Translocation of C^{14} in Sugarcane [1, 2]

Constance E. Hartt, H. P. Kortschak, Ada J. Forbes, & G. O. Burr

Physiology & Biochemistry Department, Experiment Station,
Hawaiian Sugar Planters' Association, Honolulu

Introduction

Since sugarcane plants in Hawaii translocate sucrose at the rate of over one million tons per year, translocation studies have an immense economic potential. Studies with the infrared analyzer indicate that the accumulation of sucrose in leaves during the day may inhibit photosynthesis (unpublished data), so that translocation may form a bottleneck in production. Of more importance to the general plant physiologist, sugarcane studies may contribute to the theory of translocation. In 1947 investigations on translocation of C^{14} were begun at the Experiment Station of the Hawaiian Sugar Planters' Association (*7, 12, 27*), and the results of the ensuing 15 years of research are summarized here.

The sugarcane plant possesses many advantages for studying translocation. The long symmetrical blade, with parallel venation and with no lobes or dissections, can be readily cut into uniform parts for studying velocities of movement. The large size of the plant makes it possible to take samples from uniform tissues for several types of analysis. Samples can be taken at specific positions because of the regular subdivision of the stalk into nodes and internodes. Thus, sugarcane is well constructed for determination of components moved and of rates and direction of movement. Sugarcane has a disadvantage for translocation tests because it is a monocotyledon with typical monocotyledonous structure of the stem. It is therefore difficult to separate xylem and phloem for studying tissues involved in transport; moreover, on sugarcane there is no stem-infesting aphis which could be used for sampling the phloem.

Studies of translocation of radioactive photosynthate have been summarized recently (*17, 48, 57*). Even so, Crafts (*17*) has called attention to the need for collecting more data on this important subject.

Translocation in sugarcane was studied by Hartt (*23*) who analyzed the sugars in sheaths during the day and the night and concluded that a temporary storage of polysaccharides in the sheath during the night facilitated translocation. Burr et al. (*8*) reviewed studies of translocation of C^{14} in sugarcane through October 1956, and also presented evidence of a circulatory system in the sugarcane plant (*9*). Yang (*55*) reported on studies in Taiwan on translocation of C^{14} in sugarcane. Brief presentations of some of the results reported herein are in press (*24, 27*).

This paper describes the normal course of translocation in plants grown with adequate fertilization. Effects of varying climatic conditions, deficiencies of N, P, or K, and other factors will be presented in subsequent papers.

Materials & Methods

Variety H 37-1933, generally used in this investigation, is a complex interspecific hybrid involving *Saccharum officinarum* L., *S. spontaneum* L. and *S. robustum* Brandes & Jeswiet ex Grassl. Most of the experiments were conducted with plants growing outdoors in 16-liter crocks with complete nutrient solution continuously aerated. Some field-grown plants were used.

Preparation of $C^{14}O_2$. Radioactive CO_2 was prepared from barium carbonate-C^{14}. A method was designed to prepare and store the $C^{14}O_2$, in order to obtain repeatable doses of known radioactivity. Aliquots of 2 mc, weighed with a Franz Hugershoff torsion balance, were transferred to the tube of a gas generating system. After evacuating the tube, a small amount of lactic acid, diluted approximately in half with distilled water, was admitted to the generating system a few drops at a time. The $C^{14}O_2$ so formed was transferred, by means of a mercury level, to an inverted syringe of 50-ml capacity. The $C^{14}O_2$ was washed from the generating system into the collecting syringe with air. In this way about ten ml of gas was transferred to the syringe, which was then closed with a stopcock and removed. Air was then pulled into the syringe to 50 ml. The 50 ml of gas was transferred to a storage vessel over mercury, and washed in with another 50 ml of air. The 100 ml of gas contained 2 mc of $C^{14}O_2$. In contact with only glass and mercury the gas remained unchanged for long periods. Doses of desired μc were removed by syringe immediately before starting a test. When an experiment involved more than one series, enough stored $C^{14}O_2$ was removed into a large syringe for the entire test, and aliquots for each series were transferred to a small syringe just before feeding.

---

[1] July 30, 1962.

[2] Published with the approval of the Director as Paper No. 127 of the Journal Series of the Experiment Station, Hawaiian Sugar Planters' Association.

Exposure to $C^{14}O_2$. For administering $C^{14}O_2$ to an attached leaf, in the early experiments (26) a leaf or part of a leaf was enclosed in a 1-liter graduate by means of a split rubber stopper; the needle of the syringe fitted in the stopper. In later experiments, chambers ranged in volume from 65 to 900 ml. The chamber used in most of the tests reported here had a light wood frame and light-weight plastic windows about 10 × 14 cm. The 14-cm window plus the frame resulted in an over-all length of 20 cm for the fed part. The inside depth was 6 cm and the volume was approximately 900 ml. The chamber was hinged on one side and the edges which fit on the leaf were made of rubber. The inlet was a needle held with a stopper in one edge of the chamber. The syringe containing one individual dose was fixed firmly in the inlet needle, after which the chamber was arranged over the portion of the leaf to be fed, the clasp of the chamber tightly closed, the dose of $C^{14}O_2$ injected into the chamber, the timer started, and the syringe pumped several times to mix the air in the chamber. The length of exposure was generally 5 minutes. In early tests light was described as pale, moderate, or intense; in some later tests it was measured in foot candles with a General Electric light meter. A mark placed on the leaf at the upper and lower edges of the chamber showed the fed part of the leaf. At exactly 5 minutes the chamber was opened and removed to a distance to aerate before being used for the next series.

Sampling and Preparation. Translocation time, which included feeding time, ranged from 10 minutes to 6 weeks. Translocation was terminated by cutting off the blade in very short tests, or by cutting the stalk in longer tests.

At harvest the plants were quickly separated, measured, and weighed. A large dryer with forced air current at 80 ± 1° was used for drying the parts. Blades and sheaths were dried entire. Stalk samples were either sliced with a meat cutter or ground in a Buffalo Cutter before weighed samples were taken to dry. In some tests samples were killed by boiling in 95% ethyl alcohol.

After drying, samples were reweighed and total dry weights calculated. The dried samples were milled in a Wiley mill, micro size, producing very little dust because of the fibrous nature of sugarcane. Aliquots of the Wiley mill powder were taken for counting at infinite thickness. Alcohol or other extracts were counted at infinite thinness.

Radioactive Counting Technique. Counting was done with thin-window Geiger tubes and 64 scalers from Tracerlab and Instrument Development Laboratories. Samples were counted to a total of 2000 counts, but not longer than 10 minutes, and were recounted after turning the planchet a quarter turn. Initial and final background counts were averaged and subtracted from the average gross count. Net counts under 5 per minute were calculated as zero.

Results are expressed as: RSA (Relative Specific Activity), which is the net count per minute at infinite thickness; as RTC (Relative Total Counts), which is the RSA times the total dry weight in milligrams; and as percentage of grand total counts. Grand total counts are obtained by adding the total counts in each organ of a plant. When calculated to infinite thinness, results are expressed as SA (Specific Activity = cpm/mg).

Numbering System and Definitions of Parts. The leaves and joints were numbered from the top down, counting the spindle or unrolled leaf as No. 1. The leaf, which is composed of blade and sheath, is attached at the top of the joint. The joint is composed of node and internode. Lalas are shoots which form from buds on the upper part of the stalk. Suckers are shoots which develop from buds at the base of the stalk or from the stubble. A stool of cane is an entire plant with primary, secondaries, and often other categories of stalks. The dewlap is the joint between blade and sheath. Millable cane includes all joints from No. 8 to the base of the stalk.

Sugar Analysis. Weighed aliquots of fresh sliced or ground tissues or Wiley mill powder were boiled in 95% alcohol, blended and transferred to calibrated Erlenmeyer flasks. Samples then stood at least 24 hours before being filtered.

Aliquots of alcohol extracts equal to 1 g fresh material were evaporated, transferred to water, cleared with neutral lead acetate, deleaded with potassium oxalate (dried powder), allowed to stand 2 hours, and filtered. Non-sugar reducing substances were removed by treatment with Suchar (2 mg/ml) which does not adsorb reducing sugars. The aliquot for determination of sucrose was treated with Suchar after hydrolysis with invertase to prevent loss of sucrose by adsorption on Suchar.

Reducing sugars were analyzed with alkaline ferricyanide by the method of Burr and Tanimoto (10,11). Fructose after chromatography was measured by the method of Roe (44).

Paper Chromatography. Aliquots for paper chromatography were taken without clearing with lead acetate or treatment with Suchar. The aliquot did not contain more than 2 mg of any sugar. Using Whatman No. 41 H paper, and a solvent composed of isoamyl alcohol (water saturated), isopropyl alcohol, and water (4:4:1 by volume), the samples were run, downflow, until the front was about 10 inches from the origin. At this stage, the sugars were within 2 inches of the origin. The strips were allowed to dry in air after which 1-inch sections were counted in steps of 0.5 inch. The percentage of counts in the sugar area was calculated.

The strips were then re-run in the same solvent for 24 to 36 hours, during which the solvent dripped off the ends of the strips. The approximate positions of sucrose, fructose, and glucose were determined on parallel strips of known sugars by use of a silver spray. The percentage of sugar counts in each sugar was determined by re-counting the chromatogram. From the percentage of total counts in the sugar area, and the percentage of sugar counts

in each sugar, the percentages of counts in glucose, fructose, and sucrose were calculated. Total counts in the three sugars were then calculated from total counts in the extract, and specific activity of the sugars was determined by dividing the total counts in the sugar by the milligrams of sugar determined by analysis.

Rechromatography of the sugars showed no detectable radioactive impurities and gave no further separation of the sugars.

Experimental Results & Discussion

1. Translocation in Leaf and Stalk.

A. Pathways

1. Entrance into veins. Direct counts of the living leaf made at the same position on the fed part increase after a few minutes. Because the veins project a little above the mesophyll, this increase in activity in the living fed part is thought to represent entrance into the veins (24, 27).

Belikov (3) studied the exit of assimilates from the mesophyll to the veins in soybean leaves, as affected by severing the veins, and found that each large or small vein serves a certain part of the blade collecting assimilates as a river collects water from streams. Kursanov et al. (34) demonstrated the entrance of radioactive assimilates into the leaf veins of rhubarb after only 2 minutes of photosynthesis, and stated the process could not be due to free diffusion but rather a selective process controlled by metabolism. Enriching beet leaves with ATP accelerated the movement of assimilates from the mesophyll into the conducting cells and also movement toward roots (32), which indicates that phosphorylation of sugars precedes their transfer from mesophyll to phloem, where they are rapidly resynthesized to sucrose (33).

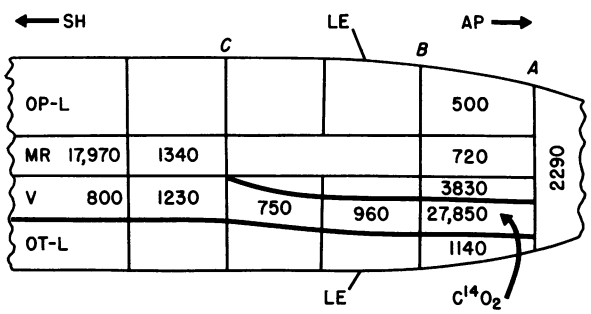

FIG. 1. Labelled photosynthate follows veins to midrib and sheath. Total counts per section, 2 hours after feeding 30 μc for 3 minutes at 9000 ft-c, using 6.5-cc chamber to small area indicated by arrow. Blanks each less than 500 TC. Strip below fed part torn down by hand, following veins. OP-L = opposite lamina; MR = midrib; V = veins below fed part; OT-L = outer lamina; LE = leaf edge; AP = apex; SH = sheath. Line A: cut across upper edge of fed part. Line B: cut across lower edge of fed part. Line C: cut across juncture between veins below fed part and midrib. The 17,970 counts in lower midrib represented 29 % of TC remaining in the blade.

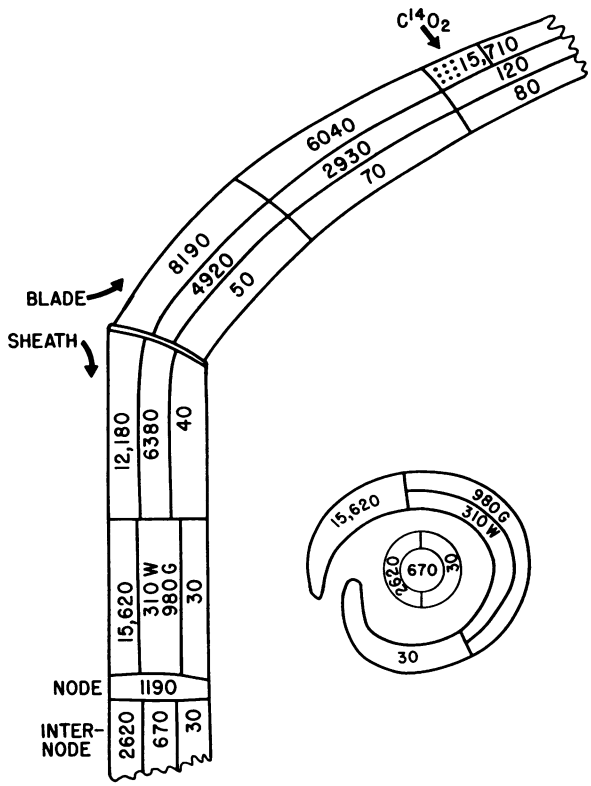

FIG. 2. Photosynthate made in one lamina moves chiefly down that side of blade and sheath to stalk. RSA 100 minutes after feeding 100 μc for 5 minutes in intense diffused light. Internal dimensions of chamber: 3.2 × 8.2 × 0.5 cm. Left: blade, sheath and joint showing fed side, center and opposite side. Central part of lower half of sheath split longitudinally into white, inner part (=W) and green, outer part (=G). Right: cross section of lower sheath and of internode showing approximate relative positions.

2. Downward

a. Down the veins to midrib and sheath. The major pathway of transport of labeled photosynthate is down the parallel veins to the midrib and sheath (fig 1). Very striking is the mass movement to the lower midrib, in which the 18,000 TC represent 29 % of the TC remaining in the blade 2 hours after feeding.

Translocation down the veins of soybean leaves was studied by Belikov (2) and demonstrated even in veins going through darkened areas of leaves.

Elegant demonstrations of detailed pathways of translocation in the phloem of bean leaves, comparing radioautographs of frozen-dried tissues with photomicrographs of fluoresced tissues, were presented by Biddulph (4).

b. Down the fed side. When $C^{14}O_2$ is fed to one lamina using a chamber reaching from margin nearly to midrib, highest RSA remains on the fed side down the sheath into the internode (fig 2). Although the lower midrib increases in activity, as in

figure 1, much of the radioactive material passes directly down the lamina and into the sheath without entering the midrib. The decrease in RSA in the center of the lower sheath may be due to increased mass in relation to bundles, and to the spreading of the bundles toward the sheath margins as reported by Artschwager (1). The outer, green part of the center of the sheath having greater radioactivity than the inner, white part is due to the fact that the bundles lie closer to the outer epidermis than to the inner epidermis.

The highest RSA remains on the fed side of the sheath and internode only when feeding is strictly unilateral, as in figure 2 where only one lamina received $C^{14}O_2$, or in the work of Yang (55) who found more activity on the fed side of the stalk than on the opposite side after feeding $C^{14}O_2$ to one lala of sugarcane. These results are comparable to those of Perkins et al. (41) who found the stem of young soybean plants had activity on the same side as that of the fed leaf. But when an entire blade section is fed $C^{14}O_2$ (table I) there is no fed side in the stalk, since the sheath wraps completely around the stalk and is attached to the entire circumference of the joint. Thus whether more counts reach the midrib side or the side of the overlapping edges of the sheath depends on the relative amounts of labelled photosynthate coming down the midrib or down the lamina. This in turn depends on how high up the blade the feeding chamber was placed, and consequently on how many of the veins from the fed part have joined the midrib.

Table I

Effect of Time on Distribution of Activity (RSA) in Parts of Internodes, Showing Labelled Components Go First to Center of Stalk

Part	Midrib side*	Opposite side**
90 minutes from blade 12***		
Internode 13		
Rind (1 mm)	0	10
Next 2 mm	10	180
Remaining center	30	480
194 minutes from blade 7†		
Internode 7		
Rind	250	110
Center	360	210
Internode 8		
Rind	10	10
Center	150	90
5 hours from blade 8††		
Internode 8		
Rind (1 mm)	1600	3000
Next 2 mm	1300	2100
Remaining center	1600	2900

* Directly below midrib of fed blade.
** Overlapping attachment of sheath edges.
*** Marks were placed on internode 13 to designate midrib side and opposite side. Ninety minutes after feeding $C^{14}O_2$ to blade 12, internode 13 was cut from the stalk. The internode was then cut vertically separating the two sides. Rind (1 mm thick) was cut off. A semicircle (2 mm thick) was next cut off. Remainder of internode was designated remaining center.
† One hundred ninety-four minutes after feeding $C^{14}O_2$ to blade 7, internodes 7 and 8 were cut from the stalk, cut vertically to separate the midrib side from opposite side, and separated into rind and center.
†† Five hours after feeding $C^{14}O_2$ to blade 8, internode 8 was cut from the plant and subdivided as in ***.

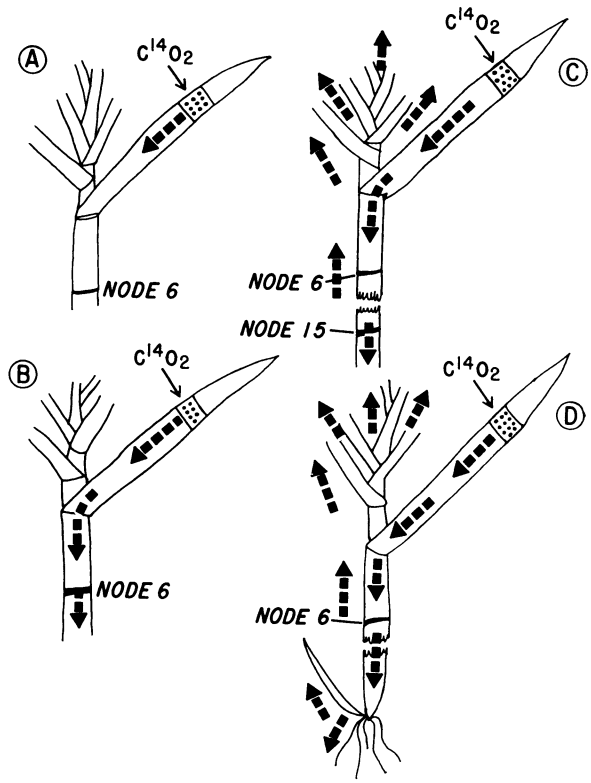

FIG. 3. Translocation for 10 to 120 minutes from fed blade 6 showing that in stalk, downward movement precedes upward movement. A: 10 minutes. Down 20 cm = 2 cm/min. 98 % of TC remains in fed part. B: 60 minutes. Down 50 cm in blade + 33 cm in sheath + 16 cm in stalk to middle of internode 6 = 99 cm total distance downward giving velocity of 1.6 cm per min. 54 % of TC in fed part. No counts up the stalk. C: 90 minutes. Down 42 cm in blade + 31 cm in sheath + 97 cm in stalk to middle of internode 15 = 170 cm total distance downward giving velocity of 1.89 cm per min. 41 % of TC in fed part. Counts in upper stalk, leaves, and spindle. D: 120 minutes. Down 42 cm in blade + 29 cm in sheath + 103 cm in stalk = 174 cm total distance downward. Counts in base of stalk, roots, and suckers as well as upper stalk, leaves and spindle. 35 % of TC in fed part.

c. To center of stalk first. When an entire blade section is fed $C^{14}O_2$, the labelled photosynthate moves down the entire blade and sheath to its node, then to the internode, going first to the center of the stalk (table I). The RSA being greater in the center of the stalk than in the rind in a test of short duration is readily explained by the course of the large leaf traces which descend from the leaf, penetrate almost horizontally to the center of the stem

and thence perpendicularly downward for as many as eight joints (1). Smaller bundles go to the periphery. After 5 hours the RSA of the rind and center is equalized (table I).

d. Direction in the stalk. Upon reaching the stalk radioactive photosynthate first turns downward (fig 3) and moves down the stalk more than one joint but less than nine joints before any finds its way into an upward-moving system. This is about the place the large, perpendicular leaf traces lose their identity. Some of the translocating material, having found its way into an upward-moving system, reaches the spindle, growing point, and young leaves before any reaches the roots. Counts are detected in the roots after two or more hours, depending upon the length of the stalk. Even the nutrient solution may have detectable counts.

Rye also had more downward translocation than upward, particularly from its lower leaves (36), while in the grape upward translocation was very rapid and basipetal translocation slower (49). In the soybean (52) translocation was predominantly basipetal.

3. Lateral. Transverse bundle connections run somewhat diagonally or at right angles between the parallel veins of a sugarcane leaf (1). Some lateral transport takes place (fig 1 & 2), going primarily toward the midrib but very little to the opposite side.

4. Acropetal. As in the soybean (50), a small percentage of radioactivity moves above the fed part to the apex of the fed blade (fig 4). This acropetal translocation is always small, generally less than 1 %, and is not affected by time. Movement to the apex of the fed blade may be due to leakage of $C^{14}O_2$ into intercellular spaces during feeding. Evidence that a small amount of leakage of CO_2 into intercellular spaces during feeding may take place, the counts moving upward, will be presented in another communication.

5. Complete picture at 90 minutes. Although 73 % of new photosynthate may leave the fed part in 90 minutes (fig 4), the fed part still has the highest RSA in the plant. The greater RSA in node than internode is accentuated down the stalk, and at the advancing front of translocation the RSA of the node is three times that of the internode. This results from 1) the greater concentration of conducting tissue in the nodes, and 2) the greater percentage of new sucrose in the conducting tissues than in the storage tissues at the front.

B. Components in Transit. Experiments with many species have shown that sucrose is the main carbohydrate translocated: willow cuttings (16); soybean (19, 52); grafts of sunflower and Jerusalem artichoke (35); grape (49); tobacco (28, 46); wheat and barley seedlings (18); bracken (22); pine and dwarf mistletoe (43); potato (37) and others. In willow cuttings, Chen (16) found fructose along with sucrose. In sugarbeet, pumpkin, cotton, and others, Kursanov (29) found a stream of sucrose, glucose, fructose, hexosephosphates, organic acids, and amino acids, with sucrose and hexosephosphates generally predominating and moving faster than the other components. On the other hand, Pristupa (42) identified stachyose and verbascose in transit in pumpkin plants; Burley (6) stated that small amounts of raffinose may also be translocated in raspberry and soybean; and Webb and Burley (53) found higher activity in sorbitol than in sucrose both in the fed leaf and at a distance of 60 cm in apple. The importance of conditions of growth in determining the nature of the components translocated was emphasized by Nelson et al. (38) who found radioactive serine in addition to sucrose in stems of soybean plants grown with adequate nutrition, but 95 to 100 % of label in sucrose in plants deficient in N; under greenhouse conditions malic acid was the only labelled compound in the stem. Since the metabolic conversion of sucrose in the stem was too slow to account for the serine and malic acid found, they concluded that translocation of the products of photosynthesis in soybean is selective, depending upon suitable conditions.

In sugarcane, counts soluble in alcohol increase toward the advancing front of translocation. In the strip torn down below the small fed part (fig 1),

Table II

*Percentage of Counts in Alcohol Extract, After Translocation for 5 Hours**

Part	Phosphates, amino acids, etc.	Sucrose	Glucose	Fructose	Malic** Acid	Lipids
Downward Translocation						
Fed blade 8, fed part	6	64	8	12	6	4
Fed blade 8, below fed part	4	84	4	5	3	1
Sheath 8	3	72	11	11	4	3
Internode 8	4	93	2	1	0	0
Internode 13	0	100	0	0	0	0
Internode 15***	0	100	0	0	0	0
Internode 19	0	100	0	0	0	0
Upward Translocation						
Internode 7	4	81	7	5	2	1
Spindle	10	66	8	6	1	8

* 400 μc fed to central part of blade 8 for 10 minutes in bright sun. For translocation curve, see fig 8.
** May include other Krebs cycle acids.
*** Alcohol extract of internode 15 contained 99.2 % of TC.

72 % of the counts remaining in the fed part were soluble in alcohol, and this percentage increased in successively lower parts to 88, 91, 92, 96, and finally 99.6 in the lower midrib. Soluble counts in the lower midrib 2 hours after feeding were 98 % sucrose. In the stalk (fig 4) 93 % of the counts in internode 5 were soluble in alcohol, 99 % in internode 8, and 100 % in internode 11.

One hundred per cent of the counts were in sucrose near the advancing front (table II). Thus, in sugarcane, sucrose is the principal compound translocated. We may, therefore, assume that most counts in other parts of the plant moved there as sucrose, which means that most of the organic matter in the plant is made from sucrose.

It was felt worthwhile to calculate the loss in radioactive sucrose in the fed part, to see if it could account for all the translocation in a given time. This can be done in an experiment in which initial and final leaf-punch samples were taken from the fed part, and their counts compared with the distribution in the entire plant at harvest. Total counts in the leaf punch samples were:

Initial	133,000
Final	50,000
Difference equals translocated	83,000 = 62 %.

Total counts in the plant at harvest were:

Entire plant	11,290,000
Still in fed part	3,500,000
Difference equals translocated	7,790,000 = 69 %.

The percentage of translocation calculated by the two methods differs by approximately 10 %.

In the initial sample, sucrose had 90 % of TC. Since all the counts in the entire plant at harvest were in the fed part at start, sucrose TC at start may be calculated thus:

TC in fed part at start	11,290,000
90 % sucrose	× .90
Sucrose TC at start	10,161,000

Analysis and chromatography of the fed part at harvest showed 2,540,000 TC in sucrose still in the fed part at harvest and thus not translocated. The loss in sucrose TC in the fed part during the 5-hour test is calculated thus:

Sucrose TC at start	10,161,000	
Sucrose TC in fed part at harvest	2,540,000	
Difference equals sucrose TC lost from fed part	7,621,000 =	98 % of TC translocated

This does not mean that 98 % of translocation was due to sucrose, because there was no measure of counts lost in respiration, and because other fractions gained in the fed part (table III). Since the gain in reducing sugars and residue equalled 12 % of the loss in sucrose, only 88 % of the loss in sucrose was due to translocation. 88 % of the loss in sucrose is 6,706,000 TC, which is 86 % of 7,790,000 (the TC translocated). 86 % differs from 100 % more than the 10 % difference in translocation measured by loss in leaf-punch counts or by distribution at final harvest, but still shows that most translocation is a transport of sucrose.

No definite evidence of translocation of other components has yet been obtained in these studies. Small amounts of radioactive glucose and fructose are often found below the fed part, not always equal in radioactivity. Labelled aspartic and malic acids are also found below the fed part. The presence of these compounds may indicate translocation or conversion. These components are not found at the advancing front of translocation. Neither are they found in upward translocation in other stalks of the stool (section II A). If they are translocated at all, it is more slowly and in smaller amounts than sucrose.

C. Destination, Use, and Storage. Shen (45) reported that the radioactive products from leaves of rice went to the centers of growth or storage. Centers of growth in sugarcane are the stem tips, root tips, and sheaths. Storage occurs in the millable cane and is most active in internodes 8 to 10 or a little below. These are the places where the newly formed photosynthate is retained.

Reducing sugars, organic acids, lipids, organic phosphates, and amino acids appear in upward translocation to the spindle (table II). As their appearance is accompanied by a decrease in percentage of sucrose counts, they are formed by conversion upon arrival at the young, growing region. Insoluble compounds are also formed in young internodes and in roots (table IV).

Age of the leaf fed determines the percentage of counts retained in its growth (tables IV & V). In mature leaves the new sucrose passes through unchanged, but in young leaves a large percentage of the new sucrose is converted to materials used in the growth of the sheath. Yang (55) found that the immature part of a blade incorporated the most C^{14} labelled translocating material; because blade 1 was most active near the apex, blade 2 at the middle,

Table III

*Total Counts in Initial & Final (5-hr) Leaf-punch Samples of Fed Part**

Sample	Sucrose	Glucose	Fructose	Residue	Other
Initial	118,000	4,000	1,000	7,000	3,000
Final	25,000	5,000	6,000	12,000	1,000
Change	−93,000**	+1,000	+5,000	+5,000	−2,000

* Data regarding application of $C^{14}O_2$ in table II.

** Excess loss in sucrose compared with gain in other components was attributed chiefly to translocation. See text for calculations comparing loss in leaf-punch counts with final distribution in entire plant.

Table IV

Distribution & Solubility of Counts After Translocation for 24 Hours From Blade 8

200 μc fed to central part of blade 8 for 5 minutes at 7000 to 8000 ft-c. Fed part at harvest still had 31,000 sucrose TC, presumably translocatable. Age of plant, 4 months.

Part	% of total counts	% soluble in alcohol
Spindle	6.0	...
Leaves 2-7	5.6	...
Joint 1-4	1.1	...
Internode 5	3.5	35.1
Internode 6	6.8	...
Internode 7	12.4	72.5
Total upward	35.4	
Fed blade 8, fed part	4.2	56.0
Fed blade above fed part	0.05	...
Fed blade below fed part	0.9	...
Sheath 8	4.4	90.3
Total in fed leaf	9.55	
Internode 8	18.4	...
Internode 9	5.9	87.8
Internode 10	1.5	...
Leaf 9	0.4	...
Stubble	4.0	...
Roots	24.8	25.3
Total downward	55.0	

Table V

Distribution of Counts After Translocation for 6 Weeks from Blade 3

Part	% of total counts	% soluble in alcohol
Spindle	0.3	
Leaves 2-6	13.4	
Joints 1-6	1.2	
Total upward	14.9	
Fed blade 7, fed part	4.1	
Fed blade above fed part	0.04	
Fed blade below fed part	2.2	
Sheath 7	23.1	6
Total in fed leaf	29.44	
Joint 7	2.0	
Leaves below fed leaf	1.1	
1 dry leaf, attached	0.2	
Leaves removed before harvest	0.1	
Joints 8-10	25.6	20
Joints 11-15	18.5	80
Joints 16-20	8.1	
Total downward	55.6	

350 μc fed to central part of blade 3 for 5 minutes in bright sun.

During the 6 weeks after feeding blade 3, four new blades had formed for which reason the fed blade had become No. 7 at harvest.

Note retention of counts by the growing sheath, chiefly insoluble components and the high solubility of labelled components in storage joints 11 to 15.

and blade 3 at the base, he concluded that the blade grows first at the apex, then in the middle, and last at the base.

Sugars increase in percentage on the dry weight basis from the fed part through the sheath to the stalk (table VI), since the analyses include stored sugars as well as those in transit. The higher percentages of sugars in the sheath and stalk than in the blade dilute the radioactive sugars to a greater extent, resulting in lower specific activities and reversing the gradient. The sugar in the transport

Table VI

*Gradients in Sugars After Translocation for 90 Minutes**

Part	Sucrose	Reducing Sugars	
	Percent dry weight		
Fed blade 5, fed part	3.72	0.52	
Fed blade, below fed part	3.92	0.64	
Sheath 5	5.78	3.12	
Internode 5	28.42	3.18	
	Specific activity, cpm/mg sugar		
Fed blade 5, fed part	191,000	112,000	
Fed blade, below fed part	32,500	12,500	
Sheath 5	10,500	1,280	
Internode 5	485	610	
	Counts/mg tissue		
	Sucrose	Glucose	Fructose
Fed blade 5, fed part	6,800	280	310
Fed blade, below fed part	1.200	40	40
Sheath 5	600	20	20
Internode 5	100	10	10

* 280 μc fed to central part of blade 5 for 10 minutes in bright sun. Relatively high SA of sucrose in sheath, primarily an organ of conduction, confirms that sucrose is chief sugar translocated.

system is moving with the gradient, but against the gradient during transfer from the sieve tubes to the storage parenchyma, the mechanism of which process has been studied by Glasziou (20, 21) and Bieleski (5). Glasziou (21) concluded that a sucrose precursor or derivative is transferred across the boundary between the outer and inner space of the tissue, and that sucrose is released into the inner space. It is perhaps this transfer step referred to by Turkina (51) when he stated that sucrose is transported to the roots of sugar beets against a concentration gradient. Importance of a positive gradient in translocation is shown by a greater velocity of translocation by day than by night, due perhaps to the washing out effect of new photosynthate (9) and in studies with detached blades (25).

The amount of new, radioactive sucrose reaching selected parts during translocation for 5 hours was estimated (table VII), and internode 19, which already contained nearly eight gm of sucrose, had taken in 0.6 mg of C^{14} sucrose. How much of this was in transit and how much in new storage is not known.

Storage in internodes 8 to 10 in 24 hours amounted to 25 % of counts, 88 % of which were soluble in

Table VII

*Amount of C^{14} Sucrose in Selected Parts After Translocation for 5 Hours From Blade 8**

Part	Sucrose mg	Sucrose cts/mg	C^{14} sucrose % of total	C^{14} sucrose mg
Fed blade, fed part	44.7**	77,000***	6.3***	2.8†
Fed blade, below fed part	147.2	8,700	0.7	1.0
Sheath 8	134.1	7,700	0.6	0.8
Internode 8	4,420	240	0.02	0.9
Internode 13	11,714	25	0.002	0.2
Internode 19	7,886	92	0.007	0.6

* Data regarding application of $C^{14}O_2$ in table II.

** Original leaf-punch sample had approximately same mg sucrose as in final leaf-punch sample.

*** Original SA of sucrose was 1,220,000. This was decreased to 77,000 by translocation of C^{14} sucrose and formation of C^{12} sucrose, or a decrease to 6.3 %.

† 6.3 % of 44.7 = 2.8 mg C^{14} sucrose. This calculation assumes that new sucrose reaching a lower part has the same SA as new sucrose in an initial leaf-punch sample.

alcohol and were, presumably, largely sugar (table IV). After 6 weeks, joints 11 to 15, which are storage joints, contained 18 % of counts, 80 % of which were soluble (table V).

D. Velocity. Swanson (48) defined velocity of transport as the lineal distance of movement per unit time. Velocities of translocation in sugarcane were calculated from the time of injection of $C^{14}O_2$ into the feeding chamber to the time of cutting the leaf or stalk from the plant: distance was measured from the bottom of the fed part (lower edge of chamber) to the advancing front of activity, which in sugarcane is very sharp (fig 4). Time of injection of $C^{14}O_2$ is close to the time translocation starts, since radioactive sucrose is made in a few seconds in a sugarcane leaf and moves down the blade 2 to 2.5 cm in 1 minute (fig 3 & 5).

Velocities range from 42 cm per hour (*26, 27*) to 150 cm per hour (fig 3, 4, & 5). Twelve estimates made with normal plants average 84 cm per hour as the usual velocity of translocation of sucrose in the sugarcane plant. Canny (13, 14) believes that the distance measured from source to front depends upon size of dose and sensitivity of detection. But we have found that increasing the dose from 40 μc to 400 μc, with an application time of 5 minutes, has no effect upon velocity. A good estimate of effect of sensitivity can be seen in figure 4. Near the front of translocation the RSA drops ten times in one joint length. Hence, zero is approached in one more joint, No. 14, and the longer the plant the nearer the approach to true velocity. It cannot be said, however, that an uncountable trace has not gone much farther.

Length of feeding, regardless of dose, may affect velocity. An average of 11 tests, each a 5-minute application, gave 96 cm per hour; 10-minute feedings averaged 72 cm per hour, and a single test at 37 minutes gave 42 cm per hour. Longer application appears to decrease velocity, although translocation time includes feeding time. Short application time is best. Five minutes is our usual time.

The velocities presented so far are all for downward movement. Velocity of translocation upward to the growing point of the stem in one test was estimated as 37 cm per hour, but this was only approximate because it was not known exactly how far the C^{14} moved down the stem before going upward. In the grape, Swanson (47) reported the velocity of upward translocation was about 60 cm per hour.

The velocities of translocation of C^{14}-photosynthate reported in the literature range from 0.24 cm per hour in Salix (15) to 5040 cm per hour in soybean (39, 40). The majority of velocities falls in

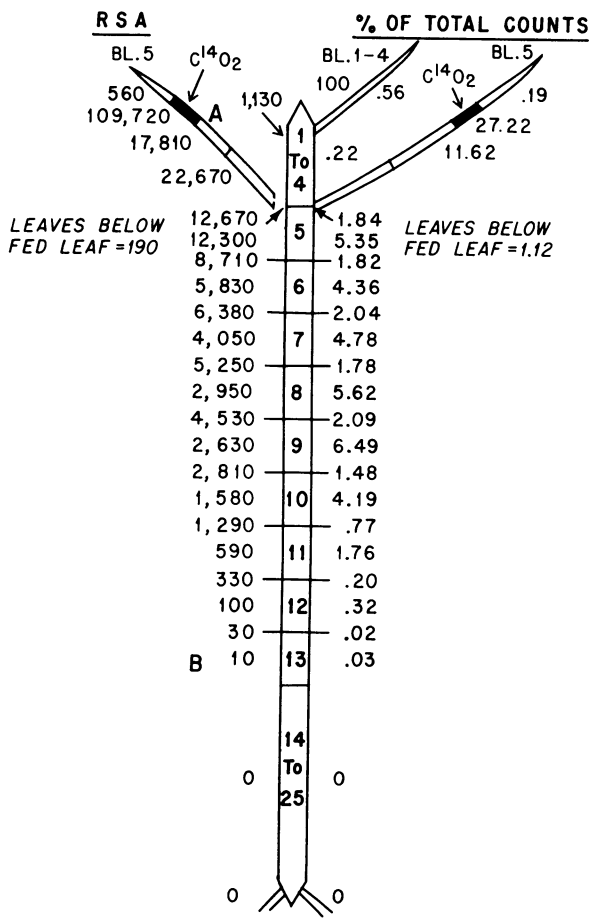

FIG. 4. RSA (left) and % of TC (right) 90 minutes after feeding 400 μc to part (black area) of blade 5 (shown twice in diagram) for 5 minutes at 3000 to 8000 ft-c. Separate counts for nodes (——— represents the nodes) and internodes. Nodes richer than internodes in RSA but lower in % because nodes weigh less. Distance A to B = 168.3 cm. Velocity = 1.87 cm per minute. In internode 8, 85 % of TC (& 92% of sugar counts) were in sucrose.

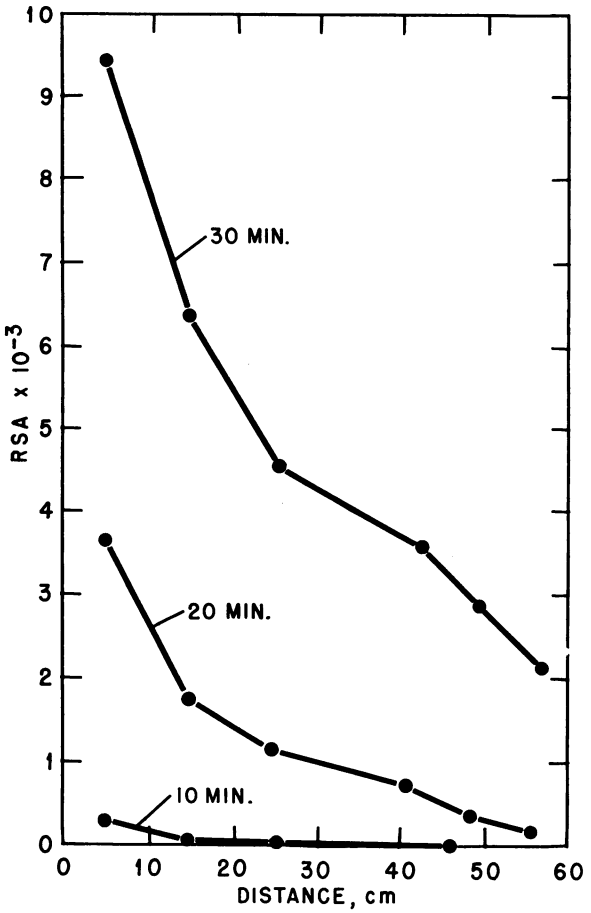

FIG. 5. Translocation in blade 5 of three stalks, each fed 40 μc for 5 minutes in bright sun, at a position 60 cm above dewlap. In 10 minutes, RSA was 13 for the section 20 to 30 cm below fed part. Velocity = 2.5 cm/min.

the intermediate range of 20 to 144 cm per hour, (19, 29, 30, 37, 40, 51, 52, 54, 56), and sugarcane belongs in this intermediate group. The results with sugarcane were obtained with large plants (stem length 200–300 or more cm), growing vigorously in full sun (10,000 ft-c on a clear day), at a favorable temperature day and night, with complete fertilization, and well watered. The velocity is decreased by unfavorable conditions of growth or by using a detached blade. What rates would be obtained with Salix if measurements were made with large shrubs or trees growing outdoors beside a stream in the summer time? The low rates obtained by Canny may be in part due to the use of small rooted cuttings in the laboratory (15).

No evidence of a very rapid rate of translocation has been obtained with sugarcane. The plant illustrated in figure 4 had lalas on joints 21 to 25, and 8 suckers growing on the stubble, none of which was radioactive. If any photosynthate had moved at the rate of 5000 cm per hour, the roots should have been active, but they were not.

E. Amounts of Translocation.

1. Measured as percentages of counts lost from the fed part, in successive punch samples. A relatively simple method of estimating the amount of translocation as distinct from velocity, is to take successive punch samples from the fed part, starting at the upper part of the fed portion and working downward so as not to interfere with translocation. The rate of transport from the fed portion diminishes with time (fig 6). In a 1-day test (fig 6, curve A), 57 % of the counts were translocated from 9:10 to

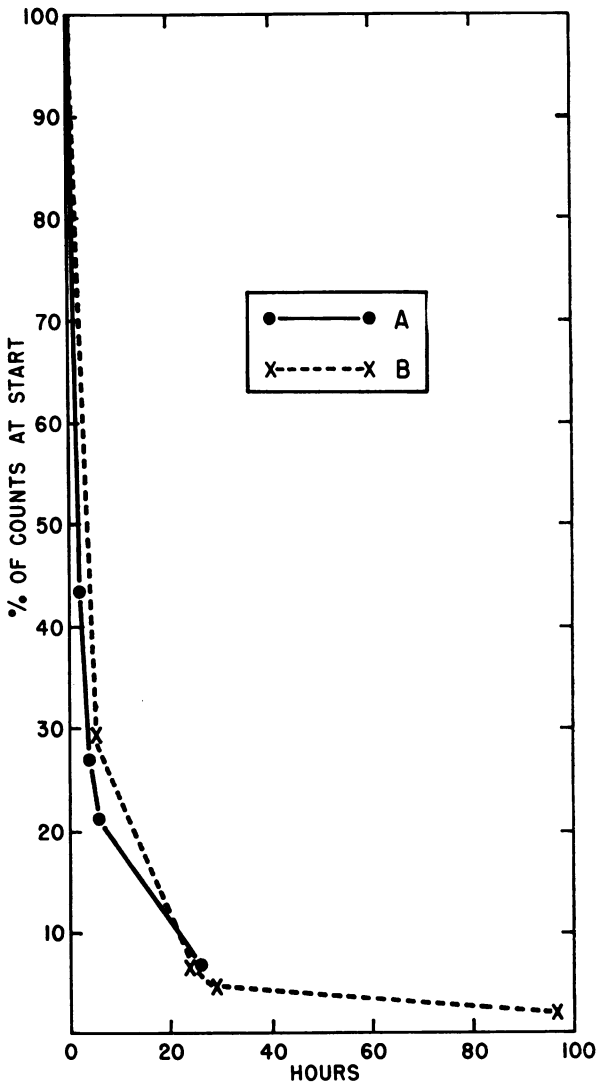

FIG. 6. Translocation measured by loss of counts from fed part, in successive punch samples. A: 10 μc fed to blade 4 for 2 minutes followed by 2 minutes in air before taking initial punch sample; average of two plants. B: same procedure with 40 μc; average of twenty plants (ten varieties, two amounts of N). All fed in the morning. Samples ground in alcohol, extract and residue plated together for counting.

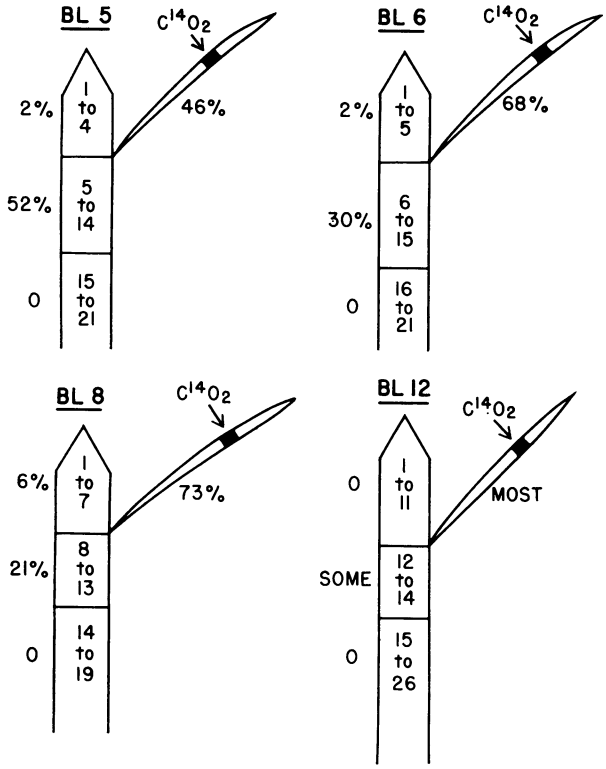

FIG. 7. Translocation for 90 minutes from blades of different rank. Rank of leaf affects total amount and velocity of translocation. Blade 5 translocated 54 % at 1.6 cm min. Blade 6 translocated 32 % at 1.9 cm min. Blade 8 translocated 27 % at 1.8 cm min. Blade 12 translocated very little at 1.1 cm min.

10:40 AM; 38 % of the remaining counts from 10:40 AM to 1:10 PM; and 20 % of the remaining counts from 1:10 to 2:40 PM. In the final period, 2:40 PM to 10:40 AM, 70 % of the remaining counts were translocated. In a 4-day test (curve B) only 2 % of the counts still remained in the fed portion after translocation for 96 hours. This may be compared with tobacco (28) in which the fed leaf still contained 32 % of activity after 96 hours, chiefly in insoluble compounds.

2. Measured by subdividing the entire plant. A more detailed picture of translocation in 90 minutes (fig 4) shows that 73 % of the newly formed sucrose may leave the fed portion in that time. Nine tests, each 90 minutes in duration, recalculated as percentage leaving the fed part per hour, ranged from 17 to 54 % per hour. Causes of this variation include climatic and edaphic factors and the presence or absence of suckers and lalas (to be reported in subsequent papers), as well as rank of the fed blade (fig 7). Old leaves, e.g., blade 12, translocate less in amount and more slowly than mature blades of middle rank, e.g., blades 5 to 8, because old blades must compete with the streams coming down from the younger leaves. The removal of competing streams by defoliating all leaves above the fed leaf greatly increases the amount of transport from the leaf (unpublished data). In studies of translocation in rye, Mayer and Porter (36) reported that the older the fed leaf, i.e., the lower down on the tiller, the greater the percentage of total counts retained by the fed leaf. A young leaf also translocates less than a mature leaf, because a young leaf uses some new photosynthate in growth. Jones et al. (28) reported that very young leaves of tobacco do not export photosynthate.

3. Calculated as absolute amount of C^{14} sucrose per hour. It was desired to calculate the rate of translocation in milligrams of newly formed C^{14} sucrose. This calculation (table VIII) was made on the assumption that all the sucrose leaving the fed part (area approximately 82 cm^2) started with a SA of at least 287,735, which was found in the fed part at harvest, since no initial leaf-punch samples were taken in this test. Actually, most of the sugar starting down had a higher SA, since the sucrose in the fed part at harvest was diluted by 90 minutes of photosynthesis with $C^{12}O_2$. Since 17.87 mg C^{14}-sucrose had left the fed part but had not left its joint, a minimum rate of 10.7 mg C^{14}-sucrose per hour is obtained for the rate of sugar movement from an area of 82 cm^2 down the leaf into the joint. This measurement comes the closest to "rate" defined by Swanson (48) as the amount (wt) of solute transported per unit time.

F. Translocation Profile Development. The translocation profile in the stem was approximately linear at 1 hour (fig 8), at which time no detectable tagged sugar had moved up the stem above the at-

Table VIII

*Translocation Rate Expressed as mg C^{14} Sucrose Per Hour**

Part	Total mg Sucrose	cpm/mg Sucrose	mg C^{14} sucrose/ mg sucrose	Total mg C^{14} sucrose
Fed part	87.2	287,735	1	87.2
Below fed part	96.15	27,866	0.0968	9.31
Sheath, upper half	55.44	21,728	0.0755	4.19
Sheath, lower half	57.82	5,564	0.019	1.10
Node 7	276.8	1,389	0.0048	1.33
Internode 7	605.09	922	0.0032	1.94
Sum below fed part = mgs sucrose that had left the fed part but not the fed joint.				17.87
17.87 mg C^{14} sucrose ÷ 1.67 hrs = 10.7 mg C^{14} sucrose per hr				

* 500 μc fed to central part of blade 7 for 10 minutes (part sunny & part cloudy). Translocation terminated at 100 minutes by cutting through internode 30. Area of fed portion: 82 cm^2. This calculation assumes that new sucrose reaching a lower part has the same SA as the sucrose in the fed part at harvest, since no initial leaf-punch samples were taken in this test. Actually, most of the sucrose leaving the fed part had a higher SA.

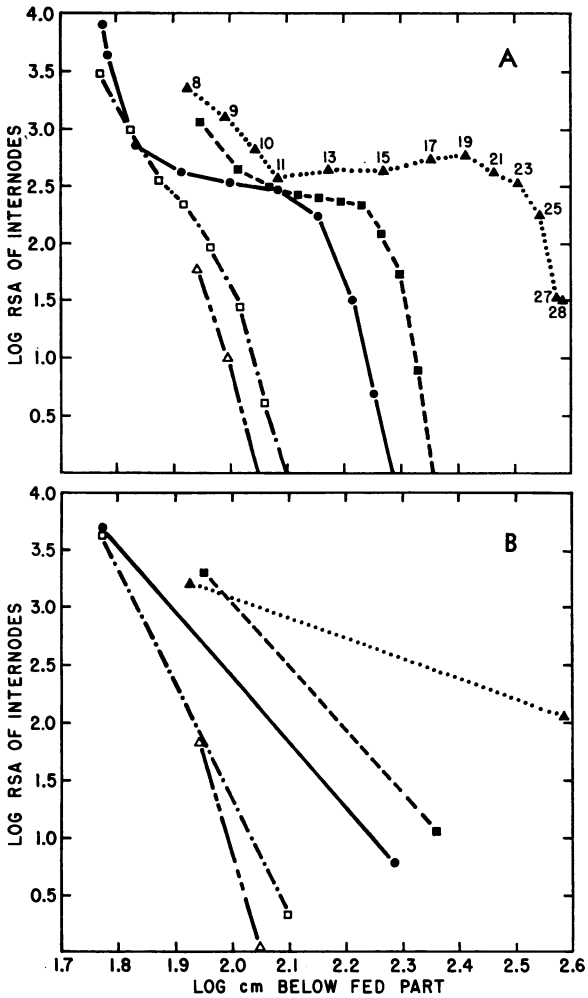

FIG. 8. RSA of internodes for translocation periods of 1 to 5 hours. A: Development of profile. Numbers on curve for 5-hour profile are those of internodes tested. Counts in internodes 13, 15, 19 were all sucrose (table II). B: Overall slope decreased with time. Regression lines calculated from A. Time: 1 hr, 1.5 hr, 3 hr, 4 hr, 5 hr. Value for b: —16.74, —10.19, —5.78, —5.49, —1.77. Designation: △ — - - — △, □ — · — □, ● ——— ●, ■ - - - - - ■, ▲ . . . . . ▲.

tachment of the fed leaf (fig 3). At 1.5 hours a deviation to the right was apparent, and further development of the profile was illustrated at 3, 4, and 5 hours (fig 8), at which times tagged sugars had definitely moved upward (fig 3). The curve for 5 hours showed a dip at internode 11 and a secondary peak at internode 19, resembling the superposition of two curves.

Since the change in shape of the profile was not discernible until after upward transport had started, and since it increased with time, it may be due to loss of sucrose in upward movement. Another factor involved may be the storage of tagged sucrose in the joints. The peak at internode 19, which was all due to sucrose, may have left the fed part at the time of maximum tagging of sucrose, before dilution by photosynthesis with $C^{12}O_2$, and may be the translocation peak detected by Canny (14), since it moved further from the fed part (down the stalk) with time.

Regression lines calculated from the data in figure 8A show the entire slope decreased with time (fig 8B). Value for b was calculated from the equation:

$$\frac{\Sigma(X-\bar{x})\ (Y-\bar{y})}{\Sigma(X-\bar{x})^2} = b$$

II. Translocation in the stool complex.

A. Interstalk Transport. Newly formed photosynthate made in a single leaf of a primary stalk found its way into lalas and suckers in two or more hours depending on length of the stalk (fig 3). When blade 3 of one stalk of a large stool (16 stalks) was fed $C^{14}O_2$, radioactive photosynthate had reached blade 2 of all stalks when sampled 20 hours later (9). At 44 hours, 68 % of counts remained in the fed stalk, 17 % in the roots, and 14 % had reached the other stalks. In another experiment 17 % of counts reached the suckers in 14 days, and were mostly in the millable cane. In millable cane, the percentages of counts soluble in alcohol were: primary, 53 %; sucker, 64 %.

Interstalk transport has also been demonstrated in rye (36) in which small amounts of activity were found in adjacent tillers after 3 weeks.

Interstalk transport in sugarcane is the movement of sucrose up the phloem. Millable cane of large suckers was inactive when sampled up to 4.5 hours after feeding 400 μc to a blade of the primary stalk. Other suckers from the same stool sampled at 5.5 and 6 hours were active, with RSA higher in the node than internode: the labelled photosynthate was, therefore, largely in transit. Counts in the alcohol extracts of node and internode were 100 % sucrose. These counts were not in the xylem, because when the xylem contents were blown out by compressed air they contained only 0.05 to 0.42 % of counts. Residues were inactive.

The results with sugarcane are thus not in agreement with the suggestion of Jones et al. (28) that sucrose moves up the stem in the xylem.

B. Contribution of Young and Old Stalks in a Stool. Young, vigorous suckers may contribute more new photosynthate to root growth and the stool

Table IX

Translocation for 3 Hours in Old and Young Stalks of Same Stool

Series	Distance to roots cm	Velocity cm/min	Time to reach roots hrs
Old	451.1	0.9	8.35
Young	234.6	1.1	3.55

200 μc fed to blade five of each stalk for 5 minutes in bright sun.

as a whole than may old stalks. An interesting difference in the time required for new photosynthate to reach the roots (table IX) may have a bearing on aging.

Old stalks contribute less new photosynthate to growth, more to storage, and new material is added daily even to the older joints (table V & fig 8).

III. Factors Affecting Translocation.

Amounts and rates of translocation in sugarcane are affected by temperature of air and roots, light, moisture, and deficiencies in potassium, nitrogen, or phosphorus (24, 27). Defoliation tests as well as studies with detached blades (24, 25) indicate that the driving force of translocation is within the leaf itself.

Summary

This paper reports studies dealing with translocation of photosynthate in sugarcane plants grown and fed $C^{14}O_2$ under normal conditions of climate and nutrition.

Sucrose is the principal compound translocated throughout the plant: down the leaf to the stalk, down the stalk to the stubble and roots, up the fed stalk to the spindle, and up other stalks in the stool of cane. No definite evidence of translocation of any other component has been obtained in these studies.

Newly formed radioactive sucrose quickly enters the veins and moves primarily downward, following the veins to midrib and sheath, at velocities ranging up to 2.5 cm per minute.

Upon arriving at the stalk, sucrose goes first to the center and then turns downward but, well before any gets as far as the roots, some sucrose finds its way into an upward-moving system and some reaches the growing point and spindle before any makes its way to the roots.

Newly formed sucrose moves into laias and suckers within a few hours and gets to the tops of all stalks in a large stool within 24 hours.

Sucrose reaching young stems, leaves, and roots is converted to other compounds used in growth of cell walls and protoplasm.

Each day's sucrose proceeds to the stem where most of it is stored in ripening joints and some is added to mature joints.

Since radioactive sucrose is made in the fed part in a few seconds, starts down the blade in 1 minute, and accounts for 100 % of radioactivity at the advancing front, it is felt that the velocity of movement from source to front reflects the velocity of translocation of sucrose in the sugarcane plant.

Velocities of translocation from the fed blade down the stalk to the advancing front range from 42 to 150 cm per hour. The average of twelve determinations is 84 cm per hour, which can be considered the average velocity of translocation of sucrose in the sugarcane plant.

Percentage of counts leaving the fed part, averaged per hour, ranges from 17 % to 54 %. Causes of this variation are discussed.

In one experiment, the radioactive sugar was moving down the leaf from approximately 82 cm^2 of fed part into the stalk at the rate of 10.7 mg C^{14} sucrose per hour.

Slope of the translocation profile in the stalk decreases with time. Shape of the profile may be explained by a separation of true transport from storage in the joints, or by a loss of sucrose due to upward translocation increasing with time, or by a combination of factors.

Factors affecting velocity of translocation in sugarcane are mentioned.

Acknowledgment

The authors are grateful to Mrs. Grace Sadaoka for constant help in these investigations.

Literature Cited

1. Artschwager, E. 1925. Anatomy of the vegetative organs of sugar cane. J. Agr. Research 30: 197–242.
2. Belikov, I. F. 1958. The translocation of assimilates in the leaf blade of soya. Dokl. Akad. Nauk SSSR, Bot. Sci. Sect. (Eng. Transl.) 120: 151–53.
3. Belikov, I. F. 1961. The effect of severing soybean leaf veins upon outflow of assimilates. Fiziol. Rast. (Eng. Transl.) 7: 429–31.
4. Biddulph, O. 1962. A histoautoradiographic study of phloem translocation. Plant Physiol. suppl. 37: xii.
5. Bieleski, R. L. 1960. The physiology of sugarcane. III. Characteristics of sugar uptake in slices of mature & immature storage tissue. Austral. J. Biol. Sci. 13: 203–20.
6. Burley, J. W. A. 1961. Carbohydrate translocation in raspberry & soybean. Plant Physiol. 36: 820–24.
7. Burr, G. O. 1954. Basic problems of sugarcane nutrition. I. Absorption & distribution of tagged elements by sugarcane. Proc. Intern. Soc. Sugar-Cane Technologists, 8th Congr. 1953: 45–51.
8. Burr, G. O., C. E. Hartt, H. W. Brodie, T. Tanimoto, H. P. Kortschak, D. Takahashi, F. M. Ashton, & R. E. Coleman. 1957. The sugarcane plant. Ann. Rev. Plant Physiol. 8: 275–308.
9. Burr, G. O., C. E. Hartt, T. Tanimoto, D. Takahashi, & H. W. Brodie. 1957. The circulatory system of the sugarcane plant. Radioisotopes in Sci. Res., Proc. 1st (UNESCO) Intern. Conf., Pergamon Press, New York. 4: 351–68.
10. Burr, G. O. & T. Tanimoto. 1949. An improved micro-method for the determination of reducing sugars. Proc. Hawaiian Acad. Sci. 24: 7.
11. Burr, G. O. & T. Tanimoto. 1950. An improved micro-method for sugars using direct colorimetry of ferricyanide. Fed. Proc. 9: 157–58.
12. Burr, G. O., T. Tanimoto, C. E. Hartt, A. Forbes, G. Sadaoka, F. M. Ashton, J. H. Payne, J. A. Silva, & G. E. Sloane. 1956. Uses of radioisotopes by the Hawaiian sugar plantations. Proc. Intern. Conf. Peaceful Uses At. Energy, 1955. United Nations, New York 12: 177–83.

13. CANNY, M. J. 1960. The rate of translocation. Biol. Rev. Cambridge Phil. Soc. 35: 507–32.
14. CANNY, M. J. 1961. Measurements of the velocity of translocation. Ann. Botany (London) 25: 152–67.
15. CANNY, M. J. 1962. The translocation profile: sucrose & carbon dioxide. Ann. Botany (London) 26: 181–96.
16. CHEN, S. L. 1951. Simultaneous movement of P^{32} and C^{14} in opposite directions in phloem tissue. Am. J. Botany 38: 203–11.
17. CRAFTS, A. S. 1961. Translocation in plants. Holt, Rinehart, & Winston, New York. 182 pp.
18. EDELMAN, J., S. I. SHIBKO, & A. J. KEYS. 1959. The role of the scutellum of cereal seedlings in the synthesis & transport of sucrose. J. Exptl. Botany 10: 178–89.
19. GAGE, R. S., & S. ARONOFF. 1960. Translocation III. Experiments with carbon^{14}, chlorine^{36}, & hydrogen^{3}. Plant Physiol. 35: 53–64.
20. GLASZIOU, K. T. 1960. Accumulation & transformation of sugars in sugarcane stalks. Plant Physiol. 35: 895–901.
21. GLASZIOU, K. T. 1961. Accumulation & transformation of sugars in stalks of sugarcane. Origin of glucose & fructose in the inner space. Plant Physiol. 36: 175–79.
22. HAMILTON, S., & M. J. CANNY. 1960. The transport of carbohydrates in Australian bracken. Australian J. Biol. Sci. 13: 479–85.
23. HARTT, C. E. 1936. The fluctuations of sugars in the leaf sheaths of the sugar cane plant during the day & the night. Hawaiian Planters' Record 40: 329–54.
24. HARTT, C. E. 1963. Tracing sugar in the cane plant. Proc. Hawaiian Acad. Sci. 37th Ann. Meet. 1961–1962. (in press).
25. HARTT, C. E. 1962. Translocation of C^{14} in detached blades of sugarcane. Plant Physiol. suppl. 37: xii.
26. HARTT, C. E. & G. O. BURR. 1951. Translocation by sugar cane fed radioactive carbon dioxide. Proc. 7th Intern. Botan. Congr. 748–49.
27. HARTT, C. E. & H. P. KORTSCHAK. 1963. Tracing sugar in the cane plant. Proc. Intern. Soc. Sugar-Cane Technologists, 11th Congr. 1962. (in press).
28. JONES, H., R. V. MARTIN, & H. K. PORTER. 1959. Translocation of ^{14}carbon in tobacco following assimilation of ^{14}carbon dioxide by a single leaf. Ann. Botany (London) 23: 493–508.
29. KURSANOV, A. L. 1956. The utilization of radioactive isotopes in biology & agriculture in the USSR. Peaceful Uses of Atomic Energy 12: 3–9.
30. KURSANOV, A. L. 1956. Analysis of the movement of substances in plants by means of radioactive isotopes. Peaceful Uses of Atomic Energy 12: 165–69.
31. KURSANOV, A. L. 1956. Radioactive elements in the study of plant life. Peaceful Uses of Atomic Energy 16: 114–20.
32. KURSANOV, A. L. 1961. The transport of organic substances in plants. Endeavour 20: 19–25.
33. KURSANOV, A. L. & M. I. BROVCHENKO. 1961. Effect of ATP on the entry of assimilates into the conducting systems of sugar beets. Fiziol. Rast. (Eng. Trans.) 8: 211–17.
34. KURSANOV, A. L., M. I. BROVCHENKO, & A. N. PARIISKAYA. 1959. Flow of assimilates to the conducting tissue in rhubarb (*Rheum rhaponticum* L) leaves. Fiziol. Rast. (Eng. Trans.) 6: 544–52.
35. KURSANOV, A. L., M. Kh. CHAILAKHIAN, O. A. PAVLINOVA, M. V. TURKINA, & M. I. BROVCHENKO. 1958. Translocation of sugars in grafted plants. Fiziol. Rast. (Eng. Trans.) 5: 1–12.
36. MAYER, A. & H. K. PORTER. 1960. Translocation from leaves of rye. Nature 188: 921–22.
37. MOKRONOSOV, A. T. & N. B. BUBENSHCHIKOVA. 1962. Translocation of assimilates in potato plants. Fiziol. Rast. (Eng. Trans.) 8: 447–54.
38. NELSON, C. D., H. CLAUSS, D. C. MORTIMER, & P. R. GORHAM. 1961. Selective translocation of products of photosynthesis in soybean. Plant Physiol. 36: 581–88.
39. NELSON, C. D., H. J. PERKINS, & P. R. GORHAM. 1958. Note on a rapid translocation of photosynthetically assimilated C^{14} out of the primary leaf of the young soybean plant. Can. J. Biochem. & Physiol. 36: 1277–78.
40. NELSON, C. D., H. J. PERKINS, & P. R. GORHAM. 1959. Evidence for different kinds of concurrent translocation of photosynthetically assimilated C^{14} in the soybean. Can. J. Botany 37: 1181–89.
41. PERKINS, H. J., C. D. NELSON, & P. R. GORHAM. 1959. A tissue-autoradiographic study of the translocation of C^{14}-labelled sugars in the stems of young soybean plants. Can. J. Botany 37: 871–77.
42. PRISTUPA, N. A. 1959. The transport form of carbohydrates in pumpkin plants. Fiziol. Rast. (Eng. Trans.) 6: 26–32.
43. REDISKE, J. H. & K. R. SHEA. 1961. The production & translocation of photosynthate in dwarf mistletoe & lodgepole pine. Am. J. Botany 48: 447–52.
44. ROE, J. H. 1934. A colorimetric method for the determination of fructose in blood & urine. J. Biol. Chem. 107: 15–22.
45. SHEN, G. M. 1960. Translocation & distribution of assimilates from the leaves of rice plant during its various developing periods—experiments with radioactive carbon (C^{14}). (Chinese with Eng. sum.). Acta Agric. Sinica 11: 30–40.
46. SHIROYA, M., G. R. LISTER, C. D. NELSON, & G. KROTKOV. 1961. Translocation of C^{14} in tobacco at different stages of development following assimilation of $C^{14}O_2$ by a single leaf. Can. J. Botany 39: 855–64.
47. SWANSON, C. A. 1957. The translocation of organic nutrients in plants. Atomic Energy & Agriculture. AAAS Pub. No. 49: 123–38.
48. SWANSON, C. A. 1959. Translocation of organic solutes. In: Plant Physiology, a Treatise. F. C. Steward, ed. 2: 481–551.
49. SWANSON, C. A. & E. D. H. EL-SHISHINY. 1958. Translocation of sugars in the Concord grape. Plant Physiol. 33: 33–7.
50. THAINE, R., S. L. OVENDEN, & J. S. TURNER. 1959. Translocation of labelled assimilates in the soybean. Australian J. Biol. Sci. 12: 349–72.
51. TURKINA, M. V. 1954. The synthesis & transportation of sucrose in the sugar beet plant. Biokhimiya 19: 357–63.
52. VERNON, L. P. & S. ARONOFF. 1952. Metabolism of soybean leaves. IV. Translocation from soybean leaves. Arch. Biochem. & Biophys. 36: 383–98.

53. WEBB, K. L. & J. W. A. BURLEY. 1962. Sorbitol translocation in apple. Science 137: 766.
54. WEATHERLEY, P. E., A. J. PEEL, & G. P. HILL. 1959. The physiology of the sieve tube. Preliminary experiments using aphid mouth parts. J. Exptl. Botany 10: 1–16.
55. YANG, T. T. 1961. A study of translocation of of photosynthate in sugar cane plants with C^{14} and P^{32}. Rept. Taiwan Sugar Exp. Sta. 23: 47–64 (Chinese with English summary).
56. ZIEGLER, H. & G. H. VIEWEG. 1961. Der Experimentelle Nachweis Einer Massenströmung in Phloem von *Heracleum Mantegazzianum* Somm. et Lev. Planta 56: 402–08.
57. ZIMMERMAN, M. H. 1960. Transport in the phloem. Ann. Rev. Plant Physiol. 11: 167–90.

Nicotinic Acid-Ricinine Relationship in Sterile Cultures of Ricinus communis L [1, 2, 3]

George R. Waller & K. Nakazawa [4]

Department of Biochemistry, Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater, Oklahoma

The role of nicotinic acid and nicotinamide as precursors for ricinine, the alkaloid produced by the castor plant, *Ricinus communis* L., is well established (6, 9, 10, 11). Present evidence indicates that the sequence nicotinic acid → nicotinamide →→→ ricinine occurs in the castor plant. The results indicated that the pyridine ring and the amide group are incorporated as a unit into ricinine with the amide undergoing an intramolecular dehydration to give the nitrile. In the course of the present study, it was observed that the ricinine content of the germinating castor seed increased markedly after 48 to 72 hours incubation at 30°. It would appear that a good way to study ricinine biosynthesis would be to add a precursor to the seed or seedling or some of its parts just before this rapid ricinine synthesis occurred. This paper reports the results obtained from studying the incorporation of nicotinic acid-7-C^{14} into ricinine using sterile excised castor embryos, excised cotyledons, and leaf discs. These experiments subsequently led us into a study of ricinine degradation by these tissues. Evidence that ricinine could be metabolized by the castor plant was obtained in 1933 by Weevers (12) when he showed that ricinine disappeared with increasing age of castor plants grown on nitrogen depleted soil.

The experiments reported in this paper establish clearly that a nicotinic acid-ricinine relationship occurs in the castor plant and that the metabolism of ricinine can be spared by the presence of higher concentrations of nicotinic acid in tissue than normally is found.

Experimental [5]

Sterile Culture Techniques. Seeds of *Ricinus communis* L., variety Cimmaron were sterilized with 0.1 % $HgCl_2$, rinsed with sterile water, and allowed to germinate for 2 days in petri dishes in the dark at 30°. Embryos were excised from the 2-day old seedlings and planted in sterile solid agar medium. They were incubated for 2, 4, 6, and 8 days in the dark at 30°. A typical experiment used 20 embryos in 100 ml of media.

Cotyledons were removed from 7 to 8-day old seedlings grown in the same fashion as described above for the 2-day old seedlings. Twenty to thirty cotyledons were cultured in 25 ml of liquid media for 48 hours at 30°.

Unless specifically mentioned, all cultures were carried out in the dark.

Culture Medium. A modified White's (7, 13)

[1] Received July 2, 1962.
[2] Supported in part by a Research Grant (GM-08624-02) from the National Institutes of Health, United States Public Health Service.
[3] For a preliminary account of this work see Federation Proc., 21, 467 (1962).
[4] Present address: Yamanashi National University, Kofu, Japan.

[5] Some data are reported as ricinine content per embryo, nicotinic acid content per cotyledon, etc. These results are from typical experiments using the number of embryos or cotyledons described. An analysis of the total amount of a component in all of the tissues used in a single experiment was made, and the results are reported on a per unit basis for clarity.