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George A. Maw[a]
[a] 23, Brooklyn Avenue, Worthing, Sussex, England

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BIOCHEMISTRY OF *S*-METHYL-L-CYSTEINE AND ITS PRINCIPAL DERIVATIVES

GEORGE A. MAW
23, Brooklyn Avenue, Worthing, Sussex, England

This review describes the natural occurrence of *S*-methyl-L-cysteine, its sulfoxide and γ-glutamyl derivative, as well as the distribution of these compounds in plants, the changes in their concentrations during plant growth and development, and the pathways so far known for their biosynthesis. The appearance of a tripeptide of *S*-methylcysteine, *S*-methylglutathione, during the metabolism of various methylated drugs and pesticides in animals and plants is also discussed.

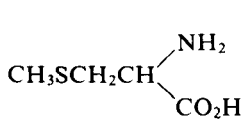
A summary is presented of the pathways of metabolism of the amino acid and its derivatives, including dethiomethylation, demethylation, transpeptidation, oxidative degradations, etc. The physiological and toxicological effects of these compounds are described, and an account is given of recent research on the identification of *S*-methylcysteine sulfoxide as the causative agent of kale poisoning in cattle. Suggestions as to the physiological role of *S*-methylcysteine and its derivatives are discussed.

I. INTRODUCTION

From a biochemical standpoint, *S*-methyl-L-cysteine **1** is of interest in being both a derivative of L-cysteine and a lower homologue of L-methionine. Historically, it was these structural relationships which in the 1930's prompted the earliest research on the biological behavior of the compound, almost a quarter of a century before the discovery that it was itself naturally occurring.

Since that time a detailed picture has been built up of the similarity of *S*-methylcysteine to cysteine and methionine in many of their respective roles. The metabolism of *S*-methylcysteine can now be accounted for in part by the extent to which it can act as a substrate for the numerous enzymes mediating in the metabolism of either cysteine or methionine (this applies particularly in animal tissues), and in part by the existence of enzymes for which *S*-methylcysteine is a normal substrate (as in plants).

The present review deals with the biochemistry of *S*-methylcysteine in organisms in which it is a natural metabolite and in those in which it must be regarded as a foreign compound. Included is an account of the naturally occurring derivatives of the amino acid, namely, its sulfoxide **2** and the dipeptide γ-L-glutamyl-*S*-methyl-L-cysteine **3**, also the tripeptide *S*-methylglutathione **4**, which is a metabolic product in certain situations. The biosynthesis and metabolism of *S*-methylcysteine was previously reviewed by Thompson.[1]



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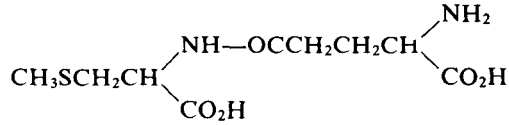

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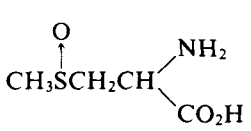

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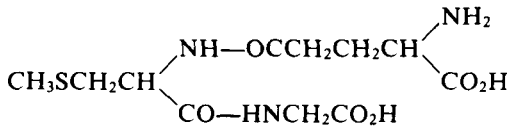

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II. NATURAL OCCURRENCE

S-*Methyl-L-cysteine and its sulfoxide*

Synge and Wood[2] in 1955 were the first to report the natural occurrence of the sulfoxide of *S*-methyl-L-cysteine as its (+) diastereoisomer in the non-protein, soluble N fraction of cabbage leaves, its isolation being achieved by ion-exchange chromatography. Almost at the same time this compound was isolated and identified as a constituent of turnip leaves by Morris and Thompson.[3] In the following year the two groups of workers described the compound in more detail,[4,5] and, on the basis of paper chromatographic studies, they produced strong evidence for its presence in other Cruciferae, such as broccoli, cauliflower, Chinese cabbage, kale and kohlrabi, also shepherd's purse (*Capsella bursa-pastoris* L), wallflower (*Cheiranthus cheiri* L) and white mustard (*Sinapsis alba* L). Its occurrence has since been confirmed in cauliflower and Chinese cabbage (*Brassica pekinensis* Rupr.).[6]

Thompson *et al.*[7] also isolated free *S*-methyl-L-cysteine as the (−) diastereoisomer from the non-protein N fraction of beans of *Phaseolus vulgaris*, and the amino acid has been reported to be a natural metabolite in *Neurospora crassa*.[8]

S-Methyl-L-cysteine sulfoxide has subsequently been established as a constituent of the common onion (*Allium cepa* L),[6,9–12] and there has been one report of *S*-methylcysteine being present in this plant.[11] Both compounds have been found in garlic (*Allium sativum*).[13] *S*-Methylcysteine also occurs in the fruit and seeds of the sea buckthorn (*Hippophae rhaminoides*)[14] and in the foliage and seeds of those species of the genus Astragalus, *e.g. A. bisulcatus*, which accumulate the corresponding selenium compound, *Se*-methylselenocysteine.[15–18] It has been detected in sonically disrupted cells of *Escherichia coli* and *Proteus mirabilis*,[19] in the haemolymph of the southern army worm (*Prodenia eridania*) fed on fresh kale,[20] in human urine[21] and in human and bovine blood.[22] The sulfoxide has also been found in human urine.[23]

There is, however, no firm evidence to suggest that *S*-methylcysteine or its sulfoxide are natural constituents of mammalian or insect tissues. Rather, they originate from the ingestion of plants containing these compounds, such as brassicas or, as described in Section V, they are formed during the metabolism of foreign organic compounds capable of acting as methylating agents.

γ-*L-Glutamyl*-S-*methyl-L-cysteine and its sulfoxide*

S-Methylcysteine and its sulfoxide have not been found as normal components of proteins in higher plants, yeasts or animals,[5,24–26] but the two compounds appear to be of fairly wide occurrence in plants as the corresponding γ-glutamyl derivatives. Both dipeptides were shown by Rinderknecht[27] to be present in the non-protein N fraction of lima

beans (*Phaseolus lunatus*) and of five other types of bean. Thompson and coworkers[28] isolated and characterized γ-glutamyl-*S*-methylcysteine **3** from kidney beans, in which it accounted for about one-third of the total non-protein N present, and they showed the natural product to be identical with the dipeptide prepared by the hydrolysis of synthetic *S*-methylglutathione **4** with carboxypeptidase.[29]

The dipeptide, alone or together with its sulfoxide, has been found in other plants, for example, sieva beans (*Phaseolus lunatus*),[30] the expressed juice from seedlings of *Phaseolus aureus*,[31] the common onion,[32] garlic,[33] the seeds and foliage of selenium-accumulating species of Astragalus,[16-18] which also contain γ-glutamyl-*Se*-methylselenocysteine, the fruit of the sea buckthorn (*H. rhaminoides*),[14] and seeds of *Vigna radiata*.[34]

III. CONCENTRATION AND DISTRIBUTION IN PLANTS

In the course of their isolation of *S*-methylcysteine sulfoxide from the expressed juice from cabbage leaves, Synge and Wood[4] estimated the compound to represent 4.4 per cent of the non-protein N of the leaf to 2.2 per cent of the total N present. This amounted to 0.57 per cent of the dry matter, equivalent to a concentration of 37.7 μmoles/g dry matter. The sulfoxide was obtained from the dialysed leaf juice by fractionation on columns of a cation-exchange resin. Morris and Thompson[5] found a comparable concentration of the sulfoxide in turnip leaves, amounting to 3.7 per cent of the non-protein N, and a value of 1.5 per cent for turnip roots. The latter workers prepared aqueous ethanol extracts of ground tissue and subjected these to cation-exchange chromatography, followed by paper chromatographic determination of the compound with ninhydrin.

Data published subsequently for the content of *S*-methylcysteine and its derivatives in other plants have been obtained by essentially similar procedures, the final analysis of the compounds being carried out by two-dimensional paper chromatography coupled with densitometry,[11,26] or by means of an amino acid analyzer.[17,18,35,36] Separation from other amino acids has also been achieved by electrophoresis on glass fibre paper, followed by a densitometric assay.[37] More recently, an automated ion-exchange chromatographic method for *S*-methylcysteine sulfoxide in freeze-dried plant samples has been described.[38]

Zacharius[35] obtained the following values for *S*-methylcysteine and its derivatives in the seeds of bush bean (*Ph. vulgaris*), expressed as μmoles/g air-dried seeds: *S*-methylcysteine, 1.25; *S*-methylcysteine sulfoxide, 0.326; γ-glutamyl-*S*-methylcysteine, 11.5; γ-glutamyl-*S*-methylcysteine sulfoxide, 1.01. An extensive survey of the content of *S*-methylcysteine in various seeds made by Evans and Boulter[36] is summarized in Table I. In bush bean seeds *S*-methylcysteine is present in greater concentration than its sulfoxide and, as seen from Table I, the amino acid occurred in appreciable amounts only in the species of Phaseolus and Vigna examined. However, the most abundant form of *S*-methylcysteine in legumes is its γ-glutamyl derivative, the molar concentration of which can be more than nine times that of the free amino acid.

The contents of *S*-methylcysteine and γ-glutamyl-*S*-methylcysteine in two species of Astragalus (*A. bisulcatus* and *A. pectinatus*) have been reported by McConnell *et al.*[17,18] and are shown in Table II.

The concentration of the γ-glutamyl derivative is highest in the seeds and falls after germination, declining further with the growth of the seedling, but rising again with the development of seed pods. Probably as a result of the hydrolysis of the dipeptide, growth of

TABLE I

S-Methyl-L-cysteine content of various legume seeds*

Legume	S-Methylcysteine (μmoles/g dry wt of seed)
Kidney bean (*Phaseolus vulgaris*)	15.61
Mung bean (*Vigna aureus*)	10.45
Cowpea (*Vigna unguiculata*)	10.90
Lima bean (*Phaseolus lunatus*)	8.07
Pea (*Pisatum sativum*	0.90
Pigeon pea (Cajanus cajan)	0.61
Field bean (*Vicia faba* minor)	0.91
Broad bean (*Vicia faba* major)	0.82
Soya bean (*Glycine max*)	0.37
Lupin (*Lupinus albus*)	0.19
Yam bean (*Sphenostylis stenocarpa*)	None detected

* Recalculated data of Evans and Boulter[36].
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the seedling is associated with an increase in the content of free *S*-methylcysteine. The concentration of the amino acid is high in young foliage but diminishes as the leaves age.

Smith *et al.*[37] have made a very detailed examination of the *S*-methylcysteine sulfoxide content of brassicas, and much of their data is summarized in Table III. As seen from the Table, the sulfoxide content varies widely and there are also large varietal differences, for example in kales, where the concentration appears to be a heritable character.[39] Only small amounts of the compound are present in the seeds, but there is an initiation of its synthesis after germination. The content of the whole plant then increases with age, a particularly rapid increase occurring at the onset of secondary growth and flower development. Flowers and secondary growth leaves were found to contain considerably more of the sulfoxide than the rest of the plant, and this is in agreement with the original findings of Morris and Thompson.[5] High levels of *S*-methylcysteine sulfoxide in young, inner leaves as opposed to older, outer leaves were also noted in Chinese cabbage.[26]

IV. BIOSYNTHESIS

S-*Methylcysteine*

Several pathways for the biosynthesis of *S*-methylcysteine are theoretically possible, including (a) the direct methylation of cysteine in the free state, (b) the methylation of cysteinyl residues in peptides or proteins, (c) *de novo* synthesis, involving the formation of the S—C skeleton by a transsulfuration mechanism, and (d) the preliminary formation of

TABLE II

Distribution of *S*-methyl-L-cysteine and γ-glutamyl-*S*-methyl-L-cysteine in two species of Astragalus*

	Concentration (μmoles/g dry matter)				
	Seedlings				
	1 wk-old	1 mnth-old	Young leaves	Flowers	Seeds
A. bisulcatus					
S-Methylcysteine	77.2	13.5	87.5	40	44
γ-Glutamyl-*S*-methylcysteine	51.5	1.1	3.3	0.7	78.2
A. pectinatus					
S-Methylcysteine	—	6.9	4.7	—	16.0
γ-Glutamyl-*S*-methylcysteine	—	3.1	trace	—	4.8

* Data of Nigam, Tu and McConnell[17] and Nigam and McConnell.[18]
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TABLE III

S-Methyl-L-cysteine sulfoxide content of various Brassica forage and root crops*

Sample	*S*-Methylcysteine sulfoxide (μmoles/g dry wt)
Kales, whole plant at harvest (Five dates of harvest)	30.09–95.38
Rapes, whole plant at harvest (Five varieties at five dates of harvest)	20.11–54.50
Turnip, leaves	13.10–52.32
roots	18.72–34.86
Brassica campestris, ssp pekinensis	26.92
ssp chinensis	38.56
ssp narinosa	46.96
Brassica oleracea Alboglabra	44.12
Raphanus sativus, leaves	20.24–25.2
roots	15.81–24.67
Raphanobrassica	22.36, 40.74
Swedes, leaves	33.67–123.75
roots	40.61–83.74

* Recalculated data of Whittle *et al.*[37]
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a derivative of *S*-methylcysteine, *e.g.* its sulfoxide or its *N*-acetyl derivative (methylmercapturic acid **9**), followed by the appropriate catabolic reaction to give the free amino acid (reduction and deacetylation, respectively, in the case of the two derivatives cited).

It is now known that enzymes exist in various organisms for the operation of pathways of types (a), (b) and (c) and that these occur *in vivo*. The evidence for pathway (d) occurring *in vivo* is less clear, although there are enzymes capable of forming *S*-methylcysteine from its sulfoxide in plants, and esterases exist which could hydrolyse the *N*-acetyl derivative.

Wolff, Black and Downey[40] first demonstrated the biosynthesis of *S*-methylcysteine by incubating L-serine with methanethiol in the presence of a partially purified enzyme preparation from yeast. This corresponds to the pathway type (c), referred to above:

$$CH_3SH + \text{serine} \longrightarrow S\text{-methylcysteine} + H_2O$$

If this reaction were to occur *in vivo*, a likely source of the methanethiol could be methionine, for fission of the latter amino acid to methanethiol occurs in many microorganisms[41,42] and has been demonstrated in animal tissues.[43,44] The above pathway was also proposed by Sugii *et al.*[45] for the formation of *S*-methylcysteine in garlic. These workers introduced ^{35}S-labeled methionine into whole plants and recovered 50 per cent of the ^{35}S in *S*-methylcysteine. As pointed out by Thompson,[1] however, although this demonstrated the transfer of the sulfur of methionine to *S*-methylcysteine, it does not constitute proof of the transfer of the intact thiomethyl (CH_3S—) group.

Methanethiol and its oxidation product, dimethyl disulfide, are well recognized as constituents of plants,[46,47] and more convincing evidence that the thiol can act as a precursor of *S*-methylcysteine in plants has been provided by Giovanelli and Mudd.[48] They obtained two enzyme fractions from extracts of spinach which could catalyse the synthesis of cysteine from inorganic sulfide and for which *O*-acetylserine was the sulfur acceptor. These two systems were also able to utilize methanethiol in place of sulfide, forming *S*-methylcysteine:

$$\text{Methanethiol} + O\text{-acetylserine} \longrightarrow S\text{-methylcysteine} + \text{acetate}$$

L-Serine, however, could not replace *O*-acetylserine as a substrate. One of the spinach fractions was appreciably more active than the other and resembled an *O*-acetylserine sulfhydrase present in microorganisms.[49,50] Similar findings were published independently by Thompson and Moore,[51] showing that extracts of *N. crassa*, baker's yeast (*Saccharomyces cerevisiae*) and leaves of turnip (*Brassica rapa* L) readily promoted *S*-methylcysteine synthesis from methanethiol and *O*-acetylserine, L-serine itself being a poor sulfur acceptor. A similar system has also been reported in onion and *E. coli*.[52] *O*-Acetylserine rather than serine is therefore most probably the normal substrate in *S*-methylcysteine biosynthesis by the transthiomethylation pathway, and this compound is also highly active as a substrate in the synthesis of cysteine from sulfide in spinach,[53] yeast[50] and bacteria.[49]

Thompson and Gering[54] were unable to obtain *S*-methylcysteine by this pathway in kidney bean or radish plants. They found, however, that in radish leaves the direct methylation of cysteine takes place (pathway (a)). DL-[1-^{14}C]Cystine taken up by intact leaves gave rise to ^{14}C-labelled *S*-methylcysteine and its sulfoxide. The source of the *S*-methyl group was shown to be methionine, for experiments with the latter amino acid labelled with ^{35}S and with ^{14}C or ^{3}H in the methyl group gave good incorporation of the methyl group but a much lower incorporation of the sulfur. This indicated that *S*-methylcysteine

formation did not involve a transthiomethylation, but was a typical enzymic transmethylation, *S*-adenosylmethionine most probably being the actual methyl donor. Supporting evidence for this pathway in plants has come from similar tracer studies in *A. bisulcatus*. McConnell *et al.*[55,56] found the *S*-adenosylmethionine was about 1.7 times as effective as methionine itself as a precursor of the *S*-methylcysteine-methyl group, and their data suggest that in this plant transthiomethylation is again an unimportant pathway in *S*-methylcysteine biosynthesis.

The dual role of methionine in *S*-methylcysteine synthesis in plants is summarized in Scheme 1. Which of the two mechanisms is physiologically the more significant may depend on the particular plant species.

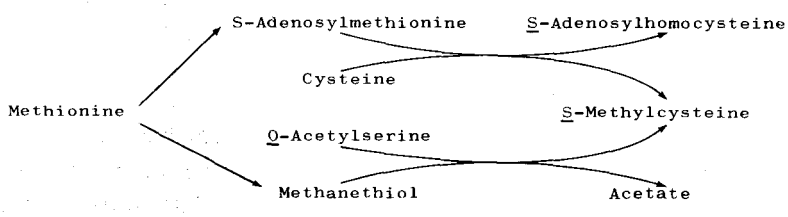
In bacteria, as already mentioned, *S*-methylcysteine may be formed from methanethiol by transthiomethylation to *O*-acetylserine. A second type (c) pathway is also possible, for purified preparations of cysteine desulfhydrase from *Aerobacter aerogenes*[57] and *Pseudomonas fluorescens*[58] form the amino acid from methanethiol, pyruvate and ammonia. However, whether this latter pathway is more significant *in vivo* than that involving *O*-acetylserine has yet to be determined. In yeasts it has not been possible to demonstrate the methylation of cysteine,[59] and the major if not the sole biosynthetic pathway for *S*-methylcysteine would appear to be one in which methanethiol is a precursor.

Formation of S-*methylcysteine from its sulfoxide and γ-glutamyl derivative.* Many plants contain predominantly *S*-methylcysteine sulfoxide or γ-glutamyl-*S*-methylcysteine, but only small or negligible amounts of the free amino acid (see Section III). These derivatives, in addition to having specific metabolic roles, may also be the forms in which *S*-methylcysteine is stored in these plants[26] and, if this is so, mechanisms should therefore exist for their conversion to the parent amino acid. The reduction of *S*-methylcysteine sulfoxide to *S*-methylcysteine has, in fact, been demonstrated in the leaves of turnip and in kidney beans.[60] The reaction requires anaerobic conditions and is of interest in that the natural (+) diastereoisomer of the sulfoxide is reduced more rapidly in bean leaves than is the unnatural (−) diastereoisomer, whereas in turnip leaves the reverse is the case.

γ-Glutamyl-*S*-methylcysteine could act as a source of the parent amino acid by losing its glutamyl group to other amino acids or peptides in the presence of a γ-glutamyl transpeptidase:

γ-Glutamyl-*S*-methylcysteine + amino acid (or peptide) $\rightleftharpoons$ γ-glutamylpeptide + *S*-methylcysteine

although the more generally recognized role for this enzyme is the reverse reaction, namely, the *synthesis* of γ-glutamyl-*S*-methylcysteine.[61]



Scheme 1. Role of methionine in S-methylcysteine biosynthesis in plants

S-*Methylcysteine sulfoxide*

All the available evidence points to *S*-methylcysteine sulfoxide being formed by the oxidation of the parent amino acid, as suggested originally by Morris and Thompson,[5] and subsequently shown to take place in tissues of Cruciferae, such as whole leaves of broccoli and discs of turnip leaf.[62] The formation of the sulfoxide required the presence of gaseous oxygen and was a stereospecific reaction, giving rise preferentially to the (+) diastereoisomer. The interconversion of *S*-methylcysteine and its sulfoxide takes place readily in plants, as indicated by the close similarity in the patterns of distribution of the sulfur of the two compounds when either was applied to the leaves of Chinese cabbage.[63]

γ-*Glutamyl*-S-*methylcysteine*

The widespread occurrence of γ-glutamyl-*S*-methylcysteine together with other γ-glutamyl dipeptides in plants prompted a study of the mode of their formation. Thompson *et al.*[61] obtained evidence that the major biosynthetic pathway is a γ-glutamyl group transfer from glutathione to the appropriate amino acid in the presence of a γ-glutamyl transpeptidase (or transferase) identified in various plant tissues:

$$S\text{-Methylcysteine} + \text{glutathione} \rightleftharpoons \gamma\text{-glutamyl-}S\text{-methylcysteine} + \text{cysteinylglycine}$$

Table IV shows the activity of the transpeptidase from a number of plant sources. The enzyme was not found in onion bulbs, as reported earlier by Virtanen.[32] However, it would appear from the work of Schwimmer[64,65] that whereas the enzyme is absent from the fleshy layers of mature dormant onion bulbs, it is present in the sprouted onion.

Numerous other L-amino acids can act as γ-glutamyl group acceptors in the presence of this enzyme, including *S*-methylcysteine sulfoxide, which has 68 per cent of the substrate activity of *S*-methylcysteine itself. This could account for the occurrence of the γ-glutamyl derivative of the sulfoxide in various legumes.

There seems to be no evidence for the direct conjugation of *S*-methylcysteine with glutamate, by analogy with the formation of γ-glutamylcysteine in bean seedlings and in wheat germ:[66,67]

$$\text{Glutamic acid} + \text{cysteine} + \text{ATP} \longrightarrow \gamma\text{-glutamylcysteine} + \text{ADP} + P_i$$

However, such an ATP-dependent formation of γ-glutamyl-*S*-methylcysteine could occur in mammalian tissues and may also be operative in yeasts (see Section V).

V. *S*-METHYLGLUTATHIONE AND *S*-METHYL-L-CYSTEINE AS METABOLIC ARTIFACTS

S-*Methylglutathione*

For more than a century it has been known that various organic compounds are metabolized in animals to mercapturic acids, or *S*-substituted *N*-acetyl-L-cysteines, which are excreted in the urine.[68] The source of the cysteine moiety of mercapturic acids has been shown to be glutathione, primarily that present in the liver, the level of which falls rapidly when precursors of mercapturic acids are administered.[69]

TABLE IV

γ-Glutamyl transpeptidase activity of various plant tissues*

Plant tissue	Transpeptidase activity†
Red kidney bean (*Phaseolus vulgaris*)	
Whole fruit	38
Immature seed	12
Leaves	16
Mature seed	4
Soya bean (*Glycine max*)	
Immature seed	25
Green pod	24
Lima bean (*Phaseolus lunatus*)	
Immature seed	61
Green pod	24
Pea (*Pisum sativum*)	
Mature seed	23
Radish (*Raphanus sativus*)	
Storage root	7
Wedgewood Iris (*Iris xithium* × *I. tingitana*)	
Bulb	1.5
Onion (*Allium cepa*)	
Bulb	0
Turnip (*Brassica rapa*)	
Storage root	0

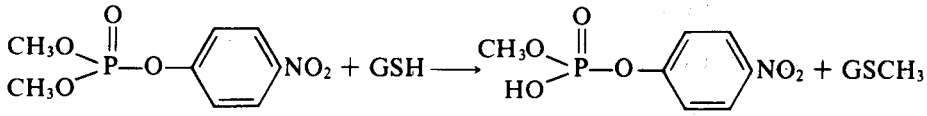
* Data of Thompson *et al.*[61]

† mμmoles aniline formed/g fresh wt/h; substrates: γ-glutamylaniline and *S*-methyl-L-cysteine.

Johnson[70] showed that the administration to rats of iodomethane, which gives rise to urinary methylmercapturic acid[71] **9**, produced this rapid depletion of liver glutathione, accompanied by the appearance of a new metabolite, identified as *S*-methylglutathione **4**, which was excreted in the bile. As much as 50 per cent of a 10–15 mg dose of iodomethane given to a 200 g rat was metabolized in this way within 1.5 h. Johnson[72] further characterized a glutathione-dependent *S*-alkyltransferase, present in the liver and to a lesser extent in the kidneys and adrenal glands of many animal species, able to catalyse the conjugation of glutathione with iodomethane.

A more recent re-examination and reclassification of the glutathione *S*-transferases has indicated, however, that these enzymes have broad and overlapping substrate specificities with regard to their donor substrate,[73,74] iodomethane being a good substrate for glutathione *S*-transferase E, but a less effective one for the *S*-transferase B.[75]

The enzyme-catalysed reaction of glutathione with compounds possessing an electrophilic center is now recognized to be an important detoxication pathway in animals and plants for the metabolism of many drugs and pesticides[76-78] and, in particular, as suggested by Hollingworth[79] it may be a major route for the *O*-dealkylation of such compounds. Thus, organophosphorus insecticides of the dimethyl triester type can be *O*-demethylated, one methyl group being transferred to glutathione (GSH), as in the case of methyl paraoxon:



The formation of *S*-methylglutathione by this pathway was observed *in vitro* in mouse liver preparations,[79] although *in vivo* no *S*-methylglutathione was found in the urine of methyl paraoxon-treated animals owing to its further metabolism (see below).

Tetrachlorvinphos ((z)-2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate) is likewise *O*-demethylated in mammalian liver preparations, glutathione acting as the methyl acceptor,[80,81] and there is some evidence that the *S*-transferase involved is identical with that catalysing the methylation of glutathione by iodomethane. The enzyme is also present in housefly tissue.[82] Other examples of this detoxication reaction in which *S*-methylglutathione has been positively identified as a product include the metabolism of DDVP (2,2-dichlorovinyl dimethyl phosphate) in rats,[83] *cis*-mevinphos (2-methoxycarbonyl-1-methylvinyl dimethyl phosphate) in mouse liver homogenates,[84] azinphosmethyl (S-(3,4-dihydro-4-oxobenzo[d]-1,2,3-triazin-3-ylmethyl) *O,O*-dimethylphosphorodithioate) in housefly tissue and mouse liver,[85,86] and dimethylvinphos (2-chloro-1-(2,4-dichlorophenyl)vinyl dimethyl phosphate) in rats and dogs.[87]

The formation of *S*-methylglutathione in the metabolism of methylparathion (*O,O*-dimethyl *O*-4-nitrophenyl phosphorothioate) is also implied from the identification of an appropriate glutathione-dependent *S*-methyltransferase in insects.[88-90] A similar detoxication mechanism appears to be operative in plants, as indicated by the demethylation of fenitrothion (*O,O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate) by seeds of white pine, white spruce and yellow birch to form desmethylfenitrothion and *S*-methylglutathione.[91]

Mammalian brain is the one tissue in which *S*-methylglutathione has been reported to be naturally occurring.[92] This tissue is also well known to contain many *O*-methylated and *N*-methylated compounds of pronounced physiological activity, and it is therefore tempting to suggest that *S*-methylglutathione may be a product of their detoxication. Its formation may even be a part of the regulatory control of their levels in brain tissue.

S-Methylglutathione was found to be the major metabolite of methyl methanesulfonate in the liver of rats and mice dosed with this compound.[93,94] Dimethylnitrosamine, another methylating agent and carcinogen, has also been reported to reduce the level of liver glutathione in mice,[95] although Craddock[24] had previously found very little methylation of glutathione by this compound in rats.

All the above instances of *S*-methylglutathione formation are examples of *S*-methylcysteine synthesis by the pathway type (b), referred to previously. As an alternative to the direct methylation of glutathione, there is evidence for *S*-methylglutathione formation from *S*-methylcysteine in the yeasts *S. cerevisiae* and *Candida utilis*.[25] In the first of these organisms the tripeptide was the major metabolite of *S*-methylcysteine. The rapidity of its

appearance in cells suspended in a medium supplemented with *S*-methylcysteine, and the non-appearance of the sulfur of the amino acid in cysteine or methionine, as checked by ^{35}S-labelling studies, suggests that under these conditions *S*-methylglutathione may have been formed by a two-step conjugation, with γ-glutamyl-*S*-methylcysteine as an intermediate. The biosynthesis of this dipeptide in plants mediated by a γ-glutamyltranspeptidase[61] has already been discussed, and in the animal *S*-methylcysteine is known to act as a γ-glutamyl group acceptor for a similar enzyme, glutathionase.[96] In animal tissues the amino acid is also a substrate for γ-glutamylcysteine synthetase:[97,98]

$$\text{Glutamate} + S\text{-methylcysteine} + \text{ATP} \rightleftharpoons \gamma\text{-glutamyl-}S\text{-methylcysteine} + \text{ADP} + P_i$$

One or other of these pathways may be operative in yeasts, and would be expected to be coupled with the further conjugation of γ-glutamyl-*S*-methylcysteine with glycine, catalysed by glutathione synthetase:

$$\gamma\text{-Glutamyl-}S\text{-methylcysteine} + \text{glycine} + \text{ATP} \rightleftharpoons S\text{-methylglutathione} + \text{ADP} + P_i$$

It has been shown that yeast cells contain the enzymes required for glutathione synthesis,[99] and highly purified preparations of the glutathione synthetase have been obtained from this source.[100]

S-*Methylcysteine and its sulfoxide*

S-Methylcysteine can arise as an artifact during the acid hydrolysis of proteins.[101] It has been obtained non-enzymically by the action of trimethyl phosphate on cysteine, a methylation which takes place under physiological conditions,[102] and it has been identified in the urine of rats and mice dosed with trimethyl phosphate.[103] The interaction of *N*-methyl-*N*-nitrosourethane with cysteine *in vitro* also gives *S*-methylcysteine.[104] Craddock[24] found traces of the amino acid in the liver protein and urine of rats dosed with dimethylnitrosamine and, following the treatment of rats with methyl methanesulfonate, *S*-methylcysteine was detected in the haemoglobin.[105] It is possible that these last two methylating agents were also reacting non-enzymically with free or protein-bound cysteine.

In several instances where *S*-methylglutathione has been formed metabolically in mammalian liver, *S*-methylcysteine and/or its sulfoxide together with the corresponding *N*-acetyl derivatives, methylmercapturic acid **9** and its sulfoxide **10**, have been detected as minor urinary constituents. An example of this is following the administration of iodomethane to rats,[70,71] although the combined methylthio compounds excreted represented only about 2 per cent of the iodomethane given. More recently, *S*-methylcysteine has been identified in the urine of workers occupationally exposed to chloromethane vapor,[106] and it is reasonable to assume that this was associated with the preliminary formation of *S*-methylglutathione in the liver. Some years earlier, Redford-Ellis and Gowenlock[107] had demonstrated the *in vitro* formation of *S*-methylglutathione after exposure of liver and kidney homogenates to chloromethane. As shown by ^{14}C-labelling studies, chloromethane was also taken up by erythrocytes, some 40 per cent of the halide absorbed forming *S*-methylglutathione enzymically.

Treatment of rats, mice and rabbits with methyl methanesulfonate similarly caused the urinary excretion of small amounts of *S*-methylcysteine,[93,94] as did doses of DDVP in rats and mice.[108] Evidence that in animals *S*-methylglutathione is the precursor of urinary *S*-methylcysteine was provided by Foxwell and Young,[109] who showed that the administration of *S*-methylglutathione directly to rats resulted in the excretion of *S*-methylcysteine and related metabolites such as methylthioacetic acid **11**, *N*-(methylthioacetyl)glycine **13** and methylmercapturic acid **9**. Furthermore, the tripeptide was converted *in vitro* to *S*-methylcysteine by rat kidney microsomes.[110]

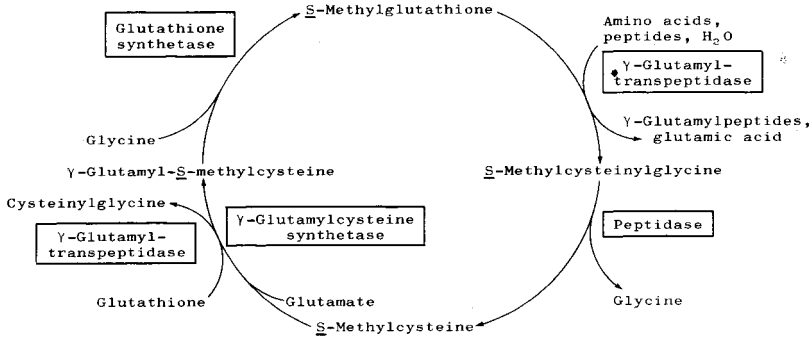
S-Methylcysteine, its sulfoxide and related methylthio compounds most probably appear under these conditions as a result of the stepwise cleavage of *S*-methylglutathione to give the free amino acid, followed by its subsequent oxidation, acetylation, etc. *S*-Methylglutathione is an effective substrate for γ-glutamyltranspeptidase, present in mammalian kidney and other tissues.[111,112] In the presence of this transferase the tripeptide can act like glutathione itself as a γ-glutamyl group donor to a number of amino acids, including asparagine, cystine, glutamine, methionine and serine, as well as to various dipeptides, *e.g.*, glycylglycine:[111,113]

S-Methylglutathione + amino acid (or peptide) $\rightleftharpoons$ *S*-methylcysteinylglycine + γ-glutamylpeptide

However, the renal γ-glutamyltranspeptidase has hydrolytic as well as transfer activity, and it has recently been stated that *in vivo* the major reaction catalysed by the enzyme is the hydrolytic fission of glutathione and its *S*-substituted derivatives and not γ-glutamyl group transfer:[114]

S-Methylglutathione + H_2O ⟶ *S*-methylcysteinylglycine + glutamic acid

The initial product of *S*-methylglutathione breakdown is *S*-methylcysteinylglycine, which is presumably further split to give free *S*-methylcysteine and glycine. The appropriate dipeptidase, referred to as cysteinylglycinase,[115,116] has been characterized in the kidney of the pig and other animals. A summary of the formation and breakdown of *S*-methylglutathione in plants, animals and microorganisms is shown in Scheme 2.



Scheme 2. Biosynthesis and metabolism of S-methylglutathione

VI. METABOLISM

Many of the early metabolic studies made on *S*-methylcysteine were concerned with its behavior in the animal, and these showed that when added to the diet in place of methionine or cysteine, it failed to induce a growth response in, for example, rabbits,[117] chicks[118] or young rats.[119,120] Neither did it yield extra urinary cystine when fed to a human cystinuric.[121] Furthermore, unlike cysteine or methionine, it was inactive in reducing the deposition of fat in the livers of animals given high fat diets.[122,123] Its inability to act as a dietary source of sulfur indicates that in the animal *S*-methylcysteine does not act as a precursor of either methionine or cysteine to any significant extent.

On the other hand, *S*-methylcysteine does show certain similarities to methionine. At high concentrations both methionine and *S*-methylcysteine depress the growth of rats,[124] although it is not certain whether the compounds act in the same manner, or whether two different mechanisms of action are involved. At high concentrations *S*-methylcysteine acts as a substrate for methionyl-tRNA synthetase of rat liver,[125,126] while it is a competitive inhibitor of cysteinyl-tRNA synthetase.[127] As will be discussed below, the compound can also serve as a substrate for a number of other animal enzymes involved in the metabolism of methionine and cysteine, and it is their action on *S*-methylcysteine which is responsible for the observed pattern of catabolism of this amino acid.

In microorganisms there are numerous instances of *S*-methylcysteine acting as a nutritional substitute for methionine and/or cysteine, indicating that it is an effective precursor of these amino acids. Thus, *S*-methylcysteine can serve as the sole source of sulfur for the growth of *N. crassa,*[8] *S. cerevisiae,*[128–131] *C. utilis,*[25] *P. mirabilis,*[19] *E. coli*[132] and the myxomycete *Physarum polycephalum,*[133] and it promotes the growth of methionineless mutants of *N. crassa,*[134–136] *P. mirabilis* and *E. coli.*[19] However, it cannot replace cystine for the growth of *Leuconostoc mesenteroides.*[137]

The biochemical similarity of *S*-methylcysteine to methionine in microbial metabolism is seen from the ability of both compounds to counteract the toxicity of cysteine and of ethionine to yeasts,[131,138] and the toxicity of cysteine to *N. crassa.*[139–141] Both amino acids competitively depressed the uptake of cysteine by *E. coli*[132] and by yeasts,[142] as well as the incorporation of sulfate-S into *E. coli,*[132] *N. crassa*[134,135] and yeasts.[145] *S*-Methylcysteine was also found to inhibit the incorporation of methionine itself into yeasts.[143]

Other examples of this similarity between *S*-methylcysteine and methionine include the ability of both compounds to protect the developing eggs of the sea urchin, *Paracentrotus lividus,* against the toxic effects of cysteine,[144] and their inhibition of cysteine transport in tobacco cells.[145] A further example in plants is the occurrence of γ-glutamyl-*S*-methylcysteine and its sulfoxide in the seeds of *Vigna radiata,* and the parallel occurrence of γ-glutamylmethionine and its sulfoxide in the seeds of *Vigna mungo.*[34]

Dethiomethylation of S-*methylcysteine and methionine biosynthesis*

The ability of *S*-methylcysteine to serve as a sole source of sulfur for the growth of various microorganisms implies a metabolic conversion of the sulfur of the amino acid to cysteine and methionine, and this has been demonstrated directly in *N. crassa*[135] and in the yeasts

S. cerevisiae and *C. utilis*[25] grown in the presence of ^{35}S-labelled *S*-methylcysteine. Doney and Thompson[146] have also shown this sulfur transfer to occur in leaves of *Phaseolus vulgaris,* but there is no evidence of such a reaction taking place in animal tissues.

To account for the formation of methionine from *S*-methylcysteine in Neurospora, Ragland and Liverman[8] originally proposed a direct transthiomethylation from *S*-methylcysteine to a four-carbon acceptor. Although the existence of this particular reaction remains unproven, there is now good supporting evidence for the participation of methanethiol as an intermediate. Methanethiol is readily formed from *S*-methylcysteine by many bacteria,[147-150] fungi[151-153] and also *S. cerevisiae.*[154] The reaction has also been observed in seeds of *Albizzia lophanta*[155,156] and in rat liver.[157]

Methionine biosynthesis in the presence of extracts of *Salmonella typhimurium* has been obtained with the thiol acting as a sulfur donor to *O*-succinylhomoserine:[158]

$$\text{Methanethiol} + \textit{O}\text{-succinylhomoserine} \longrightarrow \text{methionine} + \text{succinate}$$

In Neurospora an analogous reaction has been identified, with *O*-acetylhomoserine as the sulfur acceptor.[152]

Using ^{14}C-methyl- and ^{35}S-labelled *S*-methylcysteine, studies of the transfer of the thiomethyl group of the amino acid have been made in *N. crassa*[135] and in kidney bean leaves.[146] The methyl group and the sulfur both appeared in protein-methionine, but they did so to different extents, depending on the experimental conditions. Thompson[1,146] has interpreted this as arguing against the possibility of an intact thiomethyl transfer. However, Maw[25] has pointed out that the completeness of the transfer will depend on the extent to which methanethiol is available for metabolism by other competing pathways, such that the methyl group is lost while the sulfur is still available for methionine biosynthesis. It is therefore unreasonable in *in vivo* experiments to expect exact agreement in the amounts of incorporation of the sulfur and of the methyl group.

In experiments with the yeasts *S. cerevisiae* and *C. utilis* grown for two days on ^{3}H-methyl- and ^{35}S-labelled *S*-methylcysteine as the sole sulfur source, Maw[25] calculated that transfer of the thiomethyl group of the amino acid to protein-methionine had taken place to the extents of 21 and 35 per cent, respectively. The remainder of the sulfur incorporated by each organism was taken to originate indirectly from methanethiol or *S*-methylcysteine itself by other degradative pathways.

A second product of the cleavage of *S*-methylcysteine in bacteria was believed to be pyruvic acid,[159] and a pyridoxal phosphate-requiring *S*-alkylcysteinase, or *S*-alkylcysteine lyase, was subsequently partially purified from *Pseudomonas cruciviae.* This enzyme catalyses the stoichiometric β-elimination of *S*-methylcysteine to methanethiol, pyruvic acid and ammonia:[148]

$$CH_3SCH_2CH(NH_2)CO_2H + H_2O \longrightarrow CH_3SH + CH_3COCO_2H + NH_3$$

S-Alkylcysteine lyase has also been identified in other bacteria.[160] It was found to be inactive towards methionine as a substrate but acted on *S*-methylcysteine sulfoxide, splitting it to methyl methanethiosulfinate **5:**

$$2CH_3\overset{\overset{O}{\uparrow}}{S}CH_2CH(NH_2)CO_2H + H_2O \longrightarrow CH_3\overset{\overset{O}{\uparrow}}{S}SCH_3 + 2CH_3COCO_2H + 2NH_3$$

5

A plant *S*-alkylcysteine lyase found in the endosperm of seeds of *A. lophanta*[155,156] and which has been extensively purified from hypocotyls of seedlings of *Acacia farnesiana* Willd,[161] behaves similarly. Using the enzyme from Acacia, Mazelis and Creveling[161] found *S*-methylcysteine sulfoxide to have about 75 per cent of the substrate activity of *S*-methylcysteine itself. An interesting suggestion as to why a seedling may contain such high concentrations of the lyase in its hypocotyl and root, put forward by Mazelis and Fowden,[162] is that during the early germination period, while the cotyledon, hypocotyl and radicle are in the soil, they are very susceptible to attack by soil pathogens. Many volatile sulfur compounds, including methanethiol, are good inhibitors of fungal growth, so that the enzymic release of methanethiol at this stage of development might therefore give the seedling a better chance of establishing itself in the presence of pathogenic organisms.

The cleavage of *S*-methylcysteine to methanethiol is also a possible metabolic pathway in mammals, for Binkley[157] found that a partially purified preparation of a cystathionine-cleaving enzyme, which he termed "thionase", catalysed the hydrolytic cleavage of many *S*-alkylcysteines, including *S*-methylcysteine, to the corresponding thiols. However, other workers[163,164] have been unable to show any appreciable action of liver cystathionase on *S*-methylcysteine. The amino acid is also a poor substrate for the β-cystathionase of bacteria,[165] Neurospora[166] or plants,[167] but the cystathionine γ-cleavage enzyme of Neurospora is able to split it to methanethiol.[149]

Some bacteria, *e.g., Pseudomonas ovalis,*[168] possess a L-methionine γ-lyase which catalyses $\alpha\beta$- and $\alpha\gamma$-eliminations from methionine and several of its derivatives, also *S*-substituted cysteines, including *S*-methylcysteine. *S*-Methylcysteine is also a good substrate for tryptophanase in *E. coli,*[169] *Aeromonas liquefaciens*[170] and a marine Vibrio.[171] This enzyme mediates in a variety of other $\alpha\beta$-elimination and β-replacement reactions, the former reaction cleaving *S*-methylcysteine to methanethiol, pyruvate and ammonia, as in the *S*-alkylcysteine lyase- and L-methionine γ-lyase-catalysed reactions. Tryptophan synthetase from *E. coli*[172] and tyrosine phenol-lyase from *Aeromonas phenologenes*[173] and *Erwinia herbicola*[174] have likewise been shown to utilize *S*-methylcysteine as a substrate and to degrade it in a similar manner. There is, therefore, a variety of enzyme systems potentially capable of dethiomethylating *S*-methylcysteine, although what significance they have in methanethiol formation *in vivo* remains unclear.

Cleavage of S-*methylcysteine sulfoxide.* In addition to being a substrate for some bacterial and plant alkylcysteine lyases, *S*-methylcysteine sulfoxide is also degraded to methyl methanethiosulfinate, pyruvate and ammonia by a similar mechanism through the action of specific, pyridoxal phosphate-activated cysteine sulfoxide lyases. One of these, alliin lyase, or alliinase, is present in many Allium species,[175–179] and is so called because of its ability to cleave *S*-allyl-L-cysteine sulfoxide (alliin), the predominating sulfoxide in these plants. Similar lyases have been found in another member of the Liliaceae, *Tulbaghia violacea*[180] and in many Brassicae.[181,182] In the case of the lyase from broccoli,[181] the substrate activity of *S*-methylcysteine sulfoxide was found to be comparable with that of alliin. *S*-Substituted cysteines, such as *S*-methylcysteine, are not only inactive as substrates for these enzymes, but can be appreciably inhibitory.[183]

Demethylation

Since in plants *S*-methylcysteine can be formed from cysteine by transmethylation, attention has been given to the possibility of the operation of the reverse reaction, with *S*-methylcysteine acting like methionine as a methyl group donor. However, in animals all the evidence points to the absence of any transfer of the intact methyl group from the compound, and its consequent conversion to the demethylated product, cysteine. Transfer of the methyl group of methionine occurs via the intermediate formation of *S*-adenosylmethionine, mediated by the 'methionine-activating enzyme', ATP: L-methionine *S*-adenosyltransferase, and Cantoni[184] has shown that *S*-methylcysteine does not serve in lieu of methionine as a substrate for this enzyme; neither is *S*-methylcysteine an effective competitive inhibitor of the methionine activation.[185] Furthermore, the expected product of the reaction between the amino acid and ATP, namely, *S*-adenosyl-*S*-methyl-L-cysteine, has been synthesized and shown to be inactive as a methyl donor substrate for all the *S*-adenosylmethionine-dependent methyltransferases tested.[186] Schlenk[187] also showed that when yeasts are grown in the presence of *S*-methylcysteine, there is no evidence of *S*-adenosyl-*S*-methylcysteine formation in the cells; instead, part of the sulfur of the amino acid is utilized to form *S*-adenosylmethionine.

On the other hand, demethylation of *S*-methylcysteine by intact methyl group transfer seems to be a valid metabolic pathway in some plants. Mae *et al.*[63] found that when the ^{35}S-labelled amino acid or its sulfoxide were infiltrated into detached leaves of Chinese cabbage the labelled sulfur appeared in the neutral and acidic fractions of the tissue, and about 70 per cent of the ^{35}S in the insoluble fraction was present as cysteine. Moreover, when *S*-methylcysteine or its sulfoxide, doubly labelled with ^{35}S and with ^{3}H in the 3-position, were applied to detached leaves, the ratio of ^{3}H and ^{35}S in the cysteine isolated from the leaves was comparable with that in the compounds initially administered. This result was to be expected if demethylation of either compound had taken place, whereas if metabolism had occurred by dethiomethylation, the $^{3}H/^{35}S$ ratios in the cysteine would have been very different. Further experiments with ^{14}C-methyl-labelled *S*-methylcysteine showed that the label was incorporated into the methyl ester groups of pectin in Chinese cabbage.[188] In addition, the methyl group of *S*-methylcysteine doubly labelled with ^{14}C and with ^{3}H in the *S*-methyl group was transferred to *S*-adenosylmethionine in the leaves with little change in the original $^{3}H/^{14}C$ ratio, while the transfer to pectin methyl ester groups did involve some change in the ratio. However, the magnitude of the ratio was considerably higher than would have been expected if the methyl group had been entirely converted first to formaldehyde or formate.[189]

These findings suggest that the methyl transfer occurred by a combination of two pathways: an intact methyl group transfer through *S*-adenosylmethionine, and a transfer involving the partial degradation of the methyl group to formaldehyde or formate. The last two intermediates are known to be efficient precursors of the methyl ester groups of pectin.

Oxidation

In addition to being oxidized to its sulfoxide in plants, *S*-methylcysteine can undergo complete oxidation of its sulfur to inorganic sulfate in the animal. Administration of the

compound to dogs,[190] rabbits,[191] rats[192] and man[121] was found to cause an increase in urinary sulfate excretion. The *in vitro* degradation of the amino acid to inorganic sulfate has also been demonstrated in rat liver slices.[193] The actual pathway of the oxidation has not so far been elucidated, but it seems unlikely that cysteine or cystine are intermediates. Dethiomethylation to methanethiol has been suggested as a likely preliminary step,[68,193] for the thiol has been shown to be further oxidized to sulfate.[194]

Degradation of the *S*-methyl group to CO_2 is also an important oxidative pathway for *S*-methylcysteine in the animal. Kuchinskas[195] found that injection of the amino acid labelled with ^{14}C in the methyl group into rats gave rise to labelled CO_2 in the expired air, the maximum rate of oxidation occurring some 3–5 h after administration. Over a 24-h period, the conversion of the *S*-methylcysteine-methyl group to CO_2 in the whole rat was about twice as extensive as that of the *S*-methyl group of methionine. Cell-free preparations of rat tissues were obtained capable of carrying out the oxidation, which was stimulated by the presence of NAD and NADP, Co^{2+} and reduced glutathione.[196] The oxidation appears to require catalase and a H_2O_2-generating system.[197] More recent experiments by Case and Benevenga[198] suggest that the oxidation proceeds totally through the intermediate formation of free formate.

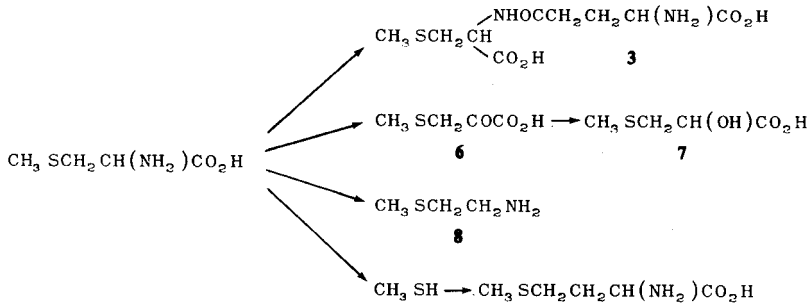
Other metabolic reactions

Studies of the metabolism of *S*-methylcysteine in yeasts and in rats have shown that the compound gives rise to a variety of other metabolites as a result of its participation in deamination, decarboxylation, acylation and conjugation reactions.

The corresponding α-hydroxy acid, methylthiolactic acid **7**, has been identified as a metabolite in the yeasts *S. cerevisiae* and *C. utilis* and was one of the main products released back into the medium following uptake of the amino acid by growing cultures.[199] Using ^{35}S-labelled *S*-methylcysteine added to the medium,[25] it was shown that two other compounds were also excreted by the cells, namely, methylthiopyruvic acid **6** and *S*-methylcysteamine **8**. The three metabolites, together with *S*-methylglutathione **4**, were also present in the sulfur pools of the yeasts after uptake of *S*-methylcysteine. *S*-Methylcysteamine, the decarboxylation product of the amino acid, was a major metabolite, representing as much as two-thirds of the non-protein S derived from *S*-methylcysteine in *C. utilis*, while *S*-methylglutathione was the principal metabolite formed in *S. cerevisiae*.

The presence of methylthiopyruvic and methylthiolactic acids is evidence for the deamination of *S*-methylcysteine, although this particular reaction does not appear to have been examined at the enzymic level in microorganisms. The amino acid acts as an efficient amino group donor to pyruvate or glyoxylate in the presence of asparagine transaminase of rat liver[200] and glutamine transaminase of brain tissue.[201] The metabolism of *S*-methylcysteine in yeasts is summarized in Scheme 3.

Detailed studies of the fate of *S*-methylcysteine in the rat and other animals have been made by Barnsley.[192,202] Subcutaneous injection of rats with the amino acid resulted in the urinary excretion of the corresponding *N*-acetyl derivative, methylmercapturic acid **9**, representing about 0.6 per cent of the administered compound, together with its sulfoxide **10**. These products are also excreted by animals dosed with methylating agents, such as iodomethane, which are believed to give rise to *S*-methylcysteine endogenously through the intermediate formation of *S*-methylglutathione (see Section V).

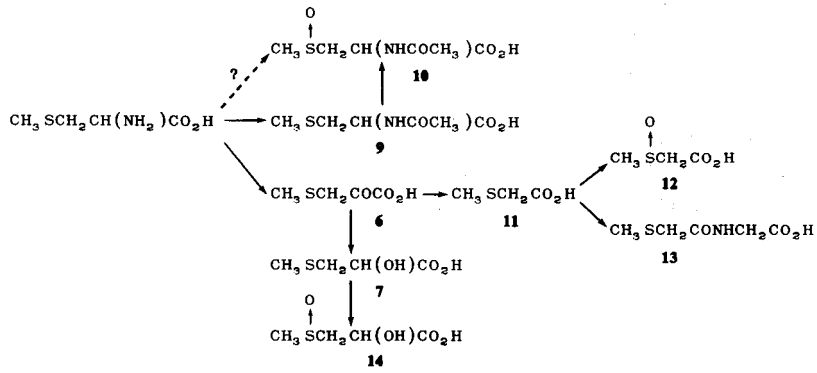


Scheme 3. Metabolism of S-methylcysteine in yeasts

Further excretion products were methylthioacetic acid **11** and *N*-(methylthioacetyl)-glycine **13,** 2-hydroxy-3-methylsulfinylpropionic acid **14** and methylsulfinylacetic acid **12.** *N*-(Methylthioacetyl)glycine and methylsulfinylacetic acid are clearly metabolites of methylthioacetic acid, and the former conjugate is excreted by rats dosed with methylthioacetic acid. The latter metabolite is the corresponding sulfoxide of methylthioacetic acid, and its appearance together with methylmercapturic acid sulfoxide and 2-hydroxy-3-methylsulfinylpropionic acid, implicates sulfoxidation as a reaction of some significance in *S*-methylcysteine metabolism. This is evident from the fact that, although inorganic sulfate was quantitatively the most important metabolite excreted, accounting for about 50 per cent of the injected compound, the three sulfoxides taken together corresponded to about 19 per cent of the dose.

2-Hydroxy-3-methylsulfinylpropionic acid is also the sulfoxide of methylthiolactic acid, a metabolite of *S*-methylcysteine in yeasts, and this suggests that deamination of the amino acid most probably occurred. The primary product of this reaction would be methylthiopyruvic acid **6,** which in addition to undergoing reduction could also be the precursor of methylthioacetic acid by an oxidative decarboxylation.

Many of the above-mentioned metabolites excreted by the rat are also formed in the guinea-pig and hamster, and their derivation and interrelationships are depicted in Scheme 4.



Scheme 4. Metabolism of S-methylcysteine in the rat and other animals.

VII. PHYSIOLOGICAL AND TOXICOLOGICAL EFFECTS

As has been discussed previously, numerous microorganisms are able to grow in the presence of *S*-methylcysteine as their sole source of sulfur, indicating that this amino acid and the methanethiol formed from it as an intermediary metabolite in methionine biosynthesis are not significantly toxic to these organisms.

In the case of animals, Pirie[190] noted that *S*-methylcysteine was appreciably toxic to dogs when given orally at doses of about 0.1 g/kg body weight. The compound is apparently tolerated in small doses by other animal species,[120,191,192,202] although high levels in the diet have caused a depression of growth.[203] Small doses of γ-glutamyl-*S*-methylcysteine given repeatedly by injection to mice and rabbits were not noticeably toxic, but at a higher dose rate of 700 mg/kg body weight it caused kidney and liver injury to rabbits.[204]

S-Methylcysteine sulfoxide appears to be quite toxic to a wide range of species. Like several other cysteine-*S*-oxides, it may act as an antibiotic in animals because of the antimicrobial properties of the methyl methanethiosulfinate formed from it in the intestine, and which inhibits growth of Staphylococci and many other bacteria at dilutions of 1:10,000 to 1:100,000.[32] Studies investigating the nutritional value of *S*-methylcysteine sulfoxide in rats have shown that when present at a level of 2 per cent or more in the diet it causes a decrease in the growth rate and food intake, coupled with other deleterious effects, including the development of anaemia and hypertrophy of the spleen, together with an abnormal deposition of iron in this organ.[205-207]

A further effect of the compound when administered in the diet is to markedly reduce the plasma- and liver-cholesterol levels in rats fed a high cholesterol diet.[208-211] A possible mechanism which has been suggested for the blood cholesterol-lowering activity of the sulfoxide is an interaction between its sulfoxide group and the sulfhydryl group of coenzyme A, leading to a disorientation of lipid biosynthesis.[212] However, whether the sulfoxide produces its effects directly or whether it is itself innocuous but gives rise to toxic metabolites is not apparent from the nutritional studies quoted above.

It is of particular interest that *S*-methylcysteine sulfoxide has been identified as the agent responsible for the condition encountered in farm animals, known as 'kale poisoning' or 'kale anaemia', which can arise when such animals are provided for long periods with a diet consisting mainly or exclusively of brassica crops. The initial recognition of this illness in cattle fed on large quantities of kale (*Brassica oleracea*) is attributed to Rosenberger.[213] Kale poisoning was subsequently studied by Greenhalgh,[214] who observed that ruminant animals, particularly cattle and goats, were more susceptible to the condition than pigs and a variety of other non-ruminant animals.

The poisoning, a severe haemolytic anaemia, develops 1–3 wk after the animals have been placed on the kale diet, and is manifested by the appearance within the erythrocytes of Heinz-Ehrlich bodies, consisting of spherical aggregates of denatured haemoglobin, followed by a pronounced fall in the blood haemoglobin level from the normal value of about 11 g/100 ml to 8 g/100 ml or less. Haemoglobinuria may subsequently result and the animals may show loss of appetite, jaundice, increased pulse rate, fall in milk production, a poor conception rate and growth retardation. If kale feeding is discontinued, haemoglobin levels gradually return to normal and the animals generally recover.

Further elucidation of this problem has been provided by the work of Smith and his collaborators.[37,212,215,216] They found that the haemolytic activity of the whole kale plant

was due to the presence of *S*-methylcysteine sulfoxide, and that the administration to experimental animals of the synthetic compound produced all the symptoms associated with excessive kale feeding. Furthermore, *S*-methylcysteine itself also produced comparable effects. Feeding experiments have shown that when administered to cattle as the chemical itself or in the form of cabbages or swedes, *S*-methylcysteine sulfoxide at an intake level of about 10–15 g/100 kg body weight elicited a mild-moderate haemolytic response, while intakes of 15–20 g/100 kg body weight gave rise to an acute response.[215]

Animals showing acute kale poisoning contained abnormally high concentrations of volatile sulfur compounds in the rumen, and *in vitro* studies in which *S*-methylcysteine sulfoxide was incubated with the rumen contents of a kale-fed goat showed the rapid evolution of dimethyl disulfide and, at a later stage, methanethiol. Subsequent feeding experiments indicated that dimethyl disulfide, like a number of other organic disulfides, is a potent haemolysin and produces the symptoms characteristic of kale poisoning.

It is now believed that dimethyl disulfide is the direct causative agent of this condition and that the compound arises from the action on *S*-methylcysteine sulfoxide of *S*-alkylcysteine sulfoxide lyases, present in a small number of the rumen bacteria, forming methyl methanethiosulfinate, which is then reduced enzymically to the disulfide. Further reduction may also occur to form methanethiol.

Smith[212] has suggested that dimethyl disulfide may exert its effect by lowering the levels of reduced glutathione (GSH) in the erythrocyte through its oxidant action:

$$2GSH + CH_3SSCH_3 \rightleftharpoons GSSG + 2CH_3SH$$

or by reacting with sulfhydryl groups on the red cell membrane, forming a mixed disulfide:

$$\text{Membrane-SH} + CH_3SSCH_3 \rightleftharpoons \text{Membrane-SSCH}_3 + CH_3SH$$

An alteration of membrane permeability would be expected to lead to a loss in the ability of the erythrocyte to retain haemoglobin.

It is not yet possible to exclude *S*-methylcysteine sulfoxide *per se* as a contributor to the overall haemolytic action, however, since by analogy with the behaviour of *S*-allylcysteine sulfoxide (alliin), the methyl compound would be expected to react with reduced glutathione as follows:

$$\textit{S}\text{-Methylcysteine sulfoxide} + 2GSH \rightleftharpoons \textit{S}\text{-methylcysteine} + GSSG + H_2O$$

These investigations on the biochemical basis of kale poisoning have an important bearing on the feeding of farm animals in that, as stated by Smith:[212,216] "because there is in practice no simple way of counteracting the toxic action of the disulfide, measures to prevent kale anaemia must be limited to those aiming to restrict the intake of *S*-methylcysteine sulfoxide or to inhibit disulfide production or absorption". The former precaution might be achieved by attention to the careful selection of kale varieties as animal foodstuffs, as studied by Whittle *et al.*[37] and to the breeding of new varieties with low *S*-methylcysteine sulfoxide contents, so as to maintain a daily intake of the compound of less than 10 g/100 kg body weight.

The *S*-methylcysteine sulfoxide content of cruciferous crops is also a factor to be considered in human nutrition. This is especially so in certain countries, for example, Japan, where Cruciferae represent a major proportion of the diet, providing an average daily intake of the sulfoxide of about 300 mg per person. At this level it is not considered that

the compound will have deleterious haemolytic effects. Rather, its effect in lowering the cholesterol content of the blood and liver could be of possible prophylactic value against hypercholesterolaemia and coronary heart disease.

The tripeptide *S*-methylglutathione, a metabolite formed during the detoxication of many methylated drugs and pesticides, may also have possible medical applications. It was first synthesized by Kermack and Matherson[217] and shown like other *S*-alkylglutathiones to inhibit the enzyme glyoxalase I, which catalyses the first step in the glutathione-requiring conversion of methylglyoxal to lactic acid. For this reason, *S*-alkylglutathiones have been proposed as possible anti-cancer drugs.[218] Clearly, more needs to be known about the biochemical and pharmacological properties of this tripeptide of *S*-methylcysteine.

VIII. PHYSIOLOGICAL ROLE OF S-METHYLCYSTEINE

It might be expected that an elucidation of the metabolism of *S*-methylcysteine and its derivatives would pinpoint its role in the physiology of the plant. However, no single, unequivocal function for the amino acid has so far emerged. Numerous possibilities have been suggested, for example, Synge and Wood[4] speculated correctly that *S*-methylcysteine sulfoxide might be a precursor of methanethiol, and they considered this to be an important role for the compound.

Following the decomposition of cruciferous plants in soil, methanethiol and its disulfide are released, and Lewis and Papavizas[219] have considered that the presence of these substances could be responsible for the reduction or suppression of some plant diseases caused by soil-borne pathogens. A protective function for the thiol formed from *S*-methylcysteine by the roots of seedlings has also been proposed by Mazelis and Fowden.[162] On account of its pronounced odor, the thiol might in addition serve an ecological function in repelling herbivores and parasites.[42] An antibacterial role for sulfoxides, such as *S*-methylcysteine sulfoxide, as a result of their ability to undergo breakdown to the corresponding alkyl alkanethiosulfinates has already been discussed.[32]

Synge and Wood[4] also suggested that *S*-methylcysteine sulfoxide might act as a precursor of the thiomethyl group in plant constituents such as sulforaphene, and for the =C=S moiety of the isothiocyanate radical in mustard oils. Baur and Yang[220] implicated *S*-methylcysteine as an obligatory intermediate in a methionine-sulfur cycle to account for the degradation of methionine to ethylene. However, more recent work on the biogenesis of ethylene[221] renders this idea unlikely. *S*-Methylcysteine has been suggested as an antimetabolite for cystine or methionine in legumes,[222] while Rinderknecht[30] postulated an oxido-reductive function for the *S*-methylcysteine:*S*-methylcysteine sulfoxide system.

Mae *et al.*[26] have attributed a more general function to *S*-methylcysteine and its derivatives as providing a soluble pool of readily available sulfur for the plant, supporting an earlier suggestion of Wiebers and Garner.[135] This would account for the relatively large amounts of these compounds in plants and for the rapid changes in their concentrations which are observed during plant growth and which accompany alterations in the nutritional supply of sulfur to the plant. These compounds have also been considered as storage forms of the methyl group.[28,188]

Thompson *et al.*[28,29] noted that γ-glutamyl-*S*-methylcysteine on demethylation would

yield γ-glutamylcysteine, the immediate precursor of glutathione in animals[223] and plants,[224] and they suggested this as an important role for γ-glutamyl-*S*-methylcysteine. In this connection, it is of interest that glutathione was found to be a major metabolite of *S*-methylcysteine in yeasts.[25]

REFERENCES

1. J. F. Thompson, *Ann. Rev. Plant Physiol.*, **18**, 59 (1967).
2. R. L. M. Synge and J. C. Wood, *Biochem. J.*, **60**, xv (1955).
3. C. J. Morris and J. F. Thompson, *Chem. and Ind.*, 951 (1955).
4. R. L. M. Synge and J. C. Wood, *Biochem. J.*, **64**, 252 (1956).
5. C. J. Morris and J. F. Thompson, *J. Am. Chem. Soc.*, **78**, 1605 (1956).
6. S. Tsuno, F. Murakami, K. Tazoe and S. Kikumoto, *Bitamin*, **20**, 93 (1960).
7. J. F. Thompson, C. J. Morris and R. M. Zacharius, *Nature*, **178**, 593 (1956).
8. J. B. Ragland and J. L. Liverman, *Arch. Biochem. Biophys.*, **65**, 574 (1956).
9. A. I. Virtanen and E. J. Matikkala, *Acta Chem. Scand.*, **13**, 1898 (1959).
10. J. F. Carson and F. F. Wong, *J. Org. Chem.*, **26**, 4997 (1961).
11. J. Kuon and R. A. Bernhard, *J. Food Sci.*, **28**, 298 (1963).
12. B. Granroth, *Acta Chem. Scand.*, **22**, 3333 (1968).
13. L. Hoerhammer, H. Wagner, M. Seitz and Z. J. Vejdelek, *Pharmazie*, **23**, 462 (1968).
14. I. E. Roponen, *Suom. Kemistilehti B*, **44**, 163 (1971).
15. S. F. Trelease, A. A. Di Somma and A. L. Jacobs, *Science*, **132**, 618 (1960).
16. P. M. Dunnill and L. Fowden, *Phytochemistry*, **6**, 1659 (1967).
17. S. N. Nigam, J.-I. Tu and W. B. McConnell, *Phytochemistry*, **8**, 1161 (1969).
18. S. N. Nigam and W. B. McConnell, *Phytochemistry*, **11**, 377 (1972).
19. W. O. K. Grabow and J. A. Smit, *J. Gen. Microbiol.*, **46**, 47 (1967).
20. F. Irreverre and L. Levenbook, *Biochim. Biophys. Acta*, **38**, 358 (1960).
21. F. Tominaga, S. Kobayashi, I. Muta, H. Takei and M. Ichinose, *J. Biochem. (Tokyo)*, **54**, 220 (1963).
22. M. D. Armstrong, *Biochim. Biophys. Acta*, **587**, 638 (1979).
23. F. Tominaga, K. Oka and H. Yoshida, *J. Biochem. (Tokyo)*, **57**, 717 (1965).
24. V. M. Craddock, *Biochem. J.*, **94**, 323 (1965).
25. G. A. Maw and C. M. Coyne, *Arch. Biochem. Biophys.*, **127**, 241 (1968).
26. T. Mae, K. Ohira and A. Fujiwara, *Plant Cell Physiol.*, **12**, 1 (1971).
27. H. Rinderknecht, *Chem. and Ind.*, 1384 (1957).
28. C. J. Morris and J. F. Thompson, *Arch. Biochem. Biophys.*, **73**, 281 (1958).
29. R. M. Zacharius, C. J. Morris and J. F. Thompson, *Arch. Biochem. Biophys.*, **80**, 199 (1959).
30. H. Rinderknecht, D. Thomas and S. Aslin, *Helv. Chim. Acta*, **41**, 1 (1958).
31. P. R. Carnegie, *Biochem. J.*, **89**, 459 (1963).
32. A. I. Virtanen, *Angew. Chem. Internat. Edit.*, **1**, 299 (1962).
33. T. Suzuki, M. Sugii and T. Kakimoto, *Chem. Pharm. Bull. (Tokyo)*, **9**, 77 (1961).
34. E. Otoul, R. Marechal, G. Dardenne and F. Desmedt, *Phytochemistry*, **14**, 173 (1975).
35. R. M. Zacharius, *Phytochemistry*, **9**, 2047 (1970).
36. I. M. Evans and D. Boulter, *Qual. Plant.-Plant Foods Hum. Nutr.*, **24**, 257 (1975).
37. P. J. Whittle, R. H. Smith and A. McIntosh, *J. Sci. Food Agric.*, **27**, 633 (1976).
38. A. F. Gosden, *J. Sci. Food Agric.*, **30**, 892 (1979).
39. A. F. Gosden and T. D. Johnston, in *Brassica Fodder Crops, Proceedings of the Conference* (J. F. Greenhalgh, I. H. McNaughton and R. F. Throw, Eds.) Scottish Development Council and Scottish Plant Breeding Station, pp. 8–12 (1977).
40. E. C. Wolff, S. Black and P. F. Downey, *J. Am. Chem. Soc.*, **78**, 5958 (1956).
41. R. E. Kallio and A. D. Larson, *A Symposium on Amino Acid Metabolism* (Ed. W. D. McElroy and B. Glass) The Johns Hopkins Press, Baltimore, pp. 616–31 (1955).
42. H. Kadota and Y. Ishida, *Ann. Rev. Microbiol.*, **26**, 127 (1972).
43. F. Binkley, *J. Biol. Chem.*, **192**, 209 (1951).
44. E. S. Canellakis and H. Tarver, *Arch. Biochem. Biophys.*, **42**, 387, 446 (1953).
45. M. Sugii, S. Nagasawa and T. Suzuki, *Chem. Pharm. Bull. (Tokyo)*, **11**, 135 (1963).
46. N. Nakamura, *Biochem. Z.*, **164**, 31 (1925).

47. D. V. Richmond, *Phytochemistry,* (Van Nostrand Reinhold, New York, 1973), Vol. 3, Chap. 2, pp. 41–73.
48. J. Giovanelli and S. H. Mudd, *Biochem. Biophys. Res. Commun.,* **31,** 275 (1968).
49. N. M. Kredich and G. M. Tompkins, *J. Biol. Chem.,* **241,** 4955 (1966).
50. J. L. Wiebers and H. R. Garner, *J. Biol. Chem.,* **242,** 5644 (1967).
51. J. F. Thompson and D. P. Moore, *Biochem. Biophys. Res. Commun.,* **31,** 281 (1968).
52. B. Granroth and A. Sarnesto, *Acta Chem. Scand., Ser. B,* **28,** 814 (1974).
53. J. Giovanelli and S. H. Mudd, *Biochem. Biophys. Res. Commun.,* **27,** 150 (1967).
54. J. F. Thompson and R. K. Gering, *Plant Physiol.,* **41,** 1301 (1966).
55. D. M. Chen, S. N. Nigam and W. B. McConnell, *Can. J. Biochem.,* **48,** 1278 (1970).
56. C. M. Chow, S. N. Nigam and W. B. McConnell, *Biochim. Biophys. Acta,* **273,** 91 (1972).
57. H. Kumagai, Y.-J. Choi, S. Sejima and H. Yamada, *Biochem. Biophys. Res. Commun.,* **59,** 789 (1974)
58. H. Yamada and H. Kumagai, *CA.,* **82,** 153761s (1975).
59. S. K. Shapiro, A. Almenas and J. F. Thomson, *J. Biol. Chem.,* **240,** 2512 (1965).
60. R. C. Doney and J. F. Thompson, *Biochim. Biophys. Acta,* **124,** 39 (1966).
61. J. F. Thompson, D. H. Turner and R. K. Gering, *Phytochemistry,* **3,** 33 (1964).
62. W. N. Arnold and J. F. Thompson, *Biochim. Biophys. Acta,* **57,** 604 (1962).
63. T. Mae, K. Ohira and A. Fujiwara, *Plant Cell Physiol.,* **12,** 881 (1971).
64. S. J. Austin and S. Schwimmer, *Enzymologia,* **40,** 273 (1971).
65. S. Schwimmer and S. J. Austin, *J. Food Sci.,* **36,** 807 (1971).
66. G. C. Webster, *Plant Physiol.,* **28,** 728 (1953).
67. G. C. Webster and J. E. Varner, *Arch. Biochem. Biophys.,* **52,** 22 (1954).
68. L. Young and G. A. Maw, *The Metabolism of Sulphur Compounds,* (Methuen, London, 1958), Chap. 2, pp. 34–37.
69. M. M. Barnes, S. P. James and P. B. Wood, *Biochem. J.,* **71,** 680 (1959).
70. M. K. Johnson, *Biochem. J.,* **98,** 38 (1966).
71. E. A. Barnsley and L. Young, *Biochem. J.,* **95,** 77 (1965).
72. M. K. Johnson, *Biochem. J.,* **98,** 44 (1966).
73. M. J. Pabst, W. H. Habig and W. B. Jakoby, *Biochem. Biophys. Res. Commun.,* **52,** 1123 (1973).
74. J. N. Ketley, W. H. Habig and W. B. Jakoby, *J. Biol. Chem.,* **250,** 8670 (1975).
75. W. H. Habig, M. J. Pabst and W. B. Jakoby, *J. Biol. Chem.,* **249,** 7130 (1974).
76. P. L. Grover, *Drug Metab.-Microbe Man Symp., 1976* (Eds. D. V. Parke and R. L. Smith), pp. 105–122 (1977).
77. L. F. Chasseaud, *Adv. Cancer Res.,* **29,** 175 (1978).
78. I. J. G. Climie and D. H. Hutson, In *Advances in Pesticide Science,* Part 3 (Pergamon Press, Oxford, 1979), pp. 537–546.
79. R. M. Hollingworth, *J. Agric. Food Chem.,* **17,** 987 (1969).
80. D. H. Hutson, B. A. Pickering and C. Donninger, *Biochem. J.,* **127,** 285 (1972).
81. M. H. Akhtar and T. S. Foster, *J. Agric. Food Chem.,* **25,** 1017 (1977).
82. F. J. Oppenoorth, L. J. T. Van der Pas and N. W. H. Houx, *Pestic. Biochem. Physiol.,* **11,** 176 (1979).
83. L. Dicowsky and A. Morello, *Life Sci.,* **10,** 1031 (1971).
84. A. Morello, A. Vardanis and E. Y. Spencer, *Biochem. Biophys. Res. Commun.,* **29,** 241 (1967).
85. N. Motoyama and W. C. Dauterman, *Pestic. Biochem. Physiol.,* **2,** 113 (1972).
86. N. Motoyama and W. C. Dauterman, *Pestic. Biochem. Physiol.,* **2,** 170 (1972).
87. M. J. Crawford, D. H. Hutson and P. A. King, *Xenobiotica,* **6,** 745 (1976).
88. J. Fukami and T. Shishido, *J. Econ. Entomol.,* **59,** 1338 (1966).
89. A. G. Clark, J. N. Smith and T. W. Speir, *Biochem. J.,* **135,** 385 (1973).
90. A. G. Clark, P. L. Cropp, J. N. Smith, T. W. Speir and B. J. Tan, *Pestic. Biochem. Physiol.,* **6,** 126 (1976).
91. D. J. Hallett, R. Greenhalgh, P. Weinberger and R. Prasad, *J. Environ. Sci. Health, Part B,* **B12,** 53 (1977).
92. A. Kanazawa, Y. Kakimoto, T. Nakajima and I. Sano, *Biochim. Biophys. Acta,* **111,** 90 (1965).
93. D. J. Pillinger, A. W. Craig and H. Jackson, *Biochem. J.,* **90,** 43P (1964).
94. D. J. Pillinger, B. W. Fox and A. W. Craig, *Isotopes Exp. Pharmacol.,* 415 (1965).
95. C. P. Siegers, A. Schuett and O. Strubelt, *Proc. Eur. Soc. Toxicol.,* **18,** 160 (1977).
96. F. Binkley, *J. Biol. Chem.,* **236,** 1075 (1961).
97. W. B. Rathbun, *Arch. Biochem. Biophys.,* **122,** 73 (1967).
98. A. Meister and S. S. Tate, *Ann. Rev. Biochem.,* **45,** 559 (1976).
99. E. D. Mooz and L. Wigglesworth, *Biochem. Biophys. Res. Commun.,* **68,** 1066 (1975).
100. E. D. Mooz and A. Meister, *Biochemistry,* **6,** 1722 (1967).

101. C. J. Calleman, L. Ehrenberg, S. Osterman-Golkar and D. Segerbaeck, *Acta Chem. Scand., Ser. B,* **B33,** 488 (1979).
102. K. Yamauchi, T. Sugimae and M. Kinoshita, *Tetrahedron Lett.,* 1199 (1977).
103. H. Jackson and A. R. Jones, *Nature,* **220,** 591 (1968).
104. R. Schoental and D. J. Rive, *Biochem. J.,* **97,** 466 (1965).
105. P. B. Farmer, E. Bailey, J. H. Lamb and T. A. Connors, *Biomed. Mass Spectrom.,* **7,** 41 (1980).
106. R. Van Doorn, P. J. A. Borm, C. M. Leijdekkers, P. T. Henderson, J. Reuvers and T. J. Van Bergen, *Int. Arch. Occup. Environ. Health,* **46,** 99 (1980).
107. M. Redford-Ellis and A. H. Gowenlock, *Acta Pharmacol. Toxicol.,* **30,** 36, 49 (1971).
108. D. H. Hutson and E. C. Hoadley, *Xenobiotica,* **2,** 107 (1972).
109. C. J. Foxwell and L. Young, *Biochem. J.,* **92,** 50P (1964).
110. T. Suga, H. Kumaoka and M. Akagi, *J. Biochem. (Tokyo),* **60,** 133 (1966).
111. S. S. Tate and A. Meister, *J. Biol. Chem.,* **249,** 7593 (1974).
112. S. Silbernagl, W. Pfaller, H. Heinle and A. Wendel, *Funct. Glutathione Liver Kidney (Pap. Konf. Ges. Biol. Chem.) 25th,* pp. 60–69 (1978).
113. S. S. Tate and A. Meister, *J. Biol. Chem.,* **250,** 4619 (1975).
114. T. M. McIntyre and N. P. Curthoys, *J. Biol. Chem.,* **254,** 6499 (1979).
115. G. Semenza, *Biochim. Biophys. Acta,* **24,** 401 (1957).
116. F. Binkley, J. Davenport and F. Eastall, *Biochem. Biophys. Res. Commun.,* **1,** 206 (1959).
117. V. Du Vigneaud, H. M. Dyer and J. Harmon, *J. Biol. Chem.,* **101,** 719 (1933).
118. C. R. Grau and H. J. Almquist, *J. Nutr.,* **26,** 631 (1943).
119. R. J. Block and R. W. Jackson, *J. Biol. Chem.,* **97,** cvi (1932).
120. M. D. Armstrong and J. D. Lewis, *J. Biol. Chem.,* **189,** 461 (1951).
121. E. Brand, R. J. Block and G. F. Cahill, *J. Biol. Chem.,* **119,** 689 (1937).
122. H. J. Channon, M. C. Manifold and A. P. Platt, *Biochem. J.,* **34,** 866 (1940).
123. M. J. Raymond and C. R. Treadwell, *Proc. Soc. Exptl. Biol. Med.,* **70,** 43 (1949).
124. N. J. Benevenga, M. H. Yeh and J. J. Lalich, *J. Nutr.,* **106,** 1714 (1976).
125. G. G. Chang, T. Z. Lin, T. C. Yu, J. Y. Guoo and F. Pan, *J. Chin. Biochem. Soc.,* **4,** 10 (1975).
126. J. M. Old and D. S. Jones, *Biochem. J.,* **159,** 503 (1976).
127. F. Pan, T. C. Yu, G. M. Duh and H. H. Lee, *J. Chin. Biochem. Soc.,* **5,** 45 (1976).
128. A. S. Schultz and D. K. McManus, *Arch. Biochem. Biophys.,* **25,** 401 (1950).
129. D. Margolis and R. J. Block, *Contribs. Boyce Thompson Inst.,* **19,** 437 (1958).
130. G. A. Maw, *J. Inst. Brew.,* **66,** 162 (1960).
131. G. A. Maw, *J. Inst. Brew.,* **67,** 57 (1961).
132. R. B. Roberts, P. H. Abelson, D. B. Cowie, E. T. Bolton and R. J. Britten, *Studies of Biosynthesis in Escherichia coli,* (Carnegie Institute of Washington, Washington, 1955).
133. J. W. Daniel and K. Babcock, *J. Bacteriol.,* **92,** 1028 (1966).
134. S. Tokuno, B. Strauss and Y. Tsuda, *J. Gen. Microbiol.,* **28,** 481 (1962).
135. J. L. Wiebers and H. R. Garner, *J. Bacteriol.,* **88,** 1798 (1964).
136. D. P. Moore, J. F. Thompson and I. K. Smith, *Biochim. Biophys. Acta,* **184,** 124 (1969).
137. L. E. Weller, R. W. Luecke and H. M. Sell, *Proc. Soc. Exptl. Biol. Med.,* **77,** 3 (1951).
138. G. A. Maw, *J. Gen. Microbiol.,* **25,** 441 (1961).
139. P. R. Adiga, K. S. Sastry and P. S. Sarma, *J. Gen. Microbiol.,* **29,** 149 (1962).
140. P. R. Adiga and P. S. Sarma, *Indian J. Biochem. Biophys.,* **7,** 141 (1970).
141. P. R. Adiga and P. S. Sarma, *Indian J. Biochem. Biophys.,* **8,** 9 (1971).
142. G. A. Maw, *J. Gen. Microbiol.,* **31,** 247 (1963).
143. G. A. Maw, *Wallerstein Lab. Commun.,* **28,** 49 (1965).
144. R. Lallier, *Acta Embryol. Morphol. Exp.,* **8,** 12 (1965).
145. H. M. Harrington and I. K. Smith, *Plant Physiol.,* **60,** 807 (1977).
146. R. C. Doney and J. F. Thompson, *Phytochemistry,* **10,** 1745 (1971).
147. S. Mitsuhashi, *Jap. J. Exptl. Med.,* **20,** 211 (1949).
148. J. Nomura, Y. Nishizuka and O. Hayaishi, *J. Biol. Chem.,* **238,** 1441 (1963).
149. M. Flavin and C. Slaughter, *Biochim. Biophys. Acta,* **132,** 406 (1967).
150. J. P. Zikarkis and R. L. Salsbury, *J. Dairy Sci.,* **52,** 2014 (1969).
151. F. Challenger and P. T. Charlton, *J. Chem. Soc.,* **424** (1947).
152. D. P. Moore and J. F. Thompson, *Biochem. Biophys. Res. Commun.,* **28,** 474 (1967).
153. I. K. Smith and J. F. Thompson, *Biochim. Biophys. Acta,* **184,** 130 (1969).
154. H. Cherest, G. Talbot and H. de Robichon-Szulmajster, *J. Bacteriol.,* **102,** 448 (1970).
155. R. Gmelin, G. Hasenmaier and G. Strauss, *Z. Naturforsch.,* **12b,** 687 (1957).
156. S. Schwimmer and A. Kjaer, *Biochim. Biophys. Acta,* **42,** 316 (1960).

157. F. Binkley, *J. Biol. Chem.*, **186,** 287 (1950).
158. M. Flavin and C. Slaughter, *Biochim. Biophys. Acta,* **132,** 400 (1967).
159. S. Mitsuhashi and Y. Matsuo, *Jap. J. Exptl. Med.*, **20,** 641 (1950).
160. F. Murakami, *Bitamin,* **20,** 126 (1960).
161. M. Mazelis and R. K. Creveling, *Biochem. J.*, **147,** 485 (1975).
162. M. Mazelis and L. Fowden, *Phytochemistry,* **12,** 1287 (1973).
163. Y. Matsuo and D. M. Greenberg, *J. Biol. Chem.*, **234,** 516 (1959).
164. J. Loiselet and F. Chatagner, *Bull. Soc. Chim. Biol.*, **47,** 33 (1965).
165. C. Delavier-Klutchko and M. Flavin, *Biochim. Biophys. Acta,* **99,** 377 (1965).
166. M. Flavin and A. Segal, *J. Biol. Chem.*, **239,** 2220 (1964).
167. J. Giovanelli and S. H. Mudd, *Biochim. Biophys. Acta,* **227,** 654 (1971).
168. H. Tanaka, N. Esaki and K. Soda, *Biochemistry,* **16,** 100 (1977).
169. Y. Morino and E. E. Snell, *J. Biol. Chem.*, **242,** 2793 (1967).
170. J. L. Cowell, K. Moser and R. D. DeMoss, *Biochim. Biophys. Acta,* **315,** 449 (1973).
171. D. D. Whitt, M. J. Klug and R. D. DeMoss, *Arch. Microbiol.*, **122,** 169 (1979).
172. H. Kumagai and E. W. Miles, *Biochem. Biophys. Res. Commun.*, **44,** 1271 (1971).
173. G. M. Carman and R. E. Levin, *J. Food Biochem.*, **1,** 285 (1977).
174. H. Enei, H. Matsui, S. Okumura and H. Yamada, *Agric. Biol. Chem.*, **36,** 1869 (1972).
175. A. Stoll and E. Seebeck, *Adv. Enzymol.*, **11,** 377 (1951).
176. S. Tsuno, *Bitamin,* **14,** 659 (1958).
177. S. Schwimmer, J. F. Carson, R. V. Makower, M. Mazelis and F. F. Wong, *Experientia,* **16,** 449 (1960).
178. F. P. Kupiecki and A. I. Virtanen, *Acta Chem. Scand.*, **14,** 1913 (1960).
179. M. Mazelis and L. Crews, *Biochem. J.*, **108,** 725 (1968).
180. J. V. Jacobsen, Y. Yamaguchi, L. K. Mann, F. D. Howard, and R. A. Bernhard, *Phytochemistry,* **7,** 1099 (1968).
181. M. Mazelis, *Phytochemistry,* **2,** 15 (1963).
182. M. Mazelis, N. Beimer and R. K. Creveling, *Arch. Biochem. Biophys.*, **120,** 371 (1967).
183. S. Schwimmer, C. A. Ryan and F. F. Wong, *J. Biol. Chem.*, **239,** 777 (1964).
184. G. L. Cantoni, *J. Biol. Chem.*, **189,** 745 (1951).
185. R. Cox and R. C. Smith, *Arch. Biochem. Biophys.*, **129,** 615 (1969).
186. R. T. Borchardt, Y. S. Wu, J. A. Huber and A. F. Wycpalek, *J. Med. Chem.*, **19,** 1104 (1976).
187. F. Schlenk and C. R. Zydek, *Arch. Biochem. Biophys.*, **123,** 438 (1968).
188. T. Mae, K. Ohira and A. Fujiwara, *Plant Cell Physiol.*, **13,** 407 (1972).
189. T. Mae and K. Ohira, *Plant Cell Physiol.*, **17,** 459 (1976).
190. N. W. Pirie, *Biochem. J.*, **26,** 2041 (1932).
191. V. Du Vigneaud, H. S. Loring and H. A. Craft, *J. Biol. Chem.*, **105,** 481 (1934).
192. N. M. Sklan and E. A. Barnsley, *Biochem. J.*, **107,** 217 (1968).
193. G. A. Maw, *Biochem. J.*, **58,** 665 (1954).
194. E. S. Canellakis and H. Tarver, *Arch. Biochem. Biophys.*, **42,** 387 (1953).
195. W. H. Horner and E. J. Kuchinskas, *J. Biol. Chem.*, **234,** 2935 (1959).
196. E. J. Kuchinskas, *Arch. Biochem. Biophys.*, **112,** 605 (1965).
197. E. J. Kuchinskas, *Arch. Biochem. Biophys.*, **112,** 610 (1965).
198. G. L. Case and N. J. Benevenga, *J. Nutr.*, **107,** 1665 (1977).
199. G. A. Maw and C. M. Coyne, *Arch. Biochem. Biophys.*, **117,** 499 (1966).
200. A. J. L. Cooper, *J. Biol. Chem.*, **252,** 2032 (1977).
201. F. Van Leuven, *Eur. J. Biochem.*, **65,** 271 (1976).
202. E. A. Barnsley, *Biochim. Biophys. Acta,* **90,** 24 (1964).
203. N. J. Benevenga, M. H. Yeh and J. J. Lalich, *J. Nutr.*, **106,** 1714 (1976).
204. G. G. Tedeschi and D. Filippi, *Boll. Soc. Ital. Biol. Sper.*, **39,** 1530 (1963).
205. H. Uchino and Y. Itokawa, *Nippon Eiseigaku Zasshi,* **27,** 248 (1972). (*CA.*, **78,** 93269t (1973)).
206. H. Uchino and Y. Itokawa, *Nippon Eiseigaku Zasshi,* **27,** 253 (1972). (*CA.*, **78,** 93270m (1973)).
207. H. Uchino, *Nippon Eiseigaku Zasshi,* **30,** 397 (1975). (*CA.*, **84,** 39514e (1976)).
208. Y. Itokawa, H. Uchino and N. Nishino, *Eiyo To Shokuryo,* **24,** 481 (1971). (*CA.*, **77,** 14029t (1972)).
209. K. Matsumoto, Y. Itokawa and M. Fujiwara, *Eiyo To Shokuryo,* **24,** 249 (1971). (*CA.*, **75,** 139265g (1971)).
210. M. Fujiwara, Y. Itokawa, H. Uchino and K. Inoue, *Experientia,* **28,** 254 (1972).
211. Y. Itokawa, K. Inoue, S. Sasagawa and M. Fujiwara, *J. Nutr.*, **103,** 88 (1973).
212. R. H. Smith, *Rep. Rowett Inst.*, **30,** 112 (1974).
213. G. Rosenberger, *Dt. tierarztl. Wschr.* **47,** 244 (1939); *ibid,* **51,** 63 (1943).
214. J. F. D. Greenhalgh, *Proc Nutr. Soc.*, **28,** 178 (1969); In *The Future of Brassica Fodder Crops,* (Eds. J. F. D. Greenhalgh and M. Hamilton), *Occ. Publ. Rowett Res. Inst. No. 2,* p. 56 (1971).

215. R. H. Smith and J. F. D. Greenhalgh, *In Brassica Fodder Crops,* (Eds. J. F. D. Greenhalgh, I. H. McNaughton and R. F. Throw), Scottish Agricultural Development Council, D.A.F.S., Edinburgh, pp. 96–101 (1977).
216. R. H. Smith, *Veterin. Sci. Commun.*, **2,** 47 (1978).
217. W. O. Kermack and N. A. Matheson, *Biochem. J.*, **65,** 45, 48 (1957).
218. R. Vince and W. B. Wadd, *Biochem. Biophys. Res. Commun.*, **35,** 593 (1969).
219. J. A. Lewis and G. C. Papavizas, *Soil Biol. Biochem.*, **2,** 239 (1970).
220. A. H. Baur and S. F. Yang, *Phytochemistry,* **11,** 3207 (1972).
221. D. O. Adams and S. F. Yang, *Proc. Natl. Acad. Sci. USA,* **76,** 170 (1979).
222. R. J. Evans and S. L. Bandemer, *J. Agric. Food Chem.*, **15,** 439 (1967).
223. J. E. Snoke, and K. Bloch, *J. Biol. Chem.*, **199,** 407 (1952).
224. G. C. Webster, *Arch. Biochem. Biophys.*, **47,** 241 (1953).