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REVIEW

UREIDE METABOLISM IN HIGHER PLANTS

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Abstract—The synthesis, transport and assimilation of the ureides, allantoin and allantoic acid, in higher plants is reviewed. Evidence indicates that in nodulated legumes ureides are synthesized from products of N_2-fixation via purine synthesis and degradation. Their synthesis in other plants also appears to be via purine degradation but is dependent on the inorganic nitrogen source fed to the plant; greatest ureide production is associated with ammonium assimilation. The use of ureides rather than amides for N-transport from the root to the shoot via the xylem stream results in an improved carbon economy of the plant. Good evidence for the transport of ureides in the phloem is lacking for most species examined although it is assumed to be important, particularly in fruit and seed development. Ureides are stored and assimilated mainly in the shoot. The precise pathways, localization and regulation of ureide assimilation are poorly understood and require further investigation. Similarities exist between the properties of the enzymes involved in ureide assimilation in higher plants and in micro-organisms. However, the evidence that light appears to be involved in ureide assimilation in green tissues suggests that different regulatory mechanisms may exist in plants compared with micro-organisms. The economically important legume crops such as soybeans, cowpeas and *Phaseolus* sp. are all ureide producers. To aid our understanding of the productivity of these plants knowledge of how ureide-N is converted into seed protein is essential.

INTRODUCTION

The ureides, allantoin and allantoic acid (Figs. 1 and 2) have long been recognized as the major nitrogenous compounds in a variety of plant species [1–6]. Other ureides, such as citrulline, are abundant in certain species [3, 4] but here the term will be restricted to allantoin and allantoic acid unless otherwise stated. The physiology and biochemistry of ureides were largely neglected until Mothes and coworkers [5, 6] in the 1950s reiterated the earlier suggestions of several French workers [1, 2] that these compounds were probably synthesized via purine degradation. A simpler pathway via the direct condensation of glyoxylate and urea could not be discounted (Fig. 1) but evidence indicated that ureides were not produced in leaves via such a route and were probably synthesized exclusively via purine intermediates [7]. Roots were considered to be the main site of ureide synthesis, even though leaves were also shown to synthesize these compounds when detached and kept in the dark [5]. The role of ureides in the N-metabolism of plants, in particular soybeans, was thoroughly investigated by a number of Japanese workers [8–23]. While this work seems to have been neglected by many Western scientists, Vogels and van der Drift [24] stressed in 1976 the importance of ureide metabolism in plants and the aim of this review is to bring the subject up to date. Wherever possible, we have outlined the areas where our knowledge is still insufficient with the hope that this will stimulate further research.

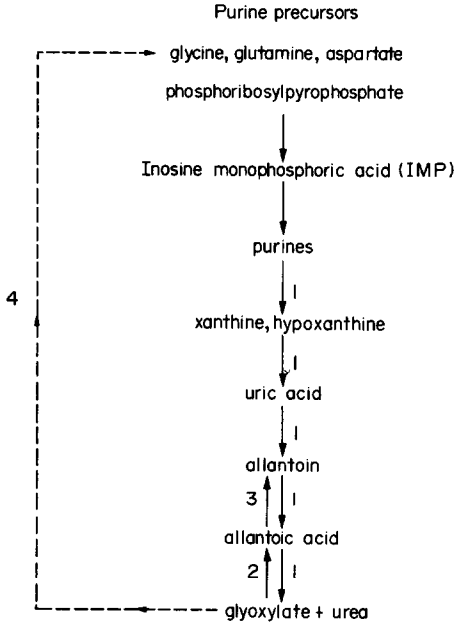

Fig. 1. Possible metabolic pathways for the synthesis of ureides. 1, Aerobic purine degradation; 2, condensation of glyoxylate–urea; 3, reverse reaction of allantoinase; 4, recycling of glyoxylate [7].

UREIDE SYNTHESIS IN ROOTS

When ureides were first detected in the xylem sap of *Phaseolus vulgaris* and *Acer pseudoplatanus* [25] and found in abundance in the roots of members of the Boraginaceae, Plantanaceae, Hippocastanaceae, Aceraceae and Leguminosae [26], the role of the root in N-metabolism was not known. In *Acer*, almost 100% of the xylem sap N was in the form of allantoin and allantoic acid during the spring [26]. In *Symphytum*, these ureides accumulated in storage organs in the autumn but it was not known whether they were formed or transported there [27]. Later, Mothes [28] showed that ureides could be produced in root cuttings of *Phaseolus* in response to C-starvation during extensive N-assimilation. When wheat seedlings were fed [2-^{14}C]-glycine for 15 min through the roots, a significant amount of radioactivity in the root extract was found in allantoin. No radioactivity was found in allantoin after feeding [^{14}C]-glyoxylic acid or [^{14}C]-urea, suggesting that the ureides were produced via purine synthesis and not from urea and glyoxylate directly ([29], see Fig. 1). Similar conclusions were drawn when [^{14}C]-glycine, [^{14}C]-hypoxanthine and [^{14}C]-urea were fed to root discs of *Symphytum* [30–34]. These labelling studies firmly established that roots could synthesize ureides, and although most of the evidence favoured synthesis via purine degradation, other pathways could not be discounted [5]. Even though it was recognized that the sap of some legumes contained ureides [25] and that allantoin, allantoic acid and allantoinase (degrading allantoin to allantoic acid) were reported in the nodules of legumes [2], the idea that ureide production might be associated with root nodules was not realized until it was suggested that the underground parts of nodulated soybeans were the main site of ureide formation [9, 35]. Since then, the evidence favouring nodules as the site of ureide synthesis can be summarized into four categories; (a) the distribution of ureides in nodulated and non-nodulated plants; (b) measurements of enzymes involved in purine degradation; (c) ^{15}N-labelling studies; and (d) inhibitor studies. These are discussed below.

Distribution of ureides in nodulated and non-nodulated plants

Nodulated soybeans were found to contain much greater amounts of allantoin and allantoic acid than non-nodulated plants [8–10, 12, 13, 21, 36]. When exogenous N-sources were fed to soybeans there was a good correlation between the decrease in allantoin content of the plants and the decrease in nodule formation [13] and N-fixing activity [21]. Similarly, it was shown that increasing concentrations of nitrate fed to nodulated soybeans decreased the ureide content of the xylem sap N, the nodule weight per plant and N_2-fixation activity per plant [37, 38]. When three varieties of soybeans were inoculated with either an 'efficient' or 'inefficient' strain of *Rhizobium* (efficiency based on the amount of H_2 gas evolved during N_2-fixation), the ureide concentration in xylem sap and estimated flux of ureides to the shoot were less in plants inoculated with the 'inefficient' strain compared with those having the 'efficient' strain [38].

Reciprocal grafts between a nodulating (A62-1) and a non-nodulating (A62-2) variety of soybeans indicated that the above-ground parts of the plants had little influence on ureide formation; stems of non-nodulated plants grafted onto the nodulated variety accumulated ureides, but little accumulated in the stems of the nodulated variety grafted onto the non-nodulated plants. In addition, removal of the nodules from the nodulated variety resulted in a rapid decrease in ureide concentration in stems and roots [15].

Measurement of enzymes involved in purine degradation

High activities of some enzymes involved in aerobic purine degradation (xanthine oxidase, uricase and allantoinase; Fig. 1) were reported in the nodules of soybeans whereas low activities were found in other plant parts [16, 17, 19]. Similarly, nodules of *Vigna* were reported to contain high activities of xanthine dehydrogenase, uricase and allantoinase [39]. Thus, nodules have the capacity to degrade purines to allantoin but do not appear to have much ability to degrade allantoic acid enzymically, since allantoicase was not detected in nodules but was present in leaves of soybeans [19].

^{15}N-*Labelling studies*

The incorporation of ^{15}N into allantoin and allantoic acid in nodules was higher than in the basal parts of enclosed roots fed $^{15}N_2$ gas even though the concentrations of ureides were higher in the roots compared with nodules [14], indicating that nodules were producing ureides. Interestingly, incorporation of the label into RNA and DNA was less than that in the ureides suggesting that there may be a non-metabolizable compartment of purine bases in roots.

By feeding nodulated and de-nodulated soybean roots with $^{15}NH_3$ it was shown that ureides had low atom % excess ^{15}N compared with amino acids [22] suggesting that NH_3 absorbed by roots was not assimilated into ureides but was readily assimilated into amino acids. The low atom % excess ^{15}N in ureides was explained by proposing that ureides were produced from N_2-fixation and would, therefore, not be expected to be labelled to any great extent after $^{15}NH_3$-feeding to roots, as the latter would be assimilated in root tissue rather than nodules.

After feeding $^{15}N_2$ to the root system of the legume *Vigna* for 1 hr, 63% of the label in the nodules was found in ureides indicating a rapid assimilation [40]. Xylem sap N was also rapidly labelled with 91% of the label in ureides after only 2 hr of exposure to $^{15}N_2$. Thus, a major proportion of the nitrogen was fixed into ureides and rapidly exported to the shoot via the xylem.

Ohyama and Kumazawa [41] detected ^{15}N in ureides after only 4 min of feeding $^{15}N_2$ to nodulated soybean roots indicating a rapid synthesis of ureides from the early products of N_2-fixation; ammonia and glutamine. In a later paper, the same authors demonstrated that little ^{15}N from $^{15}NO_3$ was incorporated into ureides as compared to the amount from $^{15}N_2$ providing good evidence that ureide synthesis takes place in the nodules from recent N_2-fixation [42].

Inhibitor studies

The hypoxanthine analogue, allopurinol [4-hydroxypyrazolo(3,4-d)pyrimidine] inhibits xanthine dehydrogenase (or oxidase) and therefore if ureides are produced via purine degradation, inhibitor treatment should result in a decrease in ureide content and an accumulation of xanthine. When nodulated soybean plants were fed the inhibitor, there was a dramatic decrease in ureides of the nodules and stem but not roots,

and an accumulation of xanthine only in the nodules [23]. In seedlings similar effects were noted. In addition azaserine, which blocks the transamidation of glutamine to formylglycinamide ribonucleotide in purine synthesis, did not affect ureide concentrations suggesting some dependence on stored RNA or DNA rather than newly synthesized purines [23]; unfortunately, this inhibitor was not used with nodulated plants.

Atkins *et al.* [39] fed allopurinol to nodulated cowpeas (*Vigna unguiculata* L.). Within 1 hr, ureide export and ureide pools decreased in nodules, but xanthine accumulated in nodules. Thus, there is now a large amount of evidence favouring the synthesis of ureides in legume nodules from N_2 fixation, via purine degradation.

The evidence for the synthesis of ureides in non-legumes and non-nodulated legumes also favours synthesis from purine degradation; much of this work has been reviewed earlier [5, 6]. Since then, little has been reported on ureide synthesis in higher plants other than that related to N_2-fixation. In non-nodulated *Phaseolus vulgaris*, ureides were the major form of reduced N in the xylem sap of plants grown with nitrate, ammonium or ammonium nitrate [43], and the addition of allopurinol to roots resulted in a marked decrease in ureide concentration in the xylem sap without affecting the concentration of other reduced N-compounds or nitrate [44]. These results are consistent with the origin of ureides via purine degradation in roots. Plants fed with ammonium salts contained more ureide-N in the xylem sap and in all plant organs except the roots compared with nitrate-grown plants, suggesting that ureides might be produced in response to ammonium toxicity in roots and transported to other plant parts [44]. Roots of non-nodulated and nodulated soybeans have some capacity to synthesize ureides, albeit small in comparison to the nodules [11, 22, 38, 42]. Ohyama and Kumazawa [42] showed that there was a lag phase in ^{15}N-incorporation from $^{15}NO_3$ into allantoin in roots and speculated that perhaps separate mechanisms exist for allantoin synthesis in roots and nodules. However, rates of purine turnover were not compared in roots and nodules and the observed lag phase may reflect differences in purine turnover rates and not necessarily different mechanisms.

In spite of numerous attempts, the only evidence for an alternative pathway to ureide formation was reported earlier when a direct condensation between urea and glyoxylate was shown to produce allantoic acid in some fungi [45] and banana leaves [46]. In micro-organisms, allantoin and allantoic acid are usually produced via purine degradation [24] but it is of interest to note that contrary to many reports the allantoinase reaction was found to be freely reversible in *Saccharomyces cerevisiae* [47]. More studies are required to determine if indeed there are alternative pathways for the synthesis of ureides in higher plants.

LOCALIZATION OF UREIDE SYNTHESIS WITHIN NODULES

There are conflicting reports on the localization of the enzymes involved in purine degradation in plant root nodules. Xanthine oxidase and uricase were reported to be associated with particulate fractions of soybean nodules [17] separated by differential centrifugation; the former was apparently tightly bound to a 'nuclear' fraction while the latter was associated with the 'mitochondrial' fraction. Some allantoinase activity was measured in 'nuclear', 'mitochondrial' and 'microsome' fractions but was mainly detected in the 100000 *g* supernatant. Unfortunately, no organelle marker enzymes were included in this work to assess the degree of cross-contamination between the different fractions. In a later paper [18], the authors separated nodule fractions, by sucrose density gradient centrifugation and uricase was, in fact, shown to be confined to the bacteroids and absent from the mitochondria. The bacteroid fraction contained no cytochrome oxidase activity, a marker enzyme for mitochondria. However, Herridge *et al.* [40] found that nearly all the uricase activity in cowpea nodules was in the 'cytosol' fraction with negligible amounts in the 'bacteroids'. In a more detailed study of cowpea nodules, xanthine dehydrogenase and uricase were found mainly in soluble extracts of bacteroid-containing tissue [39]. What activities were found in the cortical tissue were considered to be the result of contamination by bacteroids. Unlike the Japanese reports, these authors reported little uricase activity in the insoluble fraction of the bacteroids. Allantoinase appeared to be equally distributed between bacteroid and cortical tissues with a smaller but significant amount of activity in the insoluble fractions. Work is continuing in several laboratories which should resolve these apparently conflicting results.

TRANSPORT IN THE XYLEM AND PHLOEM

Much of the work on the occurrence of ureides in xylem sap has been adequately covered in a number of reviews [6, 48–51] and only a brief outline of the salient points will be discussed here. The methods used to obtain samples of xylem and phloem streams have been assessed by Pate [52], and although all the methods are not without criticism it is generally accepted that such samples provide some indication of the composition of the transport streams in the intact plant.

Ureides have been detected in both xylem and phloem saps of many higher plants [51–53]. Bollard [51] studying a wide a range of monocots, dicots and gymnosperms found citrulline in the xylem sap of 29 out of 103 species tested but it was a major constituent in only five species. Allantoin or allantoic acid or both were present in the xylem saps of 23 species of dicots and in one species of a monocot. In only four species (*Persea*, *Acer*, *Aesculus* and *Alectryon*) were these compounds the major forms of nitrogen. Concentrations of ureides per ml sap were not reported in this work but because the relationship between the concentration of N in sap samples and actual concentrations *in vivo* are likely to differ by as much as an order of magnitude; perhaps the amounts of ureides are better expressed as a percentage of the total sap nitrogen. The range of values recorded vary from 10% in *Pisum* [54] to 99% in *Acer* [26].

In non-nodulated *Phaseolus*, the percentage contribution of ureides to the total sap N varied from 13 to 42% depending on the inorganic N-source fed to the plant [43]. In soybeans, which rely solely on N_2-fixation for their N-requirement, 86% of the total sap N was in the form of allantoin and allantoic acid, whereas in plants fed 20 mM KNO_3 ureides comprised only 6% of the total sap N [38]. From these results it is clear that the amount of ureides in the xylem sap depends on the source of nitrogen available to the plant's root system. Highest amounts of

ureides were measured in sap samples when either ammonium was the N-source, compared with nitrate [43], or when N_2-fixation provided most of the plant's N [38]. In both non-nodulated *Phaseolus* [43] and soybeans [37] grown with nitrate and in nodulated soybeans [37], the percentage of ureides in the xylem sap was not altered significantly during the development of the plants from seedlings to mature plants. In nodulated cowpeas some variation was noted during development although ureides remained high, contributing between 60 and 80% to the total sap N at all stages of growth [40]. In all three legumes studied, allantoic acid was the predominant ureide. The contribution of the ureides to the xylem sap N appears then to remain fairly constant during plant development provided the source of N does not change. Van Bel has reported that amino acids can be differentially absorbed from the xylem stream during passage through the stem [55]. There have been no reports on any differential absorption of ureides from the xylem stream.

Difficulties in obtaining authentic samples of phloem sap have probably been responsible for the very limited information on the presence of ureides in phloem. Ureides were present in the phloem sap of *Acer*, *Platanus*, *Aesculus* [53], *Yucca* [56], *Phaseolus* [44] and in samples obtained from the cut ends of soybean pods (Thomas and Schrader, unpublished results). The amounts found in these legumes were small, however, and contamination from damaged cells cannot be discounted. As fruits of soybean contain large amounts of ureides predominantly in the pod wall ([12]; Thomas and Schrader, unpublished results), and because fruits in general have a relatively slow transpiration rate [50], it is likely that they receive most of their N via the phloem. Whether or not ureides are delivered to fruits via the phloem has not been conclusively proven. It seems unlikely, however, that such large quantities of ureides would be synthesized in fruits from other compounds, such as amino acids and amides, as this would appear to be an inefficient process. It is more likely that ureides themselves are transported directly to fruits via the phloem from storage pools in leaves or stems. Alternatively, ureides might be transferred from the xylem to the phloem at some point during passage from the root to the shoot. There is no direct evidence for this at present. Labelling studies could provide an answer to this question and although ^{14}C-labelled ureides are not commercially available, they can be synthesized from simpler compounds such as [^{14}C]-urea (e.g. [57]).

Nothing is known about phloem loading and unloading of ureides. Is loading an energy-dependent process similar to that demonstrated for amino acids [58, 59]? Allantoate (allantoic acid) uptake by *Saccharomyces cerevisiae* was inhibited by certain amino acids including asparagine [60]. Do such interactions occur in higher plants? A competition between asparagine (the major amino acid used in N-transport in soybeans [61]) and ureides may be important in the phloem-loading process and could conceivably be a limiting factor in phloem transport of N and possibly crop yield.

ASSIMILATION IN THE SHOOT

In plants considered to have a ureide-based metabolism rather than asparagine-based metabolism [27, 62, 63] the stem usually contains the highest amounts of ureides on an organ basis [10, 12, 40,, 64]. The greatest amounts of ureides were found in stems of nodulated soybeans, amounting to as much as 60–75% of the soluble N [12]. Similar results were reported for nodulated *Vigna* [40] and *Phaseolus* [64]. Reported values for the amount of ureide-N in stem tissue include the very high conentration of ureides in the xylem sap [11, 37, 38] and therefore overestimate the amounts of ureides stored in non-conducting stem tissue. Just how much the values are overestimated is difficult to determine but the adjustments are unlikely to alter the fact that stems contain the greatest amounts of ureides per plant. The accumulation of ureides in stems increased with nodule formation, weight and activity [10, 13, 15] and decreased rapidly when the nodules were removed [15]. Part of this decrease may be the result of a decrease in the ureide content of the xylem sap following nodule removal as it has been shown in soybeans that if N_2-fixation is inhibited then the ureide concentration in the xylem sap decreases [37, 38].

During pod formation in soybeans the amounts of ureides reached a maximum in the stems and then decreased rapidly with seed filling [10, 12, 21, 65]. This decrease may be the result of either ureide transport out of the stem (probably to the fruits which are the dominant sinks at this stage) or degradation and assimilation of ureides into other nitrogenous compounds which may be translocated to the developing fruits.

Most workers have sampled the entire stem of soybeans for their estimations of ureide content but it has been shown that the internodes do not contain equal amounts of ureides. Rather, ureide concentration increases in younger internodes with a maximum concentration at the second or third internode below the apex [10, 12]. The presence of the enzymes allantoinase and allantoicase in stems of soybeans [17, 18] and of allantoicase in *Phaseolus* [44] indicates that stems have the capacity to degrade both allantoin and allantoic acid, but it is not known to what extent stored ureides are broken down and assimilated into other compounds. Table 1 shows that both inner (pith and xylem) and outer stem parts (cortex, phloem and cambium) of soybeans contain ureides and have allantoinase activity. Indeed the measured allantoinase activity was greater than that in the uppermost fully expanded leaves on a fresh weight and organ basis. Further studies are needed to determine the fate of the stored ureides in stems, particularly as one cannot be sure from the reported work whether the observed fluctuations in the ureide content in the stems reflect changes in storage forms or are partly the result of changes in the flux of the xylem sap.

Often petioles have been included in either analyses of ureides in stems [40, 65] or in the leaves [12, 44]. The data given in Table 1 show that in pod-forming soybeans the concentration of ureides in the petioles was as high as that in the lamina but was less than that of the stems. Petioles also had *ca* 60% as much allantoinase activity as the lamina on a fresh weight basis but only around 25% on a per organ basis. In *Phaseolus* the concentration of ureides and amount per organ in petioles were less than that of stems during vegetative growth (Thomas *et al.*, unpublished results). These results do not support the suggestion that the concentration of ureides in the petioles may be greater than that in stems [65]. As noted for stems, the amounts of ureides measured in petioles are probably overestimated because of contamination from xylem sap and possibly phloem.

Table 1. Allantoinase activity and ureides in stem and leaf tissue of soybeans

Tissue	Allantoinase activity (μmol allantoic acid degraded/hr)		Total ureides (μmol)	
	g^{-1} fr. wt	$organ^{-1}$	g^{-1} fr. wt	$organ^{-1}$
Outer stem (green)	218.71	402.42	8.69	15.99
Inner stem (non-green)	64.37	—	6.59	—
Uppermost fully expanded leaf	149.42	308.55	2.26	4.68
8th trifoliolate leaf				
Lamina	191.13	391.83	0.73	1.50
Petiole	115.41	88.83	0.91	0.70

Samples for measurement of allantoinase and ureides were taken from 65-day-old nodulated soybean plants in the early stages of pod formation. Samples for stem extracts were removed from the seventh to eleventh internodes. Allantoinase activity was measured after van der Drift and Vogels [106]. Values shown are the sums of the activities measured in 50 000 g pellets and supernatants. Tissue ureides were measured in 0.05 M Tris–HCl buffer (pH 7.8) extracts after Vogels and van der Drift [87]. Results are means of duplicate samples from two plants.

Significant amounts of ureides have been measured in leaves. The concentrations of ureides are generally highest in young unexpanded leaves and decrease during rapid leaf expansion before increasing again as the leaf matures [44]. The amounts of ureides in leaves are usually much less than those measured in stems and fruits of nodulated soybeans [12, 65] but in nitrate-grown *Phaseolus* the total amount in all the leaves on a plant was as much as that found in the stems [44]. The ureide content of all the leaves on a soybean plant reached a maximum during pod formation and 1 or 2 weeks before the peak in ureide content of other organs [12]. During leaf development the ureide content of a trifoliolate leaf increased with leaf expansion amounting to approximately 40% of the soluble N and decreased just before the end of leaf expansion [12]. The peak in ureide content occurred when the concentration of soluble N had already begun to decrease on a fresh weight basis [13]. However, in field-grown soybeans ureides per g dry weight decreased in parallel with the decrease in total leaf N [61, 65]. In contrast, the ureide content of nitrate-grown *Phaseolus* did not begin to decrease until the leaf was already senescing (yellowing) [44]. Matsumoto *et al.* [12] suggested that ureides may be translocated from older leaves to younger developing leaves. The decrease in the ureide content of leaves could be the result of degradation and assimilation of N into other compounds or the export of ureides themselves. Leaves possess the ability to degrade ureides via allantoinase and allantoicase [17, 19, 40, 44]. In soybeans allantoinase and allantoicase activities were higher in leaves of a non-nodulated variety compared with a nodulated variety [19]. This indicated to the authors that ureides were perhaps formed in the leaves of the non-nodulated plants. While this may be so, the lower enzyme activity in leaves of nodulated plants, which are likely to receive large amounts of ureides via the xylem sap [37], may be the result of repression or inhibition by intermediates of ureide breakdown. Such inhibition has been demonstrated particularly with respect to allantoicase activity in bacteria and fungi [66, 67]. The exact nature of ureide assimilation (to be discussed below) is complicated by the fact that both the allantoin and allantoic acid can be degraded via a non-enzymic reaction [68, 69]. The concentration of ureides in young pods can be very high amounting to 50% of the soluble N [12] or 15% of the total N [65]. As seed-filling progresses, the ureide concentration decreases rapidly in pods [12, 21]. The total amount of ureides in all pods reached a maximum 1–2 weeks before a maximum was attained in the seeds, and pods generally contained four times as much ureides as the seeds [12]. The amount of ureides in the seeds increases only after the ureide content of the pods has reached a maximum. Thereafter, the amounts in pods decrease rapidly, whereas amounts in the seeds reach a peak before finally decreasing as fruits mature [12, 44]. Although the concentration of ureides in seeds is lower than that of the pods, allantoinase activity has been shown to be greater in the seeds than in the pods ([40]; Thomas and Schrader, unpublished results). The results suggest that ureides are first concentrated in the pods and are either degraded into other compounds such as amino acids and amides and/or transported directly to the seed where they are rapidly degraded and presumably assimilated into amino acids and proteins. The time courses of the peaks in ureide content of pods and seeds are similar to those of total N in *Phaseolus* [70] and suggest that, in both soybeans and *Phaseolus*, pods and seeds act as separate and possibly competing sinks.

The high activity of allantoinase in developing seeds support the suggestion that ureides may be degraded rapidly in seeds after translocation from the pods [21]. There is at present no evidence that distinguishes if ureides can enter seeds directly via the vascular tissue (phloem) or if they must first enter cells of the pod wall tissues before being transported to the seeds. It is conceivable that the pod wall tissues may act as 'stronger' sinks than young seeds during early pod development but as seeds develop they become the predominant sink. This could explain the pattern of ureide accumulation in fruit tissue. We still know little about the reactions involved in the assimilation of ureides in higher plants and information is

required on the site of breakdown (cellular and subcellular), the metabolic pathways involved, and on the assimilation and fate of the breakdown products.

THE PATHWAYS OF UREIDE ASSIMILATION

The discovery of the enzymes allantoinase and allantoicase in higher plants [1, 71–73] provided a scheme for an assimilation of ureides whereby purines were degraded into urea and glyoxylate. It was thought that urea would be metabolized via urease into ammonia and from there into other amino acids and proteins [1]. It was later shown that the reaction catalysed by allantoicase (EC 3.5.3.4, allantoate amidinohydrolase) is in fact a two-step reaction involving at least two enzymes, allantoicase and ureidoglycolase [74–76]. The first step (Fig. 2), catalysed by allantoicase, hydrolyses allantoate into urea and ureidoglycolic acid. The latter is then degraded via ureidoglycolase into urea and glyoxylic acid. Another enzyme, allantoate amidohydrolase (EC 3.5.3.9), was found in *Streptococcus allantoicus* which hydrolysed allantoate into ammonia, CO_2 and ureidoglycolate under anaerobic conditions [76, 77]. This enzyme was also shown to be present in aerobically grown *Pseudomonas* and *Penicillium* sp. [66, 67, 76]. Different products of allantoate degradation are formed depending on the enzyme system used (Fig. 2). Degradation via allantoicase and ureidoglycolase result in the production of urea and glyoxylate while ureidoglycolate produced via allantoate amidohydrolase can be degraded by either allantoicase or ureidoglycolase into urea and glyoxylate.

In *Streptococcus allantoicus*, an additional pathway was discovered [76, 78]. Cleavage of allantoate resulted in the production of CO_2, ammonia and ureidoglycolate via allantoate amidohydrolase as before (Fig. 2). In addition, however, a proposed intermediate in this reaction, ureidoglycine, was postulated to undergo an enzymic or non-enzymic transamination with glyoxylate to produce glycine and oxaluric acid [76]. Glycine production was demonstrated in cell-free extracts and could be enhanced by the presence of Cu^{2+} ions which are known to catalyse non-enzymic transamination reactions with glyoxylate [79–81]. The other product of the reaction, oxaluric acid, could conceivably undergo a transcarbamoylase reaction with inorganic phosphate to produce carbamoyl phosphate and oxamic acid [76]. Wu *et al.* has proposed that ureidoglycine is degraded by an enzyme other than allantoate amidohydrolase in this scheme [82]. ATP can be synthesized from carbamoyl phosphate [83]. Whether these reactions occur in higher plants is not known, although oxamate has been shown to occur with allantoin in beets [84, 85]. Both allantoin and allantoic acid can be hydrolysed under certain conditions by non-enzymic reactions [68, 69]. Allantoin is converted into allantoate which is hydrolysed into ureidoglycolate and urea or isocyanate, ammonia, and glyoxylate depending on the pH and temperature. There is no information as to whether these reactions occur *in vivo* in

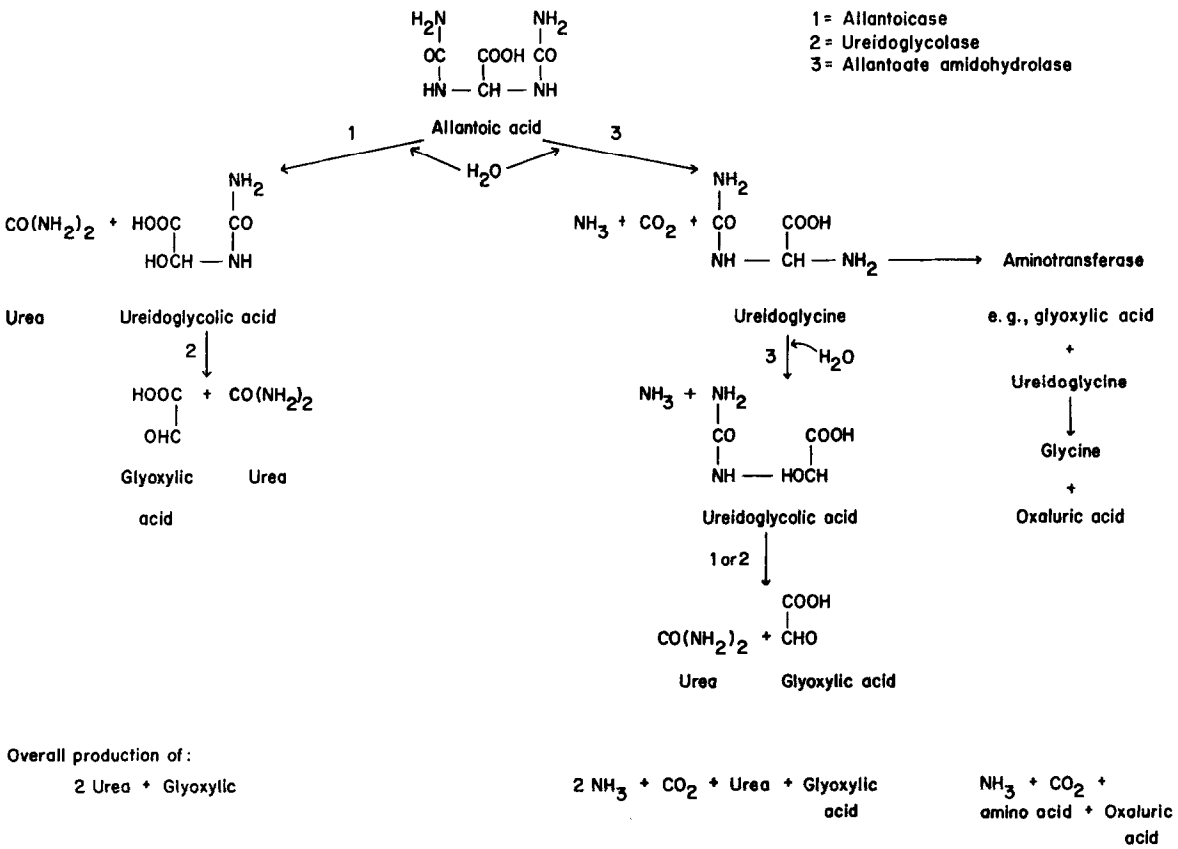

Fig. 2. Metabolic pathways for the assimilation of allantoic acid.

higher plant tissue. In all the reports of allantoicase activity in higher plants only glyoxylate has been demonstrated to be produced from allantoate [19, 26, 44, 86]. By use of a method for the differential hydrolysis of glyoxylate derivatives [87], the presence of either ureidoglycolate or ureidoglycine was suggested in extracts of leaves of *Phaseolus* incubated with allantoate although these compounds were not definitely identified [88].

PROPERTIES AND REGULATION OF THE ENZYMES INVOLVED IN UREIDE ASSIMILATION

Most of the published work on the enzymes involved in ureide assimilation has been on micro-organisms [66, 67, 74, 75, 77, 82, 89–99] with relatively few reports from higher plants. Uricase (urate: O_2 oxidoreductase, EC 1.7.3.3) activity was associated with glyoxysomes of various fat-degrading tissues [100] and peroxisomes of root tissue of *Phaseolus coccineus* [101]. The enzyme from castorbean (*Ricinus communis*) endosperm had a K_m of 7.4 μM and was inhibited by high concentrations of its substrate (uric acid) and also by xanthine and hypoxanthine [100]. In soybeans two forms of the enzyme were described, one located in the nodules and the other in the radicle. The two were distinguished by differences in pH optima and K_ms (0.56 μM in radicle and 8.1 μM in nodules) and by the requirement of the radicle enzyme, but not the nodule enzyme, for a low MW cofactor [17]. The nodule enzyme was apparantly a feature of the symbiosis between the plant and *Rhizobium japonicum* as the bacteria in pure culture had no uricase activity when assayed in the presence of uric acid, RNA or RNA degradation products [16]. Xanthine and hypoxanthine, chelating agents and high substrate concentrations inhibited both forms of the enzyme [17]. In germinating soybeans, highest activities per g fresh weight were measured in root tips, none in cotyledons or hypocotyls [16], whilst in older plants the activities in nodules were ten times that in leaves. The highest activities were measured in the larger nodules compared with the smaller nodules without leghaemoglobin and nitrogenase activity [16, 19]. Exogenous nitrogen sources have been shown to effect uricase activity. Nitrate at 50 ppm appeared to decrease the amount of uricase cofactor and hence uricase activity in radicles of non-nodulated soybeans [18], but in rooted leaf cuttings of *Phaseolus coccineus*, uricase activity increased with increasing concentrations of nitrogen fed to the plants [101]. In the latter work, however, allantoin synthesis was known to increase in the leaf cuttings under the experimental conditions used [63] while in the former work the addition of 50 ppm nitrate to non-nodulated plants had little effect on the very low amounts of ureides present [19]. These results suggest that the increase in uricase activity is correlated with increases in the synthesis of ureides.

Allantoinase from *Phaseolus hysterinus* and *Glycine hispida* (*G. max*) is inhibited by phosphate ions, cysteine and other reducing compounds, such as glutathione and thioglycolate [97, 102], and by high concentrations of urea (1 and 5 M) [98]. Lower concentrations of urea (1–20 mM) have only a slight depressing effect on allantoinase in soybean leaf extracts (Thomas and Schrader, unpublished results). Although allantoinase from several bacteria can be activated by a short term pre-incubation under acid conditions [77], the enzyme from *Phaseolus* and *Glycine* was only slightly activated [98]. Generally, Mn^{2+} ions or other bivalent cations have a stimulating effect on both plant and bacterial enzymes but the enzyme from wheat and gherkin were inhibited by Mn^{2+} [97]. The inhibition of allantoinase by phosphate ions is thought to be the result of removal of Mn^{2+} from the enzyme in bacteria but it was shown that phosphate ions inhibited the enzyme from plants in the absence of Mn^{2+} [97]. The cause of this inhibition is unknown. Reported values for the apparent K_m of the enzyme for its substrate, allantoin, are rather high; *Phaseolus* 46 mM, *Glycine* 14 mM [98], peanuts 3.8 mM [103], castorbeans 13.8 mM [104]. There have been several reports of an association of allantoinase with a membrane or microbody in fat-degrading tissues of higher plants [100, 104, 105]. In castorbeans the enzyme was present in glyoxysomes, but not mitochondria [105], and in both glyoxysomes and proplastids [100]. In soybeans the enzyme also appears to be associated with an organelle and/or membranes as over 50 % of the extractable activity was recovered in the 50 000 *g* or 100 000 *g* pellet of extracts from leaves, etiolated hypocotyls and fruits (Thomas and Schrader, unpublished results). In *Saccharomyces cerevisiae* the uptake of allantoin was shown to be energy dependent, inducible by allantoin but not allantoate or urea and inhibited by some amino acids including asparagine, aspartate and serine and by protein synthesis inhibitors [57, 60]. Do similar interactions betwen ureide uptake and amino acids occur in higher plant cells? This question may be particularly important with respect to leaf mesophyll cells in plants such as soybeans which transport most of their N from the root to the shoot in the form of ureides and asparagine. It may also be relevant to the loading of nitrogenous compounds into the phloem.

In bacteria and yeasts allantoicase activity is now thought to involve two enzymes, the first, allantoicase, catalysing the degradation of allantoate to ureidoglycolate and the second, ureido-glycolase or ureidoglycolatase, catalysing the degradation of ureidoglycolate to urea and glyoxylate ([74, 76, 99]; Fig. 2). In *Pseudomonas aeruginosa* allantoicase was inhibited by glyoxylate and some amino acids, particularly the D-forms of asparagine and glutamine, and also by products of allantoate degradation such as ureidoglycolate [66, 67, 95]. Asparagine also inhibited ureidoglycolate degradation via ureidoglycolase [95]. In the yeast *Candida utilis*, allantoicase was induced by uric acid, allantoin and allantoate, inhibited by urea and ammonia, but unlike the bacterial enzyme was not inhibited by glyoxylate or ureidoglycolate [99].

The other enzyme known to degrade allantoate, allantoate amidohydrolase, was activated 30–90-fold by acid pre-treatment in bacteria [77], by Mn^{2+} and other bivalent ions and also by reducing compounds [66, 67, 92, 94]. Ureidoglycolate inhibited the enzyme in *Pseudomonas aeruginosa* [66].

Reported values for the K_m of allantoicase for its substrate allantoate range from 9.5 to 28.6 mM in bacteria and fungi [64, 66, 67] and 5.5 mM in *Candida utilis* [99]. A value of 0.85 mM for allantoicase measured in peanut cotyledons is the only reported K_m value from higher plants [103]. In plants there appears to be some difficulty with the allantoicase assay normally used for micro-organisms with the result that there are relatively few published reports [19, 44, 86, 103, 106, 107]. Reasons for this difficulty are unknown but may be related to the

reported inhibition of the enzyme by the products of its reaction. Removal of these products may be necessary to obtain reasonable reaction rates. For example, Trijbels and Vogels [96] showed that the addition of urease or ureidoglycolase to the assay mixture for allantoicase activity in *Pseudomonas aeruginosa* stimulated the amount of allantoate degraded. A shift in the equilibrium constant was probably responsible for the observed stimulation.

Very little is known about the regulation of ureide-degrading enzymes in higher plants. There is some indication that light is either directly or indirectly involved in ureide a similation. Several reports have shown that allantoin, and more commonly allantoate, accumulate in dark-treated plants [1, 64, 106, 107]. In *Phaseolus vulgaris*, ureides accumulated not only in green tissue but also in the roots following dark treatment [64]. In the moss *Funaria hygrometrica* the total amount of allantoin and allantoate depended on the light quality with highest amounts accumulating under green and far-red light [108]. The authors suggested that purine degradation to allantoin involved two processes, one dependent on light and the other not. In darkness allantoicase activity was inhibited and this inhibition could be overcome by the addition of ATP or ATP plus glucose [107]. Ureides have also been reported to accumulate in chlorophyll-deficient parts of leaves of *Acer negundo* [109] and *Pelargonium zonale* [31, 32, 110]. The exact role of the photosynthetic apparatus in ureide metabolism is still obscure and requires further investigation. In bacteria, yeasts and fungi there does not appear to be any requirement for ATP in any of the degradation steps and if such a requirement for ATP and/or other photosynthetic reactions is found to be widespread in green tissue then the mechanism of ureide metabolism may be quite different in higher plants.

In leaves of *Phaseolus vulgaris* fed different amounts of allantoate via the petiole, the *in vivo* allantoicase activity, estimated by measuring the difference between the amounts taken up plus the small amount already in the leaf at time zero minus the amount remaining at the end of the feeding period, increased with increasing amounts of allantoate taken up but the *in vitro* activity was little changed (Table 2). Possibly the flux of allantoate into the leaves may regulate the allantoicase activity in a manner similar to the effect of nitrate flux on nitrate reductase activity [111]. Alternatively, the *in vivo* breakdown activity could, in part, be non-enzymic and depend on the concentration of allantoate present in the leaf.

Table 2. Effect of feeding primary leaves of *Phaseolus vulgaris* with different concentrations of allantoate on leaf allantoicase activity

Allantoate taken up (μmol/leaf)	Allantoicase activity (μmol allantoate broken down/hr/g fr. wt)	
	in vitro	*in vivo*
1.53	2.41	0.24
1.93	2.04	1.58
3.54	2.94	2.45
Intact plant	1.77	—

Primary leaves (12 days old) of *Phaseolus vulgaris* were fed via the petiole with an artificial sap solution based on that measured in nitrate grown plants [43] containing 2.07, 3.07 and 5.07 mM sodium allantoate. After 1 hr feeding *in vitro* allantoicase activity was measured after Thomas *et al.* [44].

OTHER NUTRITIONAL EFFECTS ON UREIDE METABOLISM

Besides N-nutrition other minerals are also known to have some effects on ureide metabolism in plants. Allantoin, but not allantoic acid, accumulated in K^+-deficient leaves of banana whereas in healthy plants or those deficient in other minerals no accumulation was observed [112]. Similarly, in wheat leaves P-deficiency resulted in an accumulation of allantoin [113].

In the tropical pasture grass *Cynodon dactylon*, allantoin accumulated in chlorotic shoots and was found to cause chlorosis when applied to the plants. The mechanism causing chlorosis was not known but leaf plastids became smaller and lost their pigments [114]. Carbon-deficient root cuttings of *Phaseolus vulgaris* assimilating large amounts of NH_4NO_3 accumulated allantoin which was thought to be a response to NH_3-toxification [5].

THE ROLE OF UREIDES IN THE CARBON ECONOMY OF PLANTS

The major N-compounds in the xylem sap of a large number of plants are the amides, glutamine and asparagine, and one of the suggested reasons for the predominance of these two compounds is that their relatively low C:N ratios minimize the loss of carbon from the root to the shoot via translocation (e.g. [49]). It would appear reasonable then to assume that plants which use ureides as the major forms of N in transport processes (C:N = 1:1) would be able to save even more carbon in transporting N to the shoot than those which use amides. In nodulated legumes which use ureides, Pate and co-workers [115, 116] have demonstrated that 'ureide' plants are indeed more efficient in C-usage in nodules compared with 'amide' plants. The improved use of carbon in the nodules was not, however, entirely due to the use of ureides for transport; it was also the result of a better conservation of C via a higher PEP carboxylase activity and a lower rate of hydrogen evolution compared with a species using mainly amides for transport [116]. The lack of knowledge regarding the precise costs of ureide synthesis in terms of ATP, NADH and carbon from either purine synthesis or some simpler pathway [115] makes comparison with the costs of an amide-based metabolism difficult. In terms of ATP consumption Atkins *et al.* [115] estimated that the use of ureides in the assimilation of NH_3 was slightly better than the use of amides. Israel and McClure [38] concluded that in terms of ATP equivalents ureide synthesis and transport were as efficient as asparagine synthesis and transport.

CONCLUSION

Even though there is now very good evidence for the synthesis of ureides in root tissue via purine synthesis and degradation we still do not know the exact pathway of N into these compounds. Is the pathway the same in plants as that in animal and microbial systems? It has been

shown that when the normal aerobic degradative pathway is blocked in chlorophyll-deficient leaves of *Pelargonium*, ureides can still be formed via an alternative cleavage of adenine [110], which involves the production of serine and hence glycine. Evidence for a recycling of the glyoxylate produced via purine degradation was claimed by studying the fate of glycine labelled with ^{14}C in two different positions in chlorophyll-deficient leaves of *Pelargonium* [7]. It was suggested that glyoxylate was reintroduced into purine synthesis via glycine and possibly serine. Further work is needed to determine if the proposed cycle is of general occurrence. Knowledge of plant purine metabolism is meagre compared with animal and microbial tissues. Is ureide synthesis the result of an overproduction of some intermediate in purine metabolism and, if so, which compound? An obvious candidate would be inosinic acid (IMP or inosine monophosphate) the compound from which all four purine bases are synthesized. Two independent pools of IMP were suggested to be present in germinating embryonic axes of soybeans, one arising from *de novo* synthesis, the other from a purine salvage pathway which are regulated independently [117]. Is the synthesis of ureides the result of a failure in the regulatory mechanism of one of these pools?

Despite the fact that ureides were recognized as important compounds in the storage and transport of N at the beginning of the century, our knowledge of how plants assimilate these compounds is minimal. In addition to the idea that the use of ureides conserves carbon, Ishizuka [118] has hypothesized that ureides arising from N_2-fixation are stored in stems and leaves as materials for use later in pod formation whereas the application of fertilizer N results in increased concentrations of amino acids and nitrate in plant organs and increased vegetative growth, but a decrease in the number of pods retained on the plant. How vegetative growth is influenced by the composition of the plant N is not known and this hypothesis needs further investigation.

The role of ureides may be important in the detoxification of ammonia in root tissues [6, 119] and it is perhaps surprising that in a recent review on ammonia detoxification ureides were not mentioned [120]. Many of the legumes which have high nitrogen requirements for seed production per g of available photosynthate [121] also use ureides as major transport forms of nitrogen (e.g. soybeans, cowpeas, *Phaseolus* sp.) suggesting that perhaps ureides in these plants are particularly important for both ammonia detoxification and the conservation of carbon.

The relationship between nodule morphology, anatomy and physiology in the major legume crops has been discussed by Sprent [122]. Species which use ureides as the major export products of fixed N belong to the tribe Phaseoleae. As pointed out by Sprent [122], both allantoin and allantoic acid have relatively low solubilities compared with the amides asparagine and glutamine. This can be considered beneficial in terms of ureides being a convenient form of storage N but may be disadvantageous if the low solubility limits nitrogen translocation and hence crop yield.

In conclusion, the importance of ureides in N-metabolism of some plants is evident. However, additional information is required on the assimilation of these compounds, particularly with respect to legume seed production.

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