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Estimation of Osmotic Gradients in Soybean Sieve Tubes by Quantitative Autoradiography

QUALIFIED SUPPORT FOR THE MÜNCH HYPOTHESIS[1]

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ABSTRACT

An attempt was made to evaluate Münch's hypothesis of osmotically generated pressure flow in soybean (*Glycine max* L.) sieve tubes from velocity measurements and calculations of pressure potentials and sieve tube resistances. Pressure potential was estimated from values for water potentials and osmotic potential. Leaf water potential measurements were made by isopiestic thermocouple psychrometry, while the water potential of the nutrient solution was made with a vapor pressure osmometer. Osmotic potential was measured by first bringing the sucrose pools in the entire plant to the same specific radioactivity by steady-state-labeling of the shoot with constant specific radioactivity $^{14}CO_2$ for 5 to 8 hours. Sucrose concentrations in sieve tubes were calculated from the disintegration rate per unit volume in sieve elements as measured by absolute quantitative microautoradiography of freeze-substituted, Epon-embedded source (leaf) and sink (root) tissues.

Conductivity of the sieve tubes was calculated from measurements of their dimensions in the petiole, stem, and root. The total pressure drop required for pressure flow at the observed velocities was calculated from the conductivity, velocity, and path length.

In all experiments, the calculated sucrose concentration in source sieve tubes was greater than that in sink sieve tubes, with an average ratio (source to sink) of 1.79:1. However, the absolute sucrose concentrations (average values of 46.4 mg cm^{-3} in the source and 23.9 mg cm^{-3} in the sink) would have been insufficient to maintain positive turgor in the sieve elements, and the expected pressure differences would not have accounted for movement at the observed velocities. However, the low values for sucrose concentrations almost certainly were due to loss of sucrose during tissue preparation but, for technical reasons, such loss could not be accurately quantified.

Assuming a sucrose concentration sufficient to maintain zero turgor in the root sieve tubes, a xylem water potential gradient (ψ_w [sink] − ψ_w [source]) of 2 bars between source and sink, and the measured ratio of sucrose concentrations in source and sink (1.79:1), the average turgor gradient between source and sink (ψ_p [sink] − ψ_p [source]) would have been about −1.6 to −3.5 bars, which compares favorably with the −1.07 to −2.41 bars average gradient that would have been required to drive translocation at the observed velocities.

The possibility that osmotically generated pressure flow might account for the movement of solutes through the phloem was proposed by Münch in 1927 (16). As several workers have pointed out, there will be an inevitable tendency for such movement to occur in a semipermeable tube in which osmotically active compounds are loaded at one end and removed at the other. There is, therefore, a basic element of acceptability to Münch's hypothesis, and this, along with various supporting experimental observations, has kept it in a position of consistent prominence among the various theories that have been advanced to explain phloem transport. That osmotically generated pressure flow must contribute to movement along sieve tubes is inevitable; the real problem is whether it must be supplemented by some other mechanism to account for the observed rates of translocation.

To evaluate the extent to which Münch-type flow might contribute to phloem transport, it is necessary to demonstrate that the existing turgor gradient from source to sink is sufficient to cause movement against the path resistance at the observed velocity. Various elements of these three measurements have been approached experimentally, although complete measurements have not been made with the same plant. In trees, the predicted presence of a decreasing osmotic gradient in the direction of translocation has been demonstrated by several workers (15). Although the occurrence of Münch-type flow must necessarily be accompanied by such a gradient, its quantitative significance is difficult to evaluate in the absence of information on water potential gradients, translocation velocities, and path resistances (*i.e.* sieve element dimensions). Data for the latter two are particularly incomplete in studies of osmotic gradients, although Zimmermann has measured the translocation velocity in white ash (22).

Several workers have investigated more specifically the problem of pressure gradients in sieve tubes. Kaufmann and Kramer (14) could not detect a turgor gradient when they used the difference between water potential and osmotic potential to calculate turgor at various positions along the phloem in red maple. However, their values for osmotic potential included solutes from the entire phloem tissue, so were not necessarily indicative of the sieve tube contents. Hammel (11) measured the turgor potentials in sieve tubes at heights of 6.3 and 1.5 m in red oak. The average turgor pressure at the upper level was 15.6 atmospheres and, at the lower level, 14 atmospheres. More recently, Shiekholeslam and Currier (19) have applied Hammel's phloem needle technique for measuring sieve tube turgor in squirting cucumber (*Ecballium elatarium*), and consistently found pressure gradients which correlated with the direction of translocation. Rogers and Peel (17) estimated turgor pressures both from the rate of flow from aphid stylets and from the differences between water potential and sieve tube exudate osmotic potential. They found a consistent negative turgor gradient of 0.5 to 4.7 atmospheres/m in the direction of translocation. None of the foregoing authors provided data for the translocation velocity or the path resistance.

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The purpose of the work reported here was to evaluate the osmotically generated pressure flow mechanism of phloem transport in soybean. Turgor pressure (ψ_p) was estimated from values for water potential (ψ_w) and osmotic potential (ψ_s). An attempt was made to measure the latter quantity by steady-state-labeling entire soybean plants with constant specific radioactivity $^{14}CO_2$ for a period sufficient to bring all of the sucrose in the translocation stream to the same specific radioactivity. Sucrose concentrations in sieve elements were then estimated by quantitative autoradiography. The velocity of translocation was measured, and resistance to flow in sieve tubes was calculated from their dimensions. The pressure drop required for flow at the observed rates was compared to the pressure drop estimated from the measured values of osmotic and water potentials.

MATERIALS AND METHODS

Growth of Plants. Soybean plants (*Glycine max* [L.] Merr. cv. 'Bragg') were grown in Hoagland solution for 5 to 8 weeks in a growth cabinet. A mixture of fluorescent and incandescent lights provided a photosynthetically active radiation (PAR) intensity of about 100 μeinsteins cm^{-2} sec^{-1} (about 5,000 lux). The plants were grown on a 14-hr photoperiod at 25 C and a night temperature of 21 C. At the time of the experiments, the total length of the plants (shoot tip to root tip) ranged from 65 to 180 cm. After the first trifoliolate leaf was mature, the two simple leaves and cotyledons were removed. Plants were normally used when the second trifoliolate leaf was mature. For longer path lengths, the plants were trimmed once a week so that the two mature trifoliolate leaves furthest from the roots were the only mature leaves on the plant.

Steady-state-Labeling, Velocity Measurement, and Plant Sampling. Forty-eight hr prior to labeling, the nutrient solution was replenished and the entire shoot of the soybean plant, consisting of two mature leaves, one to two expanding leaves and stem tip, was placed in the labeling chamber. Room air was circulated through the chamber during the prelabeling period. The plant was then labeled for 6 to 8 hr with constant specific radioactivity $^{14}CO_2$ using a closed steady-state-labeling system similar to that described by Coulson *et al.* (5). During the labeling period, the level of CO_2 was maintained at 280 ± 15 μl/l. Constant relative humidity (about 35%, entering the chamber) was maintained by circulating the air through a glass coil immersed in an ice bath. Condensate was trapped in a side arm flask which contained 5 ml of 60% perchloric acid to prevent the trapping of CO_2. The amount of $^{14}CO_2$ in the system and the arrival of ^{14}C-photosynthate in the roots were monitored by thin end window Geiger tubes connected to rate meters. The specific radioactivity of $^{14}CO_2$ in the system was calculated from the values for CO_2 concentration, volume of the system, and counting efficiency of the Geiger tube monitoring the system.

The velocity of translocation was calculated from the distance between the lowest trifoliolate leaf and the section of root being monitored by the Geiger tube divided by the time at which label was detected in the root.

After the labeling period, samples were taken for both extraction and microautoradiography by the following procedure. The chamber and room lights were turned off and the plant was taken out of the chamber. The two samples (5A and 8A in Fig. 1) to be used for microautoradiography were then frozen *in situ* by dousing them with an isopentane-methylcyclohexane mixture (9:1, v/v) cooled to about −175 C by liquid nitrogen. These samples were freeze-substituted in acetone or propylene oxide as described by Fisher and Housley (10). The total time elapsed from turning off the light until the tissue was frozen was less than 2 min. Eight different portions of the experimental plant (Fig. 1) were then extracted in boiling 80% aqueous ethanol and the extracts were concentrated for chromatography.

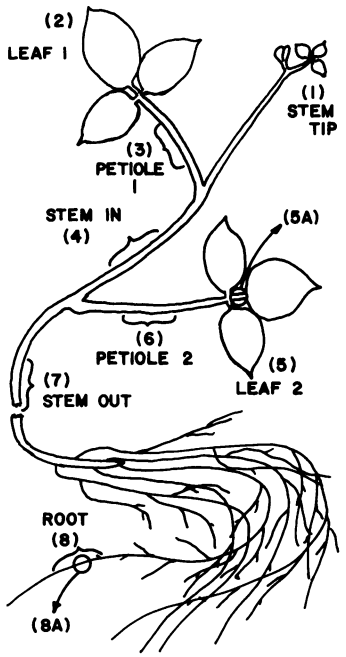

FIG. 1. Sampling positions for microautoradiography and for sugar assays. Positions 1 to 8 were used for determination of sucrose specific activities. "Stem in" indicates a stem sample from inside the labeling chamber; "stem out" indicates a sample from outside the chamber. Samples 5A and 8A were used for microautoradiography.

The concentrated 80% ethanol extracts were separated by descending chromatography on Whatman No. 4 filter paper in 1-butanol-propionic acid-water (23:12:15, v/v). Preliminary experiments with two-dimensional chromatography demonstrated that use of phenol-water in a second direction was unnecessary for adequate separation of sucrose. After autoradiography, sucrose was eluted from the paper, two aliquots were counted in a gas flow counter, and the remainder was assayed for sucrose. The sucrose to be assayed was divided into three equal aliquots. Ten μg of sucrose was added to one aliquot as an internal standard, and all three aliquots were hydrolyzed with invertase. Glucose was determined with Glucostat reagent (Worthington Biochemical Corp.). Replicate samples agreed to within 5%.

Embedding for Microautoradiography. Because embedding freeze-substituted plant tissue in Epon results in only partial retention of the sugars when the sections contact water, and this partial retention may be due to a reaction between the sugar and the resin (10), a procedure was developed which was aimed (with only partial success) at more extensive reaction of the sugar with the embedding medium. In brief, this consisted of first reacting the sugars with TDI[4] in an attempt to cross-link the sugars with the bifunctional diisocyanate, or if only one isocyanate group reacted, to react the remaining group with hydroxyl groups formed in the resin during polymerization. Both reactions were catalyzed with dibutyltin dilaurate (18).

All solvents and reagents were thoroughly dried over molecular sieves to prevent shrinkage artifacts and the vials containing the tissue were opened only in a dry box (8). The warmed, freeze-substituted tissue was first infiltrated with toluene, and TDI was then introduced in five to eight steps. Once in 100% TDI, dibutyltin dilaurate (about 0.3%) was added and the vials were capped and placed in an oven at 100 C for 12 hr. The glue and seal on the vial caps had to be removed before placing the vials into the oven or they reacted with the TDI and produced a

[4] Abbreviation: TDI:tolylene 2,4-diisocyanate.

precipitate. After the vials cooled, the TDI was replaced with toluene.

Epon embedding was carried out by infiltration with Spurr's embedding mixture (20) using boron trifluoride monoethylamine complex, thoroughly dried over molecular sieves, as the accelerator. In preliminary experiments with petiolar tissue in which more than 90% of the ^{14}C was in sucrose, this accelerator gave better retention when sections were exposed to water, although it resulted in a much shorter pot life for the resin. Five ml of the Spurr's mixture was put into a small disposable culture tube (13 × 100 mm). Enough accelerator to fill the rounded portion of the tube was added (about 0.12 g/5 ml of resin) and the tube was capped and shaken vigorously for 5 min, then centrifuged before use to remove undissolved accelerator. If the accelerator was not dry, or if too much was used, the resin would heat up and polymerize. Normally, the resin would not completely polymerize for 14 hr, but would become sufficiently thick in about 1 to 2 hr so that new batches of resin and accelerator had to be mixed two or three times during infiltration. When the monomer to toluene ratio was about 50%, dibutyltin dilaurate (1 drop/5 ml) was added along with the boron trifluoride monoethylamine complex. After infiltration was completed, the vials were rotated for 12 hr on a revolving shaker. The infiltrated tissue was put into "Beem" capsules, placed in an oven at 60 C for 12 hr, and then transferred to a 100 C oven for 3 days.

Quantitative Autoradiography. Procedures for the quantitative microautoradiography of ^{14}C in semithin plastic sections have been described elsewhere (12). The sections used in the present experiments were about 1 μm thick; the exact thickness of each section was measured by interference microscopy to allow the calculation of radioactivity per unit of sieve element volume. Several sections of ^{14}C-methyl methacrylate of known activity per unit volume were included on each slide as internal standards to determine detection efficiency. By following the exposure in test slides, the exposure times for slides actually used for grain counting were varied so as to give a grain density of about one grain/μm^2 over the sieve elements.

Water Potential Measurements. Leaf water potential measurements were made with thermocouple psychrometers, using the isopiestic technique described by Boyer and Knipling (2). Plants similar to those used for labeling were placed into the closed system the night before they were to be used. Leaf water potentials were measured the next day at the start of the light period, and at various intervals after the light period began. Leaf water potentials were measured with an estimated accuracy of ±0.3 to 0.5 bars, depending on the thermocouple. The water potential of the nutrient solution was measured by vapor pressure osmometry, with an accuracy of ±0.04 bars.

Sieve Tube Conductivities. Since the sieve plate pores and sieve tube element lumen in soybean are essentially open (9), an estimate of sieve tube conductivity can be calculated from the sieve element dimensions by use of the Hagen-Poiseuille equation (see ref. 4). The required sieve element dimensions were obtained from hand-cut fresh sections of petioles, stems, and roots which were stained with Aniline blue and examined by fluorescence microscopy (13).

RESULTS

Water Potential Measurements. The leaf water potential of plants in the steady-state-labeling apparatus was about −3 bars when the lights came on and declined slowly for 3 hr, to −4 bars (Fig. 2). The decline was more rapid during the next 4 hr. When labeling experiments were terminated between 6 and 11 hr after the lights were turned on, the leaf water potential was about −9 bars. The velocity measurement was made about 3 to 5 hr after turning the lights on, when the leaf water potential would have been about −4 to −7 bars. Labeling was started about 2 to 4 hr after turning on the lights.

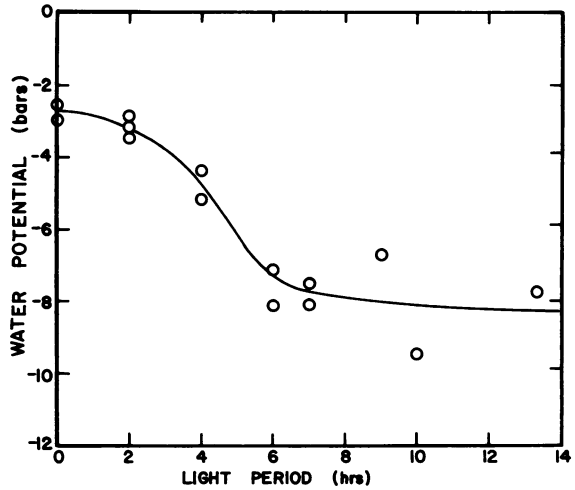

FIG. 2. Variation in leaf water potential after turning on the lights. Plants were removed from the steady-state-labeling chamber at the times indicated, and their leaf water potential was measured by thermocouple psychrometry.

The water potential of the nutrient solution, as determined by vapor pressure osmometry, was 0.60 ± 0.06 bars.

Sucrose Specific Radioactivities after Steady-state Labeling. The sucrose specific radioactivities in various parts of six experimental plants are presented in Table I. The values are presented as both the absolute specific radioactivity and as the specific radioactivity relative to that in the petiole of the lowest leaf (petiole 2). The latter value was chosen for comparative purposes because it was one of two values used for calculating sucrose concentrations from microautoradiographic data (the second value was that of sucrose specific radioactivity in the root). To correct for isotopic discrimination during photosynthesis, the theoretical maximum for sucrose specific radioactivity was calculated as 85% of the specific radioactivity of the $^{14}CO_2$ in the circulating atmosphere (3).

Because it is difficult to interpret the significance of an occasional high or low value, the following comments will refer mostly to the average values for relative specific radioactivity. However, it should be noted that the generally low relative specific radioactivities in experiment 2 could have resulted from an incorrectly high value for sucrose specific radioactivity in petiole 2.

There was no appreciable difference between the specific radioactivity of sucrose in the leaves, petiole, and roots. The most striking deviation was shown by sucrose from the step tip where specific radioactivity was higher than in any other plant part. This may reflect a more rapid turnover time for sucrose in the immature tissue or the direct assimilation of $^{14}CO_2$ might be responsible for the generally higher specific radioactivity. Although the stem tip might have had a sucrose specific radioactivity which more accurately represented the translocate specific radioactivity, there was no way of distinguishing between assimilated sucrose and translocated sucrose.

The specific radioactivity of sucrose in the stem segments appeared to be somewhat lower than in other plant parts. Sucrose in the stem could be relatively more compartmentalized because of larger pools outside the translocation stream or because of slower movement of ^{14}C-sucrose from the translocation stream. Stephenson (21) reported that depending on the growth stage of the plant, the stem may have considerable storage capacity. Fisher (6) calculated a turnover time of 154 min for sucrose outside the translocation stream in soybean petiole. It

seems reasonable that the turnover time in the stem might be longer simply due to its greater size.

The specific radioactivity of the leaf sucrose was appreciably lower than that of $^{14}CO_2$ supplied, even when isotopic discrimination was taken into consideration. This confirms an earlier observation by Fisher (7), who found that after steady-state-labeling of soybean leaves for 3 hr, the sucrose specific radioactivity had reached only 62% of the $^{14}CO_2$ specific radioactivity, or 73% of its maximum possible value when isotopic discrimination is taken into account.

Autoradiographic Estimation of Sucrose Concentrations. Sucrose concentrations in root and leaf sieve elements, calculated from sucrose specific radioactivity (Table I) and the ^{14}C per unit volume in sieve elements, are presented in Table II. The values for sucrose specific radioactivities used in the calculations were taken from the same plant part as the autoradiographs. In every experiment, the calculated sucrose concentration in leaf sieve elements was greater than that in root sieve elements. In general, however, the concentrations were low in comparison to generally reported values for phloem exudates. Only in the first experiment was the estimated concentration sufficiently high to have caused positive turgor in the sieve elements. These generally low values were due in part, at least, to loss of ^{14}C during reaction of the tissue with TDI and to loss from wetted sections. In all experiments except number 1 (and two preliminary experiments which were run to evaluate the effectiveness of TDI treatment), more than 40% of the total ^{14}C was solubilized when the sections were exposed to water. Unfortunately, it was not possible to obtain an accurate estimate of ^{14}C-sucrose loss from sieve elements, since all compounds and compartments were so extensively labeled in these experiments and the use of TDI effectively eliminated the possibility of identifying the solubilized compounds chemically.

Of more significance than the absolute values of the concentrations are their relative values in a given experiment. The ratio of sucrose concentration in leaf sieve elements to that in root sieve elements averaged about 1.79:1. It ranged from 1.14 to 4.17, without any apparent relationship to either path length or velocity.

To investigate the variability of sucrose concentrations in comparable regions of the translocation stream, a comparison was made of grain densities over sieve elements in different vascular bundles in the same petiolule and in different roots from the same experiment. Comparable parts of the translocation stream seemed similar in concentration. No difference was greater than one-third of a standard deviation for the grain counts.

Sieve Tube Dimensions and Calculated Resistances. The di-

Table I. Sucrose Specific Activity

Expt. No.	Time (hrs)	Upper left: dpm $\mu g^{-1} \times 10^{-4}$; Lower right: specific activity relative to petiole 2, %								
		Theor. Max.[a]	Stem Tip (1)	Leaf 1 (2)	Pet. 1 (3)	Stem In (4)	Leaf 2 (5)	Pet. 2 (6)	Stem Out (7)	Roots (8)
1	7	--	2.32	2.11	2.37	2.04	1.98	2.03	1.78	1.93
			114	104	117	100	96	100	88	95
2	7	--	2.01	1.37	0.98	1.10	1.07	1.63	1.01	1.43
			123	84	60	67	66	100	62	88
3	6	4.53	4.22	3.19	2.74	2.28	3.10	3.23	3.25	3.67
		140	131	99	85	71	96	100	101	114
4	6	3.96	3.86	3.35	3.48	2.65	3.12	3.48	2.39	3.08
		114	111	96	100	76	90	100	69	89
5	5	1.21	1.12	1.04	0.98	0.91	0.74	0.89	0.90	0.82
		136	126	117	117	110	102	100	101	92
6	8	1.77	1.94	1.94	1.40	1.59	1.80	1.46	1.45	1.48
		122	133	133	96	109	123	100	99	101
Average %'s		128	123	106	96	89	96	100	87	97

[a] Theoretical maximum value; obtained from specific activity of the circulating $^{14}CO_2$ multiplied by 0.85 to account for isotopic discrimination.

Table II. Sucrose Concentration, Path Lengths, Velocities and Osmotic Gradients

Expt. No.	Path Length (cm)			Velocity[b] cm min^{-1}	Sucrose Conc. in Sieve Tubes				Conc. Ratio	Gradient (Root - Leaf)		Osmotic Gradient bars m^{-1}
					Petiolule		Root					
	Shoot[a] (Petiole + Stem)	Root	Total		Sucrose mg cm^{-3}	Ψ_s bars	Sucrose mg cm^{-3}	Ψ_s bars	Leaf / Root	ΔSucrose mg cm^{-3}	$\Delta\Psi_s$ bars	
1	5+32	18	55	0.66	103.0	-8.21	52.3	-4.26	1.96	-50.7	-3.95	-7.17
2	8+32	22	62	0.88	26.6	-2.13	23.3	-1.77	1.14	- 3.3	-0.35	-0.58
3	8+30	27	65	0.66	30.4	-2.33	22.2	-1.72	1.37	- 8.2	-0.61	-0.94
4	6+35	31	72	0.80	25.9	-2.03	14.4	-1.11	1.79	-11.5	-0.91	-1.27
5	8+54	26	88	0.61	47.1	-3.75	11.5	-1.01	4.17	-35.6	-2.74	-3.11
6	10+121	30	161	0.92	45.6	-3.55	19.6	-1.52	2.33	-26.0	-2.03	-1.26
Average				0.76	46.4	-3.67	23.9	-1.89	1.79	-22.6	-1.76	-2.39
Average (Assuming sucrose specific activity in root sieve tubes = sucrose specific activity in Petiole 2 sieve tube)							23.0	-1.86	1.85	-23.4	-1.83	-2.49

[a] Measured from the blade of the lowest leaf

[b] Calculated from the path length and the time of detection of radioactivity in the roots at the level of sections taken for autoradiography

Table III. Sieve Element Dimensions and Conductivities

Plant Part	Length (μ)	Diameter (μ)	Pore Diameter (μ)	Sieve Plate Thickness (μ)	pore area / sieve tube area (pores 100% open)	Conductivity (cm min^{-1} / bar cm^{-1})	
						100% Open	70% Open[e]
Petiole[b]	125±31	8.3±1.8	0.7	1.1	0.40	21.1	8.6
Stem[c]	156±43	13.1±2.5	1.2	1.2	0.67[a]	90.7	40.1
Root[d]	137±34	10.1±4.5	1.0	1.0	0.58[a]	55.8	26.6

[a] The fraction of sieve plate area occupied by pores was about 45% in all three parts. However, fraction of sieve element area available to flow through a sieve plate was somewhat more because of frequently inclined plates.
[b] Based on 40 sieve elements.
[c] Based on 116 sieve elements.
[d] Based on 40 sieve elements.
[e] i.e., pore diameter = 0.7X the measured diameter.

mensions of sieve tube elements were quite different in the petiole, stem, and root (Table III). Sieve elements in the stem were longer than those in the root or petiole. In a single file of sieve tubes, it was not uncommon to find a few sieve tube elements half as long as the majority of the sieve elements in the file. The average diameter of each group of sieve elements indicated that stem sieve elements also had a larger diameter than those in either the petiole or root. The sieve plate thickness and the pore diameter were difficult to measure precisely, but comparisons of the values from the petiole with electron micrographs of petiolar sieve plates indicated that the measurements are probably accurate to within 0.15 μm (Table III). It should be noted that these pore diameters were made on the larger pores in the plate, each of which had a range of pore sizes down to a few tenths of a μm in diameter, although most were near the maximum diameter. The average pore diameter in the stem was appreciably larger than those in either the petiole or root. The fraction of the sieve tube cross-sectional area open for translocation through the sieve plate was greatest in the stem and least in the petiole. This arises primarily from the fact that the sieve plates in the stem and root were frequently inclined to the axis of the sieve element, whereas in the petiole they were more nearly transverse. Conductivity in the petiole was about one-fourth that in the stem and less than one-half that in the root (Table III). Particularly because of the above uncertainties in the sieve plate pore measurements and because of their variability, conductivities were also calculated for the case where the pore diameters were 0.7 times those shown in Table III. The difference in sieve tube conductivities in the three parts was even greater if the sieve plate pore diameters were smaller.

The total pressure drop required for mass flow between source and sink at the observed velocity was calculated for each experiment (Table IV) using the conductivities in Table III and the path lengths in Table II.

Table IV. Calculated Required Pressure Gradient for each Experiment [a]

Expt. No.	Required Pressure Drop (bars)	
	100% Open Pore	70% Open Pore[b]
1	-0.60	-1.36
2	-0.99	-2.25
3	-0.79	-1.78
4	-0.98	-2.19
5	-0.88	-1.99
6	-2.16	-4.88
Av.	-1.07	-2.41

[a] i.e., Ψ_p (sink) - Ψ_p (source)
[b] i.e., pore diameter = 0.7X the measured diameter.

DISCUSSION

In the present experiments, the strongest support for the Münch hypothesis comes from the observation that the calculated sucrose concentration in source sieve tubes was always greater than that in sink sieve tubes, with an average ratio (source to sink) of 1.79:1. To evaluate the significance of this difference (ignoring, for the moment, the values for absolute sucrose concentrations), some assumptions must be made about the water potential gradient between the source and sink. When the plants were harvested, leaf $\psi_w = -9$ bars and nutrient $\psi_w = -0.6$ bars. Boyer (1) estimates that the majority of resistance to water movement in soybean occurs in the root. A gradient of 2 bars between leaves and root (*i.e.* ψ_w [sink] − ψ_w [source]) would probably be the greatest that might be expected over such short distances. In that case, the water potential in the root sieve tubes would have been −7 bars (*i.e.* 2 bars greater than leaf ψ_w). Assuming zero turgor pressure in root sieve tubes, their solute potential also would have been about −7 bars. This implies a value of −12.5 bars (= −7 × 1.79) for the average solute potential in the source sieve tubes. The average turgor gradient between source and sink (*i.e.* ψ_p [sink] − ψ_p [source]) would have been −3.5 bars, adequate to cause flow at the observed rates even if the sieve plate pores were only 70% of the measured diameter (Table IV). Similar calculations may be made for higher leaf water potentials (*e.g.* earlier in the experiment). For example, with ψ_w (leaf) = −6 bars and $\Delta\psi_w = 2$ bars, $\Delta\psi_p = -1.2$ bars, which still compares favorably with the required pressure drop. As the assumed value for leaf ψ_w is increased, the calculated turgor gradient becomes less, reaching zero at about ψ_w (leaf) = −4.5 bars. At that point, the calculated osmotic potentials in the source (−4.5 bars) and in the sink (−2.5 bars) are not much different from the measured values (Table III).

However, the data are ambiguous and their interpretation depends on assumptions which must necessarily be made because of their incompleteness. Other possible interpretations are not supportive of an osmotically generated pressure flow mechanism. If the figures for sucrose concentration are taken at their face value, and the water potential gradient between the source and the sink is assumed to be 2 bars, flow would not have occurred even in the right direction, much less at the observed rates, except in experiment 1. In that experiment, the calculated pressure gradient (assuming a ψ_w gradient of 2 bars) would be −2 bars. Even in experiment 1, the concentration of sucrose was

so low that negative turgor would be expected in the root sieve tubes. In the remaining experiments, in order to have zero turgor in the sieve tubes of either the root or leaf, the sucrose concentrations need to be two to three times the measured values. Much of the uncertainty in interpretation arises from the loss of sucrose during tissue preparation, since our measurement of sucrose concentration in the sieve elements would be correspondingly lowered. Although such loss seemed substantial in all experiments except the first, it could not be measured accurately.

Taken as a whole, we believe that the data offer qualified support for the Münch hypothesis. The objective of bringing the sucrose specific radioactivity to the same value along the entire translocation path, thus allowing the calculation of sucrose concentrations from the disintegration rate per unit sieve element volume, was satisfactorily accomplished. The finding that on a relative basis, the sucrose concentration in source sieve elements was substantially greater than in sink sieve elements appears to be of greater significance than the values for absolute concentrations. The latter figures are low in comparison to published values for phloem exudates, but this may be reasonably attributed to loss of ^{14}C-sucrose during preparation of the microautoradiographs.

LITERATURE CITED

1. Boyer JS 1971 Resistances to water transport in soybean, bean and sunflower. Crop Sci 11: 403-407
2. Boyer JS, EB Knipling 1965 Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. Proc Nat Acad Sci USA 54: 1044-1051
3. Bykov OD 1970 The method of calculation of isotopic effect in photosynthesis and its value. Photosynthetica 4: 195-201
4. Christy AL, JM Ferrier 1974 A mathematical treatment of Münch's pressure-flow hypothesis of phloem translocations. Plant Physiol 52: 531-538
5. Coulson CL, AL Christy, DA Cataldo, CA Swanson 1971 Carbohydrate translocation in sugar beet petioles in relation to petiolar respiration and adenosine 5'-triphosphate. Plant Physiol 49: 919-923
6. Fisher DB 1970 Kinetics of C-14 translocation in soybean. I. Kinetics in the stem. Plant Physiol 45: 107-113
7. Fisher DB 1970 Kinetics of C-14 translocation in soybean. II. Kinetics in the leaf. Plant Physiol 45: 114-118
8. Fisher DB 1971 Artifacts in the embedment of water-soluble compounds for light microscopy. Plant Physiol 49: 161-165
9. Fisher DB 1975 The structure of functional soybean sieve elements. Plant Physiol 56: 555-569
10. Fisher DB, TL Housley 1971 The retention of water-soluble compounds during freeze-substitution and microautoradiography. Plant Physiol 49: 166-171
11. Hammel HT 1968 Measurement of turgor pressure and its gradient in the phloem of oak. Plant Physiol 43: 1042-1048
12. Housley TL, DB Fisher 1975 The efficiency of ^{14}C detection in autoradiographs of semithin plastic sections. J Histochem Cytochem 23: 678-680
13. Jensen WA 1962 Botanical Histochemistry. W. H. Freeman & Co, San Francisco
14. Kaufmann M, PJ Kramer 1967 Phloem water relations and translocation. Plant Physiol 42: 191-194
15. Milburn JA 1975 Possible mechanisms of phloem transport. Pressure flow. *In* MH Zimmermann, JA Milburn, eds, Transport in Plants. I. Phloem Transport. Springer-Verlag, Berlin pp 328-366
16. Münch E 1930 Die Stoffbewegungen in der Pflanze. Gustav Fischer, Jena
17. Rogers S, AJ Peel 1975 Some evidence for the existence of turgor pressure gradients in the sieve tubes of willow. Planta 126: 259-267
18. Saunders JH, KC Frisch 1962 Polyurethanes. Chemistry and Technology. Interscience Publishers, New York
19. Shiekholeslam SN, HB Currier 1975 Turgor pressure gradients and assimilate movement in phloem of *Ecbalium elaterium*. Plant Physiol 56: 5-18
20. Spurr AR 1969 A low-viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res 23: 31-43
21. Stephenson R 1974 Patterns of assimilate distribution and bean yield in soybean (*Glycine max* [L.] Merr.) PhD thesis. University of Queensland
22. Zimmermann MH 1969 Translocation velocity and specific mass transfer in the sieve tubes of *Fraxinus americana* L. Planta 84: 272-278