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# Amino Acid Metabolism of Pea Leaves

## LABELING STUDIES ON UTILIZATION OF AMIDES[1]

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### ABSTRACT

**Short term (2-hour) incorporation of nitrogen from nitrate, glutamine, or asparagine was studied by supplying them as unlabeled ($^{14}N$) tracers to growing pea (*Pisum sativum* L.) leaves, which were previously labeled with $^{15}N$, and then following the elimination of $^{15}N$ from various amino components of the tissue. Most components had active and inactive pools. Ammonia produced from nitrate was assimilated through the amide group of glutamine. When glutamine was supplied, its nitrogen was rapidly transferred to glutamic acid, asparagine, and other products, and there was some transfer to ammonia. Nitrogen from asparagine was widely distributed into ammonia and amino compounds. There was a rapid direct transfer to glutamine, which did not appear to involve free ammonia. Alanine nitrogen could be derived directly from asparagine, probably by transamination. Homoserine was synthesized in substantial amounts from all three nitrogen sources. Homoserine appears to derive nitrogen more readily from asparagine than from free aspartic acid. A large proportion of the pool of γ-aminobutyric acid turned over, and was replenished with nitrogen from all three supplied sources.**

The central role of glutamine in primary nitrogen metabolism of higher plants has been demonstrated by several workers. The glutamine synthetase system is present in roots (8) and leaves (7, 18, 19), and a number of experiments with $^{15}N$ have shown that glutamine is rapidly labeled in primary assimilation. In kinetic studies with $^{15}N$-nitrate (3) in young pea leaves, the amide group of glutamine had the highest rate of incorporation of ammonia, followed by glutamic acid and alanine; asparagine was extensively labeled, but at a slower rate which did not appear to be due to primary assimilation. Enzyme studies (11) suggest that the amide group of glutamine is the preferred donor which is transferred to aspartic acid for asparagine synthesis. In pea plants, asparagine is important in transport of nitrogen in the xylem (5), and is also stored in leaves, having one of the largest pools of the amino acids (10). Homoserine is also present in large amounts in pea plants (9, 10).

There are relatively few data available on utilization or degradation of asparagine (11). Asparaginase has been suggested as providing the main pathway for asparagine utilization. In developing lupin seeds, asparagine was utilized as a nitrogen source, with the nitrogen appearing predominantly in ammonia, glutamine, and alanine, and asparaginase was very active in extracts of the tissue (2).

This paper describes experiments using $^{15}N$ and $^{14}N$ labeling of growing pea leaves, which allowed a comparison of the fate of nitrogen from nitrate, glutamine, and asparagine, during a period of 2 hr. Both amides supplied nitrogen to a range of compounds, including homoserine. Nitrogen from asparagine was extensively redistributed, and it was shown that asparagine could provide nitrogen for glutamine synthesis.

## MATERIALS AND METHODS

The objective was to follow the flow of nitrogen from asparagine and glutamine. However, supplies of $^{15}N$-labeled amides are not easily obtainable, whereas $^{15}N$-nitrate is readily available. For this reason, experiments were carried out using plants grown continuously on $^{15}N$-nitrate, so that they became uniformly labeled. Expanding leaves from these plants were then supplied with unlabeled ($^{14}N$) nitrogen sources, and the elimination of $^{15}N$ from various pools was followed, in effect using $^{14}N$ as a tracer.

*Pisum sativum* (L. cv. Little Marvel) plants were grown as described earlier (3), except that the nutrient solution supplied throughout the growth of the plants contained 4 mM nitrate labeled with $^{15}N$, at 7% atom excess. Plants selected for the experiment had four fully expanded leaves, and had just reached the morphological stage known to correspond to approximately half of the attainable value for the dry weight and nitrogen content of the fifth leaf. "Expanding leaves" used in the experiment consisted of the fifth leaf and the apical bud enclosed within the stipules, and were detached from the plant by cutting the stem under water just above the point of attachment of the petiole of the fourth leaf. The cut stems of the excised leaves were placed through holes in Petri dish lids, so that the ends were supported in solutions held in the dish. The nutrient solutions contained either $^{14}N$-nitrate, $^{14}N$-glutamine, or $^{14}N$-asparagine (each at 4 mM) as the sole nitrogen source, which were drawn into the leaves by transpiration. The experiments were started in the 7th hr of the light period, and continued under normal growth conditions. At intervals from 10 to 120 min after transfer, samples (each consisting of three expanding leaves) were taken, extracted, and analyzed as described previously (3). $^{15}N$ measurements were made using a type MS-10 mass spectrometer, with small volume inlet system.

## RESULTS

The expanding leaf samples used in these experiments consisted of only leaf 5 plus apex, contrasting with the earlier work (3) in which leaf 4 was also included in the young leaf sample. The fifth leaf has higher levels of amino acids than the samples which included the fourth leaf. The experimental period was chosen to take place during the later part of the light period, when amino acid pool sizes are most stable (3).

Figures 1 to 6 show the elimination of $^{15}N$ from ammonia and the most actively metabolized amino acids, and the pool sizes for these compounds during supply of three different nitrogen

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sources. The graphs show dilutions of $^{15}N$ (already present), as $^{14}N$ from the tracer source flows into pools; however, the graphs also represent the proportion of the pool which has been synthesized from the $^{14}N$ tracer compounds (see below), and one axis of each graph is identified accordingly. For purposes of discussion, the changes will usually be described in terms of entry of $^{14}N$ tracer, which enters and dilutes the $^{15}N$ already present in the pool.

Care must be taken in interpretation of graphs of $^{15}N$ content, and simultaneous changes in pool sizes must also be considered. For example, it would be possible to observe a decrease in $^{15}N$ content even when no $^{15}N$ is moving out of the pool, if pool size increases due to an input of $^{14}N$. When nitrogen is flowing through a sequence of compounds, changes will not show rapidly in later compounds, since $^{15}N$ already eliminated from the first pool of the sequence still has to flow (undetected) through intermediate pools. Finally, it must be recognized that the whole pool measured may represent two or more pools totally unconnected in separate compartments of the intact tissue.

**Calculations.** At the beginning of the experiment, pools are uniformly labeled with 7% $^{15}N$ plus 93% "old" $^{14}N$, which is diluted by any "new" $^{14}N$ from the tracer compound. The value for $^{15}N$ can be used to calculate the proportion of new $^{14}N$ which has entered the pool. For any value for $^{15}N$

$$\% \text{ Old N} = \frac{93 \times {}^{15}N\%}{7} + {}^{15}N\%$$

$$\% \text{ New N} = 100 - \% \text{ old N}$$

For any given time

$$\text{Pool size } (\mu g \text{ N}) \times \frac{\% \text{ new N}}{100} = \mu g \text{ New N in pool}$$

The rate of increase of amount of new N in a pool during any particular time interval will give some measure of the rate of synthesis of that compound—see example of calculation in Table I. However, this value will be a minimum value, since it will not account for $^{15}N$ (plus old $^{14}N$) which continues to flow into the pool, nor for new $^{14}N$ which enters and leaves the pool between the experimental observations. A further limitation is that meaningful values are only obtained during the period of rapid dilution of $^{15}N$, and cannot be derived once the pool begins to approach saturation, even though input and turnover may continue at the same rate. In spite of these limitations, the figures are of some value, and calculations for some compounds are presented in Table I. The table also contains data for actual rates of total accumulation for some compounds, calculated for the few instances where measured pool sizes showed a sustained increase. These figures will include any kind of nitrogen entering to cause growth of the pool, but again will be minimum values which cannot account for the amount of nitrogen which flows through the pool. Where a figure for rate of synthesis from new N is greater than total measurable rate, there must be a net flow of $^{15}N$ out of the pool; when new N rate is less than total measurable rate, there must either be a net inflow of $^{15}N$ (early sampling times) or the pool is saturating (late sampling times).

The results of the experiment using nitrate as tracer (Figs. 1 and 2) are very similar to the findings for young leaves when nitrate was supplied to the whole plant (3) except that the supply of tracer directly to the expanding leaf resulted in much more rapid incorporation. There was an immediate entry of tracer nitrogen into ammonia, the first accumulating product of nitrate reduction. Most rapid incorporation into organic form was into the amide group of glutamine. Glutamic acid (which in this experiment includes the amino group of glutamine) also showed a fast initial rate of dilution of $^{15}N$, followed (in order of rate of dilution) by the amide then amino groups of asparagine, alanine (not shown), aspartic acid, homoserine, and γ-aminobutyric acid. Most curves showed a faster early decrease, then leveled

Table I. Rates of incorporation of nitrogen from nitrate, glutamine and asparagine into some amino acids in expanding pea leaves. These are minimum rates for the specified time intervals, calculated from data in Figs 1-6 as discussed in the text. Maximum for each row underlined. Figures in parenthesis show measured rates of increase in pool size for the same time intervals.

| | | Rate: μgN/g.Fresh Wt./min. | | | |
|---|---|---|---|---|---|
| | Time Interval: | 0-10 min. | 10-20 min. | 20-30 min. | 30-45 min. |
| NITRATE TRACER | $NH_4^+$ | 1.70 | S[1] | S | S |
| | Gln(amide) | 0.70 | 1.04 | S | S |
| | Asn(amide) | 0.44 | 1.30 | 0.89 | S |
| | Asn(amino) | 0.18 | 1.12 | 1.19 | S |
| | Asp | 0.06 | 0.48 | 0.58 | 0.42 |
| | HS[2] | 0.07(0.6) | 0.56(2.4) | 0.73 | 0.30 |
| | GAB[2] | 0.03 | 0.06 | 0.12 | 0.07 |
| GLUTAMINE TRACER | $NH_4^+$ | 0.44 | N[3] | 0.26 | 0.44 |
| | Asn(amide) | 1.00 | 1.97(2.1) | S | S |
| | Asn(amino) | 0.59 | 1.60(2.1) | 1.25(0.5) | S |
| | Asp | <0.01 | 0.01 | 0.54(2.3) | 0.72(1.0) |
| | HS | 0.30(0.6) | 0.66 | 0.20 | 1.25(1.2) |
| | Glu | 0.42(0.5) | 1.30(1.3) | S | S |
| | GAB | 0.25[4] | | 0.17 | 0.13 |
| ASPARAGINE TRACER | $NH_4^+$ | 0.22[4] | | 0.75 | S |
| | Gln(amide) | 0.43 | | <0.01 | 0.57 |
| | Asp | 0.28 | | 0.45 | S |
| | HS | 0.21 | | 0.65(0.8) | 0.91(1.3) |
| | Ala | 0.22 | | 0.32 | S |
| | GAB | 0.09 | | 0.13 | 0.10 |

[1] S = curve approaching saturation, value not meaningful.
[2] HS = homoserine; GAB = γ-aminobutyric acid.
[3] N = negative value due to pool size decrease.
[4] Calculated for 0-20 min.

Example of calculation: The 10 min. glutamine (amide) sample (nitrate tracer, Fig 1) had a pool size of 22 μgN (1.57 μmol)/g Fresh Wt. at 4.75% $^{15}N$ excess.
% Old N = 67.9; % New N = 32.1; New N in pool = 7.06 μgN
Similarly, at 20 min (24 μg pool, 1.9% $^{15}N$ excess), New N in pool = 17.49 μgN. Thus from 10-20 min, 10.43 μg new N entered the pool, at an average rate of 1.04 μgN/min.

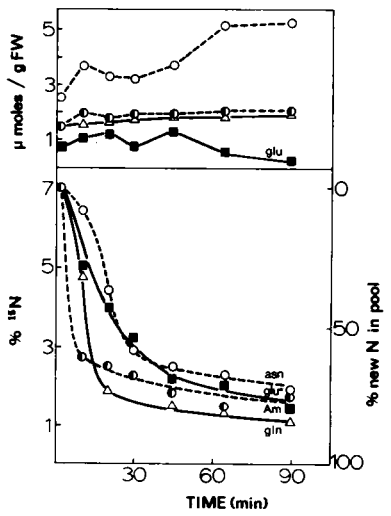


FIG. 1. Nitrate tracer. Pool sizes and levels of $^{15}N$ in various components following direct supply of $^{14}N$-nitrate to expanding leaves from pea plants previously labeled with $^{15}N$. The level of $^{15}N$ is inversely proportional to the amount of new nitrogen incorporated into the pool from nitrate. Free ammonia (Am, ◐), asparagine amide group (○), glutamine amide group (△), glutamic acid (glu, ■); the glutamic acid for $^{15}N$ analysis (glu*, ■) included the α-amino nitrogen from glutamine.

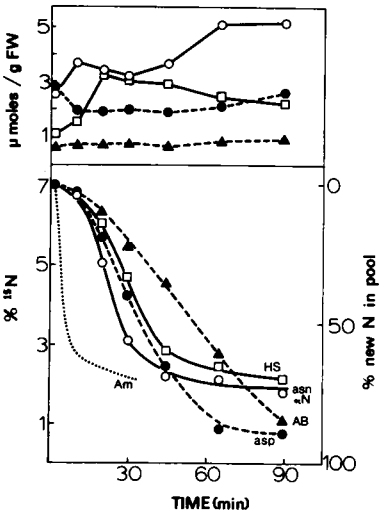


FIG. 2. Nitrate tracer. Details as in Figure 1. Asparagine α-amino group (asnαN, ○), aspartic acid (●), homoserine (HS, □), γ-aminobutyric acid (AB, ▲). Part of the curve for ammonia (Am) from Figure 1 included for comparison.

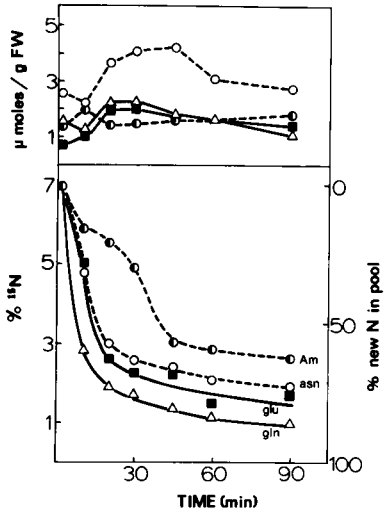


FIG. 3. Glutamine tracer. Pool sizes and levels of $^{15}N$ in various components following direct supply of $^{14}N$-glutamine to expanding leaves from pea plants previously labeled with $^{15}N$. The level of $^{15}N$ is inversely proportional to the amount of new nitrogen incorporated into the pool from glutamine. Free ammonia (Am, ◐), asparagine amide group (○), glutamine amide group (△), glutamic acid (■).

off as the active pool became saturated with new $^{14}N$, indicating the presence of a separate, less active pool. For most compounds, 20 to 40% of the pool turned over slowly or not at all. The levels of most amino acids showed only small changes during this experiment, however, there was an increase of the amount of asparagine in the tissue, and initially a sharp increase in the amount of homoserine. In the young leaves of whole plants, there is little change in these pools at this phase of the light period (3) so the increases caused by direct supply of nitrate suggest that nitrogen is entering the system somewhat faster than in the intact plant.

When glutamine was supplied to the expanding leaves (Figs. 3 and 4) the most rapid rates of incorporation from the tracer were into glutamic acid (here analyzed separately from the amino group of glutamine) and the amide group of asparagine, reaching maximum rates of synthesis after only 10 min, clearly showing that these compounds received nitrogen directly from glutamine. Free ammonia shows a complex biphasic curve, which is discussed later. However, the amount of tracer nitrogen in ammonia was quite low in the early stages of the experiment, when glutamic acid and asparagine were incorporating rapidly, and thus it cannot be regarded as the main precursor for these two compounds. There was extensive incorporation into alanine (not shown), the amino group of asparagine, homoserine, and γ-aminobutyric acid. Movement of nitrogen into free aspartic acid took place quite slowly.

Supply of glutamine appeared to disturb the metabolism, as pool sizes of many compounds showed transient or prolonged changes. Asparagine increased, while aspartic acid exhibited a large fall then recovery. There was a dramatic increase in the amount of homoserine, which increased over 5-fold in 60 min. There was a steady accumulation throughout this time, although it was 30 min before the rate of input of nitrogen into homoserine from the tracer glutamine reached a maximum (Table I). There were only small changes in the amount of glutamine itself in the tissue, and it is clear that it was rapidly metabolized and the nitrogen converted to other forms for storage.

When asparagine was used as the nitrogen source (Figs. 5 and 6) the rates of transfer of label were slower than from nitrate or glutamine. The compounds receiving nitrogen most rapidly were glutamine (amide group), alanine, and homoserine (which showed the most rapid calculated rate of synthesis). Free aspartic acid, glutamic acid (including the amino group of glutamine), ammonia, and γ-aminobutyric acid also incorporated nitrogen from asparagine. The transfer of nitrogen into free ammonia took place slowly at first, at a time when the amide group of glutamine was showing a rapid input of tracer nitrogen. Changes in pool sizes were less than when glutamine was supplied. Homoserine again showed the major increase.

## DISCUSSION

The results presented above give a considerable amount of information about the metabolism of some of the important amino acids in expanding pea leaves in the light period. Although the leaves were detached, the entry of nitrogen through the xylem would be essentially the same as in the intact plant, but any phloem transport would be disrupted. The behavior of some of the individual amino acids is discussed below. It should be remembered that we are tracing only the nitrogen groups of the compounds, and can say nothing about the corresponding carbon skeletons. Where amides were used as tracers, it was not

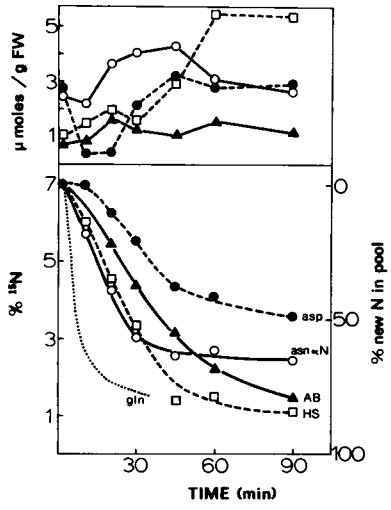


FIG. 4. Glutamine tracer. Details as in Figure 3. Asparagine α-amino group (asnαN, ○), aspartic acid (●), homoserine (HS, □), γ-aminobutyric acid (AB, ▲). Part of the curve for glutamine (amide group) from Figure 3 is included for comparison.

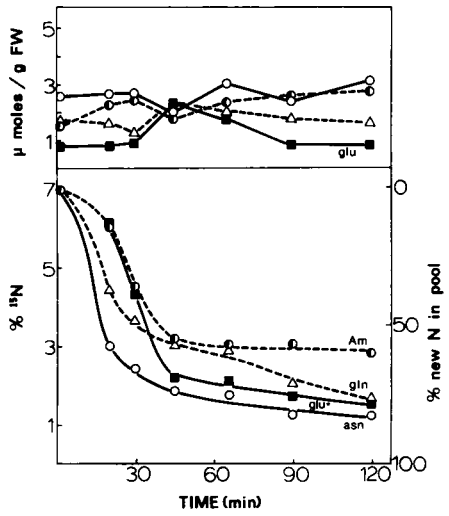


FIG. 5. Asparagine tracer. Pool sizes and levels of $^{15}N$ in various components following direct supply of $^{14}N$-asparagine to expanding leaves from pea plants previously labeled with $^{15}N$. The level of $^{15}N$ is inversely proportional to the amount of new nitrogen incorporated into the pool from asparagine. Free ammonia (Am, ◐), asparagine amide group (○), glutamine amide group (△), glutamic acid (glu, ■); the glutamic acid for $^{15}N$ analysis (glu*, ■) included the α-amino nitrogen from glutamine.

possible to determine whether nitrogen detected in a compound arose from the amide or the amino group of the tracer.

**Glutamine and Glutamic Acid.** Glutamine is one of the most active compounds in amino acid metabolism in pea leaves. The pool turns over rapidly and only a small portion of the pool (12–15%) is inactive or turning over slowly. The principal synthetic mechanism is glutamine synthetase, and these results support its role as the major pathway for assimilation of ammonia into organic form (15). Glutamine nitrogen can be transferred to a variety of compounds and the experiment with tracer glutamine shows that a considerable amount of subsequent distribution is to asparagine and to glutamate, presumably through the action of glutamate synthase. Glutamate can in turn participate in redistribution through interconversion and transamination. Some primary assimilation may occur through glutamate dehydrogenase, although this can no longer be regarded as the principal pathway for ammonia assimilation.

The experiment with tracer asparagine strongly suggests the presence of a previously unrecognized reaction, with direct transfer of nitrogen from asparagine in glutamine synthesis. Figure 5 shows a quite rapid initial synthesis of glutamine, followed by a slower but prolonged incorporation of tracer. In the early part of the experiment, the new nitrogen in ammonia is insufficient to account for the rapid initial transfer of tracer to glutamine, and in any case if the initial synthesis was from the normal accessible pool of ammonia, it would be expected to give a smooth and continuous fall to the final saturation level, as occurs with nitrate (Fig. 1). The labeling sequence for glutamine can be explained by a direct and rapid transfer of nitrogen from asparagine into one pool of glutamine, which begins to saturate, with a superimposed slower synthesis from the ammonia pool (initially containing residual $^{15}N$) which continues toward a second saturation level. Several systems might provide a mechanism for transfer of nitrogen from asparagine to glutamine. Under certain regulatory conditions, glutamine synthetase might accept asparagine in place of ammonia, in much the same way as asparagine synthetase can accept both glutamine and ammonia (12, 20). Alternatively, asparagine synthetase (utilizing glutamine) may be reversible, although no such activity was detected for the enzyme from lupin (Rognes, personal communication). Finally, a separate enzyme system may exist to carry out a specific transfer of a nitrogen group from asparagine to glutamine.

γ-Aminobutyric acid steadily incorporated tracer in all three experiments, and thus the pool must be turning over since there were no great changes in pool size. This amino acid presumably arises from decarboxylation of glutamate and certainly the labeling bears some similarity to the rate of entry tracer into glutamate, being faster in the glutamine experiment, slower when nitrate was supplied. There is no indication of the pathway of utilization of γ-aminobutyric acid, although transamination reactions have been reported (22).

**Asparagine, Homoserine, and Aspartate.** Transfer of tracer from glutamine to asparagine confirms the suggestion from enzyme observations (12, 20) that asparagine synthesis utilizes glutamine rather than ammonia as nitrogen donor.

Mitchell and Bidwell (17) suggested that there was little synthesis of homoserine in shoot tissues of young pea seedlings, but the work here shows that developing leaves of somewhat older plants are able to synthesize this amino acid. Synthesis of homoserine in microorganisms (4) involves conversion of aspartate via aspartyl phosphate and aspartyl semialdehyde; corresponding enzymes have been found in plants (1, 6, 23). However, the labeling of homoserine found in our experiments does not seem to be related to the labeling of the free pool of aspartate. In particular, with glutamine as tracer, at 10 min the pool of aspartate is very small and contains virtually no tracer at all, yet homoserine already has a considerable amount, therefore the aspartate pool cannot be the homoserine precursor. Mitchell and Bidwell (16) showed that in pea roots carbon from externally supplied aspartate did not flow directly into homoserine (or asparagine) but first entered the organic acid pool and then proceeded by a compartmented route. The results here suggest that incorporation of nitrogen into homoserine bears a closer relationship to asparagine (amino group) than to aspartate.

Asparagine and homoserine may both accumulate rapidly and function as storage compounds under some conditions. However, the balance between them is regulated in some way, since their relative proportions can vary considerably. In the nitrate experiment, asparagine rose continuously throughout the time of observations, but homoserine rose rapidly at first then slowly declined. In contrast, supply of glutamine gave a 5-fold accumulation of homoserine, while asparagine rose then decreased slightly. When asparagine was supplied, the internal levels of asparagine showed little change, yet homoserine accumulated.

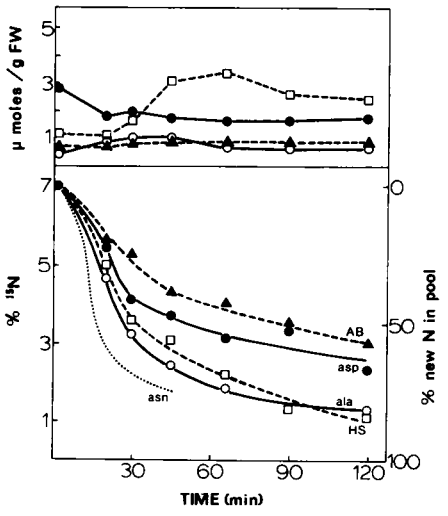


FIG. 6. Asparagine tracer. Details as in Figure 5. Alanine (○), aspartic acid (●), homoserine (HS, □), γ-aminobutyric acid (AB, ▲). Part of the curve for asparagine from Figure 5 is included for comparison.

The use of asparagine as tracer showed that it can supply nitrogen to a range of compounds. This transfer occurs in part through the contribution to glutamine, and the consequent widespread redistribution already discussed. There is also a rapid flow to alanine, and this is most readily explained by a transamination reaction; enzyme systems of this type have been reported in animals (14) and in plants (21). Formation of $\alpha$-ketosuccinamic acid (the transamination product) from $^{14}$C-asparagine has been detected in pea leaves (Joy, unpublished).

The results give little direct evidence concerning the synthesis of aspartate, but some deductions can be made. With nitrate as tracer, aspartate receives new nitrogen slowly at first, but approximately 90% of the pool is replaced in just over 1 hr, indicating that the nitrogen must flow from a precursor which itself has extensive dilution by new $^{14}$N. Ammonia, glutamine, and perhaps glutamate or asparagine would be possible sources. In the glutamine tracer experiment, during the period 20 to 45 min the pool size of aspartate rises over 6-fold yet at the end of this time the nitrogen of aspartate contains only 40% new (tracer) nitrogen, so in this case the transfer has come from a pool retaining a large proportion of old nitrogen. This appears to rule out glutamine and glutamate (and possibly also asparagine) as donors for aspartate synthesis. These results suggest that ammonia could be a nitrogen source for aspartate synthesis, but this must take place in a compartment which is separate from the site of formation and assimilation of the ammonia derived from nitrate.

**Ammonia.** Ammonia can be produced from several sources. When nitrate enters leaf tissue, reduction in the light will provide ammonia, and the main flow will be through the pool into assimilatory pathways. When amides were experimentally substituted as the nitrogen source for expanding leaves, some residual ($^{15}$N) nitrate in the tissue continued to be utilized for a short time. Nitrate content of the tissue decreased for only 15 to 20 min (unpublished results) and the nitrate then remaining appeared to be stored in a location unavailable for immediate reduction. The amides are also utilized as a source of ammonia. They may supply a pool which is separate from the nitrate-supplied pool, because the latter saturates when approximately 75% of the pool has been replaced (Fig. 1) while ammonia from each amide saturates after only 50 to 60% replacement (Figs. 3 and 5). With asparagine as tracer, there is a transfer of new nitrogen to ammonia, with a short initial delay, possibly due to continued input from residual $^{15}$N-nitrate. The release of ammonia may result from the action of asparaginase, but release of ammonia from $\alpha$-ketosuccinamic acid (the transamination product of asparagine) has been reported (14, 21). This could account for a considerable amount of the ammonia released from asparagine, providing an alternate system to asparaginase, and would also explain the results of Atkins *et al.* (2). The results here tend to confirm this, because tracer from asparagine arrives in aspartate much more slowly than into ammonia; activity of asparaginase should result in equal labeling of ammonia and aspartate.

The glutamine experiment shows a more complex situation. There is an immediate small but rapid synthesis of ammonia from glutamine, presumably due to glutaminase activity, but this soon begins to saturate, indicating the likelihood of a small separate pool of ammonia supplied from glutamine. A second phase of flow of tracer nitrogen occurs later, and it is possible that this is released from asparagine newly synthesized from tracer nitrogen.

Under normal conditions in the whole plant, production of ammonia from both nitrate and amides is likely to occur, since both are transported to young leaves.

**Position of the Amides in Nitrogen Metabolism.** For pea leaves with an adequate supply of nitrate, assimilation in the light will proceed through glutamine synthesis. Excess of assimilation over requirements for synthetic processes will lead to accumulation of nitrogen in asparagine and possibly homoserine. In the intact plant, considerable amounts of nitrogen will arrive in the young leaves in the form of asparagine, from assimilation in roots, and from assimilation and redistribution from older leaves, which receive nitrogen in xylem sap but do not accumulate it. Asparagine, and lesser amounts of glutamine, are transported in phloem (13) and xylem (3, 5) of peas. Asparagine transported to young leaves will be utilized along with the nitrogen supplied by nitrate reduced in the leaf. Utilization of asparagine can proceed through the pathways already discussed: direct transfer to glutamine, release of ammonia, transamination, and possibly conversion to homoserine. There must be a regulatory mechanism which determines whether asparagine will be stored or utilized and some aspect of asparagine regulation have been discussed (12). The storage cannot simply depend on total relative concentrations of asparagine and glutamine. For example, with nitrate and glutamine experiments, a high asparagine to low glutamine ratio still led to asparagine synthesis; with asparagine as supplied tracer, the asparagine to glutamine ratio was lower, yet there was a synthesis of glutamine from asparagine. Of course total measurement cannot distinguish between different pools in a tissue, and it is possible that relative concentrations in some specific compartments may control the storage-utilization system. Synthesis of glutamine is compartmented in plastids (7, 19) but asparagine synthetase is not associated with a particulate fraction (12, 20); little else is known about the distribution of enzymes of asparagine metabolism. It is also possible that other indicators of nitrogen status, such as free ammonia, may have a role in regulation in specific locations.

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