Plant Physiol. (1981) 68, 750–754
0032-0889/81/68/0750/05/$00.50/0

Use of Phloem Exudate Technique in the Study of Amino Acid Transport in Pea Plants[1]

Received for publication November 28, 1980 and in revised form March 23, 1981

AILEEN A. URQUHART[2] AND KENNETH W. JOY[3]
Department of Biology, Carleton University, Ottawa, Canada K1S 5B6

ABSTRACT

The phloem exudation technique using ethylenediaminetetraacetic acid (EDTA) was evaluated in studies of amino acid translocation in *Pisum sativum* L. seedlings. Exudation of phloem sap from cut petioles of fully expanded leaves was enhanced by EDTA (20 millimolar disodium salt [pH 7.0]). Amino acids (mainly asparagine, homoserine, glutamate, and also aspartate and serine) were present in petiole exudates from EDTA-treated leaves at levels which were commonly 5- to 10-fold (or more) higher compared with water-treated controls. Exudation was greater from darkened leaves, and the pattern of amino acids was markedly different from the more uniform mixture leaking from water-treated controls.

After feeding ^{14}C-labeled amino acids to the leaf blade, distribution of radioactivity in components of the exudate differed from that of the leaf tissue, suggesting selectivity of amino acid loading. [^{14}C]Asparagine was converted to 2-hydroxysuccinamic acid and to other amino acids by the leaf, but was recovered in exudate mainly as asparagine (60%) and aspartate (30%). Similarly, in the exudate, 65 to 70% of the label from [^{14}C]-aspartate was in amino acids, although in the leaf tissue 50% was in the organic acid fraction and only 11% remained as aspartate. Metabolism of asparagine and aspartate was essentially the same in intact leaf blades as in EDTA-treated leaves. Despite the possibility of EDTA damage in the petiole, phloem loading of amino acids appeared to be relatively unimpaired. Although the amount of labeled material appearing in the exudate is less than the amount translocated in the intact plant, the technique is useful in the study of amino acid transport.

Mature, transpiring leaves import nitrogen from the roots, with varying proportions of nitrate and organic nitrogen, depending on species and growth conditions (17). In *Pisum sativum*, the nitrogen level of the growing leaves stabilizes when the leaves are fully expanded (3), thus most of the incoming nitrogen must be re-exported. In a continuing study of amino acid metabolism and transport in peas (3), the redistribution of amino acids in phloem has been investigated.

Several methods have been used in the past to obtain phloem samples from leaves. Phloem sap was collected from severed inflorescences of palms and some Agavacae (22) and from cut bark of trees (5, 24) and castor bean (15). Exudate was also obtained from shallow incisions into the phloem of *Lupinus alba* (17), but cross-contamination by the contents of the xylem could occur. A few leguminous species yield an exudate from the cut, distal ends of the developing fruit (1, 18, 19), but the technique is not applicable to nonfruiting plants.

Phloem sap has been collected from the severed stylets of aphids (10) and amino acids were detected in aphid stylets exudate from willows (16, 20) and from peas (2). The quantities of sap obtained are small and the difficulty in prepositioning the aphids limit the usefulness of this method in such studies.

None of these techniques seemed suitable for collection of the phloem sap exported from fully expanded leaves of vegetative plants. Although some leaves do exude a phloem sap through the cut petiole (12), the quantities thus obtained are minute. King and Zeevaart (11) described an EDTA-promoted exudation of phloem sap from detached leaves. The method has subsequently been used with a variety of plants by other research groups (6, 8, 9, 23).

In the present study with *P. sativum* seedlings, this technique was evaluated with respect to amino acids in EDTA-promoted exudates from the phloem of fully expanded leaves. The method was also used to recover labeled material exported from the leaf blade after surface application of ^{14}C-labeled amino acids, and in a comparison of metabolism and transport of [^{14}C]asparagine and [^{14}C]aspartate in detached leaves and intact plants.

MATERIALS AND METHODS

P. sativum L. (c.v. Little Marvel) seedlings were grown in nutrient solution in lighted growth cabinets as described earlier (3) until the seedlings had four fully expanded leaves and the fifth leaf half-expanded. Plants were selected for uniform size and stage of development. The plants were not nodulated. Nitrate (4 mM) in the nutrient solution was the sole source of nitrogen.

Fully expanded leaves were detached with the base of the petiole under a solution of Na_2EDTA, 20 mM, adjusted to pH 7.0 with NaOH (11). The petioles were placed in 0.2 ml EDTA solution in small vials, so that about 1 cm of the petiole was immersed. The leaves were placed in humid chambers in either the dark (21 C) or the light (28 C); lighting was the same as that in the growth cabinets (50 $w \cdot m^{-2}$). After 30 min to 5 h the solution bathing the petioles was collected and analyzed. Samples not used immediately were stored at −20 C. Xylem sap was obtained by collecting the sap bleeding from the cut stumps of pea plants as previously described (3).

Labeling of Leaves. The fourth leaf (just fully expanded) was prepared by lightly abrading a 2 × 2 mm square of the lower epidermis with fine emery paper. Small (5 × 5 mm) squares of coverglass were sealed on three sides over the abraded area with a mixture of liquid silicone rubber and catalyst (General Electric and Dow Corning Corp.). After waiting 30 min for the silicone rubber to harden, 1 to 3 μl volumes of ^{14}C-labeled amide or amino acids were introduced with a microsyringe into the space between epidermis and coverglass.

In the experiments with intact plants, the plants were left *in situ* in the light. After 2 h, the plants were removed and the fed leaflets cut off. The coverglass chamber was peeled off and both chamber

[1] Supported by a grant from the Natural Science and Engineering Research Council of Canada.

[2] Present address: Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.

[3] To whom reprint requests should be addressed.

and leaf surface rinsed with distilled H_2O. Fed leaflets, young leaves (leaves 5 and 6), roots, and the rest of the plant were treated as separate samples; they were frozen rapidly in liquid N_2 and extracted.

In the experiments with detached (fourth) leaves, coverglass chambers were applied, and the leaves were detached and placed in EDTA solution as described above. The radioactive solutions were then added, the leaves were maintained in humid containers either in the light or in the dark for 2 h, then harvested as described. The leaf extracts and the phloem exudates were analyzed.

Extraction and Analysis. The frozen tissue was extracted by homogenizing in cold 80% (v/v) ethanol (60–80 ml/g fresh weight) in a TenBroeck homogenizer. The homogenate was centrifuged at 12,000*g* for 10 min and the pellet was washed with additional (5–10 ml, twice) ethanol. The pooled supernatant fractions were concentrated to small volume at 30 C. Water and chloroform (ratio 3:8.5 by volume) were added to the concentrated fraction and the mixture agitated vigorously. The two phases were separated by centrifugation at 17,000*g* for 10 min. The aqueous fraction was removed and the chloroform re-extracted with water. The pooled aqueous fractions were made up to a final volume containing the equivalent of 0.2 to 0.5 g fresh weight/ml.

The aqueous fractions were separated into amino acid, organic acid, and neutral fractions using Dowex 50(H^+) and Dowex 1(formate) resins (Sigma Chemical Co.) (4). Amino acids were eluted from Dowex 50 with 2 N ammonium hydroxide to avoid hydrolysis of the amides; organic acids were eluted from Dowex 1 with 0.1 to 8 N formic acid. Amino acid fractions were dried to evaporate the ammonia, and redissolved in water. Aliquots were diluted with lithium citrate buffer and adjusted to pH 2.1. These samples were separated on a Beckman Amino Acid Analyzer, model 119BL using lithium buffers. Fractions eluting from the column were split using a stream divider. Aliquots were taken for liquid scintillation counting. Peaks of radioactivity were compared with positions of amino acids detected in the stream passing through the colorimeter. Analyses were routinely continued only to alanine as most of the radioactivity was recovered in compounds eluting in the earlier amino compounds.

Phloem exudate (in the EDTA solution) was adjusted to pH 2.1 and centrifuged (15,600*g*) in an Eppendorf Micro Centrifuge (model 5412) for 2 min to remove debris and the EDTA which precipitates at that pH. The amino acid concentration of this supernatant was measured with the amino acid analyzer using lithium buffers. In the labeling experiments, the phloem exudate was first fractionated on Dowex resins then analyzed as described above. Because the samples obtained were small, all of the effluent from the analyzer was collected and radioactivity in 1-ml fractions was estimated by liquid scintillation counting. The positions of peaks of radioactivity were compared with amino acid standards or unlabeled phloem exudate samples.

Xylem sap samples were adjusted to pH 2.1 and the amino acid content analyzed as described.

Nitrate in both phloem and xylem samples was estimated by the diphenylamine method of Feller *et al.* (7). Ureides were detected by the method of Smith (21).

Radioisotopes were supplied by The Radiochemical Centre, Amersham (L-[U-^{14}C]asparagine, 105 mCi/mmol; L-[U-^{14}C]glutamine, 57.3 mCi/mmol; L-[U-^{14}C]aspartic acid and L-[U-^{14}C]glutamic acid, 10 mCi/mmol). All other chemicals used were reagent grade.

RESULTS

Amino Acid Content of Phloem Exudate. The amino acid content of phloem exudate from detached leaves (leaf 4, fully expanded) after 2 h exudation into 20 mM EDTA (pH 7.0), in the dark is shown in Table I. Asparagine, glutamine plus homoserine, and glutamic acid were highest in concentration; aspartic acid and serine were also present in high amounts. In other analyses, in which glutamine and homoserine were separated, the ratios were 1:4.

Comparison of phloem exudate with xylem bleeding sap (Table I) shows that although asparagine, homoserine, glutamine, and aspartate were major components of both xylem and phloem, the relative proportions were notably different. In addition, glutamate and serine were proportionally higher in the phloem exudate. Ureides were present in low levels (0.5–1 mM) in the xylem sap but were not detected in the phloem. Nitrate, normally about 5 mM in the xylem sap, was detected in the phloem exudate at levels of 100 nmol/leaf after 2 h.

Exudation of amino acids was dependent on the presence of EDTA, because levels of amino acids in this exudate were 5- to 10-fold higher than from water controls (Fig. 1A). EDTA-promoted exudation was also observed in light-treated leaves (Fig. 1B). Amino acid levels in the exudate were higher from darkened than from the light-treated leaves (Fig. 1, A and B). This may in part have been due to reabsorption in the xylem; although the atmosphere was water-saturated to minimize transpiration, some water uptake did occur since residual volumes of EDTA solution were always slightly less in light-treated samples. Exudation was very variable with levels of EDTA lower than 20 mM. Higher levels (25–30 mM) gave occasional signs of toxicity in the leaves.

Export of ^{14}C-Labeled Compounds from Detached Leaves. Carbon from ^{14}C-labeled asparagine, applied to the lower surface of

Table I. *Amino Acid Composition of Xylem Bleeding Sap and EDTA-Promoted Phloem Exudate*

Xylem bleeding sap was collected from the root stumps of 15 to 20 detopped pea plants (4 1/2-leaf stage) over a 30-minute period. Phloem exudates were obtained from detached, fully-expanded pea leaves (three groups of three leaves in each). Exudation into EDTA solution was over a 2-h period. Details of analyses are given in "Materials and Methods." Data are expressed in nmol per root or per three leaves and as percentage of the whole sample.

	Xylem		Phloem	
	nmoles/root	%	nmoles/3 leaves	%
Asp	22.5	11.9	8.7[a] (7.5 / 1.2)	11.3[a] (9.7 / 1.6)
BIA	6.4	3.4		
Thr	3.0	1.6	0.5	0.6
Ser	3.3	1.8	5.1	6.6
Asn	86.8	45.8	9.4	12.1
Glu	1.8	1.0	11.9	15.5
Gln	14.3	7.5	23.3[a] (4.7 / 18.7)	30.3[a] (6.1 / 24.2)
Hse	28.4	15.0		
Pro	ND[b]	-	tr[c]	-
Gly	0.7	0.4	0.3	0.4
Ala	0.8	0.4	1.9	2.5
Val	2.6	1.4	1.5	2.0
Cys	2.6	1.4	2.5	3.3
Met	0.4	0.2	0.5	0.6
Ile	1.1	0.6	0.6	0.8
Leu	0.8	0.4	0.9	1.2
Tyr	tr	-	tr	-
Phe	0.9	0.5	tr	-
Gaba	0.9	0.5	2.4	3.1
NH_3	1.0	0.5	6.3	8.2
Orn	0.7	0.4	tr	-
Lys	2.3	1.2	1.2	1.6
His	2.9	1.6	tr	-
Arg	3.5	1.9	tr	-

[a] In these analyses, Asp + β-(isoxazolin-5-one-2-yl)-alanine (BIA) and Gln + Hse were not separated. In other analyses, eluting just the first half of the amino acids, these compounds were separated and were present in the proportions indicated.

[b] ND, Not detected.

[c] tr, Trace, a detectable but unquantified amount <0.2 nmol.

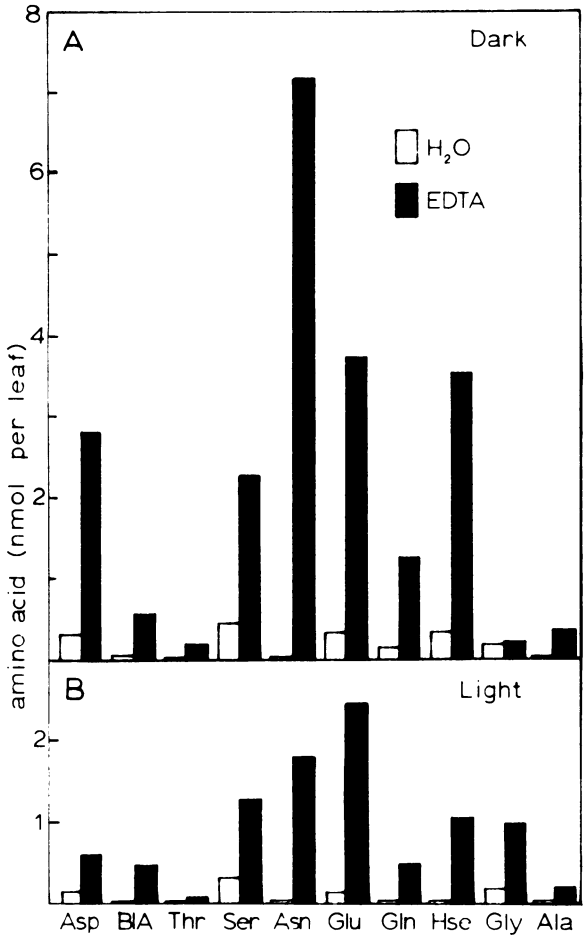

FIG. 1. Amino acid composition of phloem exudates from detached, fully expanded pea leaves. Exudation into water (□) or 20 mM EDTA (pH 7.0) (■) was over 2 h in the dark (A) or the light (B). Four leaves were used per treatment.

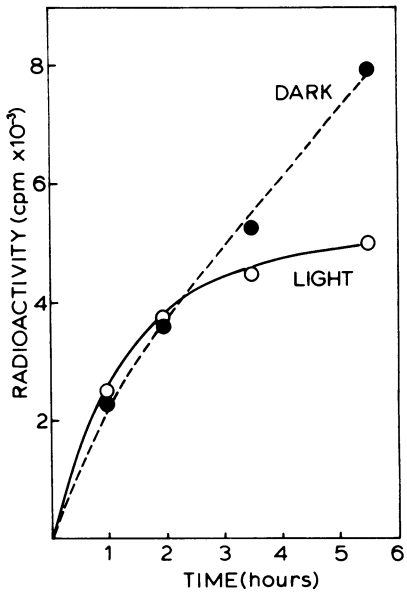

FIG. 2. Export of labeled compounds from detached pea leaves after application of [^{14}C]asparagine to the leaf surface. Leaves were detached and then [^{14}C]asparagine applied. Exudation of radioactive material into EDTA was followed for up to 6 h in the light (○) or the dark (●). Four leaves were used per treatment.

Table II. *Export of Labeled Compounds from Detached Leaves into Phloem Exudate*

Detached, fully expanded pea leaves were placed with their petioles in EDTA solution or water. Labeled amino acids were applied to lower surfaces of the leaves. Radioactivity, recovered after 2-h exudation in the dark, was measured and expressed as a percentage of the total radioactivity absorbed by the leaves. In each treatment, the results are the mean values from three leaves.

Tracer	Treatment	
	Water	EDTA
	% Exported into exudate	
Asn	0.04[a]	2.30
Gln	0[b]	4.85
Asp	0.23	0.91
Glu	0.15	0.26

[a] Radioactivity measured was 3 times background.
[b] No radioactivity detected above background.

the leaf blade, was exported out of the blade and recovered in the EDTA-promoted exudate. Export was continuous for 5 h (Fig. 2), and was linear with time when the leaves were in the dark; rate of export declined after 2 h from leaves in the light.

Labeled carbon from asparagine, glutamine, aspartate, and glutamate, fed individually as ^{14}C-tracers to the leaf blade, was exported into the phloem exudate as shown in Table II. Low levels of radioactivity were detected in the water-treated controls, but exudation was considerably enhanced by EDTA, particularly with asparagine and glutamine as tracers.

Comparison of Metabolism and Transport of Detached and Intact Leaves

Asparagine. In the detached leaf in the light, the distribution of radioactivity among soluble and insoluble compounds was similar to that in the intact plant (Table III). A major portion of the label was recovered in the fractions containing asparagine and glutamic acid, but radioactivity was also recovered in other amino acids (aspartate, glutamine, homoserine). Labeled carbon was incorporated into insoluble fractions in both detached leaves and leaves of intact plants. A considerable proportion of the labeled carbon (17–21% in detached leaves, 11.5% in leaves of intact plants) was recovered in the organic acid fractions largely as 2-hydroxysuccinamic acid in both cases. In general, although minor differences did occur, the labeling pattern in detached leaves and intact plants was similar. In the phloem exudate (from leaves fed [^{14}C]asparagine in the light), radioactivity was almost entirely in the amino acid fraction. Asparagine was the major labeled compound (about 60%); the rest of the label was mainly in aspartic acid.

In the detached leaf in the dark, less of the supplied asparagine was metabolized, and most of the label in the leaf extract was recovered still in asparagine (Table III). Again, asparagine and aspartate were the two main labeled components in the phloem exudate.

Aspartate. When aspartate was the tracer supplied to the leaf in the light, most of the aspartate was metabolized, both in the detached leaf and the intact plant (Table IV). Over 40% of the label in the fed leaflet was recovered (in several components) in the organic acid fraction, as shown in Table IV. Several amino acids were labeled (glutamate, glutamine, homoserine, alanine), but very little (about 10%) remained in aspartate; distribution of label was similar in detached leaves and intact plants. Comparison of phloem exudate and leaf extract (in the light) showed that the patterns of labeling were markedly different (Table IV), aspartate (and also glutamate and glutamine plus homoserine) being highly labeled in the exudate. Much less radioactivity (22.7%) was in the organic acid fraction of the phloem exudate, despite the large

Table III. *Distribution of Radioactivity in Leaf Tissue and Phloem Exudate following Application of ^{14}C-Asparagine to the Leaf Surface*

^{14}C-Asparagine was fed to the leaf blade of detached leaves (in the light or dark) or leaves of intact plants in the light. Phloem exudate (into EDTA) from detached leaves was collected after 2 h. The labeled leaves were removed at this time, extracted, and analysed. Four leaves or plants were used in each experiment. Two experiments are shown for detached leaves; data shown for the intact leaf were reproduced several times. The values shown are percentages of total radioactivity recovered in each fraction.

Fraction	Detached Leaf				Intact Leaf	
	Light		Dark		Light	
	Phloem exudate	Leaf extract	Phloem exudate	Leaf extract	Leaf extract	
Amino acids						
Asp	28.3 22.3	8.1 6.9	37.3 36.1	13.3 10.5	4.9	
Thr	0 0	0 0	0 0	0 0	0.3	
Ser	0 0	0 0	0 0	0 0	0.3	
Asn	58.8 60.7	37.4 } 38.7[a]	44.6 60.1	72.5 } 61.2[a]	} 66.9[a]	
Glu	0 0	6.6 }	0 0	1.7 }		
Gln	0 0	} 4.2[a] 1.1	0 0	} 0.4[a] 0.5	} 3.0[a]	
Hse	0 0	1.8	0 0	0		
Ala	0 0	0.5 0.9	0 0	0 0.2	0.5	
Others	9.4 11.6	5.9 6.6	17.2 3.5	3.4 5.4	4.1	
Neutrals	2.0 4.1	2.3 2.8	0.2 0.1	0.5 1.0	2.0	
Organic acids	1.5 1.3	17.2 21.1	0.7 0.2	1.8 5.7	11.5	
Insoluble	- -	18.0 20.0	- -	6.5 15.6	6.4	

[a] In some analyses, Asn plus Gln and Gln plus Hse were counted as single peaks owing to incomplete resolution.

Table IV. *Distribution of Radioactivity in Leaf Tissue and Phloem Exudate following Application of ^{14}C-Aspartate to the Leaf Surface*

^{14}C-Aspartic acid was fed to leaf blades of detached leaves or intact plants, as described in Table III. Four leaves or plants were used in each experiment. The values shown are percentages of total radioactivity recovered in each fraction of phloem exudate or leaf extract.

	Detached Leaf				Intact Plant
	Light		Dark		Light
	Phloem exudate	Leaf extract	Phloem exudate	Leaf extract	Leaf extract
Amino acid					
Asp	28.3	9.0	36.5	40.4	11.6
Thr	0	0.2	0	0	0.5
Ser	0	0	0	0	0.1
Asn	0	0	0	1.5	1.1
Glu	15.1	4.7	35.2	12.3	2.2
Gln	} 17.9	} 11.4	} 0	} 5.3	3.7
Hse					10.2
Ala	0	1.4	0	0	2.9
Others	2.8	8.4	3.4	5.0	8.7
Neutrals	13.2	5.5	0	0.2	4.0
Organic acid	22.7	44.1	24.9	24.2	41.3
Insoluble	-	15.3	-	11.0	13.8

proportion of label in this fraction of the leaf extract.

For detached leaves there were differences between light and dark treatments. In the dark, considerably less aspartate was metabolized (40% remaining unchanged, Table IV) and labeling of the organic acids and glutamine plus homoserine fraction was decreased. There were some differences between labeling of the leaf extract and phloem exudate, but these were less pronounced than in the illuminated leaf.

Amount of Material Exported. A comparison was made between export from detached leaves (into phloem exudate) and export from attached leaves to the rest of the intact plant (Table V). In general, the proportion of absorbed label exported from detached leaves was lower, and showed considerably greater variation, than export within the intact plant.

Table V. *Comparison of Export from Detached Leaves and Leaves of Intact Plants*

^{14}C-Labeled asparagine or aspartic acid were fed to leaf blades of detached leaves or intact plants as described in Table III. Total radioactivity recovered in the phloem exudate, fed leaves, and the rest of the intact plant was measured. The percentage radioactivity exported into phloem exudate from detached leaves, or into young leaves, roots, and other parts of the intact plant was calculated. Results from duplicate experiments are shown.

	Material Exported		
	Detached leaves		Intact plants
	Light	Dark	Light
	% total radioactivity recovered		
Asn	0.83	3.85	15.0
	2.18	4.55	19.0
Asp	0.22	0.67	7.3
	0.41	3.82	10.4

DISCUSSION

The results show that EDTA enhanced the exudation of amino acids from the petioles of detached fully expanded pea leaves. Levels of several amino acids were 5 to 10 times greater than in water-treated controls (Fig. 1), both in light-treated and darkened leaves. Levels of amino acids were not uniformly elevated by EDTA; rather certain amino acids increased (notably asparagine, homoserine, and glutamate). The spectrum of amino acids in the phloem exudate was also quite different from that in the xylem bleeding sap, (Table I) suggesting that there was little contamination from the xylem.

The presence of amino acids was demonstrated both in EDTA-promoted phloem exudates (6) and exudates obtained by other means (15, 16, 20, 22). In phloem sap from cut fruit stalks of "Meteor" peas (13), levels of total amino acids were similar to those obtained in the present study with "Little Marvel." Relative proportions of major amino acids were different, probably because of varietal differences. Amino acid concentrations were measured in aphid stylets sap from *P. sativum* c.v."Alaska" (2), but this study used sodium buffers for amino acid analyses, in which the amides and homoserine co-chromatograph with threonine and serine; a direct comparison of amino acid profiles from exudate collected by different methods cannot yet be made.

Our results also demonstrate that carbon from ^{14}C-labeled amino acids, applied to the leaf surface, was absorbed by the leaf and exported from the leaf blade (Table I). Although export was reduced compared to that in the intact plant (Table V), levels of radioactivity recovered in the exudate were nevertheless higher in EDTA-treated samples than in water-treated controls. In the feeding experiments with asparagine and aspartate, metabolism of these compounds in detached leaves did not differ markedly from that in equivalent leaves from intact plants (Tables III and IV). The composition of the phloem sap indicated selectivity in the export of labeled compounds from the leaf blade into the phloem. Asparagine was, for example, metabolized to hydroxysuccinamic acid (a major metabolite from asparagine in peas [14]) and to other amino acids, yet only asparagine and aspartate were major components of the phloem exudate. Similarly, when aspartate was the tracer used, it was greatly metabolized, both in the intact and detached leaf systems, but was a major component of the exudate. Metabolism and phloem loading, at least of asparagine and aspartate, appeared to be relatively unimpaired.

Our data (Table I) suggest cross-contamination between xylem and phloem would be slight. Nitrate is usually absent in phloem sap (13, 20, 22); indeed its absence from exudates is cited as indication of true phloem origin. In the present study, some nitrate was detected in the exudates, but most was exuded in the 1st h of experiments which were continued for 5 h (data not shown). Similar results have been reported for barley (23). There was little difference in levels of nitrate in EDTA-promoted exudate and water controls, in contrast to the large differences in amino acid exudation. The results indicate some contamination from cut cells of xylem, but nitrate levels in pea petioles are quite high (2.0–2.5 μmol/petiole, unpublished data) and no more than 5% of this is recovered in the phloem exudate.

EDTA-promoted exudation of labeled compounds has been studied in leaves which were labeled with $^{14}CO_2$ prior to detachment (6, 11). Leaching of labeled compounds already present in the petiole at the time of detachment cannot be entirely ruled out, but the exudation from detached *Perilla* leaves (11) was linear for several hours, indicating continuous translocation into the petiole. Individual leaves of intact soybean (8) were treated with short pulses of $^{14}CO_2$, and exudate was collected from the EDTA-treated stylar tips of the pods. Thus, labeled material passed through leaf, petiole, stem tissue, and pod before recovery in the EDTA. Tully and Hanson (23) treated whole plants with $^{14}CO_2$, but demonstrated that more radioactivity was removered in the EDTA exudate than had been in the sheath (section immersed in EDTA) at the time of detachment; radioactivity in the sheath increased slightly by the end of the exudation period, showing net movement out of the blade, through the sheath, and into the exudate. In the present study, leaves were detached prior to labeling; radioactive compounds were therefore translocated through leaf blade and petiole before recovery in the exudate.

In barley, EDTA-promoted exudation was stimulated by light (and also by increased CO_2 concentrations) possibly due to enhanced photosynthesis (23). No such stimulation was observed in the present work; in fact exudation of labeled compounds (Fig. 1, Table V) was slightly reduced in the light. Plausible reasons are the slight transpiration from leaves in the light even in a water-saturated atmosphere, possibly with some counter-current reabsorption into the xylem stream, or slight damage from increased EDTA levels in these leaves.

The data presented here show that EDTA promoted the exudation of amino acids from detached pea leaves. The evidence from this study and others (discussed above) demonstrates that phloem loading and translocation out of the leaf are not abolished by detachment of the leaf and EDTA treatment. When labeled compounds were used, the distance from the point of application to the end of the petiole was 25 to 30 mm, a distance too great for transport solely by diffusion. Rates of translocation are, however, clearly affected (Table V). Similar observations were made in other studies (6, 11, 23). Inasmuch as in detached leaves the phloem is disrupted, sinks removed, and transpiration essentially stopped, it is hardly surprising that export decreased. Variation in the amount of material exported into the phloem exudate in replicate experiments (Tables III, V; see also ref. 9) also indicates a need for caution and replication of experiments. However, when appropriate controls are made and the limitations of the method observed, the EDTA exudation technique of King and Zeevaart (11) is useful for studying amino acid transport from leaves. The results of such a study with pea leaves will be reported in a later paper.

Acknowledgments—The assistance of M. McLimont and C. Shay is gratefully acknowledged.

LITERATURE CITED

1. Atkins CA, JS Pate, PJ Sharkey 1975 Asparagine metabolism - Key to the nitrogen nutrition of developing legume seeds. Plant Physiol 56: 807–812
2. Barlow CA, PA Randolph 1978 Quality and quantity of plant sap available to the pea aphid. Ann Entomol Soc Am 71: 46–48
3. Bauer A, AA Urquhart, KW Joy 1977 Amino acid metabolism of pea leaves: Diurnal changes and amino acid synthesis from ^{15}N-nitrate. Plant Physiol 59: 915–919
4. Canvin DT, H Beevers 1961 Sucrose synthesis from acetate in the germinating castor bean: Kinetics and pathway. J Biol Chem 236: 988–995
5. Crafts AS, CE Crisp 1971 Phloem Exudation. *In* Phloem Transport in Plants WH Freeman and Co, San Francisco. p 65
6. Dickson RE 1977 EDTA-promoted exudation of ^{14}C-labelled compounds from detached cottonwood and bean leaves as related to translocation. Can J For Res 7: 277–284
7. Feller U, A Bauer, KH Erissman 1971 Spektrophotometrische Nitratbestimmung im Ultramikrobereich. Schweiz Landw Forsch 10: 249–257
8. Fellows RJ, DB Egli, JE Leggett 1978 A pod leakage technique for phloem translocation studies in soybean (*Glycine max* (L) Merr). Plant Physiol 62: 812–814
9. Frégeau J 1976 The effects of growth regulators on ^{14}C-assimilate transport in *Phaseolus vulgaris*. MSc thesis. University of Guelph, Guelph
10. Kennedy JS, TE Mittler 1953 A method for obtaining phloem sap via the mouth-parts of aphids. Nature 171: 528
11. King RW, JAD Zeevaart 1974 Enhancement of phloem exudation from cut petioles by chelating agents. Plant Physiol 53: 96–103
12. Leonard OA, RK Glenn 1968 Translocation of assimilates and phosphate in detached bean leaves. Plant Physiol 43: 1380–1388
13. Lewis OAM, JS Pate 1973 The significance of transpirationally derived nitrogen in protein synthesis in fruiting plants of pea (*Pisum sativum* L). J Exp Bot 24: 596–606
14. Lloyd NDH, KW Joy 1978 2-Hydroxysuccinamic acid: A product of asparagine metabolism in plants. Biochem Biophys Res Commun 81: 186–192
15. Milburn JA 1970 Phloem exudation from castor bean: Induction by massage. Planta 95: 272–276
16. Mittler TE 1958 Studies on the feeding and nutrition of *Tuberolachnus salignus* (Gmelin) (Homoptera, Aphididae). II. The nitrogen and sugar composition of ingested phloem sap and excreted honeydew. J Exp Biol 35: 74–84
17. Pate JS 1973 Uptake, assimilation and transport of nitrogen compounds by plants. Soil Biol Biochem 5: 109–119
18. Pate JS, PJ Sharkey, OAM Lewis 1974 Phloem bleeding from legume fruits - A technique for study of fruit nutrition. Planta 120: 229–243
19. Pate JS, PJ Sharkey, OAM Lewis 1975 Xylem to phloem transfer of solutes in fruiting shoots of legumes, studied by a phloem bleeding technique. Planta 122: 11–26
20. Peel AJ, PE Weatherley 1959 Composition of Sieve-tube sap. Nature 184: 1955–1956
21. Smith I 1958 Amino acids, amines, and related compounds. *In* Chromatographic Techniques, Smith, I, (ed). W Heinemann Ltd London p 72
22. Tammes PML, J van Die 1964 Studies on the phemonenon of bleeding and the composition of the exudate. Acta Bot Néerl 13: 76–83
23. Tulley RE, AD Hanson 1979 Amino acids translocated from turgid and water-stressed barley leaves. I. Phloem exudation studies. Plant Physiol 64: 460–466
24. Ziegler H 1956 Untersuchungen uber die Leitung und Sekretion der Assimilate. Planta 47: 447–500