# TRANSLOCATION III. EXPERIMENTS WITH CARBON 14, CHLORINE 36, AND HYDROGEN 3 [1,2]

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In spite of continued interest in photosynthate translocation over the years, the mechanism remains unknown and no better working hypotheses have been devised than the classical models of pressure flow (6, 15), protoplasmic streaming (7), activated diffusion (14), or interfacial flow (26).

The advent of tracer isotopes reopened many inquiries that had common meaning in terms of these theories regarding: a) the photosynthetic compounds translocated (16, 22, 26); b) the rates of movement (16, 17, 26); c) the role of respiration (10, 11, 16, 26); d) the influence of plant parts (1, 15, 19); e) the simultaneous movement of metabolites (2, 5, 23); f) the role of water (2); g) the precise translocation cross section (3, 4, 24). Unfortunately, the interpretation of such data is always difficult because of rapid (and probably differential) removal of tracers from the conducting channels and because of different absorption cross sections, in simultaneous application of tracers to leaves. It seemed desirable, therefore, to re-examine some of these aspects in short-period experiments using feeding techniques that ensured rapid penetration to phloem tissue.

In an earlier paper from this laboratory (26), it was established that sucrose is the primary component of photosynthate moving out of soybean leaves. The linear velocity of translocation was estimated to be about 100 cm/hour and was not light dependent. Temperature dependence of translocation in the stem could be interpreted as aqueous viscosity effects. In a further communication (1) it was shown that sugar gradients were not a basis for rapid movement of sucrose within the leaf. No movement occurred from a leaf with a steamed petiole or from one with a petiole excised prior to root initiation. Some upward flow to a younger trifoliate occurred when the stem was steamed below the entrance petiole. In view of these observations, it was suggested that the forces responsible for movement resided within terminal meristematic tissue.

The present paper represents a continuation of these studies employing $HCl^{36}$ and THO for simultaneous gaseous administration to leaves with $C^{14}O_2$. In some experiments, fructose-$C^{14}$ was fed to cut petioles in aqueous combination with $HCl^{36}$ or THO. Tritiated water was used for two purposes: a) an attempt to measure the simultaneity of movement of water and photosynthate, this study having begun before the pioneering work of Biddulph and Cory (2) was available; b) preliminary work in radioautographic location of photosynthetically-incorporated tritium in petioles and stems.

I. INFLUENCE OF GROWING POINT, LEAF, STEM, AND ROOT. This section describes experiments designed to examine the influence of various plant organs on the movement of photosynthate. Two different feeding techniques were employed: gaseous administration of isotope through a leaf or solution feeding of isotope through a severed petiole. The distribution of $C^{14}$-labelled photosynthate was measured following excision of various plant parts or steam treatment of the stem.

*Experimental.* 1. *Plant material.* Soybeans (*Glycine max.* var. Hawkeye) were grown in the greenhouse. During the winter months natural illumination was supplemented with about 1,000 ft-c using Daylight fluorescent lamps; the photoperiod was adjusted to 14 hours to avoid flowering. Except for the root experiments, all plants were grown in soil. The former were supported in Vermiculite over jars containing Hoagland's solution; the latter was changed daily. Plants were moved to the laboratory when the first trifoliate was fully grown (about 3 weeks after planting). In the double-feeding experiments, two plants, of similar appearance in the same pot, were selected. All plants were placed in the laboratory two or three hours prior to experiment and any pretreatment was done at least one hour in advance. Excision of leaf or stem was performed with a razor. Root tips were removed with surgical scissors just past the region of cell elongation (as judged by root hairs) while the root was immersed in culture solution.

2. $C^{14}O_2$ *administration.* $C^{14}O_2$ was administered to leaves in either of two ways. For the simultaneous exposure of two leaves to equal amounts of radioactivity, the glass chamber described by Aronoff (1) was modified to a Y. It also contained a steel needle, sealed in glass, to facilitate uniform mixing with an external magnet. For the feeding of entire trifoliates, the method of Vernon and Aronoff (26) was employed.

In the petiole feeding experiments, plants were

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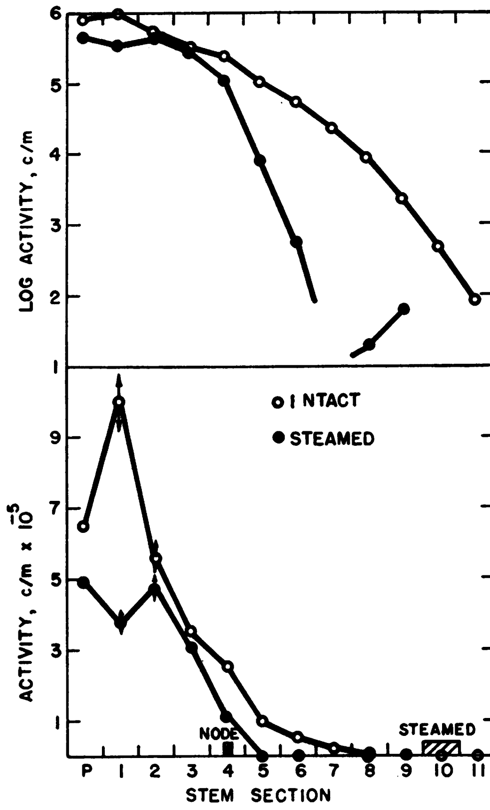
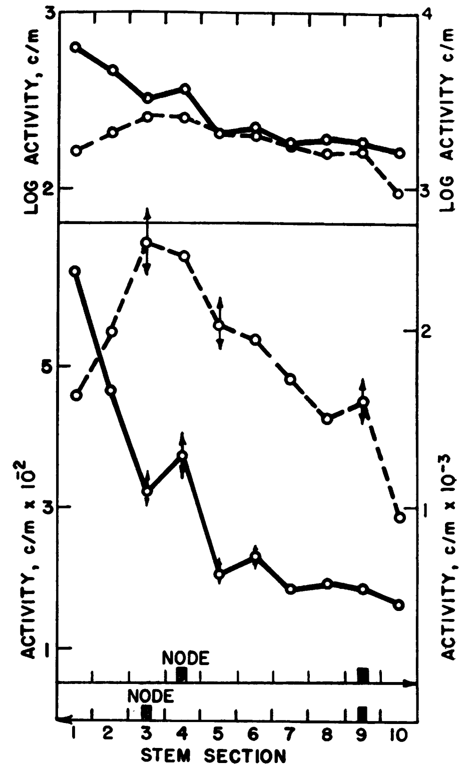
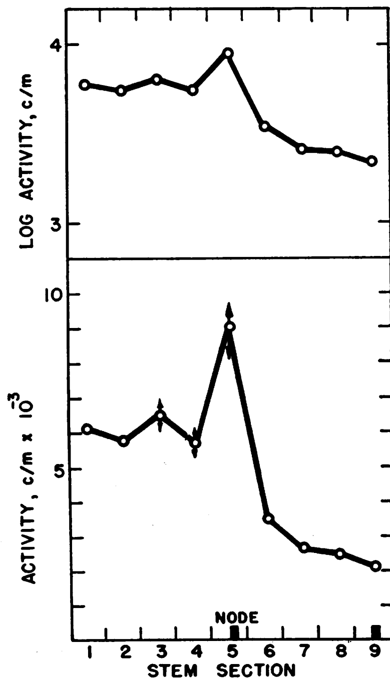


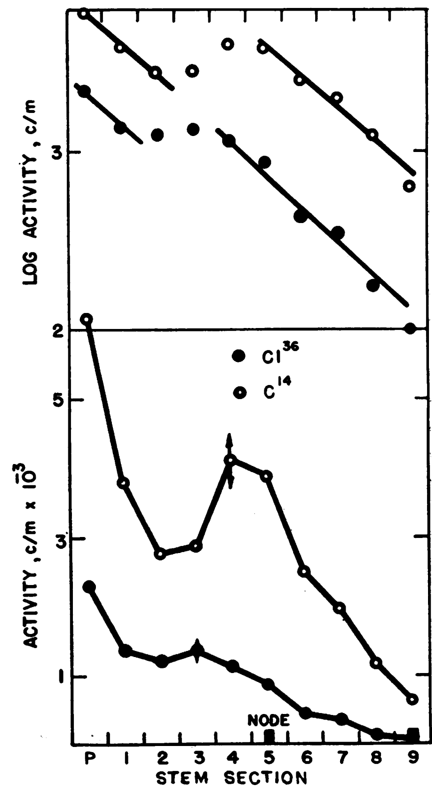
LOG ACTIVITY, c/m
ACTIVITY, c/m x $10^{-5}$
○ INTACT
● STEAMED
NODE
STEAMED
P 1 2 3 4 5 6 7 8 9 10 11
STEM SECTION

LOG ACTIVITY, c/m
LOG ACTIVITY c/m
ACTIVITY, c/m x $10^{2}$
ACTIVITY, c/m x $10^{-3}$
NODE
NODE
1 2 3 4 5 6 7 8 9 10
STEM SECTION

LOG ACTIVITY, c/m
ACTIVITY, c/m x $10^{-3}$
NODE
1 2 3 4 5 6 7 8 9
STEM SECTION

LOG ACTIVITY, c/m
ACTIVITY, c/m x $10^{-3}$
● $Cl^{36}$
○ $C^{14}$
NODE
P 1 2 3 4 5 6 7 8 9
STEM SECTION

supported horizontally, with petioles pointing downward. The leaf was removed at the petiole junction under water and a small L-shaped glass feeding tube was slipped over the cut end. All water, except for a small drop at the petiole end, was removed and the petiole was sealed to the tube with modelling clay. The radioactive solution was added through the open arm, mixed, and the open arm sealed with a stopper.

Zero time, with both feeding techniques, was taken as the time when the radioactive material was released to the tissue.

At the conclusion of the experiments, the leaves were removed, the stems were divided quickly into 2 cm lengths, and all sections were frozen in liquid nitrogen. Subsequent procedures for extraction of the tissues, for counting of radioactivity and for gross radioautography, were virtually identical to those described in earlier papers (1, 26).

*Experiment* 1. *Removal of root tips or growing point.* Exploratory tests showed that soybeans translocated radioautographically-detectable amounts of $C^{14}$-photosynthate when one $\mu c$ $C^{14}O_2$ was administered to a 1 $cm^2$ area of a leaf. Subsequently, three, 30-minute experiments were performed to determine the effect of excision of meristematic tissue. One plant, with growing point (stem tip) removed, was fed simultaneously with one which had root tips removed. Both were radioautographed on the same sheet of film. Two untreated controls were similarly fed and radioautographed. In the first trial there was considerably more activity in plant roots whose growing point had been removed, and visa versa. Two subsequent trials, however, failed to verify this finding.

*Experiment* 2. *Effect of steaming the stem.* Aronoff's qualitative experiments (1) showed that photosynthate did not move past steamed sections in petioles or in upper stem regions. It was decided to investigate this stem effect in greater detail.

Two similar plants, grown in the same pot, were administered a total of about 200 $\mu c$ of $C^{14}O_2$ by using two leaf chambers in series with the reaction vessel, the steamed plant being first in line. A 2 cm section, just below the cotyledonary node of the first plant, was steamed until it took on a watery, cooked appearance.

In three trials, no activity was found below the steamed section and total stem activity was greatly reduced compared with controls. Typical results of a 20 minute experiment are shown in table I and figure 1. Note that the stem activity in the treated

TABLE I

$C^{14}$ DISTRIBUTION FOLLOWING 20 MINUTE PHOTOSYNTHESIS OF $C^{14}O_2$*

| SECTION | ACTIVITIES (C/M) | |
|---|---|---|
| | STEAMED | INTACT |
| Leaf | 76,300,000 | 59,600,000 |
| Growing point | 1,360,000 | 723,000 |
| Petiole | 737,000 | 973,000 |
| Stem | 1,800,000 | 3,110,000 |
| Primary leaves | 510 | 846 |
| Cotyledons | 23 | 15 |

*One plant stem was steamed just below the cotyledon

plant was considerably less than that in the control even though its leaf received the greater share of $C^{14}O_2$. On the assumption that the radioactivity in the leaf is a measure of the radioactive photosynthate available for transport, the relative amounts found in the stems were 2.4 and 5.2 % for the treated and control, respectively; into the growing points, 1.8 and 1.2 %. Although there was a small increase in activity after section 7 of the treated plant, there was no major accumulation at the steamed section (compared to the gross accumulation at the girdle of a tree). It is clear that killing a lower portion of the stem affects movement from the source into sections above it.

*Experiment* 3. *Petiole feeding of fructose-U-$C^{14}$.* Sisler et al (21) found that sucrose was readily translocated in tomatoes when supplied, in solution, to cut petioles and it seemed pertinent to compare the distributions resulting from this manner of application with those of $C^{14}O_2$-fed plants. Fructose-U-$C^{14}$, specific activity 2.3 $\times$ $10^6$ c/m/mg, was fed as a 0.5 % solution to an excised petiole, for 15 minutes. The resulting distribution in the stem is shown in figure 2, where the similarity to the sucrose curve resulting for $C^{14}O_2$ feeding (see fig 5) is apparent. (The causes of the humps in the curves are not known; more than one explanation can be offered at present.)

*Experiment* 4. *Effect of excision below the cotyledon.* Four double-plant experiments were performed in which leaves and petioles were administered tracer in standard amounts for 30 minutes. One plant of each pair was severed just below the cotyledonary node; the upper portion was retained with the cut stem in tap water. Stems were cut at least one hour before the experiment; the treated plants ap-

FIG. 1. (*upper left*). Radioactive profiles along the stem following 20 min photosynthesis of $C^{14}O_2$. One plant stem was steamed just below the cotyledon.

FIG. 2 (*lower left*). Radioactive profile along the stem following 15 min petiole feeding of fructose-U-$C^{14}$, 9.3 $\times$ $10^6$ c/m/ml.

FIG. 3 (*upper right*). (–) Radioactive profile along the stem following 25 min foliar uptake of $HCl^{36}$ in the light; (- - -), radioactive profile along the stem following 15 min foliar uptake of $HCl^{36}$ in the dark.

FIG. 4. (*lower right*). Radioactive profiles along the stem following 15 min, simultaneous, petiole administration of fructose-U-$C^{14}$ and $HCl^{36}$.

peared turgid and normal. The tracers and corresponding methods of administration were: a) $C^{14}O_2$, leaf; b) $HCl^{36}$ vapor, leaf; c) fructose-U-$C^{14}$, petiole; d) $HCl^{36}$ petiole.

The results were specific. While the control plants translocated tracer in expected amounts, the cut plants were inert in each case. Small amounts of activity (from 1 to 10 % of that in controls) were recovered in petioles and first adjacent stem sections. A minute amount was observed in the growing point of the plant which was fed $HCl^{36}$ vapor, but the other three growing points showed no significant activity above background.

The distribution, amount, and rate of movement appears to be qualitatively similar whether photosynthate is supplied by the leaf or through cut petioles. Nelson and Gorham (18) have since found what appears to be different modes of transport for sucrose and fructose when they are fed through primary leaf petioles of soybeans, the implication being that fructose moves outside the phloem. There may be some question, therefore, as to whether or not the fructose measurements are comparable to those of translocate derived directly from photosynthesis. Later experiments, however, support the contention that similar mechanisms were at work and a quantitative comparison is made between the two feeding techniques. (see General Discussion).

The maxima and minima displayed in figures 1 and 2 occur commonly in experiments of this type. They are believed to be real even though the experimental errors are unavoidably large with the techniques used. The measurement error was estimated to be about ± 8 % from consideration of uncertainties in length of section, extraction, and counting, and are indicated by the vertical arrows.

The initial cutting experiments suggest that meristematic tissue in roots and growing points has no consistent influence on photosynthate translocation during these short periods. From the steaming, stem-excision and petiole-feeding experiments, it now seems certain that there is no osmotic pushing force from the leaf. The experiments, as a whole, suggest that some locus below the cotyledon has a directing influence on downward (and perhaps upward) translocation. The retardation observed by Nelson et al (17), after chilling the roots, may also be interpreted in this way.

II. Movement of $Cl^{36}$. Because of the correlation between the movements of sucrose and growth substances, the question arose as to whether or not there was a common vehicle (e.g., water or a metabolic carrier) for the movement of photosynthate and other materials. Consequently, a search was made for a radioactive non-metabolite which could enter the leaves as a gas with $C^{14}O_2$. Chlorine, in the form of $HCl^{36}$, was chosen because it is known that chloride ion has, at best, a role which is submicro, quantitatively, and dubious qualitatively, in plant metabolism. This particular isotope is easy to measure and is readily distinguished from $C^{14}$. It was felt that the acidity introduced, using only tracer quantities, would be controlled by the buffering capacity of the sap.

*Experimental.* 1. *$Cl^{36}$ Administration.* The feeding apparatus was identical to that used for $C^{14}O_2$. Thirty to sixty $\mu$l (1–2 $\mu$c) of the 1.64 $N$ acid was added to the reaction vessel; the $HCl^{36}$ was released by adding anhydrous sulfuric acid. In the dark-feeding experiment, the plant and feeding apparatus were contained within a light-tight fume hood. Plants were kept in the darkened hood for at least one hour prior to the experiment. Petioles were fed $HCl^{36}$ solutions (1–2 $\mu$c/ml, as about 0.5 $N$ acid).

2. *$C^{14}$ and $Cl^{36}$ double assay.* The highly energetic beta rays of $Cl^{36}$ permitted a rough measure of the time-course of translocation using an ordinary end-window Geiger tube. Where both isotopes were present, it was possible, because of the difference in beta energies (0.66 max. Mev for $Cl^{36}$ and 0.15 for $C^{14}$) to make differential counts of the plant extracts using a 0.07 mm aluminum filter. Placed 3 mm above the sample, this thickness of Al was found to transmit 73 % of the $Cl^{36}$ beta's and 0.5 % of the $C^{14}$ beta's. When the more sensitive windowless flow counter was used for low activity studies, the filter was held 10 mm above the sample. With this geometry, 0.04 % of the $C^{14}$ and 47 % of the $Cl^{36}$ beta's were transmitted. These relationships were very nearly linear over the range of activities employed.

3. *Chromatographic identification of chloride ion.* It was of interest to ascertain whether the $Cl^{36}$ was travelling as the free ion, or as an addition or substitution derivative of an organic compound. For this purpose the 80 % aqueous ethanol plant extract was partitioned with petroleum ether to remove plastid pigments. The ether fraction was found to have negligible activity and was discarded. The aqueous alcohol phase was concentrated and paper chromatographed in Yamaguchi's solvents (27): ethyl acetate, ethanol, 1 $N$ ammonium hydroxide (1 : 1 : 1) in one direction and acetone, water (3 : 1) in the other direction. The radioactive chloride could be detected in the usual manner; non-radioactive chloride was made visual by spraying lightly with dilute silver nitrate. The chloride spots darkened quickly when exposed to ultraviolet radiation.

All the $Cl^{36}$ activity in the stem was coincident with the chloride ion spot. Consequently, it is believed that the $Cl^{36}$ travelled primarily, if not entirely, as the free ion.

*Experiment* 5. *$HCl^{36}$, leaf, light, and dark.* It was found that soybean leaves could tolerate about 1 % by volume of the acid vapor without visible signs of injury, in the light. Movement of $Cl^{36}$ from illuminated leaves would not take place, however, unless plants were kept previously in dim light for 12 to 24 hours. On the other hand, movement from unstarved leaves occurred readily in the dark and their tolerance for HCl vapor was about three times greater.

An anomalous, ultra-rapid movement of $Cl^{36}$ was observed with the monitor. Significant, but minor, activity would appear in primary leaves within 1

TABLE II

$Cl^{36}$ DISTRIBUTION FOLLOWING FOLIAR UPTAKE OF $HCl^{36}$*

| SECTION | ACTIVITIES (C/M) | |
|---|---|---|
| | LIGHT | DARK |
| Leaf | 67,800 | 178,000 |
| Growing point | 697 | 2,780 |
| Primary leaves | 736 | 1,740 |
| Stem | 2,950 | 18,300 |

* One plant was fed 1 μc in the light (25 min); the other, 2 μc in the dark (15 min)

minute after releasing the tracer to the leaf. It is believed that this was not the result of leaks in the system because it was still observed when the system was maintained at less than atmospheric pressure and the fume hood air flow was directed upward over the plant. The results of typical light and dark feeding experiments are given in table II and figure 3.

*Experiment* 6. $HCl^{36}$ + $C^{14}O_2$, *leaf*. For simultaneous feeding, the $C^{14}O_2$ and $HCl^{36}$ were generated independently and mixed prior to administration. In three experiments, plants which had been kept in subdued light for 48 hours were given the following amounts of the two tracers for 15 minutes: a) 2 μc $HCl^{36}$, 2 μc $C^{14}O_2$; b) 2 μc $HCl^{36}$, 125 μc $C^{14}O_2$; c) 1 μc $HCl^{36}$, 150 μc $C^{14}O_2$. In each case, although expected amounts of $Cl^{36}$ were found in the various stem sections, the quantities of $C^{14}$ were too small to warrant detailed counting, as is shown in table III.

*Experiment* 7. $HCl^{36}$ + *fructose*-U-$C^{14}$, *petiole*. Preliminary experiments with petiole feeding of $HCl^{36}$ showed that 15 minutes was adequate time for tracer to reach the hypocotyl. In this experiment, a cut petiole was fed a solution of $HCl^{36}$, and fructose-U-$C^{14}$. The data are given in table IV and figure 4. A 3.2 cm length of petiole adjacent to the stem was also analysed. In figure 4, its activity has been normalized to make it comparable to the 2 cm stem sections.

## DISCUSSION

It is clear that chloride ion moved readily from the leaf but independent of photosynthate, under conditions of low light intensity or in the dark. This is in marked contrast to the known requirement of sucrose,

TABLE III

$C^{14}$ AND $Cl^{36}$ DISTRIBUTIONS FOLLOWING 15 MINUTE, SIMULTANEOUS, FOLIAR APPLICATION OF $C^{14}O_2$ (125 μc) AND $HCl^{36}$ (2 μc)

| SECTION | ACTIVITIES (C/M) | |
|---|---|---|
| | $C^{14}$ | $Cl^{36}$ |
| Leaf | 328,000 | 239,000 |
| Growing point | 174 | 1,100 |
| Primary leaves | 0 | 1,916 |
| Stem | | |
| Section 1 | 20 | 1,870 |
| Section 2 | 0 | 2,200 |

either added or photosynthetic, for auxin movement. Chloride movement was also distinguished from that of photosynthate by finding relatively greater activity in the primary leaves. This agrees qualitatively with the observations of Swanson and Whitney (23) who noted different rates of movement into kidney bean leaves (P > Cs > K).

The rapid appearance of the *minor* amount initially moved into primary leaves indicated a velocity of at least 7 m/hour. This same order of magnitude was found by Nelson et al (19) for a small component of $C^{14}$ activity following $C^{14}O_2$, leaf administration. It seems highly probable that there may be a small, rapid component of translocation which is overridden by the main phase.

Unfortunately, the inhibition of photosynthesis by HCl vitiates much of its value in this type of experiment. The inhibition is evident in the data of table III. The amount of $C^{14}$ incorporated in the leaf, relative to the amount applied, was about a thousand times less than that for the singly-applied $C^{14}O_2$ in experiment 2. The increase in HCl tolerance in the dark suggests that the inhibition may lie within the

TABLE IV

$C^{14}$ AND $Cl^{36}$ DISTRIBUTIONS FOLLOWING 15 MINUTE, SIMULTANEOUS, PETIOLAR ADMINISTRATION OF FRUCTOSE-U-$C^{14}$ ($5.75 \times 10^6$ C/M/ML) AND $HCl^{36}$ ($2.28 \times 10^6$ C/M/ML)

| SECTION | ACTIVITIES (C/M) | |
|---|---|---|
| | $C^{14}$ | $Cl^{36}$ |
| Growing point | 4,520 | 1,040 |
| Petiole | 9,850 | 3,700 |
| Stem | 33,540 | 10,710 |

photochemical phase. This, of itself, does not explain the lack of chloride movement from the unstarved leaves, at normal light intensities unless, under these conditions, the ion is incorporated into some system in an irreversible manner.

The movements of chloride and fructose were, however, unrestricted in experiment 7, the relative amounts transported being virtually identical with those in other petiole experiments (see table IX under Discussion). It is felt, therefore, that there was ample justification for comparing the distributions of the two tracers in this experiment. The noncoincidence of the maxima in figure 4 suggests that different transport mechanisms were at work, although it may correspond merely to the differences corresponding to counter-current distribution of diverse compounds. On the other hand, the similarity in the semi-log slopes has intriguing theoretical inference.

Horwitz's recent elegant analysis (8), for the comparable situation, has shown that the slope of such a plot is determined by the three parameters: k, the diffusion constant or first order rate constant for irreversible removal of activity from the flowing

stream: $A_p$, the cross section of flow and v, the linear velocity of fluid flow. Consequently, similarity in semi-log slopes cannot be construed to mean similar modes of transport without fixing at least two of these parameters.

One might conclude, from the analysis in table IX, that the volume rate of flow, in experiment 7, was the same for chloride as for fructose. To assume similar v's, however, one must say that $A_p$ was identical for each and of this there is, of course, no knowledge at present. Even with the a priori assumption that the v's and $A_p$'s were common, one must account for similar k's. Horwitz (8) used the published k value for sucrose (2 $mm^2/hr$) for his model and obtained curves that agreed with the empirical data of Biddulph and Cory (2) for $P^{32}$, the inference being that removing $P^{32}$ from the translocation stream was conditioned by the same rate constant as sucrose. It seems possible, then, that there is a common vehicle (e.g., an ionic protein carrier) for transporting materials across the pipe membrane; but such an hypothesis (however attractive) constitutes an additional assumption in the present argument. Consequently, it would be unsafe to advocate the same transport mechanism for chloride and fructose, solely on the basis of the similar semi-log slopes found in experiment 7.

III. Movement of THO. When this study was begun there were no reported measurements of simultaneous movement of water and photosynthate using isotopic water. This is, of course, a crucial aspect of the mass flow theory. The use of THO in photosynthetic experiments will result in tritium-labelled photosynthate and one can, in this manner, measure the movement of water and photosynthate simultaneously, without employing isotopic carbon.

*Experimental.* 1. *THO administration.* Soybean plants used were similar in age and development to those used in the other experiments, and were watered 10 to 15 minutes prior to feeding in an attempt to eliminate internal water tensions. Plants were either petiole-fed, as previously, or leaf-fed by THO vapor. For the latter, the special bubbling apparatus, shown in figure 5, replaced the reaction flask of earlier sections. The entire assembly was enclosed in a completely darkened fume hood and the THO vapor-saturated air was cycled over the leaf for 1 hour in the dark. Then a small amount of $CO_2$, sufficient to give about 0.1 % by volume, was introduced into the system with a hypodermic syringe via the interconnecting tubing. After a few minutes the leaf was given about 1,000 ft-c of illumination. Zero time was taken as the time of illumination.

After treatment, the plants were sectioned quickly and frozen in liquid nitrogen. Water was removed from each section as follows: a) the frozen section was transferred quickly to one arm of the distilling apparatus, shown in figure 5e, which was immersed in liquid nitrogen; b) the system was then evacuated and the Dewar transferred to the other arm; c) the arm containing the tissue was then placed in a steam

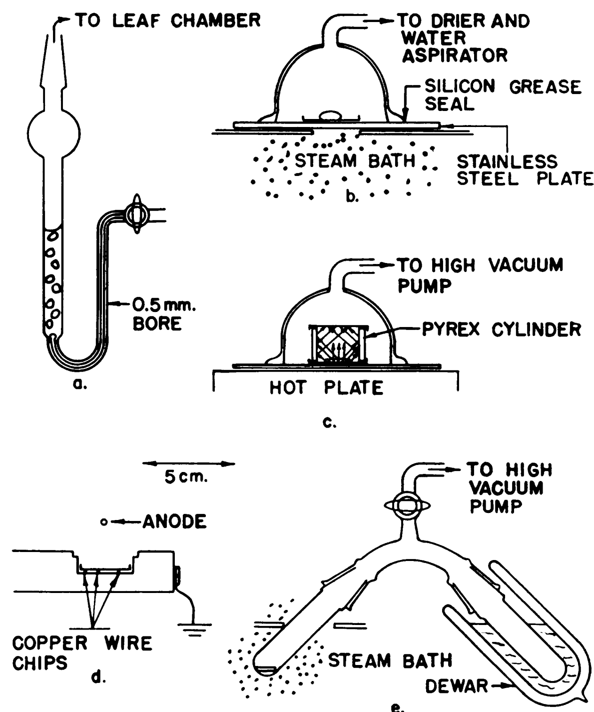



Fig. 5. Apparatus for administration and analysis of THO. a. THO vapor generator. b, c. $NH_3TCl$ sample planchet preparation. d. grounding of planchet to avoid counting hysteresis. e. distillation of THO from plant sections.

bath and the distillation allowed to proceed for 1 hour; d) the arm containing the distillate was capped quickly; the sample was allowed to attain room temperature and was then assayed for THO activity.

2. *THO assay.* THO activity was measured by a modification of the $NH_4Cl$ exchange method described by Jenkins (9) in the following sequence: a) $NH_4Cl$ (200 mg/ml) was dissolved in the THO sample and allowed to equilibrate for 10 minutes; b) 0.1 ml of this solution was placed on an aluminum planchet in a small bell jar (fig 5b) and evaporated to dryness at steam temperature; c) a Pyrex cylinder with a clean upper planchet was placed over the sample (fig 5c) and the system was evacuated to about $10^{-3}$ mm Hg for 10 minutes; d) while the vacuum was maintained, a hot plate, previously heated to about 200° C, was moved under the steel plate. The $NH_3TCl$ then sublimed slowly, condensing first on the cool cylinder and finally, as the cylinder heated from the bottom, on the upper planchet. This procedure produced uniform, infinitely thick samples that could be counted in a windowless flow counter.

During counting, these samples showed the hysteresis effect observed by Jenkins. The indicated disintegration rate would decrease progressively to about 70 % of the initial rate after 10 minutes. If the plates were allowed to stand in the air or in the flow counter, with the anode potential reduced to

zero, the initial rate was again obtained. It seemed reasonable that this was a space-charge effect, the ionized $_2He^3$ disintegration product of tritium having insufficient recoil energy to escape the electrostatic field of the anode. The ions would then accumulate in a layer just above the sample and some of the beta's would be captured before they could produce the necessary geiger avalanche. It was finally found that the difficulty could be overcome entirely by ensuring good contact between the bottom of the metal planchets and the grounded plate-holder, as in figure 5d.

The assay procedure was standardized so that successive sample planchets, made from the same THO sample, gave counts that varied less than ± 3 % from the mean. The counting rates obtained by this procedure were proportional to the molar concentrations of the THO samples. Points had been obtained from a dilution series starting with THO of a specific activity of 10 μc/ml.

3. *T-photosynthate assay.* Photosynthetically-incorporated tritium was measured in each section following the THO analyses. The dried sections were extracted with 80 % ethanol and the extracts were assayed in a manner similar to the previous $C^{14}$ extracts. The dried sample planchets ranged in cross section from 0.1 to 0.4 mg/cm² and were therefore corrected for self-absorption. A self-absorption curve, constructed from energy relationships suggested by Libby (12), proved to be reasonably valid over the range measured.

*Experiment* 8. *THO, petiole administration.* Assuming the same recovery ratio for water as for other translocates, it was estimated that detectable amounts of THO should be found in plant sections. For example, in experiment 3, the fructose-$C^{14}$ molar ratio between the upper stem sections and fed solution was about 0.6 %. On this basis, a feeding solution containing 50 μc/ml of THO (about 12,000 c/m) should yield about 70 c/m in the upper stem. Two 15 minute experiments were performed using 50 μc/ml THO. In both cases, no detectable THO could be found in any of the plant sections.

*Experiment* 9. *THO + fructose*-U-$C^{14}$, *petiole administration.* Two trials were carried out. In the first, a mixed solution containing fructose-U-$C^{14}$ (same as expt. 3) and THO (50 μc/ml) was petiole-fed in the usual manner. The results of the THO and $C^{14}$ assays are presented in table V. The $C^{14}$ stem distribution is shown in figure 6.

TABLE V

$C^{14}$ AND THO DISTRIBUTIONS FOLLOWING 15 MINUTE, PETIOLAR ADMINISTRATION OF FRUCTOSE-U-$C^{14}$ ($1.4 \times 10^7$ C/M/ML) DISSOLVED IN THO ($1.2 \times 10^4$ C/M/ML)

| SECTION | ACTIVITIES (C/M) | | RECOVERY RATIOS* (%) | |
|---|---|---|---|---|
| | $C^{14}$ | THO | $C^{14}$ | THO |
| Growing point | 5,080 | 21 | 0.2 | 0.2 |
| Stem | 51,700 | | | |
| 1 | 7,220 | 12 | 0.5 | 0.1 |
| 2** | 9,950 | 0 | | |

* (c/m/gm of section)/(c/m/ml of fed solution).
** No THO activity found below this section.

As a check on the analytical procedure, the following was performed: a) 30 μl of THO solution (10 μc/ml) were added to the distillate from an entire stem section (about 1 ml). This gave the expected THO assay; b) 30 μl each of two THO solutions (1 and 10 μc/ml, resp.) were added to two fresh, 2 cm stem sections (each contained about 0.1 ml of $H_2O$). The sections were sealed in small vials and, after 2 hours, frozen and analyzed for THO. The distillates gave counts of 42 and 394 c/m, respectively.

As a check on the possibility of dilution of any transported THO by transpiration, the second trial was performed in *still* air, with a slightly older (5-week) plant having two fully-expanded trifoliates spaced about 5 cm apart. The younger trifoliate was left intact and the oldest petiole was fed the fructose-$C^{14}$, THO mixture, as previously, except that the THO activity was increased to 5 mc/ml and the feeding time was reduced to 6 minutes. The fed petiole was included in the subsequent assay as two, 6 cm lengths designated $P_1$ and $P_2$. $P_1$ dipped into the fed solution and was rinsed quickly with water prior to freezing. The results are given in table VI and figure 7. In the figure, $P_1$ and $P_2$ have been normalized to 2 cm lengths by dividing the activity found in each by three.

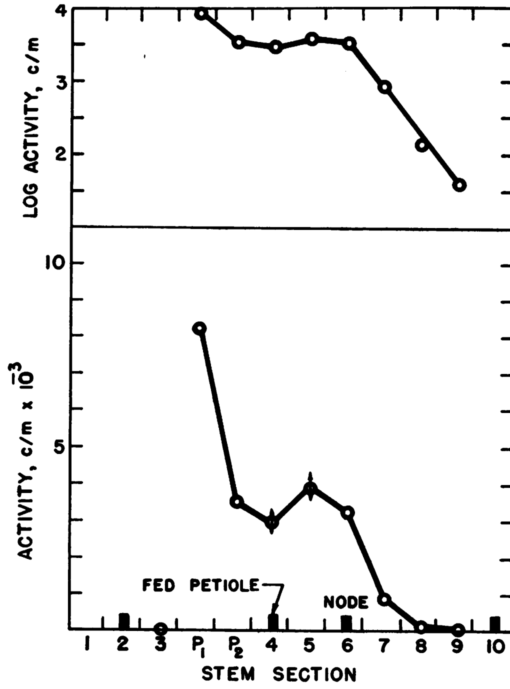
TABLE VI

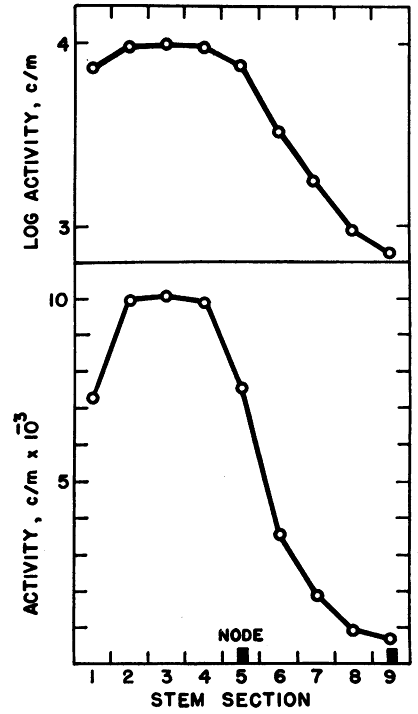
$C^{14}$ AND THO DISTRIBUTIONS FOLLOWING 6 MINUTE, PETIOLE ADMINISTRATION OF FRUCTOSE-U-$C^{14}$ ($1.4 \times 10^7$ C/M/ML) DISSOLVED IN THO ($1.2 \times 10^6$ C/M/ML)

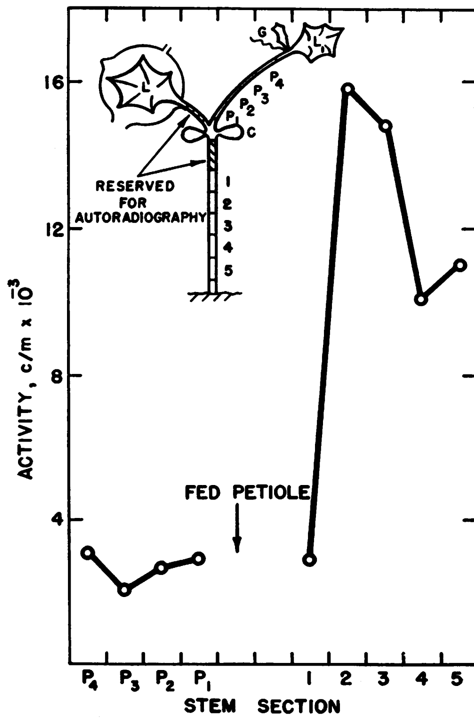
| SECTION | ACTIVITIES (C/M) | | RECOVERY RATIOS (%) | |
|---|---|---|---|---|
| | $C^{14}$ | THO | $C^{14}$ | THO |
| Petiole 1 | 24,600 | 8,240 | 2.5 | 0.7 |
| 2 | 10,600 | 79 | 1 | 0.007 |
| Stem 1 | 0 | 0 | | |
| 2 | 0 | 0 | | |
| 3 | 120 | 0 | | |
| 4* | 2,960 | 3 | 0.2 | 0.0003 |

* Petiole entrance. (No THO activity found above or below this section.)

*Experiment* 10. *THO vapor, leaf administration.* In these experiments, leaves were illuminated following equilibration with THO vapor for 1 hour in the dark. During the illumination period, part of the tritium of the water was incorporated into sucrose. The sucrose formed during this time presumably had a specific activity identical with the water from which it was formed (neglecting possible isotope effects). However, part of these tritiums–the sucrose hydroxyls–are exchangeable. The activity found in the


LOG ACTIVITY, c/m
ACTIVITY, c/m x $10^{-3}$
FED PETIOLE
NODE
STEM SECTION
1 2 3 $P_1$ $P_2$ 4 5 6 7 8 9 10


LOG ACTIVITY, c/m
ACTIVITY, c/m x $10^{-3}$
NODE
STEM SECTION


ACTIVITY, c/m x $10^{-3}$
RESERVED FOR AUTORADIOGRAPHY
FED PETIOLE
STEM SECTION
$P_4$ $P_3$ $P_2$ $P_1$ 1 2 3 4 5

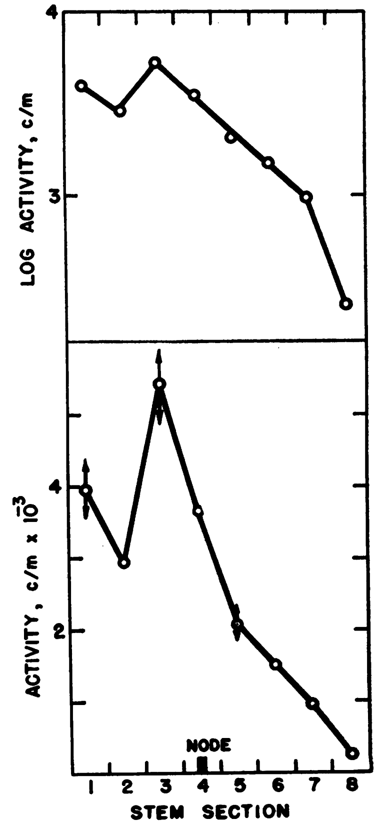

LOG ACTIVITY, c/m
ACTIVITY, c/m x $10^{-3}$
NODE
STEM SECTION

TABLE VII

DISTRIBUTIONS OF T-PHOTOSYNTHATE (T-PH) AND THO FOLLOWING 15 MINUTE PHOTOSYNTHESIS BY SOYBEAN TRIFOLIATE, AND PRECEDED BY ONE HOUR'S EQUILIBRATION WITH VAPOR FROM THO (10 MC/ML) IN THE DARK

| SECTION | ACTIVITIES (C/M) | | RECOVERY RATIOS* (%) | |
|---|---|---|---|---|
| | T-PH | THO | T-PH | THO |
| Fed leaf | 689,000 | 398,000 | | |
| Growing point | 3,500 | 175 | 2.7 | 0.04 |
| Stem | 20,800 | | | |
| 1 | 3,950 | 19 | 7.6 | 0.005 |
| 2 | 2,950 | 7 | 5.7 | 0.002 |
| 3** | 5,420 | 0 | | |

* c/m/gm (section/fed leaf). The fed-leaf activity was reduced to 62 % for sucrose (28).
** No THO activity found below this section.

sucrose would then vary according to the degree of exchange of these groups with non-equilibrated water (if any).

Results for the usual 3-week-old soybean are presented in table VII and figure 8. An 18-day-old cucumber, *Cucurbita sativus* L., was used for radioautography. Its analysis is also included in table VIII and figure 9.

## DISCUSSION

If solvent water (THO) and solute (i.e., photosynthate or petiole-fed fructose-$C^{14}$) move en masse, then the solute can move no faster than the solvent. Indeed, since the solute will be subject to adsorptive effects en route (probably specifically for each solute), as if it were moving down a column during adsorption chromatography, one might expect accumulative retardation of the solute. Consequently, if the radioactive solvent and solute are confined to phloem tubes, the recovery ratio (activity per unit volume, source/stem-section) for solvent should remain the same, or should decrease less rapidly, than the solute recovery ratio. The data of these experiments show that, except for a few sections immediately adjoining entrance petioles, the THO recovery ratios were always two or three *orders of magnitude less* than those of the solute. With this reasoning alone, one is led to the conclusion that there appears to be insufficient movement of water to meet the requirements of the mass flow theory.

Casual contemplation of the Horwitz model (8) could suggest that the disparity between sugar and THO movement results from more rapid removal of THO from the phloem system and its subsequent dilution by transpiration water. Actually, as mentioned previously, the diffusion constant employed in this model is that of free diffusion (aqueous solution into water). The diffusion constant for sucrose into water is approximately the same as the self-diffusion constants of the various H- and O-isotopes of water. The latter differ from each other, at most, by 15 %.

It does not seem possible that there was appreciable irrigation of the downward translocation stream by the upward stream of transpiration or guttation, on the basis of the following analysis. A conservative estimate of the functional xylem cross section[5] for 5-week-old soybeans, similar to that used in experiment 9 (6 min trial), was found to be $4 \times 10^{-8}$ cm$^2$. Under the most favorable conditions, the maximum expected transpiration rate would be about 0.02

[5] Appreciation is expressed to Mr. J. P. Miksche, Department of Botany, Iowa State University, for allowing examination of unpublished, soybean material.

TABLE VIII

DISTRIBUTION OF T-PHOTOSYNTHATE (T-PH) AND THO FOLLOWING 30 MINUTE PHOTOSYNTHESIS BY CUCUMBER LEAF, AND PRECEDED BY ONE HOUR'S EQUILIBRATION WITH VAPOR FROM THO (10 MC/ML) IN THE DARK

| SECTION | ACTIVITIES (C/M) | | RECOVERY RATIOS (%) | |
|---|---|---|---|---|
| | T-PH | THO | T-PH | THO |
| Leaf fed (L) | 875,000 | 702,000 | | |
| Leaf (upper) ($L_1$) | 19,200 | 22 | 4 | 0.003 |
| Growing point (G) | 7,700 | 27 | 10 | 0.004 |
| Cotyledon (C) | 198 | 163 | 0.09 | 0.02 |
| Petiole 1 | 2,940 | trace* | | |
| 2 | 2,680 | trace | | |
| 3 | 2,030 | 0 | | |
| 4 | 3,110 | 0 | | |
| Stem 1 | 2,920 | 8 | 2 | 0.001 |
| 2 | 15,800 | trace | | |
| 3 | 14,800 | 10 | 10 | 0.001 |
| 4 | 10,500 | trace | | |
| 5 | 11,000 | trace | | |

* difference from background (0) depends upon level of significance used in counting.

FIG. 6 (*upper right*). Stem $C^{14}$-profile following 15 min, simultaneous, petiole administration of fructose-U-$C^{14}$ and THO.

FIG. 7 (*upper left*). Stem $C^{14}$-profile following 6 min, simultaneous, petiole administration of fructose-U-$C^{14}$ and THO.

FIG. 8 (*lower right*). Distribution of T-photosynthate along the stem following 15 min photosynthesis by soybean trifoliate, and preceded by 1 hour's equilibration with THO vapor in the dark.

FIG. 9 (*lower left*). Distribution of T-photosynthate following 30 min photosynthesis by cucumber leaf, and preceded by 1 hour's equilibration with THO vapor in the dark.

ml/hr/cm$^2$ of leaf area (measured on one surface). The total remaining leaf area of the plant used in this experiment did not exceed 50 cm$^2$. Consequently, the maximum transpiration rate expected would be 1 ml/hour. Using the above estimate for the xylem cross section, one obtains a maximum linear velocity of 250 cm/hour. On the other hand, the solute was moving downward with a velocity of at least 120 cm/hour (obtained by extrapolating the linear portion of the semi-log plot of figure 7 to the abscissa). Assuming then, that THO entered the stem in amounts comparable with the solute, and that it was removed by the upward-moving transpiration stream at the petiole exit, one would expect a maximum dilution factor of two in the upper stem. As may be seen from table VI, no THO activity was found *above* the entrance petiole which was at section 4. Similarly, in the soybean trial of experiment 10, the THO recovery ratio in the growing point was about 1/100 of the same ratio for T-photosynthate in the adjoining stem sections 1 and 2 which were immediately beneath the entrance petiole. Since the only trifoliate was maintained in a water-saturated atmosphere, the transpiration rate would have been greatly reduced (at least 10-fold) and there should have been considerable accumulation of THO in the growing point. Finally it should be noted that, in the 6-minute trial of experiment 9, the THO activity in $P_2$ was less than 1/100 that of $P_1$ whose end dipped directly into the radioactive solution.

Despite these arguments, it is difficult to understand why so little THO was recovered, since a concentrated sucrose solution might be expected to actually drag solvent with it (20).

Biddulph and Cory (2) have reported data on translocation of THO from the leaves of kidney beans that would appear to be at variance with the present observations, although their method of application was somewhat different. THO, in a concentration of $1.39 \times 10^{-3}$ M (about 40 mc/ml), was applied as a liquid spray in solution with $NaH_2P^{32}O_4$ to a 1 inch diameter circle on the under side of a trifoliate leaflet. $C^{14}O_2$ was released, simultaneously, to the upper surface of the sprayed area. THO entered the stem in detectable quantities although the amount moved per unit activity applied, during 15 minutes, was only 1/30 of the $P^{32}$ and 1/50 of the $C^{14}$. Although the amount of THO incorporated into the leaf was not specified, it seems reasonable, despite the larger concentration applied, that it was no greater than in the present experiments where leaves were equilibrated with 10 mc/ml THO. Nevertheless, they obtained THO concentrations of $5 \times 10^{-11}$ m/ml in stem sections 6 inches below the entrance node. This level of activity (about 1.4 $\mu$c/ml) is well within the range of our less-sensitive assay method and would give about 300 c/m. The discrepancy between the results of the investigations, therefore, appears to be real and must arise either from the method of tracer application or from differences in hydrostatic conditions between the two plant systems. The latter seems a possibility. In all of their experiments, Biddulph and Cory found that the THO front contained an unexpectedly large amount of tracer. This, they suggested, undoubtedly indicated that the application of tracer water to the leaf epidermis affected a local reduction of tension at that spot which facilitated the movement. In the present experiments, any such tensions would have been relieved by the initial equilibration with pure water in the petiole experiments and by uniform addition of water in the leaf-vapor experiments.

## Discussion

Petiole-Versus Leaf-Feeding. Table IX summarizes all relevant data regarding the two feeding methods. Note that the leaf and stem activities are expressed on a gm$^{-1}$ basis. Leaf photosynthate is expressed as sucrose activity assuming the 62 % incorporation found by Vernon and Aronoff (26) for their 20 minute feedings. It was assumed that the source concentration, in all cases, remained invariant and that the amounts transported were directly time-dependent. Accordingly, the stem activities were normalized to a 15 minute translocated period.

Table IX

Comparison of Leaf-versus Petiole-Feeding Methods

| Experiment no. | Recovery ratios* (%) |
|---|---|
| *Leaf* | |
| 2. (intact). $CO_2$, 20 min | 3.1 |
| 5. (light, intact). $HCl^{36}$, 25 min | 1.3 |
| 5. (dark, intact). $HCl^{36}$, 15 min | 5.2 |
| 10. (intact). THO, 15 min | 4.0 |
| *Petiole* | |
| 3. Fructose-U-$C^{14}$, 15 min | 0.47 |
| 7. Fructose-U-$C^{14}$, 15 min | 0.58 |
| 7. $HCl^{36}$, 15 min | 0.47 |
| 9. Fructose-U-$C^{14}$, 15 min | 0.37 |

* Leaf: c/m/gm fresh (stem/fed leaf); petiole: (c/m/gm fresh stem)/(c/m/ml of fed solution). The fed-leaf activities, in expts. 2 and 10, were reduced 62 % for sucrose (28) (see text for explanation).

The molar ratios, within each feeding method, were surprisingly similar. On this basis alone, it would seem that the same mechanism was at work for transporting photosynthate and chloride because about the same relative amounts were transported into the stem per unit time. The lower value for experiment 5 (light) is undoubtedly due to the inhibition of chloride movement by light as noted earlier.

The distinct reduction in these ratios for the petiole experiments is not surprising. The severed petiole undoubtedly presents a much smaller entrance cross section for tracer than the leaf. Stem activity profiles are qualitatively similar for the two feeding methods showing (except for the discontinuities) a general, downward decay. A priori, these comparisons might seem invalid because, in the leaf-feeding

method, the source activity does not acquire the instantaneous steady state that is characteristic of the petiole method. However, the theoretical semi-log curves obtained by Horwitz (8), for a time-variable source of activity, show a departure from linearity, the slope decreasing rapidly in the lower stem sections. This situation is depicted almost ideally in the semi-log curve for figure 1 (intact). Indeed, it seems significant that this departure from linearity in the lower stem is about the only notable difference between the stem profiles of the two feeding methods (compare, e.g., figs 1, 3, and 8 with figs 2, 4, 6, and 7).

From these experiments, therefore, there would seem to be justification for ascribing similar mechanisms in the transport of tracers either from solution through cut petioles or from leaves after gaseous incorporation. As noted earlier, this conclusion is not compatible with that of the Canadian group (16) which found evidence for different stem pathways of hexose and sucrose when fed through primary-leaf petioles. Recently (18), they made radioautographic comparisons of stems following similar petiole administrations of fructose and sucrose, and after photosynthetic leaf-incorporation. While there was precise phloem localization in the latter case, the petiole-fed activity was more widely distributed in phloem and xylem and the Canadian group was convinced that its petiole- and leaf-fed soybeans translocated materials in different fashions. While one cannot refute this conclusion, it seems highly probable that our respective plant systems were not physiologically comparable. They observed a continuous uptake of solution by the primary petioles (1.0–1.5 µl/min) whereas, in the present experiments, only negligible amounts of THO were taken up by the trifoliate petioles. It is possible, therefore, that the former solution-cultured plants were in a water-deficient state and the latter (soil-grown, at field capacity) were in a water-saturated condition[6]. Alternatively, it is conceivable that physiological uptake by primary and trifoliate petioles may not be the same. In any event, the ultimate decision for or against similar transport modes with the two feeding techniques would be greatly enhanced by further radioautographic studies using soil-grown plants[7].

*Stem profile discontinuities.* In every stem analysis, the orderly distributions were disrupted by activity peaks part way down the stem. Similar variations are evident from the data of others (2, 16, 26). Some have been attributed to accumulations at the stem nodes. In the present experiments, a peak coincided with a node in one experiment only (fig 2) and it is felt that these peaks must have some other meaning in experiments of this type.

## Summary

A study has been made of short-period translocation in soybeans, *Glycine max.*, comparing the rates of movement of labelled photosynthate (from leaves) or fructose-U-$C^{14}$ (from petioles) with $HCl^{36}$ and THO.

1. Efforts to determine any directing influence from plant parts were unsuccessful. Removal of root tips and growing points gave variable results; steaming the lower stem reduced the rate of photosynthate into stems; excision below the cotyledon completely stopped the downward movement of all leaf- and petiole-applied tracers.

2. When $HCl^{36}$ is applied chlorine appears to move as chloride ion. At moderate light levels, photosynthesis is inhibited in the presence of non-wilting amounts of hydrogen chloride as shown by double-labelling experiments with $C^{14}O_2$. Chloride movement is also reduced in the light compared to the dark.

3. Simultaneous petiole feeding of THO and fructose results in the expected movement of fructose but, essentially, no movement of THO. T-photosynthate, from leaf-vapor feedings, also moves at expected rates but is accompanied with essentially no THO. Similar results for leaf-vapor feeding were obtained for cucumber. The data are best explained by a process involving movement of solute, without corresponding movement of water as the solvent.

4. On the basis of quantitative comparisons, it was concluded that the tracers (except for THO) may be transported by the same type of mechanism whether fed from solution through cut petioles or through leaves after gaseous application.

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[6] It is worthy of note that the same explanation would account for the greater movement of THO observed by Biddulph and Cory (2) who also used solution-cultured plants.

[7] The authors are grateful to Doctors H. J. Perkins, C. D. Nelson, and P. R. Gorham, National Research Council, Ottawa, Canada, for a preview of their manuscript and for a valuable interchange of views.

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