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Transport, Metabolism, and Redistribution of Xylem-Borne Amino Acids in Developing Pea Shoots[1]

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ABSTRACT

Amino acid metabolism and transport was investigated in the leaves of 3-week-old nonnodulated seedlings of *Pisum sativum* L. Xylem sap entering the shoot contained nitrate (about 5 millimolar), and amino compounds (11 millimolar) of which 70% was asparagine plus glutamine; aspartate and homoserine were also present. Mature leaves showed stable nitrogen levels and incoming nitrogen was redistributed to growing leaves. Younger leaves, still enclosed in the stipules, showed negligible rates of transpiration, suggesting that most of their nitrogen must arrive in the phloem.

^{14}C-Labeled amides and amino acids were supplied to detached shoots through the xylem, and metabolism and redistribution were followed over 12 hours in light. Asparagine entering mature leaves was reexported directly to young leaves, with relatively little metabolic conversion. Substantial amounts of glutamine were converted to glutamate, which was exported (with unchanged amide) with little further conversion. The pattern of redistribution was confirmed when ^{14}C-labeled amino acids were applied directly to the under surface of mature leaves. Labeled compounds were found in the phloem exudate from treated leaves, and the composition resembled the pattern of labeling in the compounds arriving in the young developing leaves.

Nitrogen required for growth of plant shoots is transported from the roots via the xylem as a mixture of nitrate and organic nitrogen, the proportions varying with species and conditions (17). Mature leaves (vigorously transpiring) receive most of this xylem-borne nitrogen (8, 19). Nitrogen levels stabilize in fully-expanded leaves (4) and therefore much of the incoming nitrogen must be reexported. Developing leaves, vegetative apices, and fruiting structures are major sinks for nitrogen for amino acid and protein synthesis but, since they have relatively small surface areas, the requirements cannot be met by transpirationally-derived nitrogen. They must depend on amino acids transported from the mature leaves, presumably in the phloem.

Transfer of nitrogen from mature leaves to fruiting structures was demonstrated in *Pisum sativum* (14) and *Lupinus albus* (18) using [^{15}N]NO_3 supplied to cut ends of stems. Redistribution of amino acids from leaves to fruiting structures has also been shown (3, 14) and transfer of amino acids from xylem to phloem of *L. albus* pods was studied in detail (16, 23). In nonfruiting plants transfer of ^{14}C-labeled amino acids from older to younger leaves was demonstrated in *Pisum arvense* (19) and beet (11). In these studies with vegetative plants, the redistribution of amino acids from mature to developing leaves was not followed in detail, nor were labeled compounds recovered in the developing leaves identified. Export from the mature leaves of label from amino acids (applied as tracers to the leaf surface) has been demonstrated in several plants (11, 20, 22).

In *P. sativum* seedlings, the xylem sap contains a mixture of nitrate and organic nitrogenous compounds, mainly asparagine and glutamine, but also homoserine, asparate, and a few other amino acids (4, 25). Experiments with ^{15}N-labeling showed that pea leaves utilize the nitrogen from nitrate and from asparagine and glutamine for further amino acid metabolism (5). In these pea seedlings, the young leaves of the vegetative apex are enclosed within the stipules of the next oldest, expanding leaves, and there is little transpirational flow to the apical leaves. Young pea leaves are therefore a useful system in which to study redistribution of nitrogen from the mature leaves.

Uptake and redistribution of ^{14}C-labeled amides, the major nitrogenous compounds in the xylem of pea (25), was followed in mature, expanding, and immature leaves of nonnodulated plants over 12 h in the light, after supply to the shoot in the transpiration stream. Movement of amino acids from mature leaves to immature leaves and other parts of the plant was investigated by application of ^{14}C-labeled amino acids to the lower surface of mature leaves.

MATERIALS AND METHODS

Pisum sativum L. (c.v. Little Marvel) seedlings were grown in nutrient solution in lighted growth cabinets as previously described (4, 25). Light sources (Westinghouse 9GT 12 Daylight, High Output tubes and 100 w incandescent bulbs) supplied a total of 50 w/m^2 at the level of the plants. The temperature was 26 to 28°C in the 12-h photoperiod and 18°C in the dark. The seedlings were used when they had four fully expanded leaves and the fifth leaf half-expanded. The plants were not nodulated; nitrate (4 mM) was the sole source of nitrogen.

Transpiration rates were estimated by measuring water loss from shoots whose stems were detached under water at the lower end of the epicotyl. Cut ends were inserted through slits in parafilm covering small vials of water. Vials and shoots were weighed at intervals. No water loss was detected from similar vials without shoots. Comparison was made with water loss from intact plants growing in nutrient solution. Transpiration from detached leaves was estimated similarly. The effect of removing young leaves at various stages of development was observed by measuring water loss from shoots for 1 h, then excising the young leaf and measuring water loss from the remainder of the shoot for a further hour.

Labeling Experiments I. Shoots were selected at a uniform stage of development. At the start of the light period, stems were detached as described above and the cut ends placed in vials containing feeding solution. This consisted of nutrient solution (diluted to give 1.5 mM NO_3), 1.5 mM asparagine, and 1.5 mM glutamine. The shoots were allowed to adapt for 30 min, then

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transferred to the same feeding solution, containing L-[U-^{14}C] asparagine, or L-[U-^{14}C]glutamine. The amounts of tracer amide were adjusted so that three shoots absorbed approximately 1 μCi over a 30-min feeding period in the light. The labeled solution was chased by unlabeled feeding solution and uptake and redistribution of radioactivity was followed in samples of individual leaves over 12 h in the light. Three shoots were sampled at each time interval; leaves (including stipules) were separated and frozen in liquid N_2. Prior to use, asparagine and glutamine (labeled and unlabeled) were separated from contaminating aspartate and glutamate by passing them over Dowex 1 (Cl form, Sigma), the amides being recovered in the effluent (21).

Labeling Experiments II. ^{14}C-Labeled amino acids were applied individually to a 2 × 2 mm area of the lower epidermis of leaf 4 (Stage 7, just fully expanded) as described (25). Both detached shoots (with cut ends of shoots in unlabeled feeding solution) or intact plants (left *in situ* in nutrient solution) were used, four plants per treatment. After 2 h feeding in the light, shoots or plants were removed and the fed leaflet cut off. The fed leaflet, young leaves (leaves 5 and 6), roots (of intact plants), and rest of shoot or plant were separated and frozen.

Phloem Exudate. An EDTA[3]-promoted exudate (12) was collected from fully expanded leaves. Replicate groups of four leaves were analyzed after 2 h exudation. Details are given elsewhere (25).

Extraction and Analysis. Frozen tissue was extracted in cold 80% (v/v) ethanol (25) and ethanol-soluble and ethanol-insoluble fractions obtained. Lipids and pigments were removed from the ethanol fraction by extraction with chloroform and water (8.5:3, by volume). Aqueous fractions were made up to final volume equivalent to 0.2 to 0.5 g fresh weight/ml. Amino acid, organic acid, and neutral fractions were separated using Dowex resins (Sigma) (7). Amino acid fractions were further separated on a Beckman Amino Acid Analyzer Model 119 BL, using lithium buffers, or by high-voltage electrophoresis and ascending paper chromatography (4). Organic acids were separated by ascending paper chromatography on Whatman 3MM in 1-butanol:acetic acid:water (12:3:5, by volume) and visualized colorimetrically with potassium permanganate (9).

The ethanol-insoluble pellet remaining after ethanol extraction was routinely extracted in 1 N NaOH, and residual material was solubilized in NCS (Amersham/Searle):water (9:1 by volume). In some samples the ethanol-insoluble pellet was digested with protease (Type V, Sigma) in 0.1 M Tris buffer (pH 7.7), 5 mM $CaCl_2$ at 30°C for 24 h (M. M. Smith, personal communication).

Total organic nitrogen was estimated by micro-Kjeldahl digestion (6). Ureides were detected by the method of Smith (24). Chl

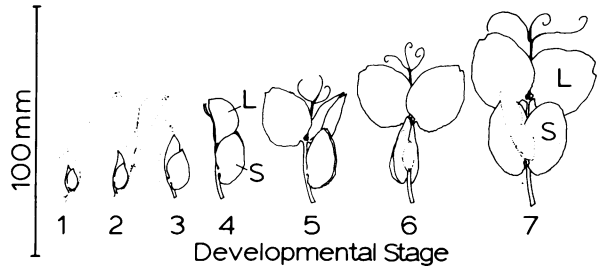

FIG. 1. Stages of development of the expanding leaves of pea plants, characterized by the unfolding of leaflets (L) and stipules (S). In 3-week-old seedlings grown in nutrient solution with a 12-h photoperiod, these stages were 1 day apart. (——), the expanding leaf; (-----), the next older or younger leaf.

[3] Abbreviations: EDTA, ethylenediaminetetraacetic acid; GABA, γ-amino butyric acid; HSA, 2-hydroxysuccinamic acid.

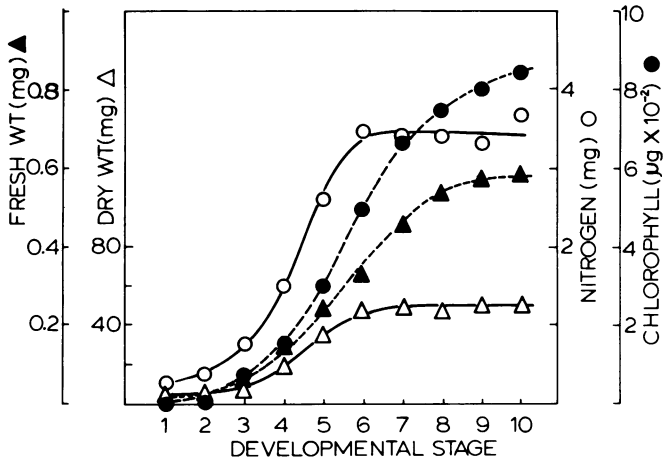

FIG. 2. Fresh weight (▲),dry weight (△, organic nitrogen (○), and Chl (●) content of pea leaves during leaf expansion. The fourth leaf from four plants was used for each determination. Developmental stages 1 to 7 are illustrated in Figure 1. Values are expressed per leaf.

was determined after extraction in 80% (v/v) acetone (1). Aqueous samples were counted by liquid scintillation spectroscopy. Radioactive compounds on paper were located with a Packard Radiochromatogram Scanner, Model 7201, and were eluted with 60% (v/v) ethanol for estimation of radioactivity. Radioisotopes were supplied by The Radiochemical Centre, Amersham. HSA was prepared from [U-^{14}C]asparagine (15). All other chemicals used were reagent grade.

RESULTS

Growth and Expansion of Leaves. Pea leaves (leaves 4–7) developed through a series of morphologically distinct stages characterized by unfolding first of the stipules then of the leaflets (Fig. 1). Each stage was 1 d apart under the described growth conditions. At Stage 3, the young leaf becomes visible, partially exposed by the opening stipules of the older leaf; at Stage 4 it is fully exposed; by Stage 7 it is fully expanded. Although no further visible change took place after Stage 7, an age could be assigned to it by referring to the development of the next leaf. Thus, when Leaf 5 was just fully expanded (Stage 7), Leaf 4 was at Stage 10, and Leaf 6 at Stage 4.

Leaf 5 was sampled at each developmental stage; fresh and dry weights, organic nitrogen content, and Chl content were measured (Fig. 2). Dry weight and nitrogen content reached maxima at Stage 7 when the leaves were just fully expanded. Nitrogen levels were stable for a further 3 d.

Transpiration Rates. Rates of water loss from detached shoots over 2 to 3 h in the middle of the light period were 0.4 to 0.7 ml/h per shoot. These rates were only slightly less than for intact shoots over the 12-h light period (8–10 ml/shoot). Mean water loss from detached leaves was 0.097 ml/h for half-expanded leaves (Stages 4–5) and 0.14 ml/h for fully expanded leaves. The summed rates of the detached leaves equalled the total water loss from the shoot. Comparison of rates of water loss, initially and after excision of the young leaf, showed that when the young leaf was still completely enclosed within stipules of the older leaf, its removal gave no change in transpiration rate of the shoot. On the other hand, when the young leaf was partially or fully exposed (Stage 3 onward), there was, after its excision, marked reduction in overall water loss from the shoot.

Xylem-borne Amino Acids. In the first labeling experiments, either asparagine or glutamine was fed as a 30-min "pulse" via the transpiration stream. In the fully expanded leaves (Leaves 3 and 4), the rapid initial rise in label in the ethanol-soluble fraction

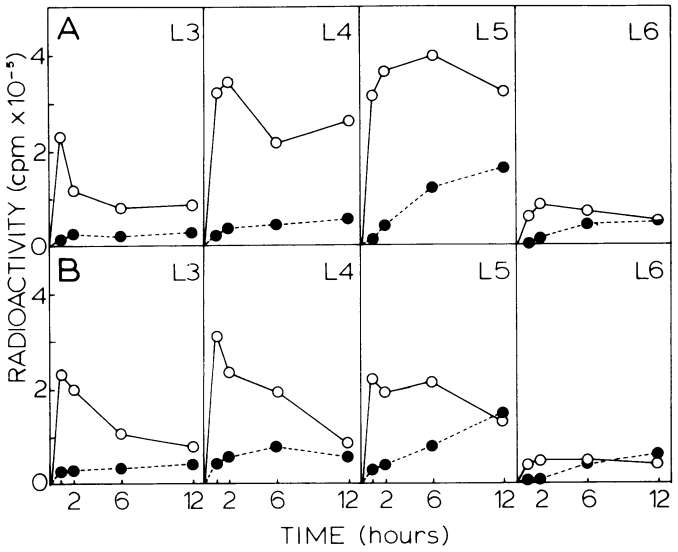

FIG. 3. Distribution of radioactivity in ethanol-soluble (○) and ethanol-insoluble (●) fractions of leaves 3 to 6 of pea shoots fed with [^{14}C] asparagine (A) or [^{14}C]glutamine (B) via the xylem as a 30-min pulse. Shoots were maintained in the light and samples taken at intervals. Three shoots were used for each sample. Leaves were extracted and analyzed separately. Values shown are total radioactivity recovered in each sample.

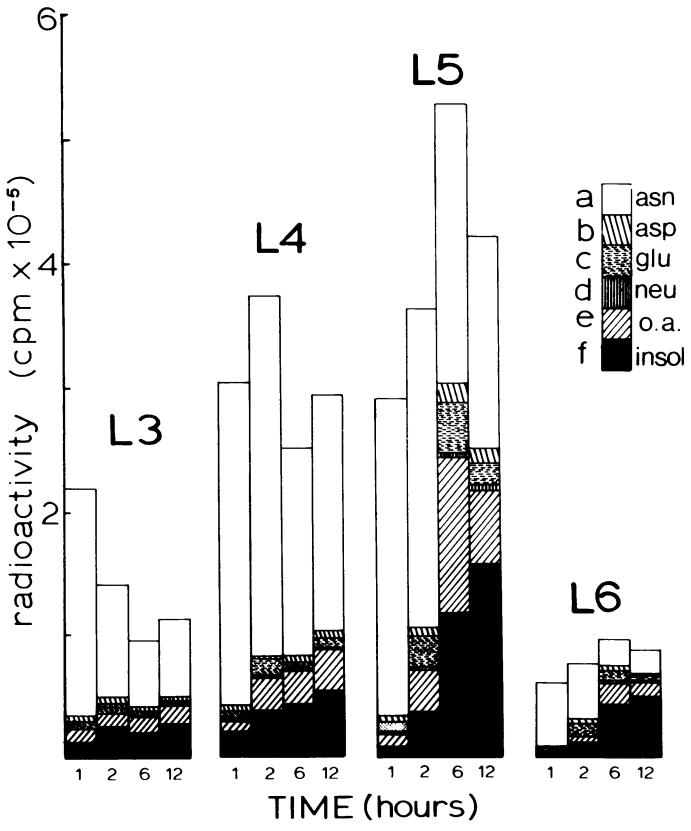

FIG. 4. Distribution of radioactivity in soluble and insoluble compounds of leaves of pea shoots fed through the xylem with [^{14}C]asparagine as described in Figure 3. Values shown are total radioactivity recovered in each fraction. (a) asparagine; (b) aspartate; (c) glutamate; (d) neutral compounds; (e) organic acids; (f) insoluble (mainly protein).

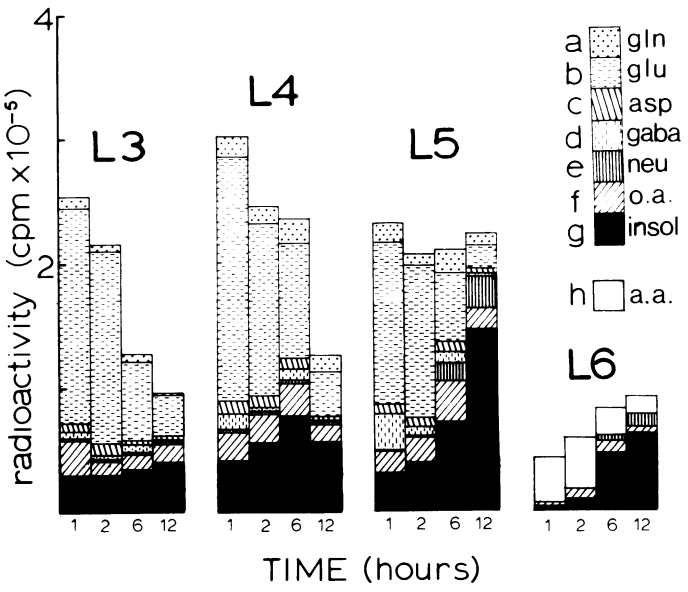

FIG. 5. Distribution of radioactivity in soluble and insoluble compounds of leaves of pea shoots fed through the xylem with [^{14}C]glutamine as described in Figure 3. Values are for total radioactivity recovered in each fraction. (a) glutamine; (b) glutamate; (c) asparatate; (d) GABA; (e) neutral compounds; (f) organic acids; (g) insoluble (mainly protein); (h) amino acid fraction, not further analyzed in Leaf 6.

Table I. *Distribution of Radioactivity in Pea Plants Following the Supply of Labeled Amino Acids to the Surface of a Mature Leaf*

^{14}C-Labeled amino acids were fed to the fourth leaf of intact plants for 2 h in the light. Treated leaflets, young leaves (L5, L6), rest of shoot, and root were sampled separately. Radioactivity in ethanol-soluble and insoluble fractions was determined. Four plants were used for each experiment. The data shown were reproduced 4 to 6 times. The values shown are percentages of total radioactivity recovered in the plants.

	Asn	Gln	Asp	Glu
	cpm × 10⁻⁵			
Radioactivity				
Amount supplied	10.4	9.2	13.01	9.56
Total recovered	10.22	8.12	9.56	7.98
Washed off	0.44	1.24	0.15	0.27
Recovered in plant	9.78	6.89	9.41	7.71
	% of total recovered in plant			
Soluble				
Treated leaflet	69.3	76.4	77.8	83.9
Leaf 6	1.9	1.8	1.1	0.7
Leaf 5	4.9	1.8	2.4	3.0
Rest of shoot	6.2	7.0	2.9	2.5
Root	6.6	6.7	2.4	2.0
Insoluble				
Treated leaflet	8.7	4.4	11.3	6.6
Leaf 6	0.3	0.4	0.4	0.2
Leaf 5	0.8	0.3	0.6	0.2
Rest of shoot	0.5	0.2	0.6	0.3
Root	0.6	0.8	0.4	0.4

was followed by a decline within 6 h to about half of the maximum level (Fig. 3). A very small amount of label was recovered in the insoluble fraction of these leaves. In the expanding leaves (Figs. 3, A and B, Leaf 5), a similar, rapid rise in radioactivity in the soluble fraction occurred in the first hours. The level of radioac-

tivity did not subsequently decline, however, but remained high for the next 5 h. Incorporation of radioactivity into the insoluble fraction of Leaf 5 was considerable, the levels rising steadily during 12 h. In the immature leaves still enclosed within stipules, radioactivity did enter these leaves, mainly in the soluble fraction in the first 2 h (Fig. 3, Leaf 6); thereafter, label in the insoluble fraction increased gradually while that in the soluble declined slowly. The amount of radioactivity liberated from the insoluble fraction by protease digestion or 1 N NaOH extraction was identical, and indicated that protein accounted for 90% of the insoluble label in mature leaves and 95% in young leaves.

When asparagine was tracer, label recovered in the ethanol-soluble fraction of fully expanded leaves (L3, L4) was mainly still asparagine (Fig. 4). Changes in total radioactivity in these leaves was almost completely a reflection of change in asparagine labeling. In the expanding leaf (Fig. 4, L5), asparagine initially (1 h) accounted for 89% of the total. Although asparagine remained the main labeled compound, there was with time considerable incorporation into protein, some other amino acids, and the organic acid fraction. HSA was the main compound labeled in the latter fraction. In the young leaf (L6), over 90% of the radioactivity initially entering the leaf was asparagine. Later there was incorporation of label into other soluble compounds and protein.

When glutamine was the tracer, the amide was largely metabolized to glutamate in all samples analyzed (Fig. 5). In mature and expanding leaves (L3, L4, L5), most of the label in the first hours was glutamate (plus amide) and these components subsequently decreased; label was also incorporated into some other soluble compounds and protein. In the immature leaf (L6), 87% of the label was in the amino acid fraction in the first hour. Radioactivity in the insoluble fraction increased later.

Fate of Leaf Amino Acids. When asparagine, glutamine, aspartate, and glutamate were applied as tracers to the surface of fully expanded leaves for 2 h, most of the radioactivity supplied was recovered in the plant (98, 88, 74, and 84% respectively). Little was recovered in washings from the leaf surface (4, 15, 2, and 4% of the supplied activity respectively). In all four cases, 80 to 90% of the label recovered in the plant remained in the treated leaflet (Table I). Most of this was in the soluble fraction, but 5 to 15% was recovered in the insoluble fraction of the fed leaflet. Some radioactivity was also recovered in the young leaves (L5, L6), the root, and the rest of the plant.

The data shown are for intact plants growing in nutrient solution. For comparison with the xylem-fed shoots of the first group of experiments, detached shoots were also used. The results (not shown) were almost identical; most of the applied label was recovered still in the treated leaflet, but some was transported to the young leaves and the rest of the shoot. Since the lack of roots eliminated one of the main sinks, there were of course slight differences in proportions of label recovered in the young leaves and other parts of the shoot.

Further analysis of the soluble compounds of the treated leaflet and the young leaves showed (Table II) that when asparagine was the tracer, most of the label in the treated leaflet after 2 h was still in asparagine (66.9%), the rest in a few other amino acids and HSA. In the young leaves, again most of the label was in asparagine (58.4%). In the roots (data not shown) almost all of the soluble radioactivity was asparagine (92%).

With glutamine as tracer, most of the labeled glutamine was metabolized in the treated leaflet. Label was mainly recovered as glutamate, a few other amino acids (notably GABA), and the organic acid fraction (five compounds, not identified). Label recovered in the young leaves was again mainly glutamate, and radioactivity was also present in the organic acid fraction and protein. In the roots, 79 to 80% of the soluble radioactivity was in amino acids; 16 to 20% organic acids. Of the amino acids, 42% was in glutamate, 19.3% in glutamine plus homoserine, 13.2% in GABA, 10% in asparagine, and 9.6% in asparatate.

Aspartate fed to mature leaves was extensively metabolized. Label in these source leaves was recovered mainly in the organic

Table II. *Labeling of Pea Leaf Components Following the Supply of Labeled Amino Acids to the Surface of a Mature Leaf*

^{14}C-Labeled amino acids were fed to the fourth leaf of intact plants for 2 h in the light. Samples were taken and extracted as described in Table I. Radioactivity in the soluble compounds was estimated. Data are shown for (T) treated leaves (Leaf 4), and (Y) young leaves (Leaves 5 + 6). Amino acids in Asn- and Gln-labeled leaves were separated with an amino acid analyzer, and in Asp- and Glu-labeled leaves by paper electrophoresis and chromatography. The values are percentages of radioactivity recovered in soluble plus insoluble fractions of each leaf sample.

	Asn		Gln		Asp		Glu	
	T	Y	T	Y	T	Y	T	Y
	% total radioactivity recovered in leaf sample							
Amino acids								
Asp	4.9	4.9	0.2	3.5	11.6	5.3	3.0	0
Thr	0.3	0	0.1[b]	0	0.5	ND[a]	0.2	ND
Ser	0.3	0		0	0.1	ND	0.6	ND
Asn	66.9[b]	58.4	0.1	0	1.1	ND	0.2	ND
Glu		0	49.2	38.6	2.2	13.3	22.9	15.3
Gln	3.0[b]	0[b]	1.4[b]	4.7[b]	3.7	ND	3.5	ND
Hse					10.2	ND	3.7	ND
Pro	0.3	0	0.1	0	0.1	ND	0.2	ND
Gly	0	0	0.4	0	0.2	ND	0	ND
Ala	0.5	0.4	0.7	0	2.7	ND	0.4	ND
GABA	ND	ND	6.7	5.1	2.9	0	23.7	0
Others	4.1	6.1	2.3	1.1	1.7	27.0	2.8	26.3
Neutral compounds	2.0	2.5	4.0	7.3	9.3	5.2	19.0	18.4
Organic acids[c]			21.5	25.3	43.1	32.0	16.2	21.7
HSA	9.3	3.9						
Others	2.2	8.1						
Insoluble	6.4	15.7	9.6	14.3	12.7	18.6	7.8	18.4

[a] Not determined separately.

[b] Counted as single peak owing to incomplete resolution.

[c] Radioactivity in 4 or 5 compounds counted together except in Asn-labeled samples in which labeled HSA was determined separately.

Table III. *Labeling of Leaf and Phloem Components Following the Supply of Labeled Asparagine to Pea Leaves*

[^{14}C]Asparagine was supplied to the mature leaf blade of detached leaves or intact plants. Phloem exudate was collected from detached leaves for 2 h. Labeled leaves and the young leaves of intact plants were extracted after 2 h. Four leaves or plants were used in each experiment. Values are percentages of the radioactivity recovered in ethanol soluble fractions.

	Detached Leaves		Intact Plants	
	Treated leaf	Phloem	Treated leaf	Young leaves
	% radioactivity in soluble fraction			
Amino acids				
Asp	9.23	25.3	5.2	5.8
Thr	0	0	0.3	0
Ser	0	0	0.3	0
Asn	43.3	59.7	62.3	63.5
Glu	7.6	0	9.3	6.0
Gln	1.7	0	0.4	0
Hse	2.7	0	2.8	0
Ala	0.9	0	0.5	0.5
Others	7.7	10.5	4.7	7.2
Neutral compounds	3.2	3.1	2.1	3.0
Organic acids	23.6	1.4	12.3	14.3

Table IV. *Labeling of Leaf and Phloem Components Following the Supply of Labeled Aspartate to Pea Leaves*

[^{14}C]Aspartate was supplied to plants and samples collected as in Table III. Values are percentages of the radioactivity recovered in ethanol soluble fractions.

	Detached Leaves		Intact Plants	
	Treated leaf	Phloem	Treated leaf	Young leaves
	% radioactivity in soluble fraction			
Amino acids				
Asp	10.6	28.3	13.0	6.4
Thr	0.2	0	0.5	N[da]
Ser	0	0	0.1	ND
Asn	0	0	1.2	ND
Glu	5.5	15.1	2.5	16.1
Gln	13.4[b]	17.9[b]	4.1	ND
Hse			11.4	ND
Ala	1.6	0	3.0	ND.7
Others	9.9	2.8	5.5	32
Neutral compounds	6.5	13.2	10.4	6.3
Organic acids	52.0	22.7	48.3	38.7

[a] Not determined; radioactivity in these compounds is included in "others."

[b] Gln plus Hse were counted as a single peak owing to incomplete resolution.

acid fraction (43%, five major compounds), homoserine, glutamine, alanine, and a few other compounds. In the young leaves, very little label was found in aspartate, but 45% of the label was in amino acids (of which 13% was glutamate), 32% was in organic acids, and 18.6% insoluble material. Metabolism of applied glutamate was also quite extensive. In the treated leaflet, only 22.9% of the radioactivity remained as glutamate. GABA was a major metabolite, compounds in organic acids and neutral fractions also becoming labeled. In the young leaves, about 15% of the label was in glutamate, and no label was recovered in GABA.

Phloem Exudate. Asparagine or aspartate (^{14}C-labeled) was fed to detached pea leaves, and phloem exudate collected after 2 h exudation. Tables III and IV show analyses of soluble fractions of the fed leaf and phloem exudate and compare fed leaf and young leaves of intact plants. Small differences were apparent but, for both asparagine and aspartate, the labeling patterns of the fed leaves in both types of experiments were very similar. Phloem exudate and young leaves were also alike. In asparagine-feeding experiments (Table III), the major labeled compound in both phloem and young leaves was asparagine, 59.7 and 63.5% of the soluble fraction respectively. Aspartate was labeled in the phloem, 4- to 5-fold higher than in the young leaves. About 14% of the soluble label was in organic acids in the young leaves, compared to only 1.4% in the phloem. Little else was labeled in either phloem or young leaf. When aspartate was tracer (Table IV), most of the aspartate was metabolized and about 50% of the soluble label was in organic acids after 2 h. Comparison of phloem and young leaves showed that aspartate was 4-fold more strongly labeled in the phloem. Although radioactivity was recovered in organic acids in the phloem, label was 2-fold higher in the young leaves.

DISCUSSION

Nitrogen Requirements for Developing Leaves. Nitrogen status and other growth parameters in pea leaves (Fig. 2) were readily correlated to the developmental stages shown in Figure 1. Since mature (fully expanded, Stage 7 onwards) and half-expanded leaves (Stages 4–5) were shown to transpire vigorously, they would receive nitrogen from the xylem. In peas of this age, the xylem bleeding sap was a mixture of amino acids (11 mM N, 70% amide) and nitrate (4 to 5 mM N), a total of 0.015 μmol N/μl xylem sap (25). The following calculations did not include ureides, present only in trace amounts (25).

Fully expanded leaves reached maximum nitrogen levels (Fig. 2) at Stage 7; these levels were stable for a further 3 to 4 d. From Figure 2 we calculated that the nitrogen content of expanding leaves (Stage 4–5) increases by 1.10 mg N/d; young leaves by 0.15 mg N (Stage 1–2) and 0.3 mg N (Stage 2–3). Although young leaves still enclosed in stipules (Stages 1–3) transpired negligible amounts of water, a net increase in water content of 10 μl (Stage 1–2) and 38 μl (Stages 2–3)/d occurred (calculated from fresh and dry weight changes). The xylem therefore contributed at most 2 to 4 μg N and 8 to 15 μg N/d, respectively. From the estimated transpiration rates for expanding and mature leaves (0.1 and 0.14/h), we calculated an approximate daily supply of nitrogen to the leaves of a 4½-leaf shoot (Table V). These estimates show that transpiration could provide only about one-third of the nitrogen required for expanding leaves and very little of that for young leaves. The balance must come from mature leaves by redistribution, as amino acids, present in the phloem (25). Since the nitrogen

Table V. *Nitrogen Requirements and Supply for Pea Leaves*

Nitrogen requirements for immature, expanding, and mature leaves were calculated from the daily nitrogen increments shown in Figure 2. Supply of nitrogen to these leaves was estimated from the xylem sap contents and measured transpirational flow to the leaves. Details of calculations in text.

Leaf		Nitrogen		
Number	Stage of Development	Requirements	Supply[a]	Balance
		μg N/day		
6	1–2 (immature)	−150	+2 to +4	−146 to −148
5	4–5 (expanding)	−1100	+381	−719
4	7 (mature)	0	+534	+534
1–3	(mature)	0[b]	+1182	+1182

[a] By transpiration or water (sap) absorption.

[b] Older leaves may show net decrease in nitrogen content and could hence contribute additional exported nitrogen.

levels in mature leaves are stable (Fig. 2), all of the nitrogen entering these leaves is available for redistribution; a total of 1.716 mg N/d would be available from leaves 1 to 4 (Table V), an amount in excess of that required by the growing leaves (5 and 6) together (866 μg N/d). The calculations are approximations; they assume no selective absorption during transport, and a nitrogen content of the transpirational xylem sap similar to that of the slower bleeding sap. A lower concentration of nitrogen in the faster transpiration stream would decrease the amount of nitrogen available thus giving closer correspondence with the actual requirements of the younger leaves.

Metabolism of Labeled Amino Acids in the Shoot. In the present study with peas (nonnodulated and nonfruiting), differences in the extent of metabolism and reexport of different amino compounds were noted, following their supply via the xylem or directly to mature leaves. There was relatively little metabolism of asparagine in mature leaves (Fig. 4, Tables II and III), although metabolism, particularly to organic acids, was more pronounced in rapidly expanding leaves (Fig. 4, L5). In both mature and rapidly expanding leaves, the major organic acid, HSA, presumably resulted from the action of asparagine transaminase which is present in pea leaves (10, 15). In the expanding leaves, there was considerable incorporation of label from asparagine into protein, which become the major labeled component of younger leaves (Fig. 4, L6).

Glutamine, fed through the xylem or applied to the leaf surface of peas, was converted largely to glutamate in all ages of leaves (Fig. 5) but was more extensively metabolized in expanding and young leaves (Fig. 5, Table II). Glutamine is probably metabolized by glutamate synthase, shown to be present in pea leaves (13).

Aspartate and glutamate, supplied to the leaf surface, were extensively metabolized in mature and young leaves. Label was transferred in the treated leaves from aspartate to homoserine, and in particular to the organic acid fraction, evidence of aspartate transamination (Tables II and IV). Glutamate, in pea, was readily metabolized, largely to GABA, the organic acid fraction, and neutral compounds.

These results were similar to those reported for fruiting lupin (14, 16, 23) where xylem-borne amino acids have been grouped according to the rapidity of transfer from xylem to phloem and the extent of metabolism to other compounds. Asparagine is rapidly transferred to phloem with little or no conversion. Aspartate and glutamate belong to the group from which carbon is transferred more slowly to phloem, with little as the original tracer compound. Glutamine is intermediate in both rapidity of transfer and extent of metabolism in lupin (2, 16, 23) and in soybean (22).

Uptake and Redistribution of Xylem-borne Amino Acids. The first feeding experiments, in which ^{14}C-labeled amides were supplied to the shoot in the transpiration stream, showed that the xylem-borne amides were rapidly transported into transpiring leaves, and were then reexported from the mature leaves (Fig. 3). The mature leaves did not accumulate nitrogen (Fig. 2) but some turnover or exchange with the existing leaf pools occurred, since radioactivity was not totally flushed from the leaf after the initial "pulse" of label had passed. These leaves appear to play a considerable role redistributing the amino compounds that arrive from the root. The largest of the expanding leaves rapidly accumulated labeled carbon, presumably due to the direct arrival of xylem-borne amides. Levels of soluble radioactivity, however, remained high while extensive incorporation into insoluble material occurred, suggesting a later continuing influx from the older leaves. The younger leaves were not transpiring but nevertheless accumulated radioactivity while labeled carbon was being exported from mature leaves. The second group of experiments, in which label was supplied directly to the surface of mature leaves, verified amino acid export to young leaves, roots, and stems (Table I). Again, asparagine was not extensively metabolized in the mature leaf and was the main compound recovered in the young leaves, sugggesting that it was reexported directly. Glutamine was metabolized extensively to glutamate in the supplied leaf and the recovery of glutamate as the main component of the young leaves suggested transport in this form.

Previous work suggests that metabolism and phloem loading of labeled amino acids is relatively unimpaired when phloem exudation from detached leaves is promoted by EDTA treatment (25). Analysis of such phloem exudates indicates a selective loading and transport of amino acids. When labeled asparagine was the tracer,there was selective accumulation of asparagine (and aspartate) in the phloem, whereas labeling of the organic acids was low compared to the content of the supply leaf (Table III). Label from aspartate accumulated in the leaf as organic acids, yet aspartate and other amino acids were enriched in the phloem (Table IV). With both tracers, labeling of the phloem exudate resembled the pattern in the young leaves (Tables III and IV).

Serine, a major phloem component (14, 25) was scarcely labeled from any of the amino acids tested. The usual source of carbon for serine is, however, CO_2-fixation (14). Homoserine is also present in both xylem and phloem (25) and some label from aspartate was recovered in homoserine (leaves) or in the homoserine plus glutamine peak (phloem) (Table IV). Analysis of phloem exudates and young leaves (Tables II, III, and IV) strongly suggests that transfer of label from the xylem-borne amides (and related amino acids) was mainly to asparagine, aspartate, and glutamate, the main amino compounds in the phloem (25).

The observations and calculations therefore indicate that much of the xylem-borne amino acids (particularly amides) arriving from the root first enter the mature leaves. They are then reexported in phloem to contribute to the nitrogen requirements of the growing leaves, either relatively unchanged (asparagine) or after some metabolic interconversion (glutamine).

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