Phytochemistry, Vol. 21, No. 12, pp. 2771–2781, 1982.
Printed in Great Britain.

0031-9422/82/122771-11$05.50/0

REVIEWS

GLUTATHIONE METABOLISM AND POSSIBLE BIOLOGICAL ROLES IN HIGHER PLANTS

HEINZ RENNENBERG

Botanisches Institut der Universität Köln, Gyrhofstr. 15, D–5000 Köln 41, West Germany

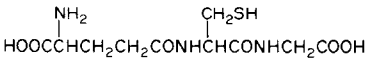
(*Revised received* 12 *May* 1982)

Key Word Index—Glutathione; sulphur metabolism; hydrogen peroxide; long-distance transport; pesticides.

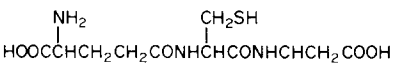
Abstract—Synthesis of glutathione in plants seems to proceed in the same series of enzyme catalysed reactions observed in animal cells; the pathway of glutathione degradation, however, has not yet completely been eludicated in plants. Whereas γ-glutamylcyclotransferase and 5-oxo-prolinase activity seem to be involved in degradation of glutathione in plants, the participation of a γ-glutamyltranspeptidase is uncertain. First observations indicate a separation of glutathione synthesis and degradation by compartmentation in green cells. As the reaction catalysed by 5-oxo-prolinase is regulated by sulphur nutrition, it might be the rate limiting step in glutathione catabolism in plants as observed in animal cells. From investigations with plant tissue cultures and from transport studies, glutathione appears to be the main long-distance transport form of reduced sulphur translocated from mature leaves to the roots and to other parts of the plant. In addition, glutathione seems to be used as a storage form of reduced sulphur in plant cells supplied with excess inorganic sulphur. Whereas a role of glutathione in the detoxification of pesticides can be regarded as definitely established, a function of this peptide in the detoxification of hydrogen peroxide in chloroplasts is evident from biochemical investigations only. Further studies are needed to show whether glutathione is indeed used as reductant for the detoxification of hydrogen peroxide in chloroplasts *in vivo*.

INTRODUCTION

Although the tripeptide glutathione (γ-L-glutamyl-L-cysteinylglycine) (**1**) is thought to be a constituent of all living cells [1–3], convincing evidence for its wide distribution has not been established in the Plant Kingdom (cf. ref. [4]). Plant cells contain considerable amounts of free, low MW thiol, and it is generally assumed that glutathione is a major component of this fraction; however, in most of the investigations performed only the free thiol content was measured, although the determination of glutathione was claimed. An unequivocal proof by rigorous chemical methods that glutathione is indeed the compound determined, has only been achieved for a few plant species (cf. ref. [4]). In several of these species glutathione seems to be the major free thiol, and the incorporation of sulphide into glutathione seems to be a major path of reduced sulphur; in tobacco cells, cultured for 16 days under photoheterotrophical conditions up to 40% of the sulphur offered to the cells as sulphate is found to be present in glutathione [5]. Cucumber leaf discs exogenously supplied with sulphate incorporate 46–74% of the sulphur reduced by the cells into glutathione [6]; in spinach leaf discs 64–83% of the low MW thiol was confined to glutathione [7]; and in leaves of trees up to 95% of this fraction accounted for this peptide [8]. However, glutathione does not appear to be an essential component of plant cells. Most of the Gram-positive bacteria tested contain, if any, only small amounts of glutathione, although high amounts of free, low MW thiol have been demonstrated in these organisms [9]. The chemical nature of the free thiol in Gram-positive bacteria has only been analysed in *Bacillus megaterium*, where coenzyme A has shown to be the main contributor to this fraction [10]. Also in higher plants, glutathione is not generally the major free, low MW thiol. Price [11] has shown that several legumes, namely *Phaseolus vulgaris*, *P. limensis*, *Glycine max* and *Trifolium repens* contain a free thiol different from glutathione and suggested the name 'phaseothione' for this compound. 'Phaseothione' has been isolated from seedlings of *Phaseolus aureus* [12] and characterized as γ-L-glutamyl-L-cysteinyl-β-alanine



(1) γ-L-Glu-L-cys-gly
reduced glutathione (GSH)



(2) γ-L-Glu-L-cys-β-ala
reduced homoglutathione (homo-GSH)

(homoglutathione) (2) [13]. In all species where homoglutathione was present it was found to be the major free, non-protein thiol, whereas at most trace amounts of glutathione were detected [11]. Twenty-five other legumes investigated, however, lacked measurable amounts of homoglutathione, but contained instead substantial amounts of glutathione [11]. Therefore, homoglutathione (2) does not appear to be the general free, non-protein thiol in leguminous plants. As it cannot be excluded that homoglutathione is also present in non-leguminous plants, further investigations of thiol containing γ-glutamyl peptides and other free, low MW thiols are necessary to establish their degree of heterogeneity and their distribution among plants. Recently, a fast and sensitive HPLC method with high resolution for low MW thiols has been developed by Fahey *et al.* [14–16]; therefore, considerable progress in this area can be expected during the coming years.

Despite these difficulties in the interpretation of the available data, an estimation of the concentration of glutathione in subcellular compartments of plant tissues can be performed. Glutathione concentrations of *ca* 100 μM have been reported for various plants, although concentrations as high as 700 μM have been determined [5, 7, 17–20]. In chloroplasts, however, glutathione concentrations of 1–3.5 mM have been found [21–23]. Bergmann and Ulbrich [23,24] analysed the glutathione content of protoplasts, vacuoles and chloroplasts isolated from tobacco mesophyll cells that contained glutathione in concentrations of *ca* 100 μM/kg fr. wt. 76% of the glutathione in the protoplasts were found in the chloroplasts, 17% in the vacuoles and 7% in the cytoplasm. Considering that the vacuole accounts for more than 85% of the volume of the protoplasts and that the chloroplasts account for 25% of the volume of the protoplasm, it can be calculated that the concentration of glutathione is in the range of 20 μM in vacuoles, 60 μM in the cytoplasm, and 2000 μM in chloroplasts of tobacco mesophyll cells. These data suggest that there might be a considerable concentration gradient for glutathione between cytoplasm and vacuole, and that there is a high concentration gradient for this peptide between chloroplasts and cytoplasm. The present review deals with recent developments in our knowledge of glutathione metabolism and functions in higher plants, with special reference to the question of whether the compartmentation of glutathione inside plant cells can be related to a compartmentation of glutathione metabolism and to possible physiological roles of this peptide.

GLUTATHIONE METABOLISM

Biosynthesis of glutathione

Synthesis of glutathione in animal and bacterial cells as well as in yeast has been shown to be a two-step process (cf. ref. [3]). In the first step, the dipeptide γ-L-glutamyl-L-cysteine is synthesized by the γ-glutamylcysteine synthetase (EC 6.3.2.2) in an ATP-dependent reaction from L-glutamate and L-cysteine. In the second step, glycine is added to the *C*-terminal end of this dipeptide to yield glutathione. This reaction is catalysed by the glutathione synthetase (EC 6.3.2.3) and is also dependent on ATP (Fig. 1). Green plants most likely synthesize glutathione by the same two-step process. In *Chlorella sorokiniana*, supplied with ^{35}S-sulphate, γ-L-glutamyl-L-cysteine was found to be radioactively labeled with kinetics consistent with a function as an intermediate in glutathione synthesis [25]. Comparable investigations with higher plants have not been published, but γ-L-glutamyl-L-cysteine, or the corresponding disulphide, has been isolated from wheat germ [26], garlic [27] and seeds of chives [28]. The existence of a γ-glutamylcysteine synthetase is implicated by the capability of homogenates from corn roots [18, 29] and tobacco suspension cultures [30, 31] to synthesize glutathione from the constituent amino acids in the presence of Mg^{2+} and ATP *in vitro*. The enzyme has been demonstrated in seedlings of *Phaseolus vulgaris* [32], wheat germ [33] and cultured tobacco cells [Bergmann, L., personal communication]. Whether the presence of γ-glutamylcysteine synthetase in *Phaseolus vulgaris* indicates that the formation of γ-L-glutamyl-L-cysteine is also a step in homoglutathione biosynthesis has not been investigated.

The catalytic properties of plant γ-glutamylcysteine synthetase have only been analysed in a 50-fold purified enzyme preparation from wheat germ [33]. The enzyme exhibited optimal activity in the presence of Mg^{2+} and K^+ at pH 7.5. The affinity for the substrates (L-glutamate, K_m 1.2 mM; L-cysteine, K_m 4.5 mM; ATP, K_m 1.4 mM) of the wheat germ enzyme are comparable with those observed in highly purified kidney enzyme preparations (cf. ref. [3]). The reaction mechanism of γ-glutamylcysteine synthetase and the regulation of this enzyme have been extensively studied with enzyme preparations from animal cells (cf. ref. [3]). Animal γ-glutamylcysteine synthetase does not catalyse ATP/ADP or ATP/P_i exchanges. It is inhibited by methionine sulphoximine, similar to glutamine synthetase, in a reaction associated with the synthesis of methionine sulphoximine phosphate. This compound appears to be a transition state analogue in the reaction of γ-L-glutamylphosphate with L-cysteine on the enzyme, indicating that the formation of γ-L-glutamylphosphate is part of the enzymes normal catalytic mechanism. As the synthesis of glutathione from the constituent amino acids in homogenates from cultured tobacco cells is also inhibited by methionine sulphoximine [31], biosynthesis of γ-L-glutamyl-L-cysteine in plants might proceed via enzyme-bound γ-L-glutamylphosphate as in animal cells. Webster and Varner [33] proposed on the basis of ATP/ADP exchange studies that wheat germ γ-glutamylcysteine synthetase initially reacts with ATP to form a phosphorylated enzyme, which subsequently reacts with L-glutamate to yield a γ-glutamyl–enzyme intermediate. However, the observed ATP/ADP exchange could be due to contaminating enzymes in the γ-glutamylcysteine synthetase preparation. In purified γ-glutamylcysteine synthetase preparations from rat kidney non-allosteric feedback inhibition by glutathione (K_i 2.3 mM) has been observed [34]. In homogenates from tobacco cells synthesis of glutathione from the constituent amino acids is inhibited to 50% by glutathione concentrations of 30 μM [Bergmann, L.,

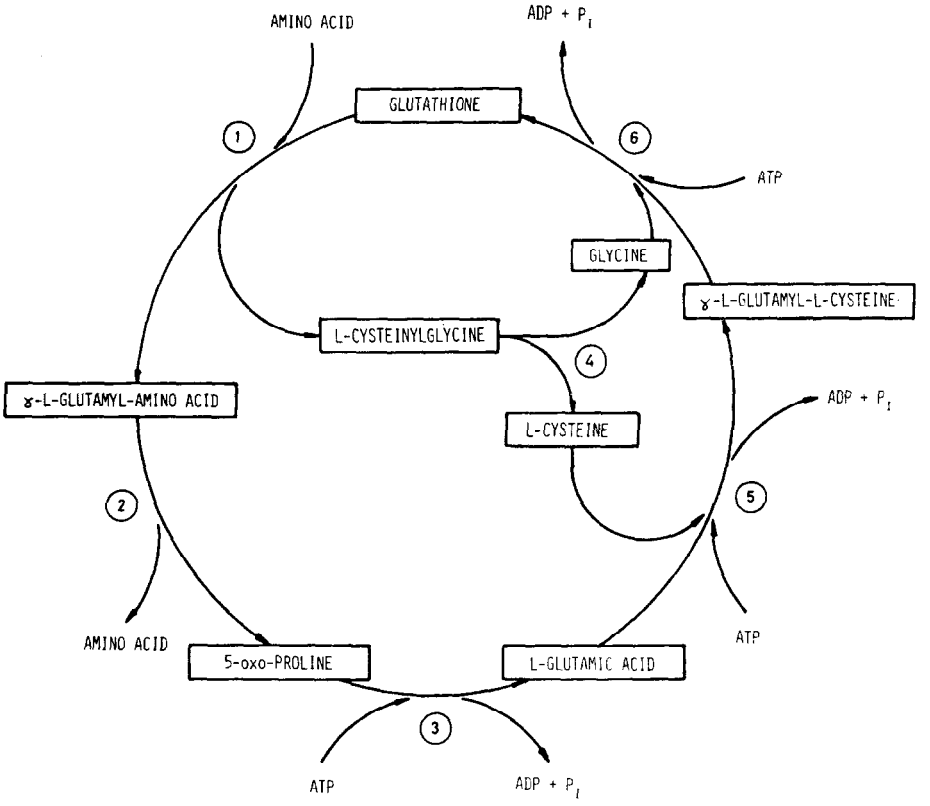


Fig. 1. Synthesis and degradation of glutathione in the γ-glutamyl-cycle. 1, γ-Glutamyl transpeptidase; 2, γ-glutamyl cyclotransferase; 3, 5-oxo-prolinase; 4, dipeptidase; 5, γ-glutamylcysteine synthetase; 6, glutathione synthetase.

personal communication] under standard conditions [30, 31]. Therefore, tobacco γ-glutamylcysteine synthetase appears to be about 75-fold more sensitive to the feedback inhibitor glutathione than the rat kidney enzyme.

Little is known about glutathione synthetase in plants. The enzyme has been demonstrated in acetone powder of seedlings from *Phaseolus vulgaris* [35] and in homogenates of tobacco cells [Bergmann, L., personal communication]. As for the γ-glutamylcysteine synthetase, the presence of Mg^{2+} and K^+ is necessary to obtain optimal enzyme activity. Whether the finding of glutathione synthetase in *Phaseolus vulgaris* [35] does mean that this enzyme is also participating in the synthesis of homoglutathione remains unresolved.

Green tobacco cells cultured under photoheterotrophical conditions release up to 30 times as much glutathione into their culture medium as chloroplast-free, heterotrophically growing suspensions [5]. After transferring green tobacco cells into darkness, the efflux of glutathione proceeds for several days at a high rate, although the capability of the cells to perform photosynthesis is reduced by more than 50% within 48 hr [5; Bergmann, L., personal communication]. Efflux of glutathione stops when the ultrastructure of the chloroplasts of the transferred cells exhibits a clear differentiation towards etioplasts–leucoplasts [5; Bergmann, L., personal communication]. Thus, synthesis and efflux of high amounts of glutathione into the medium of cultured tobacco cells seems to be restricted to chloroplast-containing cells without depending on photosynthesis. These data indicate that synthesis of glutathione in green tobacco cells at least partially proceeds in the chloroplasts. This conclusion is supported by the observation of a fast incorporation of 'rapidly turning over', soluble L-cysteine into glutathione in *Chlorella sorokiniana* and *Lemna paucicostada* [25, 36].

When crude plastid preparations of green and chloroplast-free tobacco cells in suspensions culture were homogenized and centrifuged, the supernatant fractions of both preparations exhibited comparable capabilities to synthesize glutathione from the constituent amino acids. Upon treatment of the pellets with EDTA additional activity to synthesize glutathione *in vitro* was solubilized, accounting for 70–75% of the total activity to catalyse glutathione synthesis in chloroplast preparations, but for only 30% in chloroplast-free plastid preparations [Bergmann, L., personal communication]. Thus, the membrane-bound activity to catalyse glutathione synthesis is considerably higher in chloroplast preparations than in chloroplast-free plastid preparations, whereas both fractions do not differ significantly in their proportions of soluble catalytic activity. The percentage of the cells total activity to synthesize glutathione present in the chloroplasts is not yet known;

however, an enhanced amount of membrane-bound enzymes for glutathione synthesis seems to allow green tobacco cells a high production and efflux of glutathione [5], probably because of the special proximity to the pathway of sulphate assimilation into L-cysteine [37, 38]. The high concentration of glutathione and the observed feedback inhibition of γ-glutamylcysteine synthetase by much smaller glutathione concentrations [Bergmann, L., personal communication] should prevent the synthesis of large amounts of glutathione in chloroplasts *in vivo*. However, the real impact of the glutathione concentration in chloroplasts on the regulation of glutathione synthesis is unclear, since the sensitivity of the chloroplast membrane-bound γ-glutamylcysteine synthetase to the feedback inhibitor glutathione has still to be elucidated.

Although the steady state concentration of L-cysteine in plant cells has not been extensively studied, the available data indicate concentrations considerably smaller than the apparent K_m value of γ-glutamylcysteine synthetase (4.5 mM [33]) for this amino acid [17, 25, 36, 39], especially if the observation that less than 2% of the cell's total soluble L-cysteine constitutes the rapidly turning-over pool of L-cysteine [25, 36] is taken into consideration. Therefore, the rate of glutathione synthesis *in vivo* may be significantly influenced by the intracellular L-cysteine concentration, as observed in animal cells [40].

Degradation of glutathione

In animal cells glutathione is assumed to be degraded in the series of steps outlined in Fig. 1 [3, 41]. In this pathway the γ-glutamyl moiety of glutathione is transferred by a γ-glutamyl transpeptidase (EC 2.3.2.2) to an amino acid acceptor. Whereas the remaining L-cysteinylglycine is hydrolysed by a dipeptidase (EC 3.4.13.6), the γ-glutamyl moiety of the γ-glutamyl amino acid is cyclized by a γ-glutamyl cyclotransferase (EC 2.3.2.4) to 5-oxo-proline, the cyclic lactam of glutamic acid. The 5-oxo-proline synthesized in this way is hydrolysed in an ATP-dependent reaction to glutamic acid. Hydrolysis of 5-oxo-proline, catalysed by 5-oxo-prolinase, seems to be the rate limiting step in degradation of glutathione in animal cells [41, 42]. From this pathway of glutathione degradation and the path of glutathione synthesis Meister *et al.* defined the γ-glutamyl cycle (Fig. 1), and proposed that the uptake of amino acids via membrane-bound γ-glutamyl transpeptidase is the main function of this cycle [3, 41, 43–45]. Despite intensive work on this model, including estimation of glutathione turnover, experiments with labeled metabolites, *in vivo* and *in vitro* studies with specific inhibitors of enzymes of the cycle and investigations of specific enzyme deficiencies (cf. refs. [3, 41]), such a function of the γ-glutamyl cycle in animal cells has still to be considered as tentative. Most of the criticism of the cycle is related to the function of the γ-glutamyl transpeptidase *in vivo*. In animals, γ-glutamyl transpeptidase is predominantly localized in epithelial cells which are involved in transport phenomena [41, 46]. The major source of γ-glutamyl transpeptidase is the brush border membrane of the proximal tubule of kidney [47]. This membrane also contains substantial amounts of aminopeptidase M, which can hydrolyse L-cysteinylglycine and its *S*-substituted derivatives (see below), and another peptidase capable of hydrolysis of the reduced, as well as the oxidized, form of this dipeptide [46, 48]. From the physical properties, especially the large carbohydrate content [49], of γ-glutamyl transpeptidase and from experiments on the localization of γ-glutamyl transpeptidase and aminopeptidase M (cf. ref. [46]), it has been suggested that the catalytic domains of both enzymes are restricted to one side of the brush border membrane, most likely the exterior surface. Therefore, it was concluded that γ-glutamyl transpeptidase is predominantly involved in the utilization of extracellular glutathione. Animal, as well as plant, γ-glutamyl transpeptidase is able to catalyse both transpeptidation and hydrolysis of the γ-glutamyl moiety of glutathione *in vitro* [50–52]. However, comparison of the apparent K_m values and pH optima for transpeptidation and hydrolysis [50, 53] with the glutathione concentration in the plasma and the pH in the proximal tubule [54] strongly suggests that hydrolysis rather than transpeptidation takes place during utilization of extracellular glutathione in kidney. This conclusion is supported by the observation that radioactively labeled glutathione is rapidly degraded in the lumen of kidney tubules in the absence of amino acid acceptors [55]. Although these observations provide evidence against a function of the γ-glutamyl cycle in degradation of extracellular glutathione, this cycle might still be responsible for the turnover of glutathione inside cells. However, there is indeed another plausible pathway of glutathione degradation that does not require the action of a γ-glutamyl transpeptidase and a dipeptidase, but carboxypeptidase activity (Fig. 2). In this pathway, first the glycine moiety, rather than the γ-glutamyl moiety is split from glutathione by a carboxypeptidase; the remaining γ-L-glutamyl-L-cysteine may then be further degraded to L-cysteine and L-glutamate via 5-oxo-proline as suggested in the γ-glutamyl cycle. There is indeed evidence that this alternative pathway might be responsible for at least the degradation of glutathione conjugates in plants (see below). The information available about the degradation of glutathione itself in plants is so far insufficient to indicate whether this alternative cycle or the γ-glutamyl cycle is responsible for catalysing this process.

The γ-glutamyl transpeptidase activity is not only widely distributed among animals and microorganisms (cf. ref. [3]), but also has been detected in higher plants (cf. ref. [4]). Several roles have been proposed for the plant enzyme. However, data concerning a participation of γ-glutamyl transpeptidase in degradation of glutathione inside plant cells have not been published. A function of γ-glutamyl transpeptidase in degradation of extracellular glutathione, as suggested for animal cells (cf. refs. [3, 46]), appears to be very unlikely in plants, as glutathione is taken up by plant cells as the intact molecule [24, 56], and is not degraded extracellularly to its constituent amino acids. Many plants contain high amounts of γ-glutamyl compounds, mainly γ-glutamyl derivatives of amino acids and amines (cf. ref. [4]). Therefore, it has been suggested that plant γ-glutamyl transpeptidase plays an important role in

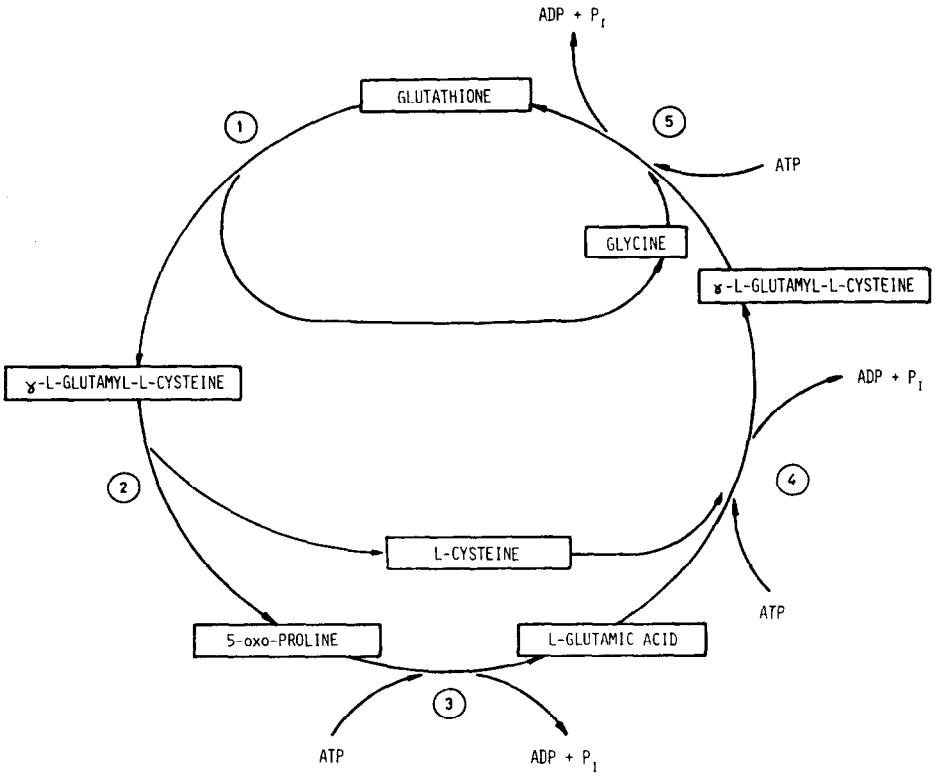


Fig. 2. Synthesis and degradation of glutathione in an alternative cycle. 1, Carboxypeptidase; 2, γ-glutamyl cyclotransferase; 3, 5-oxo-prolinase; 4, γ-glutamylcysteine synthetase; 5, glutathione synthetase.

the biosynthesis of γ-glutamyl dipeptides [57]. As plant γ-glutamyl transpeptidases have been reported to catalyse hydrolytic cleavage of γ-glutamyl residues (cf. ref. [4]), a participation in the degradation of γ-glutamyl derivatives has also to be considered. As outlined in a recent review [4], transpeptidation using glutathione as γ-glutamyl donor seems to be responsible for the formation of several γ-glutamyl peptides in plants; however, it is impossible with our present knowledge to generally explain the presence of γ-glutamyl compounds by γ-glutamyl transpeptidase activity. Thus, so far neither a role of plant γ-glutamyl transpeptidase in the degradation of glutathione inside or outside plant cells, nor a general function of this enzyme in biosynthesis and degradation of other γ-glutamyl compounds has been established.

The fate of glutathione in plant cells has been investigated in tobacco suspension cultures [56, 58], supplied with this peptide as sole sulphur source. Under these conditions, glutathione was utilized by the cells as sulphur source for protein synthesis [56]. Therefore, degradation of glutathione to the constituent amino acids has to be assumed to make the sulphur moiety of glutathione available for protein synthesis. It is evident from feeding experiments with ^{35}S-glutathione, in the presence of methionine sulphoximine concentrations inhibiting the *de novo* synthesis of this peptide [31], that glutathione is taken up by tobacco cells as an intact molecule. Degradation of glutathione must therefore take place inside the tobacco cells [56]. When photoheterotrophic tobacco suspensions, precultured under sulphur starvation conditions, were exposed to glutathione specifically labeled in the γ-glutamyl moiety of the peptide, a substantial amount of the radioactivity inside the cells was found in 5-oxo-proline as well as in glutamate. When glutamic acid labeled glutathione was added to cell homogenates prepared from green tobacco cells, labeled 5-oxo-proline was again detected [58]. These observations indicate that in tobacco cells the γ-glutamyl moiety of glutathione is cyclized to 5-oxo-proline, which is subsequently converted to glutamate. They are consistent with both the path of glutathione degradation via the γ-glutamyl cycle (Fig. 1) and via the alternative cycle in Fig. 2.

These results are the only indications of the existence of a γ-glutamylcyclotransferase in plants; L-cysteinylglycine-specific dipeptidase activity has not yet been reported in plants. However, plant 5-oxo-prolinase has been extensively studied and is by far the best characterized enzyme of glutathione metabolism in plant cells. In 1976 Mazelis and Pratt [60] reported the *in vivo* conversion of 5-oxo-proline to glutamic acid by detached leaves of several plant species. Subsequently, the existence of 5-oxo-prolinase was demonstrated in a broad spectrum of plants and the properties of a 59-fold purified enzyme from wheat germ was analysed in the same laboratory [61]. In addition, the catalytic properties and the subcellular localization of tobacco 5-oxo-prolinase has been studied [62]. From these investigations we know that plant 5-oxo-prolinase is a soluble enzyme pre-

dominantly localized in the cytoplasm. To obtain optimal enzyme activity, the presence of the monovalent ammonium cation and the divalent cations Mg^{2+} and Mn^{2+} in the assay mixture are necessary. Plant 5-oxo-prolinase has an extremely alkaline pH (9.5–10.5) and a high temperature optimum (55°). In contrast to animal 5-oxo-prolinase, where heat stabilization by 5-oxo-proline was observed [42], the high temperature optimum of the tobacco enzyme is due to stabilization by ATP [62]. High 5-oxo-prolinase activity in plant cell homogenates was not only observed with the co-substrate ATP, but also with other purine nucleotides [61, 62], although ATP was the best co-substrate of the compounds tested. Thus, the specificity for the co-substrate ATP is low for plant 5-oxo-prolinase, whereas a high specificity for this nucleotide has been observed in 5-oxo-prolinase preparations from animal cells [63]. Substrate affinity of the plant enzyme (5-oxo-proline: K_m 14 and 30 μM [61, 62]) was found to be comparable with animal 5-oxo-prolinase (5-oxo-proline: K_m 50 μM [63]). In bacteria, however, a lower substrate affinity was observed (5-oxo-proline: K_m 140 μM [64]), accompanied by a reduced substrate specificity. Using the 5-oxo-proline analogue 2-imidazolidone-4-carboxylic acid, in bacterial enzyme preparations competitive inhibition was obtained with K_I values of 30 mM [64]. Whereas tobacco 5-oxo-prolinase was shown to be much more sensitive to competitive inhibitors (2-imidazolidone-4-carboxylic acid, K_I 14.5 μM; dihydro-orotic acid, K_I 2mM [62]), an intermediate figure was observed for the mammalian enzyme, where K_I values of 120 μM and 10 mM, respectively, were determined [63].

If during degradation of glutathione in plants the reaction catalysed by 5-oxo-prolinase is the rate limiting step as observed in animal cells [41, 42], regulation of the activity of this enzyme by sulphur nutrition would be expected. Furthermore, if glutathione is synthesized in the leaves, translocated to the roots and degraded to make reduced sulphur available for protein synthesis, as proposed by investigations of the long-distance transport of sulphur in higher plants (see below), regulation of 5-oxo-prolinase activity should be different in leaf and root cells. When 5-oxo-prolinase activity was investigated in 45-day-old tobacco plants [Rennenberg, H. and Polle, A., unpublished results], the lowest activity was always observed in the leaves and the highest activity in the roots; the apex exhibited an intermediate activity. These differences observed in whole tobacco plants are reflected by the 5-oxo-prolinase activities in photoheterotrophically and heterotrophically grown tobacco cells in suspension culture. When cultured with sulphate as sole sulphur source, 5-oxo-prolinase activity was more than twice as high in dark-grown as in green tobacco cells, although both cultures were derived from the same clone. Throughout the culture cycle of both green and dark-grown tobacco suspensions, 5-oxo-prolinase activity remained constant, as long as the cells were sufficiently supplied with sulphate; under sulphur starvation conditions, 5-oxo-prolinase activity declined [Rennenberg, H. and Polle, A., unpublished results]. As these observations indicate that tobacco 5-oxo-prolinase is indeed regulated by sulphur nutrition, we recently investigated the influence of glutathione on the activity of this enzyme in the presence and absence of sulphate. Feeding of glutathione to green and to dark-grown tobacco cells affects their 5-oxo-prolinase activities in completely different ways [Rennenberg, H. and Polle, A., unpublished results]. When green tobacco cells were transferred from a culture medium with sulphate to a culture medium with glutathione as sole sulphur source, the 5-oxo-prolinase activity decreased to the level observed under sulphur starvation conditions. This decrease is accompanied by a slow uptake of glutathione. When sulphate is added to the suspension under these conditions, 5-oxo-prolinase activity increased again, the uptake of glutathione stops and sulphate is taken up very fast. However, when dark-grown tobacco cells were transferred from a medium with sulphate into a medium with glutathione as sole sulphur source, 5-oxo-prolinase activity increased as long as there was glutathione available in the culture medium. Glutathione was taken up much faster by the dark-grown than by the green tobacco cells. The increase in 5-oxo-prolinase activity was also observed when sulphate was added to the glutathione-containing culture medium. Under these conditions the high uptake of glutathione proceeds, and sulphate is not taken up by the cells as long as there is glutathione present in the medium [Rennenberg, H. and Polle, A., unpublished results]. These differences in the regulation of sulphate and glutathione uptake and 5-oxo-prolinase activity in dark-grown and green tobacco cells are consistent with the idea that green cells are equipped for synthesis and export of glutathione, whereas dark-grown cells are equipped for uptake and degradation of this peptide. Therefore, green and dark-grown tobacco cells in suspension culture respond to sulphate and glutathione as sulphur sources, as one would expect from leaf and root tissues of whole plants in which glutathione is the main transport form of reduced sulphur from the leaves to the roots. Leaf cells that reduce more sulphur than necessary for their own needs, incorporate the surplus into glutathione and translocate it into the phloem (see below); these cells should exhibit an uptake of sulphate that is preferred to the uptake of glutathione, and should show a low rate of glutathione degradation. Root cells that are not able to reduce enough sulphur for their own needs, but take up glutathione from the phloem and degrade it to the constituent amino acids, should exhibit an uptake of glutathione that is preferred to the uptake of sulphate and should show a high rate of glutathione degradation. These observations indicate that regulation of 5-oxo-prolinase activity plays an important role in the degradation of glutathione. The impact of the regulation of the enzymatic hydrolysis of 5-oxo-proline on this process, however, can only be established when the complete pathway of glutathione degradation in plants is eludicated. In addition, further investigations are necessary to show whether the regulation of 5-oxo-prolinase activity and the regulation of sulphate and glutathione uptake in green and dark-grown tobacco cells can be verified in leaf and root tissues of whole plants.

POSSIBLE BIOLOGICAL ROLES OF GLUTATHIONE

Functions of glutathione in the chloroplast

The high concentration of glutathione in the chloroplast [21–23] suggests that there might be a special function(s) of glutathione in this organelle. In chloroplasts glutathione is present predominantly in the reduced form (GSH) [65] and most of its proposed functions are related to the thiol group and its use as a reductant. It is generally assumed that glutathione maintains protein, cysteine and homocysteine in the reduced, i.e. the metabolically active, form. Such a function of glutathione only appears to be likely if the oxidized glutathione (GSSG) formed by the use of GSH as reductant is rapidly re-reduced in an enzymatic process to keep the GSH–GSSG ratio at the observed high level. A high GSH–GSSG ratio seems to be necessary not only for a role of glutathione as reductant, but also to achieve optimal protein synthesis in animal [66, 67] as well as in plant cells [68, 69]. In animal cells GSSG has been shown to inhibit protein synthesis, whereas the amount of GSH present did not affect this process. It is supposed that GSSG converts an initiation factor of protein synthesis into an inactive form [66, 67]. The GSSG content of dry wheat embryos [69] and of conidia of *Neurospora crassa* [68] is high, but declines early during germination. As the incorporation of [^{35}S] methionine into protein in extracts from wheat embryos is inhibited by GSSG [69], GSSG seems to accumulate during seed ripening to keep protein synthesis at a low level. A decrease in the GSSG content appears to be necessary early in germination to obtain optimal protein synthesis during growth and development.

Glutathione reductase (EC 1.6.4.2), catalysing the reduction of GSSG at the expense of oxidizing NADPH, has been identified in both photosynthetic and non-photosynthetic tissues of many plants [22, 65, 70–81]. There is substantial evidence that glutathione reductase is present in chloroplasts [22, 78, 79, 81], but it appears that this enzyme is about equally distributed between chloroplasts and cytoplasm [80]. Differences between cytoplasmatic glutathione reductase and glutathione reductase in chloroplasts have so far not been reported. Catalytic properties as well as physical properties seem to be very similar for plant, yeast and mammalian glutathione reductase [80].

The presence of glutathione in chloroplasts might be of importance for a participation of glutathione in the degradation of hydrogen peroxide. Although chloroplasts contain, if any, only minute catalase or peroxidase activity [82, 83], they produce considerable amounts of several toxic oxygen-derived species in the light that give rise to the generation of hydrogen peroxide (cf. refs. [82, 83]). Reduction of hydrogen peroxide in animal cells is catalysed by glutathione peroxidase (EC 1.11.1.9) using GSH as reductant [84–87]. Most of the animal glutathione peroxidases investigated were demonstrated to be selenium-dependent enzymes [84, 85, 87] which seems to be due to a selenocysteine in the active centre of the enzyme [88]. However, recently a considerable fraction of animal glutathione peroxidase was shown to be selenium-independent [86]. In plant cells glutathione peroxidase has been reported in crude homogenates from spinach [21, 89] and maize [90–91]. Other investigators, however, provided evidence that glutathione peroxidase activity is neither present in cells of higher plants, nor in microorganisms [87]. Thus, the presence of selenium-dependent or independent glutathione peroxidase in plants remains a controversial issue.

Degradation of hydrogen peroxide in chloroplasts can apparently take place independent of glutathione peroxidase activity. The path of detoxification of hydrogen peroxide that might proceed in the chloroplast is the coupling of the reduction of hydrogen peroxide to the oxidation of glutathione via the ascorbate–dehydroascorbate system. Hydrogen peroxide may be reduced by ascorbate, and the dehydroascorbate produced this way may be re-reduced to ascorbate using GSH as reductant. The GSSG synthesized upon reduction of dehydroascorbate may be reduced by glutathione reductase. In the light, this reduction may proceed by oxidation of photosynthetically generated NADPH. The enzymological equipment necessary to operate this series of reactions, originally proposed by Foyer and Halliwell [22], has not only been demonstrated in *Euglena*, which does not contain catalase activity [92, 93], but also in chloroplasts of higher plants. Although reduction of hydrogen peroxide by ascorbate may proceed non-enzymatically at alkaline pH [83], ascorbate peroxidase activity catalysing this process has been discovered in spinach chloroplasts [94]. Glutathione dehydrogenase activity (EC 1.8.5.1) catalysing the reduction of dehydroascorbate by oxidation of GSH has repeatedly been demonstrated in leaf tissues, but was not localized in the chloroplast [81, 95–98]. The observation, however, that illuminated, ruptured chloroplasts catalyse dehydroascorbate-dependent oxygen evolution and the generation of ascorbate in the presence of NADP(H) and glutathione (GSH or GSSG) [81], strongly supports the idea that glutathione dehydrogenase is also present in this organelle. Dehydroascorbate-dependent oxygen evolution in ruptured pea chloroplasts only proceeds under these conditions in the presence of the high glutathione concentrations [79, 81] seen in chloroplasts *in vivo* [21–23]. Although these observations indicate that chloroplasts are equipped for the reduction of hydrogen peroxide by GSH, it still has to be shown whether this reaction mechanism is indeed responsible for the detoxification of hydrogen peroxide in this organelle *in vivo*.

Functions of glutathione in the chloroplast do not appear to be restricted to the reduced form of this peptide. GSSG has been shown to deactivate three enzymes of the Calvin cycle *in vitro*; these enzymes were also found to be deactivated in darkness, and to be activated in the light [21, 99, 100]. As thiol-containing proteins seem to be required for the light activation of these enzymes of the Calvin cycle [82, 101], it has been suggested that GSSG is also involved in dark deactivation of the enzymes *in vivo* [21, 99, 100]. Other investigators, however, found that practically all the cellular glutathione exists as GSH, irrespective of whether or not leaves are kept in light

or darkness [22, 80, 102]. From these observations Halliwell and Foyer [65] concluded that the GSSG concentration in the chloroplasts is insufficient for the postulated deactivation in darkness. As exact data about the GSSG concentration in the chloroplasts are not available, the possibility of a function of glutathione in deactivation of enzymes of the Calvin cycle has still to be considered.

Glutathione as a storage and transport form of reduced sulphur

Tobacco cells in suspension culture grown under photoheterotrophic conditions release high amounts of glutathione into their culture medium [5, 59, 103], when supplied sufficiently with sulphate and ammonium. Up to 40% of the sulphate offered to the cells is incorporated into glutathione, 99% of which is translocated out of the cells [5]. Glutathione seems to be released as GSH, but is partially present in the medium as GSSG due to autoxidation [5]. The high efflux of glutathione is not directly dependent on photosynthesis but is restricted to chloroplast-containing cells [5]. When the sulphate supply in the medium is exhausted, released glutathione is taken-up and re-utilized as a sulphur source for protein synthesis [56, 59]. As tobacco cells are also able to grow with glutathione as sole sulphur source [56, 104], these observations have led to the conclusion that glutathione may function as a storage and transport form of reduced sulphur in plants [5, 59]. The use of glutathione rather than L-cysteine might have the advantage that glutathione does not participate in the regulation of sulphur assimilation to such a great extent as L-cysteine does (cf. refs. [36–38]). From this point of view, glutathione might be an 'inert' form of reduced sulphur (sulfur in the oxidation state -2) more suitable for storage and transport than L-cysteine. Such a function of glutathione has also been proposed for animal cells, although conclusive evidence for such a function in animal tissues has not been provided [3].

The idea of a function of glutathione as a storage form of reduced sulphur in plants is supported by observations from several investigators; Smith [17] has reported that under sulphur starvation conditions the intracellular glutathione pool of tobacco cells declines before the L-cysteine pool is affected. In spinach leaf discs supplied with high concentrations of sulphate, a 4-fold elevated glutathione content was measured [7]; accumulation of glutathione was also observed, when leaves of trees were fumigated with sulphur dioxide [8]. From the seasonal variation of glutathione and glutathione reductase in needles of spruce and other conifers which have elevated levels during winter, it has been suggested that GSH is able to increase frost-tolerance [19]. Such a function of glutathione would be consistent with the proposal of Levitt [105] that freezing injury might be caused by changes in the conformation of proteins due to oxidation of thiol groups by freeze dehydration. However, as an increased glutathione content in spinach leaf discs did not result in an enhanced frost tolerance, the high glutathione content of conifers during winter may well be explained as a storage of reduced sulphur in autumn [7]. This interpretation is supported by the rapid decrease in the glutathione content during the period of intensive growth in early spring [19].

The role of glutathione in the long-distance transport of reduced sulphur has been analysed in tobacco plants [106]. When tobacco plants were fed with ^{35}S-sulphate via a leaf in the middle of the stem, radioactivity was translocated along the stem toward the apex as well as toward the roots. A clear gradient of labeled sulphate and of reduced sulphur compounds was observed from the source leaf in an acropetal and a basipetal direction. Therefore, sulphate seems to be reduced in the leaf supplied with radioactivity, and reduced sulphur compounds seem to be translocated to other parts of the plant. A similar distribution was observed when all mature leaves were cut off, except the one or two in the middle of the stem fed with ^{35}S-sulphate. This finding indicates that the observed basipetal translocation cannot be due to a gradient in water potential between the source leaf and the leaves at the lower part of the stem. Thus, only phloem transport can account for the long-distance transport of sulphate and reduced sulphur toward the root. When labeled sulphate was fed to the roots of a tobacco plant, from which all the mature leaves were cut off except two in the middle of the stem, a slight gradient of reduced sulphur was established from the mature leaves in basipetal direction toward the root, i.e. against the direction of sulphate transport. This observation indicates that mature leaves are able to reduce more sulphur than necessary for their own needs and that this surplus reduced sulphur can be translocated in the phloem. The root system, however, although directly exposed to sulphate, is probably not able to reduce enough sulphur for its own needs. Separation of the reduced sulphur compounds translocated from mature tobacco leaves to the roots revealed that 67% of the label in this fraction consists of glutathione, *ca* 27% of methionine and 2–4% of cysteine. This distribution did not change along the translocation pathway. Therefore, glutathione appears to be the predominant long-distance transport form of reduced sulphur in tobacco plants. [106].

Similar results were obtained, when the experiments performed with tobacco plants were repeated with *Ricinus communis* [107]. Collection of the phloem sap from castor bean [107] and cucurbit plants [Rennenberg, H. and Schmitz, K., unpublished results] that were supplied with ^{35}S-sulphate via a mature leaf, again showed that glutathione was the main reduced sulphur compound present in the phloem sap. Glutathione accounted for 80% of the reduced sulphur in the phloem sap of *Ricinus*, and for 49% of this fraction in cucurbits. Thus, a function of glutathione as major transport form of reduced sulphur seems to be a general phenomenon in higher plants. This conclusion is supported by the observation that in leaves of soybean plants fumigated with $^{35}SO_2$, besides sulphate, glutathione was the main labeled compound translocated into the petiole [108]. Also, in extracts of aphids applied to *Vicia faba* plants that were supplied with $^{35}SO_3^{2-}$ via a leaf, glutathione accounted for a considerable fraction of the radioactivity; however, in these experiments trans-

location of labeled L-cysteine and its use for glutathione synthesis inside the aphids cannot be excluded [109].

Conjugation of pesticides with glutathione

From investigations performed during the last 10 years it is clear that many pesticides, including chlorotriazines, thiocarbamates, chlorinated nitrobenzenes and others, are converted *in vivo* to the corresponding glutathione conjugates in higher plants [cf. ref. [110]). Although spontaneous conjugation of pesticides with glutathione has been observed *in vitro* [111], under physiological conditions this process appears to be catalysed by glutathione-*S*-transferases (EC 2.5.1.18). Glutathione-*S*-transferases have been identified and analysed in animal tissues (cf. ref. [112]) and also in several higher plants [110, 113–117]. The wide distribution of glutathione conjugates among higher plants suggests that the ability to catalyse conjugation with glutathione is a general phenomenon of these organisms.

In animal cells, L-cysteinylglycine- and L-cysteine-derivatives have shown to be breakdown products of glutathione conjugates [113]. These observations indicate that upon degradation of glutathione conjugates in animal cells first the γ-glutamyl moiety and subsequently the glycine moiety are cleaved from the conjugates. This series of reactions is consistent with the γ-glutamyl cycle for the degradation of glutathione itself (Fig. 1). However, upon breakdown of different glutathione conjugates in different plant species the γ-glutamyl-L-cysteine- and L-cysteine-derivatives, but never the L-cysteinylglycine-derivative, have been detected [110]. Therefore, during degradation of glutathione conjugates in plants, the glycine moiety seems to be split off first, probably by the action of a carboxypeptidase, and then the γ-glutamyl moiety is cleaved. Whether the γ-glutamyl moiety is removed from the conjugates by the action of a γ-glutamyl transpeptidase, as proposed by Lamoureux and Rusness [110], or by a γ-glutamyl cyclotransferase has so far not been investigated. Thus, degradation of glutathione conjugates in plant cells cannot be explained by the series of reactions proposed in the γ-glutamyl cycle (Fig. 1). However, the observed degradation products of glutathione conjugates in plants are consistent with the alternative cycle of glutathione synthesis and degradation shown in Fig. 2. Whether these differences between the degradation of glutathione conjugates in animal and plant cells reflect a difference in the degradation of glutathione itself has still to be eludicated.

The conjugation of pesticides with glutathione prompts the question: is conjugation with glutathione responsible for the detoxification of these compounds? Evidence that detoxification of pesticides is indeed a function of glutathione comes from investigations performed with the thiocarbamate herbicide *S*-ethyl dipropylthiocarbamate (EPTC) and the antidote to this herbicide *N,N*-diallyl-2,2-dichloroacetamide (R-25788) [114]. In corn plants, containing relatively high glutathione concentrations (0.4 mM) [18], the herbicide EPTC causes less damage than it does in weeds that contain lower glutathione concentrations [118]. When EPTC is applied together with the antidote, herbicide injury to corn plants is prevented without reducing the activity of the herbicide to the weeds [114]. However, not EPTC itself, but the corresponding sulphoxide, seems to be the phytotoxic compound that is conjugated with glutathione in corn [118]. Lay and Casida [18] have shown that the already high glutathione concentration in corn is further increased upon treatment with the antidote R-25788, and that this enhanced glutathione content is accompanied by an enhanced glutathione-*S*-transferase activity. The enhanced glutathione content of R-25788 treated corn plants seems to be due to an enhanced ability to synthesize this peptide from the constituent amino acids [18]. Heterotrophic tobacco cells treated with the antidote R-25788 release almost twice as much glutathione into their culture medium and contain an activity for the synthesis of glutathione that is *ca* twice as high as that in untreated controls [30]. The *in vitro* stimulation of glutathione synthesis upon addition of R-25788 to the reaction mixture, as reported by Carringer *et al.* [29], was not detected with the tobacco system [30]. From these observations it can be concluded that in the presence of the antidote R-25788 detoxification of EPTC via conjugation with glutathione occurs, while still sufficient glutathione remains to participate in other metabolic processes. Therefore, the data available support the idea of a function of glutathione in the detoxification of pesticides, although the protective mechanism of the antidote R-25788 might be more complex.

CONCLUSIONS

Even though our present knowledge about the metabolism of glutathione in plants is very poor, functions of glutathione in the detoxification of pesticides and in the long-distance transport and storage of reduced sulphur have been established during the past few years. In addition, evidence is accumulating that detoxification of hydrogen peroxide in the chloroplasts proceeds via ascorbate–dehydroascorbate using GSH as reductant. There is an urgent need to fill the numerous gaps in our information about glutathione synthesis and degradation and the regulation of these processes before further progress in the understanding of the functions of glutathione in plants can be expected.

Acknowledgements—I would like to thank Drs. Bergmann, Fahey and Halliwell for providing data so far unpublished and/or manuscripts of papers in press and Drs. Basic, Bergmann and Haworth for critical reading of the manuscript.

REFERENCES

1. Waley, S. G. (1966) *Adv. Protein Chem.* **21**, 1.
2. Kosower, E. M. (1976) in *Glutathione: Metabolism and Function* (Arias, J. M. and Jakoby, W. B., eds.) p. 1. Raven Press, New York.
3. Meister, A. and Tate, S. S. (1976) *Annu. Rev. Biochem.* **45**, 559.
4. Kasai, T. and Larson, P. O. (1980) in *Progress in the Chemistry of Organic Products* (Herz, W., Grisebach, H. and Kirby, G. W., eds.) Vol. 39, p. 243. Springer, Wien.
5. Bergmann, L. and Rennenberg, H. (1978) *Z. Pflanzenphysiol.* **88**, 175.

6. Sekiya, J., Schmidt, A., Rennenberg, H., Wilson, L. G. and Filner, P. (1982) *Phytochemistry* **21**, 2173.
7. de Kok, L. J., de Kan, P. J. L., Tánczos, O. G. and Kuiper, P. J. C. (1981) *Physiol. Plant.* **53**, 435.
8. Grill, D., Esterbauer, H., and Klösch, U. (1979) *Environ. Pollut.* **19**, 187.
9. Fahey, R. C., Brown, W. C., Adams, W. B. and Worsham M. B. (1978) *J. Bacteriol.* **133**, 1126.
10. Setlow, B. and Setlow, P. (1977) *Biochem. Biophys. Res. Commun.* **75**, 45.
11. Price, C. A. (1957) *Nature (London)* **180**, 148.
12. Carnegie, P. R. (1963) *Biochem. J.* **89**, 459.
13. Carnegie, P. R. (1963) *Biochem. J.* **89**, 471.
14. Fahey, R. C., Newton, G. L., Dorian, R. and Kosower, E. M. (1980) *Analyt. Biochem.* **107**, 1.
15. Fahey, R. C., Newton, G. L., Dorian , R. and Kosower, E. M. (1981) *Analyt. Biochem.* **111**, 357.
16. Newton, G. L., Dorian, R. and Fahey, R. C. (1982) *Analyt. Biochem.* **114**, 383.
17. Smith, I. K. (1975) *Plant Physiol.* **55**, 303.
18. Lay, M. M. and Casida, J. E. (1976) *Pestic. Biochem. Physiol.* **6**, 442.
19. Esterbauer, H. and Grill, D. (1978) *Plant Physiol.* **61**, 119.
20. Gressel, J., Zilkah, S. and Ezra, G. (1978) in *Frontiers of Plant Tissue Culture* (Thorpe, T. A., ed.) The International Association for Plant Tissue Culture, Calgary.
21. Wolosiuk, R. A. and Buchanan, B. B. (1977) *Nature (London)* **266**, 565.
22. Foyer, C. H. and Halliwell, B. (1976) *Planta* **133**, 21.
23. Bergmann, L. and Ulbrich, J. (in preparation).
24. Bergmann, L. (1981) in *Biology of Inorganic Nitrogen and Sulfur* (Bothe, H. and Trebst, A., eds.) p. 341. Springer, Berlin.
25. Giovanelli, J., Mudd, S. H. and Datko, A. H. (1978) *J. Biol. Chem.* **253**, 5665.
26. Tkachuk, R. and Mellish, V. J. (1977) *Can. J. Biochem.* **55**, 295.
27. Suzuki, T., Sugii, M. and Kakimoto, T. (1961) *Chem. Pharm. Bull.* **9**, 77.
28. Matikkala, E. J. and Virtanen, A. I. (1963) *Acta Chem. Scand.* **17**, 1799.
29. Carringer, R. D., Rieck, C. E. and Bush, L. P. (1978) *Weed Sci.* **26**, 167.
30. Rennenberg, H., Birk, C. and Schaer, B. (1982) *Phytochemistry* **21**, 5.
31. Rennenberg, H. and Uthemann, R. (1980) *Z. Naturforsch. Teil C* **35**, 945.
32. Webster, G. C. (1953) *Plant Physiol.* **28**, 728.
33. Webster, G. C. and Varner, J. E. (1954) *Arch. Biochem. Biophys.* **52**, 22.
34. Richman, P. G. and Meister, A. (1975) *J. Biol. Chem.* **250**, 1422.
35. Webster, G. C. (1954) *Arch. Biochem. Biophys.* **48**, 241.
36. Giovanelli, J., Mudd, S. H. and Datko, A. H. (1980) in *The Biochemistry of Plants* (Stumpf, P. K. and Conn, E. E., eds.) Vol. 5, p. 453. Academic Press, New York.
37. Schmidt, A. (1979) in *Encyclopedia of Plant Physiology* (Gibbs, M. and Latzko, E., eds.) New Series, Vol. 6, p. 481. Springer, New York.
38. Anderson, J. W. (1980) in *The Biochemistry of Plants* (Stumpf, P. K. and Conn, E. E., eds.) Vol. 5, p. 203. Academic Press, New York.
39. Brunold, C. and Schmidt, A. (1978) *Plant Physiol.* **61**, 342.
40. Tateishi, N., Higashi, T., Shinya, S., Naruse, A. and Sakamoto, Y. (1974) *J. Biochem.* **75**, 93.
41. Meister, A. (1981) in *Current Topics in Cellular Regulation* (Estabrook, R. W. and Srere, P., eds.) Vol. 18, p. 21. Academic Press, New York.
42. van der Werf, P. and Meister, A. (1975) in *Advances in Enzymology* (Meister, A., ed.) Vol. 43, p. 519. John Wiley, New York.
43. Meister, A. (1976) in *Glutathione: Metabolism and Function* (Arias, I. M. and Jakoby, W. B., eds.) p. 35. Raven Press, New York.
44. Meister, A. (1974) in *Glutathione* (Flohé, L., Benöhr, H. Ch., Sies, H., Waller, H. D. and Wendel, A., eds.) p. 56. Georg Thieme, Stuttgart.
45. Meister, A. (1978) in *Functions of Glutathione in Liver and Kidney* (Sies, H. and Wendel, A., eds.) p. 43. Springer, Berlin.
46. McIntyre, T. M. and Curthoys, N. P. (1980) *Int. J. Biochem.* **12**, 545.
47. Glossman, H. and Neville, D. M. (1972) *FEBS Letters* **19**, 340.
48. Hughey, R. P., Rankin, B. B., Elce, J. S. and Curthoys, N. P. (1978) *Arch. Biochem. Biophys.* **186**, 211.
49. Tate, S. S. and Meister, A. (1975) *J. Biol. Chem.* **250**, 4619.
50. McIntyre, T. M. and Curthoys, N. P. (1979) *J. Biol. Chem.* **254**, 6499.
51. Goore, M. Y. and Thompson, J. F. (1967) *Biochim. Biophys. Acta* **132**, 15.
52. Kean, E. A. and Hare, E. R. (1980) *Phytochemistry* **19**, 199.
53. Tate, S. S. and Meister, A. (1974) *J. Biol. Chem.* **249**, 7593.
54. Gottschalk, C. W., Lassiter, W. E. and Mytle, M. (1960) *Am. J. Physiol.* **198**, 581.
55. Silbernagl, S., Pfaller, W., Heinle, H. and Wendel, A. (1978) in *Functions of Glutathione in Liver and Kidney* (Sies, H. and Wendel, A., eds.) p. 60. Springer, Berlin.
56. Rennenberg, H. (1981) *Z. Pflanzenphysiol.* **105**, 31.
57. Thompson, J. F., Turner, L. K. and Gering, R. K. (1964) *Phytochemistry* **3**, 33.
58. Rennenberg, H., Steinkamp, R. and Polle, A. (1980) *Z. Naturforsch. Teil C* **35**, 708.
59. Rennenberg, H. and Bergmann, L. (1979) *Z. Pflanzenphysiol.* **92**, 133.
60. Mazelis, M. and Pratt, H. M. (1976) *Plant Physiol.* **57**, 85.
61. Mazelis, M. and Creveling, R. K. (1978) *Plant Physiol.* **62**, 798.
62. Rennenberg, H., Steinkamp, R. and Kesselmeier, J. (1981) *Physiol. Plant.* **52**, 211.
63. van der Werf, P., Griffith, O. W. and Meister, A. (1975) *J. Biol. Chem.* **250**, 6686.
64. van der Werf, P. and Meister, A. (1974) *Biochem. Biophys. Res. Commun.* **56**, 90.
65. Halliwell, B. and Foyer C. H. (1978) *Planta* **139**, 9.
66. Kosower, N. S. and Kosower, E. M. (1974) in *Glutathione* (Flohé, L., Benöhr, H. Ch., Sies, H., Waller, H. D. and Wendel, A., eds.) p. 276. Georg Thieme, Stuttgart.
67. Kosower, N. S. and Kosower, E. M. (1976) in *Glutathione: Metabolism and Function* (Arias, I. M. and Jakoby, W. B., eds.) p. 159. Raven Press, New York.
68. Fahey, R. C., Brody, S. and Mikolajczyk, S. D. (1975) *J. Bacteriol.* **121**, 144.

69. Fahey, R. C., Di Stefano, D. L., Meier, G. P. and Bryan, R. N. (1980) *Plant Physiol.* **65**, 1062.
70. Mapson, L. W. and Goddard, D. R. (1951) *Nature (London)* **167**, 975.
71. Conn, E. E. and Vennesland, B. (1951) *Nature (London)* **167**, 976.
72. Anderson, D. G., Stafford, H. A., Conn, E. E. and Vennesland, B. (1952) *Plant Physiol.* **27**, 675.
73. Young, L. C. T. and Conn, E. E. (1956) *Plant Physiol.* **31**, 205.
74. Mapson, L. W. and Isherwood, F. A. (1963) *Biochem. J.* **86**, 173.
75. Ida, S. and Morita, Y. (1971) *Agric. Biol. Chem.* **35**, 1542.
76. Ida, S. and Morita, Y. (1971) *Agric. Biol. Chem.* **35**, 1550.
77. Drumm, H. and Mohr, H. (1973) *Z. Naturforsch. Teil C* **28**, 559.
78. Schaedle, M. and Bassham, J. A. (1977) *Plant Physiol.* **59**, 1011.
79. Jablonski, P. P. and Anderson, J. W. (1978) *Plant Physiol.* **61**, 221.
80. Wirth, E. and Latzko, E. (1978) *Z. Pflanzenphysiol.* **89**, 69.
81. Jablonski, P. P. and Anderson, J. W. (1981) *Plant Physiol.* **67**, 1239.
82. Halliwell, B. (1978) *Prog. Biophys. Mol. Biol.* **33**, 1.
83. Halliwell, B. (1982) *Adv. Chem.* (in press).
84. Flohé, L. and Günzler, W. A. (1974) in *Glutathione* (Flohé, L., Benöhr, H. Ch., Sies, H., Waller, H. D. and Wendel, A., eds.) p. 132. Georg Thieme, Stuttgart.
85. Flohé, L., Günzler, W. A. and Ladenstein, R. (1976) in *Glutathione: Metabolism and Function* (Arias, I. M. and Jakoby, W. B., eds.) p. 115. Raven Press, New York.
86. Lawrence, R. A. and Burk, R. F. (1978) *J. Nutr.* **108**, 211.
87. Smith, J. and Shrift, A. (1979) *Comp. Biochem. Physiol.* **63B**, 39.
88. Forstrom, J. W., Zakowski, J. J. and Tappel, A. L. (1978) *Biochem.* **17**, 2639.
89. Flohé, L. and Menzel, H. (1971) *Plant Cell Physiol.* **12**, 325.
90. Neubert, D., Rose, T. H. and Lehninger, A. L. (1962) *J. Biol. Chem.* **237**, 2025.
91. Neubert, D., Wojtczak, A. B. and Lehninger, A. L. (1962) *Proc. Nat. Acad. Sci. U.S.A.* **48**, 1651.
92. Shigeoka, S., Nakano, Y. and Kataoka, S. (1980) *Biochem. J.* **186**, 377.
93. Shigeoka, S., Nakano, Y. and Kataoka, S. (1980) *Arch. Biochem. Biophys.* **201**, 121.
94. Groden, D. and Beck, E. (1979) *Biochim. Biophys. Acta* **546**, 426.
95. Yamaguchi, M. and Joslyn, M. A. (1952) *Arch. Biochem. Biophys.* **38**, 451.
96. Gero, E. and Candido, A. (1967) *Bull. Soc. Chim. Biol.* **49**, 1895.
97. Mapson, L. W. and Moustafa, E. M. (1976) *Biochem. J.* **62**, 248.
98. Foyer, C. H. and Halliwell, B. (1977) *Phytochemistry* **16**, 1347.
99. Wolosiuk, R. A. and Buchanan, B. B. (1978) *Arch. Biochem. Biophys.* **189**, 97.
100. Breazeale, U. D., Buchanan, B. B. and Wolosiuk, R. A. (1978) *Z. Naturforsch. Teil C* **33**, 521.
101. Anderson, L. E., Nehrlich, S. C. and Champigny, M.-L. (1978) *Plant Physiol.* **61**, 601.
102. Halliwell, B., Foyer, C. and de Rycker, J. (1977) *Abstracts of the 4th International Congress on Photosynthesis* Reading, England, p. 144.
103. Rennenberg, H. (1976) *Phytochemistry* **15**, 1433.
104. Hart, J. W. and Filner, P. (1969) *Plant Physiol.* **44**, 1253.
105. Levitt, J. (1972) in *Responses of Plants to Environmental Stresses* (Levitt, J., ed.) p. 188. Academic Press, New York.
106. Rennenberg, H., Schmitz, K. and Bergmann, L. (1979) *Planta* **147**, 57.
107. Bonas, U., Schmitz, K., Rennenberg, H. and Bergmann, L. (1982) *Planta* **155**, 82.
108. Garsed, S. G. and Read, D. J. (1977) *New Phytol.* **79**, 583.
109. Garsed, S. G. and Mochrie, A. (1980) *New Phytol.* **84**, 421.
110. Lamoureux, G. L. and Rusness, D. G. (1981) in *Sulfur in Pesticide Action and Metabolism* (Rosen, J. D., Magee, P. S. and Casida, J. E., eds.) ACS Symposium Series Vol. 158, p. 132. American Chemical Society.
111. Leavitt, R. and Penner, D. (1979) *J. Agric. Food Chem.* **27**, 533.
112. Jakoby, W. B. (1978) in *Adv. Enzymol.* **46** (Meister A., ed.), p. 383. John Wiley, New York.
113. Shimabukuro, R. H., Lamoureux, G. L. and Frear, D. S. (1978) in *Chemistry and Action of Herbicide Antidotes* (Pallos, F. M. and Casida, J. E., eds.) p. 133. Academic Press, New York.
114. Lay, M. M. and Casida, J. E. (1978) in *Chemistry and Action of Herbicide Antidotes* (Pallos, F. M. and Casida, J. E., eds.) p. 151. Academic Press, New York.
115. Guddewar, M. B. and Dautermann, W. C. (1979) *Phytochemistry* **18**, 735.
116. Diesperger, H. and Sandermann, H. (1979) *Planta* **146**, 643.
117. Neben, I., Sahm, H. and Kula M.-R. (1980) *Biochim. Biophys. Acta* **614**, 81.
118. Casida, J. E., Gray, R., and Tilles, A. (1974) *Science* **184**, 574.