nuclei, and displayed active destruction of glycoproteins and lipoproteins. Fibrogenesis and thickening of the tunica

propria were clearly discernible. This atrophy of the gonads led to reduced fecundity, many animals being unable to reproduce.[211]

Similar effects on the ovaries and general gametogenesis have been reported in varying degrees in flesh-eating flies (*Sarcophaga ruficornis* Fabr.),[212] the catfish (*Pimelodus sp.; Heteropneustes fossilis* Bloch)[213] and the fireball gudgeon (*Gobio sp.; Hypseleotris galii*).[214] Sea urchin (*Arbacia punctulata*) eggs treated with thiourea in solution show a cortical response similar to that brought about by sperm cells. Following this phenomenon, which is visible at the surface of the egg, the process of cleavage is initiated. However, an identical response is seen with other chemicals (eg. urea, glycerol, sucrose) and as such may be somewhat non-specific.[215] Indeed, another study has reported that thiourea treatment inhibited the initial cleavage of sea urchin eggs, with more dilute solutions arresting development at the gastrula stage. These effects were reversible on washing with fresh water.[216]

5.3. *Enzyme Inhibition*

Thiourea has a general effect on enzyme systems, particularly in plants, where it can activate amylase catalysed hydrolysis of soluble starch (diastase of potato pulp)[217] as well as modulating respiration and photosynthesis in potato and algae.[218,219] However, its most prominent effects are towards oxidising enzymes,[220] particularly the peroxidases. The compound has been shown to poison cucumber and potato oxidases[221] and to decrease the catalase activity of cabbage plants[222] as well as inhibiting thyroid peroxidase,[201,223] myeloperoxidase (verdoperoxidase)—a peroxidase occurring in leukocytes that contains a greenish ferriporphyrin (as does lactoperoxidase)—,[224] lactoperoxidase (milk catalase),[220,225] chloroperoxidase[226] and salivary gland peroxidase.[227]

The peroxidases (EC class 1.11. hydrogen peroxide oxidoreductases) are haemoproteins which are widely distributed throughout animal and plant tissues. They are multi-substrate enzymes which catalyse the dehydrogenation (oxidation) of a variety of substances that are suitable electron donors such as phenols, amines and halide ions.[228,229] They employ hydrogen peroxide as a hydrogen acceptor which is converted to water in the process. The first stage in the reaction sequence is where hydrogen peroxide interacts with the peroxidase to form a reactive enzyme species which is the active oxidising agent. A sequential transfer of electrons from the donor substrate (compound to be oxidised) then occurs, the first electron reduces the active enzyme to an intermediate state which requires the input of a second electron to regenerate the initial native peroxidase. This cyclic sequence may then reoccur.[230]

Thiourea may bring about both reversible and irreversible inhibition. The former is thought to occur when thiourea either competes with the natural substrate for the activated peroxidase or interacts with the putative reactive oxidising species (see thyroid section). Irreversible inhibition takes place if the localised concentration of thiourea is high relative

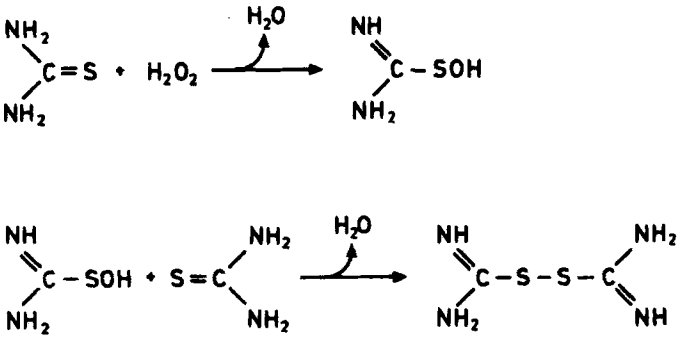
to the natural substrate, when the compound binds to and inactivates the reactive oxidised enzyme, thereby acting as a suicide substrate.[100,108,109,223,231–234] In both cases the thiourea is oxidised. Inorganic sulfate is one of the end products of peroxidase catalysed oxidation of thiourea,[201,235] and formamidine disulfide and formamidine sulfinic acid have been identified as products of chloroperoxidase[100] and myeloperoxidase[224] mediated oxidation.

6. MOLECULAR MECHANISMS OF INTERACTION

The thiocarbamides are a class of compounds which appear not to participate in mammalian biochemistry. Ergothioneine, the betaine of thiolhistidine, present in ergot (fungus—*Claviceps purpurea*), has been found in appreciable amounts in mammalian blood and seminal fluid,[236] and has been identified in extracts of the cerebellum, optic nerves and dorsal roots where it has been mooted as a putative excitatory transmitter. However, current evidence suggests that ergothioneine is not synthesised in the body but is of dietary origin.[229,237] Benzylthiourea[238,239] and butylthiourea[240] have been found in the seeds of papaya and scurvy grass, respectively, whilst thiourea itself has been detected in tissues from the laburnum tree.[241] Thiourea can also be produced by some fungi (eg. *Botryomyces, Verticillium*) when fed asparagine and amino salts.[242] Consequently, it is not difficult to appreciate that the presence of a foreign compound amidst the awesome catalytic array of intermediary metabolism may produce unwelcome effects.

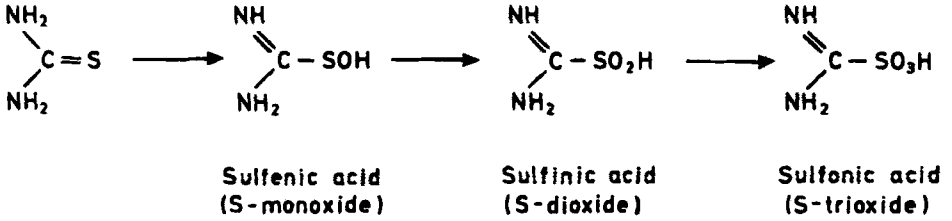
It is generally agreed that thiourea is a reactive molecule and that this reactivity lies with the sulfur within the the thiocarbonyl group. Indeed, compared with thioamides (eg. thioacetamide CH_3CSNH_2), the thiocarbamides, of which thiourea (NH_2CSNH_2) is the progenitor, are more nucleophilic owing to increased π-electron density arising from the donation of electrons by the second nitrogen atom adjacent to the thiocarbonyl group.[49] However, toxic responses do not appear to be elicited by thiourea itself but arise as a consequence of its metabolic activation.[180,243–245]

Clues may be gleaned from studies involving the chemical oxidation of thiourea and related thiocarbamides and the toxicity of related thioamides. Thiourea may be oxidised to its corresponding disulfide in aqueous solutions containing cupric ions, this reaction being pH sensitive and probably involving the initial formation of a thiourea-metal ion complex prior to (electrochemical) oxidation.[198] Such reactions may also proceed to the formation of stable complexes with (first-row) transition metal ions.[246–248] This is of interest as a radical mechanism may be involved, as proposed for thiols[249] and methimazole (a substituted thiourea).[250,251] Subsequent radical scavenging may disrupt enzyme activity by complexation of metal ion cofactors[252,253] and/or combination with activated substrate intermediates. When hydrogen peroxide is employed it has been proposed that the thiocarbamide moiety acts as a nucleophile towards the electrophilic oxidising agent, firstly producing a sulfenic acid which may then react with another thiocarbamide molecule to give the disulfide (Scheme 1).[254] The formation of an unstable sulfenic acid (or a sulfenyl iodide) is also postulated during the iodine mediated oxidation of thiourea to its disulfide.[195–197] These reactive sulfenic acids (*S*-monoxide) are readily oxidised to the corresponding sulfinic (*S*-dioxide) and sulfonic (*S*-trioxide) acids (Scheme 2).



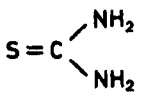
SCHEME 1

Sulfenic acids are notoriously unstable. However, whilst formamidinesulfenic acids are quite reactive they are more stable than alkanesulfenic acids.[255] In contrast, certain sulfinic acids including that of thiourea are isolable. The thiocarbamide sulfinic acids are moderately strong reducing agents[256] and in aqueous alkaline conditions decompose to urea whilst in acid solution they eliminate sulfur dioxide, yielding a formamidine.[161,229,257]

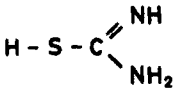


SCHEME 2

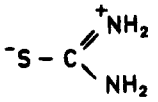
During their interaction with living systems, the thiocarbonyl groups of related thioamide compounds undergo two sequential oxidations to produce the *S*-oxide and the *S,S*-dioxide as major metabolites. Molecular orbital calculations suggest that these are reactive species.[258] In particular, the *S,S*-dioxide is extremely reactive chemically and acylates water (to form amides) or cellular nucleophiles (to form covalently bound materials), the latter of which presumably interferes deleteriously with cellular function.[259] Acylation is not an option open to thiourea. In a similar manner, it has been proposed that the *S*-oxide of methimazole (a substituted thiourea) decomposes to the imidazole carbene plus sulfur monoxide, and that the disproportionation of sulfur monoxide yields sulfur dioxide and reactive atomic sulfur.[260] A mechanism involving the formation of an oxathiirane intermediate is thought to contribute to the desulfuration of carbon disulfide[261] and phosphorothionates[262] but probably is not involved in the desulfuration of thioamide or thiocarbamide *S*-oxides.[259]



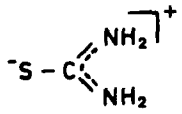
I



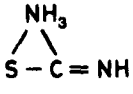
II



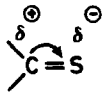
III



IV



V



VI

For thioureas, the sequential formation of sulfenic, then sulfinic acids can be catalysed by the flavin-containing monooxygenase derived from hog or hamster hepatic microsomes (endoplasmic reticulum).[263] The sulfenic acids (formanidinesulfenic acids) formed may react with protein thiols to give mixed disulfides and this disulfide linkage could account for the observed covalent binding of thiocarbamide metabolite(s) in microsomal fractions.[244] However, in the intact living system, low-molecular weight thiols such as glutathione would preferentially reduce sulfenic acids and any thiocarbamide-protein mixed disulfide formed; stable mixed-disulfide adducts would be present only at low concentrations of glutathione.[264,265]

In addition, the sulfinic acid group of formamidinesulfinic acids is subject to nucleophilic displacement by peptide amino groups to yield stable *N*-substituted guanidine adducts[244,256] and this could also explain the observed thiocarbamide-dependent covalent binding.[244] However, in this instance the sulfur of thiocarbamides is lost during the binding of metabolites to cellular macromolecules.[244] Anyway, only a fraction of the sulfinic acid would react in this manner, most would decompose in water to urea with the liberation of the sulfur moiety.[264]

Covalent binding to macromolecules with its subsequent disruption in function and potential immunogenic sequelae is advocated as the major mechanism of toxicity, but it appears uncertain as to whether or not the sulfur is necessary. The suggestions outlined above give two schemes, one where the sulfur is part of the bound molecule (disulfide linkage) and the other where it is not (guanidine adduct). It is certainly true that thiourea itself can be desulfurated to urea and that this sulfur (either alone or as part of a molecule) can be transferred to the protein in the thyroid gland and to other tissue macromolecules.[180,201,202] It may be that several mechanisms are operating, with liberated (reactive) sulfur also playing a role in toxicity.

For instance, it has been suggested that a reductive desulfuration liberating hydrogen sulfide was responsible for the toxicity of thiocarbamides.[266] Although this feeling appears

to have been overtaken by the view that it is an oxidative attack on the sulfur which produces reactive metabolites which associate with macromolecules,[244,267,268] the idea of the liberation of hydrogen sulfide, a more potent inhibitor of the cytochrome oxidase system than cyanide,[269] has many intriguing aspects. Insidious toxicity may occur at levels far below those generally regarded as dangerous. In the field of neurology this compound, perhaps absorbed from the gastrointestinal tract and not efficiently trapped by methaemoglobin, has been suggested as causing neurological problems at concentration levels far below those which produce overt brain damage.[270–274] An open mind should always be kept with respect to new and alternative ideas, even if they are a resurrection of older contemned ones.

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