Kinetics of photosynthate translocation

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KINETICS OF PHOTOSYNT HATE TRANSLOCATION

by

Donald Boyd Fisher

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Biochemistry

Approved:

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1965
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I. INTRODUCTION AND LITERATURE REVIEW

The transport of substances in plants takes place largely through two sets of conducting tissues, the xylem and the phloem. The non-living nature of the conducting cells in the xylem and their obvious tubular form resulted in a more ready understanding of transport in that tissue than has been the case with phloem. In the phloem the question of how and where materials are transported is greatly complicated by the fact that all of the cells involved are maintained in a living state, and must remain alive in order for solute transport to occur (28).

A number of mechanisms have been proposed to account for transport in the phloem, and a myriad of experiments have been performed to test those proposals. In the work to be presented here, the main interest has been concerned with those experiments which have employed radioactive tracers, and their mathematical interpretations according to one transport mechanism or another.

One of the most attractive hypotheses offered as an explanation for phloem transport has been Münch's theory of mass flow between tissues of different osmotic pressures (72). The modern version of this theory envisions a solution of high sugar concentration being contributed to sieve tube endings in the leaf by the secretory activity of the cells in the vein
endings. At points of sugar utilization in the other parts of the plant, the loss of sugar from the sieve tubes causes a decreased osmotic pressure. The sugar solution is forced through the connecting sieve tubes along the pressure gradient caused by the difference in osmotic pressure. The principal difficulty with this mechanism is the question of whether there is an adequate pressure gradient generated to cause the observed flow rates (27, 54).

Spanner (90) has advanced a theory which also calls for the bulk flow of a solution in the sieve tubes. In this model, however, the motivating force is electroosmosis, caused by the cyclic pumping of potassium ions through the sieve plates in the direction of flow.

On the basis of their work with the cotton plant, Mason and Maskell (67) and Mason and Phillis (68) proposed that movement takes place by a process of "activated diffusion" through a stationary cytoplasm. To account for the transport rates observed, the apparent diffusion coefficient of sucrose must be about 40,000 times that of the usual coefficient. They did not suggest how this might be achieved.

Van den Honert (99) proposed that materials might be transported along a diffusion gradient at an interface in the sieve tube. He proposed that the tonoplast was the site of this interface, but sieve cells apparently do not have a tonoplast (e.g., 38), and the location of the proposed inter-
face is presently a matter of conjecture.

Several workers have suggested that protoplasmic streaming plays a key role in phloem transport. Curtis (30) suggested that cyclosis in the sieve elements accompanied by diffusion of sugar across the end-walls of adjacent sieve elements might account for the transport. However, streaming of the type he hypothesizes has not been observed in mature sieve elements (28), and the commonly observed translocation rates (about 1 cm min$^{-1}$) are an order of magnitude greater than cytoplasmic streaming rates in higher plants (59).

Thaine (95, 96) proposed a translocation model based on his observations of cytoplasmic tubules which, he says, traverse the sieve element lumen from one sieve plate to the other. The contents of some of the strands move up the sieve element while those of the others move down carrying carbohydrates which are in a dynamic equilibrium with sucrose in the sieve element vacuoles. This model is quite similar to one proposed by Canny (19), also based on Thaine's observations. These observations have been disputed by Esau et al. (36) and, although strands within sieve tubes have been described by others (27, 34, 39, 78), they often seem to be quite different from those described by Thaine. According to Evert and Derr (39), they are more fibrillar in nature, and he has not seen any motion associated with them. A recent description of streaming in phloem fibers (69) resembled the descriptions
made by Thaine but motion pictures of the streaming showed more bulk movement of protoplasm than Thaine reported. Also, Thaine did not observe reversal of streaming, but this was clearly demonstrated in the case of the fibers.

Radioactive tracers have been employed relatively recently in translocation studies, and the data available from them has encouraged the development of a number of mathematical models of translocation (22, 37, 54, 91). Since, at least in some cases, the proposed theories for phloem transport would be expected to result in different kinetic observations, the models have been largely concerned with mechanisms in the stem which would account for the data obtained.

Horwitz (54) considers six basic models for translocation, four of which (Models 1, 2, 5, and 6) assume a constant rate of tracer influx into the stem. In consideration of the large sugar and starch pools in the leaf in relation to the rate of carbon dioxide fixation, this almost certainly is an incorrect assumption, as Horwitz himself realized. Model 4 is not concerned with the movement of tracers. Model 3, in which he considers a time-variable source of activity, treats only the case where the rate of tracer influx into the stem increases according to the equation \( C_0 = k(1 - e^{-\gamma t}) \), where \( C_0 \) is the concentration of tracer leaving the leaf, and \( k \) and \( \gamma \) are constants. Although Horwitz rejects this model because it apparently cannot generate the exponential translocation
profile reported at that time (11, 102), the model has been treated more completely by Evans, Ebert and Moorby (37). They have shown that the exponential portion of the translocation profile in soybean is followed later in time by a more nearly linear rise in activity. Their equations are applicable to a chemical steady or unsteady state, but in the chemical steady state, which they consider to be most likely, the equations for the influx of tracer into the stem reduce to the same form as for Horwitz' Model 3. Vernon and Aronoff (102) have also demonstrated that the profile in soybean is linear at longer times (i.e., 45 minutes), but this is often overlooked because the data was plotted semilogarithmically.

Spanner and Prebble (91) have studied the transport of $^{137}$Cs in the petiole of Nymphoides peltatum, and presented a mathematical interpretation of their findings. They point out the difficulty of determining a translocation velocity when there is appreciable movement of tracer out of the translocation stream into the surrounding tissues, as obviously occurred in their experiments. The application of the conclusions drawn from experiments with $^{137}$Cs to the movement of normal translocates is of questionable value, however. Leakage of Cs, an ionic material, from the translocation stream into surrounding tissues, or adsorption within the stream itself, especially if tracers of high specific activity are employed, cannot be expected to reflect the behavior of sugar, the
material of primary interest.

Canny (19) has proposed a model for translocation based on Thaine's (96) observations on transcellular strands in sieve tubes, and he and Phillips present a mathematical treatment of the model (22). They assume that the downward moving strands are loaded with sugar at a source. This sugar is slowly exchangeable with the vacuolar sugar, which in turn slowly exchanges with the sugar in the upward moving strands. The questions to be raised concerning their model are numerous, however. In the first place it apparently cannot account for the rates of transport observed. Canny himself (19) has pointed out that sugar must be transported in the phloem at a rate of 3-24 gms cm\(^{-2}\) of sieve tube area per hour. If it is assumed that the positive strands occupy one-fourth of the sieve tube area, and their velocity is 60 cm hr\(^{-1}\), the concentration difference between the upward and downward moving strands must be 0.2 gms cm\(^{-3}\) of sugar in order to effect a transport rate of only 3 gms cm\(^{-2}\) hr\(^{-1}\).

It is also difficult to rationalize the water relations which must exist in the strands and vacuole. If the sucrose in the strands is in an osmotically active form, and the membranes bounding the strands are extensible, it would seem that the concentrations would be rapidly equalized in all compartments simply due to the diffusion of water between them. After the concentrations become the same, there cannot
be any net transport of sucrose.

Several of the boundary conditions for the partial differential equations in this paper are incompletely stated and are sometimes misleading when they are given. For example, in the discussion of the general properties of the system (p. 390), the initial distribution of sucrose along the strands is said to apply to $S$, the sum of sucrose concentrations in the two sets of strands. In fact, in order to arrive at the solutions given, the condition must apply to $s_1$, the downward moving strands. The concentrations in the upward moving strands and vacuole, $s_2$ and $s_0$, respectively, must be zero.

Further, in the discussion of diffusion profiles, the statement is made that intake into the sieve tubes is maintained constant after a time $t = 0$, and therefore $s_1$ will be constant after $t = 0$. This cannot be true, however, since the model is concerned with infinite strands, but in the leaf the actual loading mechanism for the strands must be considered. Presumably the upward moving strands would be loaded with sugar and reverse to become downward moving strands. In this case, $s_1$ at $x = 0$ would actually be the sum of $s_2$ at $x = 0$ plus whatever sugar was deposited by the loading mechanism. In an unsteady state, which is the condition under discussion, this would require that the actual rate of loading of radioactive sugar must decrease with time in order for $s_1$ to remain
constant.

The plots of curves obtained from the mathematical model are not compared fairly with experimental results. Only the solution for $s_1$ is plotted, but supposedly the experimentally obtained values for the distribution would be $s_1 + s_2 + 2s_0$. Although this may be justified in the case of "wave profiles," it cannot be in the case of "diffusion profiles" in which, by definition, significant amounts of radioactivity are contained in the terms $s_2$ and $s_0$.

A final criticism, which can be made to varying degrees for all presently available mathematical models, is that there is virtually no experimental data available concerning the actual kinetics of tracer efflux from the source. Horwitz's (54) and Evans, Ebert and Moorby's (37) time-variable source depends on a constant rate of tracer incorporation into the source pool, which is completely mixed and drained at a constant rate by the translocation stream. There are, however, a number of possibilities which would invalidate this model. The most obvious of these are the questions of intermediates, compartmentation, interconversions between the sugars and other metabolites, especially starch, and of the rate of movement of translocate from its area of production to the source pool.

If the movement of labelled photosynthate is being studied, the label must pass through intermediate sugar
phosphate pools before appearing as the free sugar. This alone will cause a delay of several minutes before the labelling of the translocate reaches a maximum. Burma and Mortimer (15) have shown this delay clearly in the case of sucrose labelling in sugar beet, a common subject of translocation studies.

Intracellular compartmentation of metabolites is commonly invoked to explain kinetic data, and has been well documented for amino acids (8), organic acids (66), and phosphorylated intermediates (82). In the case of sugars, the most obvious possibilities for compartmentation in the cell are the vacuole and cytoplasm. By isolating chloroplasts in a non-aqueous medium, Heber (49) has shown that, at least during some stages of growth of the wheat plant, the sugar concentration in the chloroplasts may be considerably higher than in the vacuole. By following the rates of labelling of compounds in the chloroplast and non-chloroplast fractions of spinach leaves, he and Willenbrink (50) conclude that reduced carbon is transported from the chloroplasts as sugar phosphates. Glucose diphosphate, uridine diphosphate glucose, and possibly sucrose are formed in the cytoplasm. Tolbert, however, has proposed that glycolate is the form in which reduced carbon is exported from the chloroplasts (97). He bases his hypothesis on observations of glycolate excretion from algae and from chloroplast preparations, and on the very high metabolic
activity shown by glycolate and related compounds.

A second possibility for compartmentation arises from the characteristics of the tissues within the leaf. The veins and their border parenchyma are often essentially non-photosynthetic compartments, because the chloroplasts in the border parenchyma are often small and few in number (4), at least in dicotyledons. The epidermal layers of the leaf also offer a distinct possibility for tissue compartmentation. Yemm and Willis (112) have shown that on a residual dry weight basis, the lower epidermis may contain about twice as much sucrose as the mesophyll. These concentrations could, of course, be more similar on a fresh weight basis. They also showed that the changes in the sucrose levels in the mesophyll and epidermis do not always occur in a similar manner, which would imply a slow rate of exchange between the two.

Interconversions between sugars and other metabolites are numerous. Starch is potentially the most important in this respect. Chan and Bird (23) have shown that in tobacco leaves an exchange of radioactive carbon can occur between sucrose and starch even during periods of net starch synthesis. Loss of radioactivity from starch was especially noticeable at low light intensities.

Finally, there is the question of the rapidity of movement of the translocate from its site of production to the veins. Little is known concerning this rate, and Evans, Ebert, and
Moorby (37) propose that a slow rate might account for the initial exponential portion of the translocation profile in soybean. It is pertinent to note in this connection that on the basis of the comparative anatomy of many plants, Haberlandt has proposed a "theory of expeditious translocation" (44). The essence of this theory is that plant photosynthetic structures are specialized in such a way as to provide a ready path for removal of photosynthate to the conducting tissues. Wylie (110) has shown that the spongy parenchyma, a laterally oriented structure, takes up a larger portion of space in the leaf as the distance between veinlets increases.

Since there are so many factors which might affect the rate of tracer efflux from the leaf, the present study was undertaken in order to provide some experimental data and theoretical interpretations of these factors. Several recent publications suggest that the shape of the translocation profile is actually generated within the leaf. Mortimer (71) has shown that in the sugar beet petiole the profile moves as an advancing front with little change in shape. Moorby, Ebert and Evans (70) have provided a similar picture from their excellent experiments with soybean. The shape of the translocation profile showed no systematic change in shape or height as it progressed down the soybean stem.
II. ANATOMIC OBSERVATIONS

In order to provide some basic anatomical data on soybean, a few observations were made on the general features of the soybean leaf and conducting system, and some measurements were made of the phloem.

A. Observations on the Leaf Structure

Observations of mature soybean leaves revealed the presence of an unusual layer of mesophyll, one cell thick, extending horizontally between the vascular bundles in the plane of the phloem. This layer consists of a regular network of cells in contact with the lower layer of palisade parenchyma. Because of its relation to the veins, it will be referred to as "paraveinal mesophyll." Figure 1 shows the paraveinal mesophyll in a paradermal section of a soybean leaf which was fixed in formalin-acetic acid-alcohol (FAA), sectioned at 10 μ and stained with iron hematoxylin-safranin (83).

In contrast with the spongy parenchyma cells, which have arms extending in all directions, forming a three-dimensional network of cells, the arms of the paraveinal mesophyll cells extend only in a horizontal plane, forming a two-dimensional network. The border parenchyma cells at the level of the phloem have extensions which contact adjacent paraveinal

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1 The writer is indebted to Dr. N. R. Lersten for his interest and criticisms concerning the anatomical observations on soybean leaf.
mesophyll cells (Figure 1). The paraveinal mesophyll cells contain chloroplasts which are of a smaller size than those of the other mesophyll cells, and in this respect the paraveinal mesophyll resembles the border parenchyma. The cells are fairly large, extending over a distance of more than 100 μ, and, except for the largest interveinal areas, all of them are in contact with a cell of the border parenchyma. This could greatly facilitate horizontal conduction within the leaf.

The appearance and location of the paraveinal mesophyll in relation to the other tissues of the leaf were confirmed by examining soybean leaves which had been cleared in sodium hydroxide and chloral hydrate, dehydrated in alcohol, and stained with safranin (modified from (5)). In such preparations the appearance of this layer was fully in agreement with the foregoing description, and thus ambiguities which might arise in reconstructing a three-dimensional picture from sections were eliminated.

In cross sections of fixed material, a distinctive paraveinal mesophyll layer is difficult to recognize, but may be distinguished by its general horizontal orientation and small chloroplasts (Figure 2). A small veinlet is in the center of the figure. The paraveinal mesophyll contacts it precisely at the level of the phloem. Comparison of Figures 1 and 2 shows its spatial relationship to the other leaf tissues, particularly to the palisade parenchyma and veins.

In hand-cut sections of fresh leaves the lack of chlorophyll in the paraveinal mesophyll makes that layer much more
Figure 1. Paradermal section of a soybean leaf, at the level of the paraveinal mesophyll (1 cm = 65 μ). Palisade parenchyma appears in the top of the figure, and spongy parenchyma in the lower right hand corner.
Figure 2. Cross-section of a soybean leaf (1 cm = 35 µ)  
Two layers of palisade parenchyma appear under the upper epidermis. The paraveinal mesophyll appears directly under the second layer of palisade cells. Spongy parenchyma cells are immediately beneath the paraveinal mesophyll.

Figure 3. Hand-cut cross-section of a fresh soybean leaf (1 cm = 27 µ)
noticeable than in chemically-fixed material (Figure 3). Treatment of sections of fresh leaves with I$_2$-KI showed that the paraveinal mesophyll contained far less starch than the remainder of the mesophyll. In all mesophyll cells the starch was confined to the chloroplasts.

This layer of cells was of particular interest in relation to the studies on the translocation of photosynthate since it appears that carbon fixed in the photosynthetic cells must pass through the layer to reach the veins. Although photosynthate is known to be transported to the veins quite rapidly (62), cells which might be specialized for this intermediate step of transport have not been described previously. Modifications in the paraveinal region of the mesophyll have been described (44), but the degree and character of differentiation found in soybean is apparently unique. Photographs showing paraveinal mesophyll in soybean have been published (32), but the special features of the layer were not mentioned.

In proposing that the paraveinal mesophyll in soybean has a special function of horizontal conduction in the leaf, it is enlightening to compare the interveinal distances in soybean to those obtained by Wylie (110). In support of his hypothesis that the laterally oriented tissues (spongy mesophyll and epidermis) are important in horizontal conduction, he studied the relationship between the intervascular
distances and the amounts of palisade parenchyma, spongy parenchyma and epidermis. He found that, in general, leaves with large interveinal distances had more laterally oriented tissues and less vertically oriented tissues. Although the correlation is not strong, and his method of measuring intervascular distances seems to the writer to be somewhat arbitrary, the relationship does seem to exist. In soybean the veinal pattern is net-like and the average dimensions of ten interveinal areas was 157 $\mu \times 239 \mu$. Even the smaller dimension is large enough to place it among the herbaceous plants listed by Wylie as having the largest interveinal distances. However, the amount of palisade parenchyma in soybean (50%) is exceeded by only three species on his list, all of which have intervascular distances of less than 80 $\mu$. The specialization of the paraveinal mesophyll in soybean could account for this apparent deviation from Wylie's findings.

B. Observations on the Phloem

Examination of soybean petioles and stems fixed in FAA and stained with iron hematoxylin-safranin showed a typical organization of the phloem (35). The sieve plates are simple, with approximately 50-70 pores occupying an estimated 60% of the sieve plate. Measurements with an ocular micrometer showed that the pores were about 0.5 $\mu$ in diameter although
they were too small to measure accurately, and it was not possible to tell whether this actually was the diameter of the perforation. The thickness of the sieve plate was about 1-2 μ. The length of the sieve elements was usually about 125 μ. The diameter of the sieve elements varied, particularly between those in the petiole and those in the stem. The average diameter, that is, the average of two mutually perpendicular distances across the sieve plate, of thirty sieve plates in the petiole was 5.1 μ, with extremes of 4 μ and 6 μ. Corresponding values for the stem of 33 sieve plates were 6.5 μ average diameter, and extremes of 5 μ and 7.5 μ.

The cross-sectional area of the translocation stream was also of interest from two points of view. In the first place, the mathematical model for tracer efflux from a leaf required that the cross-sectional area of the translocation stream be proportional to the amount of leaf area which had exported sugar to it. The cross-sectional area of the translocation stream leaving the leaf was also desired for other calculations concerning the model.

To accomplish these two aims, hand-cut cross sections of the veins in soybean leaflets were stained with I₂-KI, which variously stained the tissues surrounding the phloem but did not stain the phloem itself, that is, the sieve tubes, companion cells and phloem parenchyma. The leaflet area upstream from each section, as indicated by the leaflet venation, was
outlined on a piece of paper. The phloem area was outlined by tracing the image projected on the viewing screen of a Zeiss photomicroscope. The areas of the leaf and phloem were determined by using a planimeter.

The fraction of the phloem area occupied by the sieve tubes was estimated to be about 20% by staining other fresh sections with aniline blue and examining under a fluorescence microscope (29). The sieve plates and often the sieve tube walls fluoresced yellow after this treatment. This figure is a minimum value, since it is possible that the sieve plates of some tubes were missed in cutting.

The results of the measurements are shown in Figure 4. As hypothesized, the phloem cross-sectional area is a linear function of the leaf area which contributes translocate to it. The slope of the graph indicates that each square centimeter of leaf area is drained by 740 \( \mu^2 \) of phloem tissue, or 148 \( \mu^2 \) (740 \( \mu^2 \times 0.2 \)) of sieve tubes. However, although this relationship held within the leaf itself, the cross-sectional area of phloem in the petiole, which supported a leaf of 36.1 cm\(^2\), was 33,500 \( \mu^2 \), or 928 \( \mu^2 \) of phloem per square centimeter of leaf surface. Either the structure of the phloem is different there, or there is a real increase in the cross-sectional area of the phloem where it passes out of the leaf.

The values of phloem area per square centimeter of leaf surface are about one-fourth of those reported by Crafts (26)
Figure 4. Relationship of phloem area to leaf area
in similar measurements of the phloem area in petioles of *Tropaeolum* and *Phaseolus*. This may be a real difference or may be due to the low light intensity used to grow the present plants (280 foot-candles; see Section III, A1), or to differences in the procedure of measuring the phloem area, since he was not using iodine-stained sections.
III. ISOTOPIC EXPERIMENTS

A. Materials and Methods

1. Plant material

Soybean plants (*Glycine max*, var. Hawkeye) were grown in soil in a Scherer growth chamber under 280 foot-candles of light (measured by a Gossen Tri-Lux foot-candle meter) supplied by Gro-Lux fluorescent bulbs. A low light intensity was used to develop plants with stems long enough to be suitable for translocation studies. The plants were grown in a cycle of 14 hours of light at 29°C and 10 hours of dark at 18°C. Experiments were run with plants between 26 and 30 days old, when they had two or three mature trifoliate leaves. The first trifoliate leaf, which was used for all experiments, had an area of about 35-40 cm², and a fresh weight of about 0.5-0.6 grams. The distance from the leaf to the roots was usually more than 30 cm. Before the experiment, the shoot was excised just above the node of the first trifoliate leaf. This was done to provide a simple transport pattern and to make the plants comparable to those being used simultaneously in other experiments with tritiated water.
2. **General feeding and sampling procedures**

Depending on the purpose of the experiment, the first trifoliate leaf was fed in one of a number of feeding chambers. In each of these chambers the petiole was sealed with modeling clay and stopcock grease at the point where it passed into the feeding chamber. Radioactive carbon dioxide was generated by injecting 10% aqueous perchloric acid into a generator well containing the barium carbonate-$^{14}$C with a hypodermic syringe inserted through a rubber nipple. The atmosphere was circulated by a finger pump at a rate of 1200 ml per minute.

All feeding experiments were run in a fume hood with a strong blower. Pulse-labelling experiments were terminated by stopping the pump and clamping off the hose connections to the feeding chamber, which was then opened in the draft of air provided by the hood blower.

When feedings were carried out over long periods of time, an empty chamber was included in the system to minimize changes in the carbon dioxide level, and so approach a chemical steady state. A chemical steady state was an important requirement for these experiments, since the data would otherwise be difficult to interpret. If such a buffering chamber were included in the system, the $^{14}$CO$_2$ was generated before the start of the experiment and circulated for at least 30 minutes through the system, which did not include the feeding
chamber. The latter was spliced into the system at zero time. The amount of Ba\(^{14}\)CO\(_2\) used in such experiments was never sufficient to change the CO\(_2\) level by more than 10%.

The circulating atmosphere was monitored in several experiments with a thin end-window G. M. tube (6) attached to a ratemeter and recorder. The G. M. tube was always placed so as to monitor the atmosphere coming from the buffering chamber. A separate experiment showed that even when the buffering chamber was quite large (about 85 liters), the time for the activity distribution in the system to equilibrate following \(^{14}\)CO\(_2\) generation was only a few minutes. It was therefore unnecessary to stir the chamber.

Except for a few early experiments, light was provided by a bank of two 24-inch Gro-Lux bulbs which were adjusted to provide the same light intensity at the leaf surface as existed in the growth chamber (280 fc). The light distribution beneath the bulbs was fairly uniform except at the ends, where it could be raised to the desired level by placing pieces of aluminum foil over the bulbs to act as reflectors.

In some experiments the leaf was sampled by taking a punch from the leaf with a paper punch. The punches were taken at random, except that no punch was ever taken "upstream" from a previous punch, since in that case the translocation stream from the punch would have been severed by the previous punch. The positions of the samples taken for
four experiments are indicated in Figure 5. The area of the punches was found to be 0.334 cm$^2$ by weighing a number of punches from a piece of paper whose weight per unit area had been previously determined.

Stems were cut into 2-cm sections, starting at the petiole. A holder for the sections was constructed by folding a two-inch wide piece of heavy plastic lengthways and stapling across it at intervals. This provided a sort of "ammunition belt" for the sections. The stem sections could be inserted into successive slots and the whole belt rolled up and plunged into liquid nitrogen, where it remained until the sections were extracted. If the staples were placed fairly close together, the sections would not fall out.

3. Extraction

The samples taken during the experiment were extracted three times for at least forty minutes during each extraction in hot 80% aqueous ethanol. In experiments where the lipids were also chromatographed, 0.005% BHT (4-methyl-2, 6-di-tert.-butyl phenol) was added to prevent oxidation of the lipids (109).

When small samples such as leaf punches and stem sections were being extracted, the extract was placed in a side arm test tube (25 mm x 125 mm) in a 50°C water bath and taken to dryness by blowing through a stream of air. A length of glass
Figure 5. Sequence of samples taken during experiments

A. Section III, B3b
B. Section III, B3a
C. Section III, B5
D. Section III, B7
tubing, which extended to within a few centimeters of the surface of the extract, was held in place by a cork stopper. The air stream entered through this tube and exited via the side arm of the test tube. If lipids were to be chromatographed, nitrogen was used. By arranging the gas stream to pass through several tubes in succession, a number of samples could be taken to dryness at one time. When large samples were extracted, the extract was taken to dryness in a rotary flash evaporator, in a water bath at 35°C.

Lipids were removed from the dried extract with Skelly F. In the case of small samples, two aliquots of about one milliliter each removed virtually all of the color. When working with larger quantities of tissue, some of the lipid remained underneath encrusted matter. The latter had to be redissolved in ethanol or acetone, taken to dryness again, and re-extracted. When the lipids were to be chromatographed, 0.005% BHT was included in the Skelly F.

4. Chromatography of water soluble materials

The water soluble materials from the dried extract in the side-arm test tubes were transferred to the origins of unwashed Whatman No. 1 filter paper by dissolving them in about fifteen microliters of water. The test tubes were then centrifuged briefly to collect the remaining solution at the bottom, which was also spotted on the chromatogram. This was
repeated twice more, washing the sides at the bottom of the tube with each aliquot. Recovery of the radioactivity remaining in several tubes showed that more than 95% of the activity had been removed from the tubes. Chromatography and autoradiography of this activity showed no qualitative differences between it and the bulk of activity which had been removed from the tubes by the routine washing procedure described above.

Depending on the purpose, the chromatograms were developed in two dimensions, ascendingly or descendingly. Generally speaking, ascending chromatography was used when maximum resolution was not required. In this case, they were run in wide-mouth jars, as described by Aronoff (6). The first solvent was 80% aqueous phenol (v/v) (100), and the second was a solution of n-butanol-propionic acid-water (similar to (9)). The latter was made up by preparing two solutions, one of n-butanol-water (624:42, v/v), and the other of propionic acid-water (335:88, v/v). The chromatographic solvent was prepared just before use by mixing equal volumes of these two solutions. A map of the compounds separated by these solvents is shown in Figure 6.

For descending chromatography, the extract was spotted on large sheets of filter paper in a corner 2½ inches from each edge. The chromatogram was first developed in the shorter direction to the edge of the paper in aqueous phenol. After
Figure 6. Autoradiogram of water-soluble materials separated by ascending chromatography
drying, it was developed in the long direction for thirty hours in the butanol-propionic acid-water solvent. The chromatograms were dried and trimmed to 14 inches x 17 inches for autoradiography with Kodak No-Screen Medical X-Ray film. Descending chromatography resulted in some loss of activity, since some solvent dripped off the edge of the paper during development in the second solvent. However, the loss was slight, as may be seen by comparing these autographs (Figure 7) with those of the ascending chromatograms (Figure 6).

For several purposes, it was desirable to have a third solvent for water soluble materials. Descending chromatography in ethyl acetate-pyridine-water (2:1:2, v/v) (57) was used to separate sucrose and asparagine in a few cases where they were incompletely resolved, and to separate the water-soluble products resulting from the hydrolysis of polar lipids. It was also used as an auxiliary solvent for the identification of some radioactive compounds on the chromatograms.

5. Chromatography of polar lipids

Glass plates, 20 cm x 20 cm, were coated to a depth of 0.25 mm with Silica Gel G (E. Merck Co., Darmstadt, Germany)

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1The writer is indebted to Dr. C. L. Tipton for his interest and suggestions concerning the investigation of the soybean lipids.
Figure 7. Autoradiogram of water-soluble materials separated by descending chromatography.
applied as a suspension of 30 gm gel in 60 ml distilled water. The plates were activated by a final drying at 120°C for 30 minutes. The Skelly F extract, containing the lipids, was taken to a small volume under a stream of nitrogen and spotted on the thin-layer plate, which was then developed one-dimensionally in chloroform-methanol-water (65:25:4, v/v) (104). A map of the polar lipids separated in this system is shown in Figure 8. The relative Rf's of sulfolipid and Lipid #2 were dependent on the amount of lipid chromatographed. When large amounts were chromatographed, as here, sulfolipid ran ahead of Lipid #2. At intermediate concentrations, they were virtually superimposed.

The lipids were autoradiographed by placing the thin-layer plate, gel side down, on X-ray film. The position of the plate on the film could be marked by scratching the emulsion along the edge of the plate with a sharp instrument. Radioactive spots were located on the thin layer plate by fastening a piece of Saran Wrap tightly over the autoradiogram with tape, and outlining the spots with a ballpoint pen. The Saran Wrap was then similarly fastened over the thin-layer plate, and the radioactive areas were outlined by pricking the silica gel with a needle.

Since it is known that sugars may be carried into non-polar solvents in the presence of lipids (63), it seemed desirable to determine how much of the activity present in the
Figure 8. Autoradiogram of lipids separated by thin-layer chromatography.
Skelly F extract was due to non-lipid materials. For this purpose, a soybean leaf was fed radioactive carbon dioxide for 50 minutes, extracted, dried, and delipidated with Skelly F. A similar Skelly F extract from a "cold" leaf was added to the delipidated materials from the "hot" leaf and dried. Some 80% aqueous ethanol was added, and the solution was refluxed with boiling chips for 45 minutes. This mixture was dried and extracted with Skelly F. The amounts of activity in the fractions are shown in Table 1.

Table 1. Amount of non-lipid radioactivity in Skelly F extracts

<table>
<thead>
<tr>
<th>Fraction</th>
<th>cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skelly F soluble from &quot;hot&quot; leaf</td>
<td>$2.83 \times 10^4$</td>
</tr>
<tr>
<td>Skelly F soluble from &quot;cold&quot; leaf</td>
<td>$1.44 \times 10^4$</td>
</tr>
<tr>
<td>Water soluble from &quot;hot&quot; leaf</td>
<td>$3.23 \times 10^5$</td>
</tr>
</tbody>
</table>

The lipids from both the "hot" and "cold" leaves were chromatographed and autoradiographed. The autoradiograms of the "cold" lipids showed about as much activity within 0.16 $R_f$ unit of the origin as that of the "hot" lipids. A second much lighter band of activity appeared at $R_f$ 0.47 (i.e., about the same as that of sulfolipid), and two very faint bands at $R_f$'s 0.59 and 0.62. The latter three bands were not
comparable in activity to the authentic lipids of the "hot" extract with $R_f$'s above 0.5.

The materials close to the origin were eluted with water and chromatographed descendingly in ethyl acetate-pyridine-water (2:1:2, v/v). After autoradiography, the chromatogram was sprayed with p-anisidine (6). The results of this showed that most of the activity present was sucrose, with some fructose, and probably glucose. (P-anisidine is very insensitive to glucose.) From these results, one can calculate that half of the activity in the Skelly F extract from the "hot" leaf was non-lipid material. The amount of "water soluble" activity carried into the non-polar solvent represented almost 5% of the total water soluble activity. This was due to the presence of lipids, because re-extraction of delipidated extracts with Skelly F resulted in only slight amounts of radioactivity in the Skelly F.

6. Identification of water soluble compounds

Tentative identification of most of the spots could be made from published chromatographic maps produced by similar solvents (9). The identities were confirmed by spotting about 50-100 micrograms of a known compound at the origin of a chromatogram along with some crude radioactive extract. The specific activity of the extract was great enough so that none of the compounds present would be detected by the sprays
used. The chromatogram was developed two dimensionally and sprayed to detect the reference compound. Benzidine acetate (9) was used to detect reducing sugars, p-anisidine (9) to detect raffinose, sucrose and fructose, 0.25% ninhydrin in acetone to detect the amino acids (9), and a modified Riegler's nitrite test (85) to detect organic acids.

In case of ambiguity, the radioactive spot was also co-chromatographed with standards in ethyl acetate-pyridine-water. This was particularly important in confirming the identities of serine, glycine and glutamate, since radioactive glycine was absent from the chromatograms after short labelling times.

Several additional tests were made to establish the identity of some of the other compounds. An extract was deionized by passing it through the strong anion and cation exchangers IRA 400 and IE 120 and chromatographed two-dimensionally. The autoradiograms showed spots corresponding to glucose, fructose, sucrose, unknown a, unknown R, a compound directly below fructose, and one just below alanine. The latter two compounds were not considered important since they only appeared after long periods of photosynthesis, and were lightly labelled. From published maps, the spot below alanine may be ribose. Maltose and raffinose did not appear in chromatograms of the crude extract used for deionization, which explains their absence in the deionized extract. In
addition to co-chromatography, maltose was identified by hydrolysis to glucose with amyloglucosidase (Section III, A10).

The identity of unknown $g$ was of special interest because of the kinetics it exhibited in the labelling experiments, and because it had previously been identified by Vernon (100) as raffinose. Co-chromatography of "raffinose" from one of his chromatograms with authentic raffinose showed that it actually was the compound designated here as unknown $g$. To test the possibility that it was an oligosaccharide, it was subjected to invertase ($0.03\%$, $35^\circ C$, for one hour), and hydrolysis in $2N \text{ HCl}$ at $100^\circ C$ for 3 hours. Neither treatment changed its $R_f$ in ethyl acetate-pyridine-water, nor did it co-chromatograph with stachyose, melibiose, or lactose on two-dimensional chromatograms.

No attempt was made to identify unknown $g$. On the basis of published maps, it seems quite possible that it may be either citric acid or isocitric acid.

7. **Identification of lipids**

Tentative identifications of several of the polar lipids were made by comparing the autoradiogram of a two-dimensional thin-layer plate, developed as described by Lepage (64) with his map of the polar lipids from plants. Treatment of the plates with iodine vapor and various sprays also assisted in
determining the identity of some of the lipids, especially the sterols. Iodine vapor\(^ {89}\) revealed a number of leaf lipids, including all of those which are identified here, except sterol glucoside and esterified sterol glucoside. In the stem, where there was relatively more sterol glucoside, it, too, gave a faint spot, although it is possible that this was due to another lipid with the same \( R_f \). Lepage reported that sterol glucoside was not detected with iodine. Esterified sterol glucoside was not detected with iodine, but it was present in quite small quantities in both the leaf and the stem.

Treatment of the plates with antimony pentachloride in carbon tetrachloride (1:4, v/v)\(^ {81}\) revealed the galactolipids, sulfolipid, and sterol glucoside.

Spraying the plates with 20% aqueous sulfuric acid\(^ {81}\) followed by heating at 120\(^\circ\)C for about five minutes gave light grayish-brown spots for all lipids except sterol glucoside and its ester, which gave distinctive reddish-purple spots. The formation of colored compounds on treatment by sulfuric acid is characteristic of sterols\(^ {87}\). A reddish-purple color was also given by some substances which had run close to the front. Those lipids which had begun to char at this stage showed considerable orange-yellow fluorescence under ultraviolet light. This was not true of the sterols, however. Further heating produced more charring of all of the lipids.
including the sterols, which then fluoresced yellow. The other lipids did not show as strong a fluorescence as after the five-minute heating.

Heating the plate to 120°C for five minutes, followed by spraying lightly with 20% aqueous perchloric acid (64) resulted in brown spots for most of the lipids, and a reddish spot for sterol glucoside. On standing, sterol glucoside turned purple. All of the spots fluoresced orange-yellow in ultraviolet light, except sterol glucoside, which was more yellowish. In general, the results were similar to those obtained with sulfuric acid, except the colors were considerably paler, and the spray seemed generally less sensitive.

Further identification was made by hydrolysis of the lipid in acid and base, and chromatography of the water soluble products in ethyl acetate-pyridine-water (2:1:2, v/v). The lipids were eluted from the silica gel using a procedure described by Abramson and Blecher (2). The powder was suspended in a few milliliters of chloroform-methanol-water-formic acid (97:97:4:2, v/v) and mixed with a Vortex Jr. mixer for about one minute. The suspension was centrifuged, the supernatant transferred to a test tube and the procedure repeated twice more. The supernatant was taken to dryness by blowing a stream of nitrogen through the test tube, which was in a 50°C water bath. Before adding acid or base for hydrolysis, about 100 microliters of methanol was added to
dissolve the lipid.

Basic hydrolysis was carried out by adding 2.0 ml of 2N NH₄OH and heating for three hours on a steam bath, in a test tube with a spiral condenser. The hydrolysate was taken to dryness in a rotary flash evaporator at 40°C, and then taken up in a few microliters of water and spotted on the origin of a chromatogram. The use of ammonium hydroxide for hydrolysis eliminated the necessity of neutralizing the hydrolysate, since the base was lost during evaporation. The procedure worked quite well for the galactolipids, but the hydrolysis of the other lipids seemed to be incomplete. The reasons for this apparent difference were not investigated, since the galactolipids were quantitatively more important.

Acid hydrolysis was carried out in a manner similar to that for basic hydrolysis, except that 2.0 ml of 1N HCl was used. The hydrolysate was taken to dryness by placing it in vials over sodium hydroxide pellets in a vacuum desiccator. This drying procedure was employed, hopefully, to reduce the degradation of sugars by the acid, but analysis of known amounts of galactose (25 micrograms) taken through the drying procedure showed a recovery of only 80% (20 micrograms).

If it was desired to ascertain the distribution of radioactivity between the fatty acids and water soluble portions of the lipid, the partitioning solvents described by Dawson (31) were employed. The solvents are prepared by equilibrat-
ing one volume of water with two volumes of isobutanol-chloroform (1:2, v/v). One milliliter of the upper (aqueous) phase and two milliliters of the solvent (lower) phase were added to the dried acid hydrolysate, which was then shaken and centrifuged to separate the resulting emulsion. Aliquots were taken from each phase for counting.

The galactolipids were identified primarily by their hydrolysis products. Acid hydrolysis yielded one compound which contained the bulk of radioactivity and which co-chromatographed with galactose. Basic hydrolysis of monogalactosyl diglyceride gave a compound which had an $R_f$ slightly lower than galactose, and was presumed to be monogalactosyl glycerol (84). Digalactosyl diglyceride gave a radioactive compound which had a considerably lower $R_f$ than galactose, and was presumed to be digalactosyl glycerol (84).

Acid hydrolysis of sterol glucoside produced a compound which contained the bulk of radioactivity, and which co-chromatographed with glucose.

Acid hydrolysis of sulfolipid produced a compound which contained the bulk of radioactivity and had a low $R_f$ in both phenol-water and butanol-propionic acid-water, and was therefore presumed to be glucose sulfate (10).
8. **Quantitative analysis of sugars**

Wager's (103) modification of Nelson's (75) method for determining reducing sugars was employed for glucose, fructose, galactose, and sucrose. This method is based on the reduction of copper sulfate and development of a blue color with arsenomolybdate. The procedure was modified slightly in that the final dilution to 25 ml was not made, thus increasing the sensitivity. Sucrose was hydrolyzed with 0.01% invertase for 10 minutes at 30°C. To obtain consistent results, it was found necessary to heat the copper solutions for 20 minutes rather than 10, as described by Wager. A five microgram standard was included during each run of unknowns as a check on the procedure.

All of the sugars were rechromatographed one-dimensionally in butanol-propionic acid water on commercially acid-washed filter (Schleicher & Schuell, No. 398) before determination. A standard curve prepared for sucrose by chromatographing the standards separately, eluting them, and determining them by Wager's method is shown in Figure 9. The amount of interference from the paper is quite small. This was also true of blanks from unwashed Whatman No. 1 paper, although there seemed to be more variation in the blanks from that paper.

The sensitivity for glucose, fructose, and sucrose was identical, at 12.5 Klett units per microgram. For galactose
Figure 9. Standard curve for sucrose
the sensitivity was less, at 7.3 Klett units per microgram. From the results of many standard curves, the error of the method was judged to be about ± 15%. Above 20 Y, the error was somewhat less, and below 5 Y, more.

9. Radioactivity determinations

Samples in solution were counted by drying an aliquot on a copper planchet and counting in a Nuclear-Chicago flow gas counter with a Micro-Mil window. The counting efficiency was determined by counting a standard planchet, and varied during the course of the experiments between 30-33%, because of different windows, cleaning, etc. Samples too radioactive to be counted directly were shielded by one or more thicknesses of aluminum foil and counted. A correction factor was obtained by counting a known sample which was also shielded.

Combustion of samples was carried out as described by Jeffay and Alvarez (56), except that the carbon dioxide was trapped in 10 ml of the absorbing solution, and 1.0 ml of this was added to 19.0 ml of the liquid scintillant. The overall counting efficiency of the procedure was determined by combusting two samples of standard \( { }^{14} \text{C} \)-benzoic acid (Nuclear-Chicago, 5080 dpm/mg). The values obtained were 60.3% and 59.7%, for an average of 60%. The value obtained by counting standard \( { }^{14} \text{C} \)-toluene (Nuclear-Chicago, \( 4.38 \times 10^5 \) dpm/ml) in 19.0 ml of liquid scintillant plus 1.0 ml of absorbing
solution was 62.5%.

Radioactive spots on the chromatograms were cut out and counted in one of two different ways. In the first, the paper was counted in the Nuclear-Chicago flow gas counter. Spots which were too large were either cut into smaller pieces or eluted and counted. Counting efficiency was determined by counting several spots, eluting, and recounting. The corrections for absorption by the paper in these cases were 2.96, 3.07, and 2.86, for an average of 2.97.

The second method of counting chromatographic spots was to place them in counting vials containing about 20 ml of the liquid scintillant described by Jeffay and Alvarez (56). Although the results from this method were felt to be satisfactory, there was more variation in the counting efficiencies, probably due to differences in solubilities of the compounds in the liquid scintillant (105). The efficiencies for a number of the compounds is given in Table 2. The spot was counted first in the flow gas counter to determine the total activity.

Lipids were counted on the thin-layer plate by placing a thin end-window Geiger-Muller tube over the spot, which had been located as described in the section on lipid chromatography. The window was shielded from other radioactive areas nearby by covering those parts with six layers of aluminum foil. Counting efficiency was determined by counting several
Table 2. Counting efficiencies obtained by counting radioactivity on paper in the liquid scintillation counter

<table>
<thead>
<tr>
<th>Compound</th>
<th>Counting efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine</td>
<td>53.5</td>
</tr>
<tr>
<td>Fructose</td>
<td>62.5</td>
</tr>
<tr>
<td>Aspartate</td>
<td>55.4</td>
</tr>
<tr>
<td>Alamine</td>
<td>55.5</td>
</tr>
<tr>
<td>Glucose</td>
<td>62.2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>58.5</td>
</tr>
<tr>
<td>Malate</td>
<td>56.2</td>
</tr>
</tbody>
</table>

spots in this manner, scraping them from the plate, and combusting them to determine total activity. Counting efficiencies determined in this way for three spots were 2.96%, 3.06%, and 2.55%, for an average of 2.86%.

10. Starch hydrolysis

Leaf starch becomes labelled quickly and heavily during photosynthetic assimilation of radioactive carbon dioxide (16). Since Chan and Bird (23) have shown that in tobacco leaves there is a carbon exchange between starch and sucrose, even during net starch assimilation, it was desirable to follow the kinetics of labelling in the starch, as well as in ethanol soluble compounds. However, a procedure for the
selective extraction of starch from samples as small as those used in the present experiments was not available in the literature. Because it was not necessary to maintain the starch molecule intact, it seemed that a procedure which would hydrolyze the starch from the extracted samples selectively would be simplest. The one employed in these studies used salivary amylase, followed by amylglucosidase, to hydrolyze the starch completely to glucose, which could then be quantitatively determined. The technique is simple and may be used with large numbers of samples of any size. Since the analytical procedure for glucose allows the determination of less than five micrograms of glucose, extremely small amounts of starch can be determined quantitatively.\textsuperscript{1}

Salivary amylase was obtained by collecting saliva in a beaker in an ice bath. Calcium acetate was added at a final concentration of 10mM, and the precipitated mucopolysaccharides were removed by centrifuging at 12100 x g for 20 minutes.

A commercial preparation, "Agidex 3000," of the amyloglucosidase from \textit{Aspergillus niger} was obtained from Glaxo Laboratories, Ltd., Middlesex, England. This enzyme will hydrolyze \(\alpha-1,6\)-, as well as \(\alpha-1,4\)-linked glucose (1).

\textsuperscript{1}The writer is indebted to Dr. W. J. Whelan for suggesting the use of amyloglucosidase, and to Drs. J. F. Robyt and M. Abdullah for their advice and comments on this procedure.
To establish the conditions adequate for complete hydrolysis, and to check for the presence of non-glucose carbon-14 released during the procedure, a preliminary experiment was run in which three samples of ethanol-extracted leaf tissue were incubated with the enzymes. The leaf had assimilated $^{14} \text{CO}_2$ at a steady state for three hours, and had been in atmospheric (i.e., unlabelled) $\text{CO}_2$ for 15-20 minutes before extracting.

Each sample was dried, weighed, and placed in a 25 mm x 125 mm test tube with some acid washed sand. A few drops of water were added, and the mixture was ground with a stirring rod for about a minute. Debris clinging to the end of the stirring rod was washed into the test tube with a small amount of water. The homogenate was made up to about two milliliters with water, and autoclaved at 235°F for 15 minutes to swell the starch grains to render them more susceptible to hydrolysis by the enzymes.

The supernatant from one tube was removed after centrifugation, and the residue washed twice with 3 ml aliquots of water, and recentrifuged. Both washings were added to the original supernatant, and made up to 5.0 ml. Unfortunately, the sample pan prepared to determine the total activity in this fraction was lost. However, from the activity on the chromatograms it was estimated to be about one-fourth that in the starch. This is in agreement with a later experiment
(Section III, B6), where about one-third of the activity was soluble after autoclaving. Since its specific activity was the same as the starch (Table 4), this would indicate that the oligosaccharides are a major carbohydrate pool in the leaf.

After taking the supernatant to near dryness, one aliquot was subjected to two-dimensional ascending chromatography, and another was hydrolyzed with amyloglucosidase and also chromatographed. The activity in the unhydrolyzed supernatant was distributed in four main areas on the autoradiogram. Two distinct spots of about equal activity appeared in the area usually occupied by organic acids in this system, with Rf's in phenol of about 0.14 and 0.23, and in butanol-propionic acid-water of about 0.30 and 0.41, respectively. Considerable activity remained at the origin, and more was streaked from the origin up to about Rf 0.38 in the phenol direction and to 0.10 in the butanol-propionic acid-water direction. Sucrose was not present.

The hydrolyzed supernatant showed large amounts of glucose, and considerably less material at the origin and in the streak from the origin. The two "organic acids" were unaffected. The relative amounts of the various compounds on this chromatogram are shown in Table 3. It was concluded from this work that the supernatant following autoclaving contained considerable amounts of starch-like oligosaccha-
Table 3. Distribution of $^{14}$C in an amyloglucosidase hydrolyzate of the water-soluble materials released by autoclaving ethanol-extracted leaf tissue

<table>
<thead>
<tr>
<th>Compound</th>
<th>Per cent of total activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Organic acid&quot; 1</td>
<td>7.2</td>
</tr>
<tr>
<td>&quot;Organic acid&quot; 2</td>
<td>8.0</td>
</tr>
<tr>
<td>Origin + streak</td>
<td>19.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>66.0</td>
</tr>
</tbody>
</table>

Oligosaccharides. This was confirmed by Linden and French¹ who, by chromatography of these same oligosaccharides in a solvent more suitable for separating maltodextrins and by treatment with $\beta$-amylase, showed that at least the lower molecular weight oligosaccharides (i.e., up to $G_{13}$) are linear chains of $\alpha-1,4$-linked glucose units. A similar series of oligosaccharides has been reported in algae (16), green gram (Phaseolus radiatus) (76), and in liver (88), where they were thought to be concerned with glycogen synthesis. In his studies on the intracellular localization of sugars, Heber (49) reported "tri-, tetra- and pentasaccharides" which were present in the chloroplasts but not the cytoplasm.

The glucose from the hydrolyzed oligosaccharides was prepared for quantitative analysis by rechromatography in butanol-propionic acid-water on the acid washed S&S filter paper. The autoradiogram showed small amounts of a second compound with about half the $R_f$ of glucose. There was no indication of the possible presence of this compound in the original two-dimensional chromatogram. It was not sucrose, since some radioactive sucrose had been chromatographed next to it on the same chromatogram, and its $R_f$ was definitely higher than the unknown. No attempt was made to identify this compound.

To hydrolyze the starch in the autoclaved samples, an equal volume of the undiluted salivary amylase was added to each tube, along with a few grains of thymol. They were then stoppered and incubated for 24 hours at 37°C with occasional swirling. In another experiment, tests of the residue for starch with $I_2$-KI at this stage were negative. Aliquots from each tube were chromatographed ascendingly in two dimensions. The autoradiograms showed large amounts of maltose and glucose, and a fairly prominent spot about halfway between maltose and the origin which quite probably represented branched-chain oligosaccharides from amylopectin. These were the only spots appearing on the autoradiogram of the sample from which the supernatant had been removed following autoclaving. In the other autoradiograms the non-oligosaccharide
spots mentioned earlier also appeared in small amounts.

Following the treatment with salivary amylase, 0.33 ml of a solution of amylglucosidase (1 mg "Agidex 3000" per ml in 0.25M sodium acetate buffer, pH 4.5) was added to the tubes, and the temperature was raised to 55-57°C. The tubes were restopped after reaching this temperature, and incubated for another 24 hours. Aliquots of 5 microliters each were removed from the samples to follow the course of the hydrolysis, and chromatographed twice ascendingly in butanol-propionic acid-water. The autoradiogram for one of the samples from which the supernatant following autoclaving had not been removed is shown in Figure 10. Virtually all of the activity has been converted to glucose, and in the autoradiogram of the sample from which the supernatant had been removed, there was no other activity at all.

Aliquots were taken from each sample for counting, and another aliquot was chromatographed to isolate the glucose and determine its specific activity. The quantitative determinations for glucose were run in duplicate for each sample, and all of the duplicates agreed within 3%. Direct determinations of glucose on aliquots from samples from a different experiment gave amounts of glucose which were higher than those obtained by chromatography of the glucose by about 30%. The results are shown in Table 4, along with the specific activity of the oligosaccharide glucose, sample weights, total activity,
Figure 10. Progress of hydrolysis by amylglucosidase

A. Origin
B. Maltose
C. Glucose
D. "Organic acid 1"
E. "Organic acid 2"
Table 4. Total activities, specific activities, and amounts of starch and maltodextrin oligosaccharides in soybean leaf samples

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Dry wt. (mg)</th>
<th>Total activity (dpm)</th>
<th>Gluc. spec. act. (dpm/γ)</th>
<th>Total glucose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.415</td>
<td>6.13 x 10^7</td>
<td>2.54 x 10^4</td>
<td>2.42</td>
</tr>
<tr>
<td>2</td>
<td>10.236</td>
<td>4.49 x 10^7</td>
<td>1.99 x 10^4</td>
<td>2.26</td>
</tr>
<tr>
<td>3</td>
<td>15.940</td>
<td>8.23 x 10^7</td>
<td>2.15 x 10^4</td>
<td>3.83</td>
</tr>
<tr>
<td>2 (Oligosacch.)</td>
<td>1.1 x 10^7 (est.)</td>
<td>2.20 x 10^4</td>
<td>0.6 (est.)</td>
<td></td>
</tr>
</tbody>
</table>

and total glucose, calculated on the basis that all of the activity is in the glucose. The total activity will be somewhat low because of the aliquots removed, but the total fraction removed was less than 10%.

Combustion of the washed residue from one of the samples showed an amount of activity remaining which amounted to about 4% of that which had been released by the procedure for starch hydrolysis.

Except, of course, for the checks during hydrolysis, the procedure followed in later experiments was largely the same as given above. Twenty-four hour hydrolysis periods were used because it was convenient to leave the samples overnight. The supernatant, however, was not removed following autoclaving, with the exception of one experiment. This was initially
felt to be unnecessary, since there was so little non-glucose activity present, and the specific activity of the oligosaccharide fraction was so close to the starch that it seemed very possible that they were at least quite closely related metabolically. It also seemed possible that the oligosaccharides were actually degradation products of starch caused by the extraction procedure (47). Several later observations made this interpretation doubtful, however. In the first place, Gibbs (42) has reported quite different labelling patterns in dextrins and in starch isolated from sunflower leaves following short periods of photosynthesis in radioactive carbon dioxide. The distribution of label in the carbon atoms of dextrin glucose was virtually the same even after the shortest photosynthetic period, whereas the glucose from starch was labelled much more strongly in carbon atoms 3 and 4, and it took several minutes for the label to become uniformly distributed.

In the second place, in the only experiment where the kinetics of labelling in the starch and oligosaccharides was followed separately, the curves obtained for the two were quite different (see Figure 33). It also seems likely that the type of degradation to be expected during extraction would be more or less random from sample to sample, and would not give the regular kinetic curves found for the oligosaccharides (Figure 33) or for maltose (Figures 22 and 35). For these
reasons, it does not seem likely that the oligosaccharides are artifacts created during extraction.

B. Results

1. Progression of the translocation profile down the conducting tissue

   a. Experiments with soybean  At the time these experiments were begun, neither Moorby, Ebert and Evans' (70) nor Mortimer's (71) results had been published. The data in the literature available at that time, however, was sufficient to strongly suggest that the translocation of solutes could be described by a single velocity, and that there was very little loss from the translocation stream. It was felt that if one could demonstrate that the translocation profile did not change in shape as it passed down the stem, this would be strong evidence against both a velocity gradient and removal of translocate from the translocation stream. The following experiments were performed to test this hypothesis.

   Six plants were prepared for feeding in the manner described in the previous section, except that the side leaflets of the experimental leaves were excised and only the center leaflet was fed. The leaflets were fed simultaneously in a rectangular plexiglass feeding chamber, 24" x 3" x 3/4",
which had a removable top, with notches along one side for the petioles. A twenty-liter bell jar was included in the system to maintain an approximate steady state. The stems were excised at intervals, sectioned, and stored under liquid nitrogen. The leaves could not be harvested, since there was no way to remove them from the feeding chamber.

Two experiments were run. In one of them, one of the plants was inadvertently injured, and another was used for another purpose. The data are shown in Figures 11 and 12. There is no obvious difference in the shapes of the profiles at different times, and the pattern resembles the results one would expect if all of the translocate moved with the same velocity. A similar picture is obtained from a plot of cpm vs. section number. The average velocities calculated from the intercepts at log cpm = 1.0 are 0.84 cm min⁻¹ from Figure 11, and 1.05 cm min⁻¹ from Figure 12. These values are in good agreement with rates reported by other authors for soybean (70, 100).

The profiles from the plants harvested at the shortest times showed a quite irregular distribution of radioactivity ahead of the main "front". Even the sections at the bases of the stems showed slight amounts of activity. This has been reported by other authors (73, 74), who felt that it might be due to a "fast" component of translocation which accounted for the movement of minor amounts of materials.
Figure 11. Advance of radioactive profiles in soybean stems; first experiment
Figure 12. Advance of radioactive profiles in soybean stems; second experiment
b. Experiments with barley

Monitoring the translocation stream at various distances from the source should give similar curves at each position, if the assumptions made for the mathematical models are correct. In order to test this hypothesis, and to test the model for a linear leaf, barley plants (*Hordeum vulgare*) were grown under the same conditions as those described for soybean. The plant used was 42 days old, and the terminal 9 cm of a mature, healthy leaf was fed in the hemispherical feeding chambers described by Aronoff (6). The remainder of the leaf was supported and flattened by threads criss-crossed between two pieces of wood. Illumination was provided by two 250-watt tungsten lights which were placed above and below the leaf to supply uniform lighting. A thin end-window G. M. tube, shielded with aluminum foil except for a \( \frac{1}{4} \)"-wide slot, could be accurately positioned at any of three positions, 1.7, 3.7, and 15.7 cm from the feeding chamber, along the upper surface of the leaf. The number of counts registered during one minute periods was recorded for each position.

The feeding of \(^{14}\text{CO}_2\) was terminated after 30 minutes by opening the hemispheres. At the end of the experiment, the sections of the leaf which had been monitored were extracted and counted. The extracted section was counted while still wet to obtain a rough correction to the final count rate observed at each position. This was done in order to deter-
mine the count rate due to soluble materials, most of which are presumably translocated, while insoluble ones are not. The corrections amounted to about 15% of the final count rate. All of the data was corrected in this manner for insoluble material by assuming that it had been accumulated linearly during the experimental period. The count rate due to the soluble materials was then calculated. The results are plotted in Figure 13. Two-dimensional ascending chromatography of the ethanol-soluble extract showed that about 95% of the activity was in sucrose.

It is obvious that the data obtained from this experiment is not consistent with the concept of a single velocity of translocation without loss of translocate. Instead, it appears that there has been considerable exchange, at least, of the translocate. The reasons for the difference in the behavior of this system and that of soybean and sugar beet are not apparent. The results are given here in order to illustrate the fact that there may very well be exceptions to the assumptions made in the models. The experiment was an early one, which was the reason for the use of incandescent rather than Gro-Lux bulbs. In some later experiments with soybean leaves in which a small area of the leaf was fed by sealing the end of a piece of glass tubing against lower surface (similar to the experiment by Aronoff (7)), only 1% or less of the ethanol soluble radioactivity fixed was
Figure 13. Kinetics of $^{14}$C accumulation at various points along a barley leaf following feeding of the leaf tip.
translocated in 25 minutes. When the entire leaf is fed, 3-6\% is translocated in that time. It may be possible that stopcock grease seals applied directly to the leaf surface cause some anomalies in translocation. (The soybean experiments are not reported for this reason. It was also difficult to prevent the seals from leaking slightly.) Nevertheless, it is obvious that translocation did occur in this experiment and that either there was a velocity gradient or loss of material from the translocation stream, or both.

2. **Carbon-14 kinetics in the soybean leaf during steady state labelling: An exploratory experiment**

One of the first attempts at following the $^{14}C$ kinetics in the leaf during steady state labelling provided an excellent example of how the sucrose concentration in the leaf may change quite rapidly for no obvious reason.

A feeding chamber similar to the one described for the second steady state labelling experiment (Section III, B7, and Figure 34) was used in this experiment. This chamber was an earlier version of the other one, and differed essentially in that the top was a petri dish cover, and the sides were of a heavy plastic wrapping material. Single samples were taken at each sampling time by a paper punch which was inside the chamber and could be manipulated through the loose plastic bag on the side of the chamber.
Illumination was provided by a 200 watt tungsten light placed about 12 inches above the feeding chamber, with a \( \frac{1}{2} \)" water shield between the two. An 83-liter plexiglass chamber was placed in the system to maintain a steady state. At the end of the feeding period the hose connections to the feeding chamber were clamped and disconnected. They were then connected to a CO\(_2\) trap containing 100 ml of CO\(_2\)-free 0.1N NaOH. The remaining \(^{14}\)CO\(_2\) was circulated through the trap until virtually all of the activity had disappeared from the system. The carbonate was precipitated with saturated BaCl\(_2\), dried and weighed (6). The specific activity of the \(^{14}\)CO\(_2\) was calculated from the amount used for the experiment minus the fraction which had been lost during the experiment, as was indicated by the difference in the count rates in the atmosphere at the beginning and end of the labelling period. The rate of \(^{14}\)CO\(_2\) disappearance during the experiment was very nearly linear with about 25\% of the activity finally disappearing.

The CO\(_2\) absorption and weighing procedure was checked by generating the CO\(_2\) from 296 mg BaCO\(_3\) in a smaller system (about one liter). The CO\(_2\) was absorbed, precipitated, dried and weighed as before. The amount recovered was 308 mg, or 104\% of the initial amount.

To provide assurance that translocation actually was occurring, the amount of activity in the ethanol-extractable
materials in the stem and root was determined and found to be $1.5 \times 10^6$ cpm, indicating that a substantial amount of movement had occurred.

The samples were chromatographed in two dimensions using ascending chromatography. Quantitative determinations were run on sucrose and fructose. Some of the results from this experiment are shown in Figures 14-17. The last samples came from a side leaflet and this may be the reason for the difference shown by the last few points. The data for sucrose is particularly interesting since the amount per sample almost triples during the experiment. The reason for this is not clear, but the most obvious difference between this and later experiments is the lighting employed. In order to assure a steady state in later experiments, the plant was removed from the growth chamber, subjected to surgery, etc., and kept under the experimental lighting conditions in the hood for at least two hours before feeding.

The amount of barium carbonate collected from this experiment requires some comment. If it is assumed that the CO$_2$ concentration at the start of the experiment was 0.03\% (52), then only about 170 mg of BaCO$_3$ should have been collected. Instead, 1.475 gm was collected, or almost nine times as much as expected. The part of the system included during the CO$_2$ absorption period apparently does not leak seriously, since monitoring of the system after $^{14}$CO$_2$ generation and
Figure 14. Carbon-14 kinetics in some sugars and organic acids during steady state labelling of a soybean leaf.
Figure 15. Carbon-14 kinetics in some amino acids during steady state labelling of a soybean leaf.
Figure 16. Sucrose and fructose concentrations during the steady state labelling experiment.

Figure 17. Specific activity of sucrose and fructose during steady state labelling of a soybean leaf.
before feeding in a similar experiment showed a loss of activity of about 2% after one hour. Furthermore, the specific activity of the sucrose at the end of the experiment is only about two-thirds that of the Ba$^{14}$CO$_3$, and has finished rising. This is comparable to the situation found in the second steady state labelling experiment (Section III, B7), where more than the expected amount of Ba$^{14}$CO$_3$ was also recovered. The only possible explanation of this would appear to be that the CO$_2$ concentration in the laboratory air actually was higher than normal when the system was set up.

3. Quantitative behavior of the sucrose pool size

   a. Daily variations in the sucrose pool in a soybean leaf

   In view of the results obtained from the first steady state labelling experiment, it seemed advisable to follow the level of the sucrose pool during the day to determine whether a chemical steady state was attainable at all. For this purpose, an experiment was run in which single punches were taken from a plant in the growth chamber at approximately hourly intervals, extracted and chromatographed descendingly for 30 hours in butanol-propionic acid-water. The pattern of sampling is shown in Figure 5B. The sucrose spots were located by chromatographing several other spots of crude extract which could be sprayed with p-anisidine to reveal the sucrose. Variation in the distances traveled by the samples
could be compensated for by the behavior of naturally fluorescent compounds which ran close to sucrose.

The sucrose was determined quantitatively, and the dried, extracted punch was weighed on a microbalance. By weighing several samples more than once, the weighing error was judged to be ± 15 micrograms.

The results of this experiment are shown in Figures 18 and 19. There is apparently little variation in the amount of sucrose present during the day. (The "day" was shortened in order to observe the effect of turning off the light.) Even after the lights were turned off, the amount remained fairly constant for several hours. The dry weight of the extracted punches, however, increased constantly during the day until the lights were turned off, when it apparently started to decrease. This may represent starch deposition and dissolution. By using the slope of the graph, a rate of starch deposition can be calculated on this assumption, and gives a figure of about 7 g hr\(^{-1}\) per punch or about 21 g hr\(^{-1}\) cm\(^{-2}\) of leaf surface. This may be compared to the value of 30 g hr\(^{-1}\) cm\(^{-2}\) obtained from a later steady state labelling experiment (Section III, B7).

b. The effect of excision on the sucrose pool size in a soybean leaf

The effect on the sucrose pool of interrupting the translocation stream was of interest for two reasons. First, it was desirable to have some idea of what might happen
Figure 18. Sucrose concentration in a soybean leaf during the day.
Figure 19. Extracted dry weight of samples taken from a soybean leaf during the day.
to the pool size if translocation were blocked by taking a
large number of punches from a leaf. Second, if the primary
effect were simply an accumulation of sucrose, the rate of
accumulation might be taken as an indication of the rate of
sucrose translocation from the leaf.

Starting about six hours after the lights had come on in
the growth chamber, single punches were taken from the center
leaflet of a trifoliate leaf at 15-minute intervals. After
five samples had been taken to determine the level of sucrose
prior to excision, the leaf was excised and its petiole placed
in a vial of water at the same height in the growth chamber as
the leaf had been while on the plant. The major veins were
also cut at their juncture with the midrib to prevent loading
of the veins with sucrose. Sampling was continued for two
hours following excision. The pattern of sampling is shown
in Figure 5A.

The results are shown in Figure 20. Before excision, the
sucrose level was about four micrograms per sample. Following
excision the level doubled within two hours, increasing to
about eight micrograms. The effect of excision is largely
what one might expect as a result of blocking the transloca-
tion of sucrose from the leaf. The rate of accumulation is
about two micrograms per hour or, since the leaf area was
about 35 cm$^2$, 210 micrograms per hour for the entire leaf
($2 \gamma \, hr^{-1} \times 35 \, cm^2 / 0.334 \, cm^2 = 210 \, \gamma \, hr^{-1}$). This is probably
Figure 20. Behavior of the sucrose pool in an excised soybean leaf
a minimal value since it is quite probable that some of the carbohydrate which normally would have been translocated was converted into starch and/or hexoses. Geiger (41) found that when translocation was blocked in sugar beet, there was a considerable increase in the amount of radioactivity in glucose and fructose.

4. The kinetics of $^{14}$C accumulation in the petioles of soybean and kidney bean leaves following pulse labelling

In order to have additional data on the efflux of tracer from leaves beyond that which was implied by the translocation profiles, a soybean leaf was pulse-labelled, and the upper side of its petiole was monitored with a G. M. tube connected to a ratemeter and recorder. For comparison of this data with that obtained from a leaf without a specialized paraveinal mesophyll, a kidney bean leaf (Phaseolus vulgaris) was also pulse-lavelled and its petiole monitored in a similar manner in another experiment.

Two hours before the labelling period, surgery was performed on the plant, which was then placed under the experimental lighting conditions (Gro-Lux lamps) in the hood. Radioactive carbon dioxide was fed to the leaf for about three minutes. About two centimeters of the petiole were monitored in each case, and the G. M. tube was two centimeters away from the edge of the leaf.
The results are shown in Figure 21. The count rates were converted to a common scale by normalizing the curves to a similar maximum count rate, which was about 4000 cpm in both cases. The curves have been smoothed to eliminate the random variations in the count rate, as registered by the recorder. Due to a technical difficulty, the soybean experiment was not continued for as long as the kidney bean experiment.

The curves are remarkably similar, except for the first parts. Although the lag shown by the soybean curve certainly cannot be said to prove that the paraveinal mesophyll represents an intermediate pool of sucrose, it is at least consistent with that viewpoint and offers some circumstantial support for it.

5. Carbon-14 kinetics in the soybean leaf following pulse-labelling

In order to provide experimental data on the kinetics of various compounds, and possibly identify some of them with the source pool in this way, a soybean leaf was pulse labelled with \(^{14}\text{CO}_2\) and sampled at intervals over a three-hour period. Pulse labelling offers a number of attractive features both from theoretical and experimental points of view. In the first place, it has a reasonably sound theoretical interpretation (see Section IV, F). Also, since translocation continues over long periods of time, those compounds which are
Figure 21. Carbon-14 kinetics in soybean and kidney bean petioles following pulse-labelling.
primarily associated with the photosynthetic cycle will largely disappear from the chromatograms after the first few samples, thus simplifying their interpretation. Finally, it is much easier to maintain a chemical steady state throughout the experiment, and sampling is considerably simplified.

Two hours before feeding, surgery was performed on a soybean plant which was then placed under the experimental lighting conditions (Gro-Lux lamps) in the hood. Carbon dioxide from 5.0 mg Ba$^{14}$CO$_3$ (0.271 mc/mg) was fed using the hemispherical feeding chambers. Feeding was terminated by stopping the motor and clamping the hose connections to the hemispheres, which were then opened and removed as far as possible from the leaf. Samples of one punch each were taken from the leaf at various time intervals. The pattern of sampling is shown in Figure 5C. Only the first six samples could be extracted immediately, and the others were boiled in ethanol for about a minute and a half, allowed to cool, and placed in a deep freeze at -20°C until extraction could be completed.

a. Carbohydrates Data for the carbohydrates is shown in Figures 22-26. The sugar phosphates almost disappeared within fifteen minutes after labelling, but faint traces remained even after 100 minutes. Several of the samples were hydrolyzed with alkaline phosphatase (Intestinal alkaline phosphatase, Worthington Biochemical Corp.) and the hydrolysis
Figure 22. Carbon-14 kinetics in sugars following pulse-labelling of a soybean leaf.
Figure 23. Specific activity of sucrose following pulse-labelling of a soybean leaf
Figure 24. Sucrose concentration in the soybean leaf during the pulse-labelling experiment.
Figure 25. Carbon-14 kinetics in starch following pulse-labelling of a soybean leaf.
Figure 26. Carbon-14 kinetics in the residue following pulse-labelling of a soybean leaf.
products separated by two-dimensional ascending chromatography. Except for the first two samples (1 and 4 minutes), where other minor areas of activity appeared, almost all of the activity was in the aldohexose area of the chromatogram. Since there was obviously more than one compound there, this activity was re-chromatographed in ethyl acetate-pyridine-water. Three spots appeared, two of which were labelled strongly and ran quite close together with an Rf similar to that of glucose, which undoubtedly was the actual identity of one of them. A third spot, not labelled as strongly as the other two, had an Rf which was about two-thirds that of glucose.

Maltose and raffinose disappeared rapidly from the chromatograms. This behavior would seem to exclude their importance in relation to translocation but is of interest from the point of view of their metabolism. Raffinose behaves entirely differently from sucrose, which may be taken as a possible indication of sucrose compartmentation. Maltose, the lowest member of the maltodextrin series of oligosaccharides, behaves entirely differently from glucose, and from the oligosaccharide series taken as a whole (see Section III, B6 and Figure 33). Both raffinose and maltose appear to be turning over quite rapidly.

Unknown β is included with the data on sugars because it apparently is a neutral compound. Since its identity is
unknown, little can be said about its metabolism. The fact that it becomes labelled quite rapidly, increases for about 20 minutes and then decreases slowly makes it of interest from the point of view of translocation, since this is at least roughly the behavior to be expected of the source pool.

The behavior of sucrose, glucose and fructose is markedly similar to that reported by Geiger (41) following pulse labelling of sugar beet leaves, except that the changes are spread over a longer period of time. Under comparable conditions, the sucrose in his experiments disappeared with a half-time of about 20 minutes, and the hexoses were labelled maximally after about 30 minutes. In the present experiments, sucrose decays with a half-time of about 60 minutes, and the hexoses stop increasing after about 60 minutes. He also found the same slight difference in the behavior of glucose and fructose as that shown here. The kinetics of sucrose labelling strongly imply that it serves as the ultimate source, at least, for translocated sucrose.

The analytical data revealed a somewhat different picture for the specific activity curve for sucrose (Figure 23). The peak shown by the curve for total activity at about twenty minutes does not occur in the curve for specific activity. The only indication of a peak in the latter curve came from the first point. In order to clarify the kinetics for times less than 20 minutes, a second pulse-labelling experiment was
run in which three punches were included in each sample, and samples were taken more frequently. The details of that experiment are given in Section III, B6. At this time, it will be sufficient to refer to Figure 32, which indicates that for the present experiment, the data for specific activity (Figure 23) gives a more realistic picture of the kinetics than does the curve for total activity. The peak at 20 minutes shown by the total activity in this experiment is therefore regarded as either a brief chemical unsteady state or a misleading statistical fluctuation. Largely on the basis of the data obtained in Section III, A6, the specific activity curve in Figure 23 has been drawn to indicate a brief increase following the labelling period.

If it is assumed that the sucrose is in a single compartment which is drained by the translocation stream at a steady rate and constantly replenished at the same rate, its turnover time can be calculated from the rate of disappearance of label from the sucrose pool. By plotting the natural logarithm of the specific activity against time, a straight line should be obtained, the slope of which is the reciprocal of the turnover time (86). The data shown here does give a straight line in such a plot, and its slope indicates a turnover time for the sucrose pool of 90 minutes.

The analytical data for sucrose indicates that a fairly steady state was maintained during the experiment. In
particular, there was no increase in the amount of sucrose per
punch, as was observed in the first kinetics experiment and
which might indicate a cessation of translocation.

Both the starch and insoluble residue showed an increase
in activity during the experiment. (The insoluble residue is
included under the category of "carbohydrates" for convenience
only.) The starch increased by a sizeable amount, about $3.1 	imes 10^5$ dpm. This activity almost certainly comes largely from
sucrose, since it is the only other compound present which
loses enough activity to account for such an increase.
Unfortunately, these starch samples were ruined after count­
ing, and could not be analyzed for their glucose content.

b. Lipids  The data for the lipids is shown in Figure
27. Almost all of the lipids showed roughly the same label­
ing pattern, with the exception of sterol glucoside. In
addition, although it was not counted because it ran so close
to the pigments at the front, the kinetics of esterified
sterol glucoside appeared qualitatively similar to that of
sterol glucoside in that it increased at first then decreased
at later times. The amount of activity present in the
esterified sterol glucoside was considerably less than in the
sterol glucoside.

The behavior of sterol glucoside was highly interesting
for two reasons. In the first place, the shape of the curve
shown here is virtually identical to that of the curve in
Figure 27. Carbon-14 kinetics in polar lipids following pulse-labelling of a soybean leaf.
Figure 21 for the $^{14}$C kinetics in the petiole. Furthermore, the similarity of it and the curve obtained for sucrose is obvious, at least during later times. In the third place, sterol glucoside is the only lipid which becomes labelled in the stem during translocation (Section III, B8d). Although the exact significance of these observations is uncertain, their importance is obvious and will be discussed further in a later section (Section V). It seems quite likely that sterol glucoside and its ester are identical to the steroids reported by Biddulph and Cory in their studies on translocation in kidney bean (12).

Since the radioactivity in a complex lipid will be the sum of that contained in all of its parts, it is necessary to have information concerning the relative amounts of activities in the carbohydrate and fatty acid (or sterol) portions of the molecule. Accordingly, some of the monogalactosyl diglyceride samples were hydrolyzed with 1N HCl, and the dried hydrolysate was partitioned as described in Section III, A7. These hydrolyses were the first performed, however, and were carried out in 80% aqueous ethanol, which was found to cause considerable degradation of the galactose. Nevertheless, the results are felt to be valid indications of the relative amounts of activity in the water soluble and fatty acid portions of the lipid, and are in agreement with later data (Section III, B7b). After 45, 95, and 155 minutes, the amount
of activity recovered in the aqueous phase was 92%, 88%, and 84%, respectively, of the total activity. All of the original lipid activity was accounted for by the total recovered from the aqueous and non-aqueous phases. Data from later hydrolyses showed that almost all of the water soluble activity was present as galactose. A similar picture emerged for digalactosyl diglyceride and sterol glucoside (Section III, B7b). It is apparent, therefore, that the kinetics observed here are due largely to radioactivity incorporated into the hexose moieties of the lipids.

c. Amino acids and organic acids Little comment will be made on the amino acids and organic acids since their direct involvement in translocation is quite improbable, at least under the conditions of these experiments. The data is reported here (Figures 28 and 29) primarily for the sake of completeness. One point which is of interest, however, is the absence of radioactive glycine from the compounds appearing among the early products of photosynthesis. In view of Tolbert's suggestions (97) concerning the role of glycolate in the transport of reduced carbon from chloroplasts, this would be of particular significance since it suggests that the glycolate pathway is only of minor importance in these experiments. However, the presence of a small, rapidly turning-over pool would not be ruled out. Furthermore, Vernon and Aronoff (101) have identified considerable quantities of glycine
Figure 28. Carbon-14 kinetics in amino acids following pulse-labelling of a soybean leaf
Figure 29. Carbon-14 kinetics in organic acids following pulse-labelling of a soybean leaf.
among the products of short-term photosynthesis in soybean.

For these reasons, some further checks were made on the spots appearing in this area of the chromatogram of the sample taken one minute after the labelling period. Both the serine and glutamate spots were eluted and re-chromatographed in ethyl acetate-pyridine-water, but no radioactive glycine appeared. Furthermore, in later work where radioactive glycine did appear, it always separated satisfactorily from the two other amino acids, and spraying with ninhydrin showed that it was present in amounts at least roughly comparable to serine.

The area around the glutamate spot appeared somewhat distorted in the last twelve autoradiograms, suggesting incomplete resolution of two or more spots, and they were eluted and re-chromatographed in ethyl acetate-pyridine-water. At the time, it was thought that glycine might be the cause of the odd appearance of the spot, and the chromatogram was developed with this in mind. Unfortunately, the method used to separate glycine and serine required that the solvent drip off the end of the paper for some time, and apparently some other radioactive compound was lost. Neither glycine nor serine was present in significant amounts at these times, and there was no indication on the initial autoradiograms of a sharp change in the amount of glutamate between samples six and seven.

The initial sharp drop in activity in the "Malic-
Glyceric" curve is due to the disappearance of glyceric acid from the chromatograms. This was apparent from the autoradiograms, although the two acids often did not separate well enough to provide separate data.

d. Total activity The total activity in each sample was computed by totalling all of the activity in each compound for each sample. The error accumulated by determining total activity in this way is not as great as it might appear since almost 90% of the activity was in the starch, sucrose, and residue. Activity which was run off the paper in the butanol-propionic acid direction was not accounted for, of course, but in a steady state labelling experiment where all of the compounds were retained on the chromatogram (Section III, B2), compounds with Rf's above that of malic acid accounted for only about 1% of the water-soluble activity after 120 minutes.

The data for total activity is plotted in Figure 30. Although the curve for the data is drawn as indicating a decrease in activity, unfortunately it cannot be said to demonstrate this unequivocally. If the decrease occurs as shown, it amounts to a loss of about 10% of the total activity. Later experiments with pulse-labelled soybean indicated that this would be about half the amount of export to be expected (Table 5).
Figure 30. Total $^{14}$C per sample following pulse-labelling of a soybean leaf.
6. **Carbon-14 kinetics in soybean leaf following pulse-labelling, with special reference to the specific activity of sucrose during short times**

The ambiguity of the data for sucrose obtained from the first pulse-labelling experiment made it desirable to obtain additional data for times up to 20 minutes. Accordingly, a soybean leaf was pulse-labelled with $^{14}\text{CO}_2$ for one minute in a manner similar to the previous experiment. To reduce the amount of variation, especially in the analytical determinations for sucrose, three punches, one from each leaflet, were included in each sample. Total ethanol-soluble activity was determined by making up the extract to 5.0 ml and counting an aliquot.

The water-soluble activity following autoclaving of the extracted samples was analyzed separately from the starch in this experiment. The tubes were centrifuged after autoclaving, and the supernatant removed. The residue was washed twice by adding several milliliters of water, re-centrifuging, and removing the wash water, which was then added to the original supernatant. This solution was made up to 10.0 ml, an aliquot was removed for counting, and the remainder was incubated with amyloglucosidase. About two milliliters of this hydrolysate were taken to dryness in a side-arm test tube under an airstream, and chromatographed ascendingly in butanol-propionic acid-water. Only three areas of radioactivity
appeared on the chromatograms. Most activity was contained in glucose, and some was at the origin. A third compound was found only in the first two samples, and appeared just above glucose. No attempt was made to identify it. The total amount of activity in each of the three compounds was determined by counting all three and computing the fraction of the total activity accounted for by each.

The results of this experiment are plotted in Figures 31-33. The analytical data for sucrose is not shown, since there was little variation from sample to sample, with no systematic changes evident. The average amount of sucrose per sample was 15.0 micrograms, with extremes of 16.6 and 13.2 micrograms. The specific activity of sucrose reaches a maximum at about six minutes, and is fairly constant afterwards.

The behavior of the starch and maltodextrins is quite interesting. Like sucrose, starch continues to increase in activity after the labelling period, but the maltodextrins do not show a systematic change in either direction. This could be taken to indicate some differences in the synthetic pathways of these compounds, especially when considered together with Gibbs' (42) data on the distribution of $^{14}C$ in the hexoses from these compounds in sunflower plants.

The kinetic behavior of the unknown compound and the material remaining close to the origin of the chromatogram of the maltodextrin hydrolysate is also interesting. Although
Figure 31. Kinetics of total $^{14}$C and sucrose-$^{14}$C following pulse-labelling of a soybean leaf.
Figure 32. Specific activity of sucrose following pulse-labelling of a soybean leaf
Figure 33. Carbon-14 kinetics of ethanol insoluble materials following pulse-labelling of a soybean leaf. "Origin" refers to activity left at the origin of the chromatograms of the amylglucosidase digest of the ethanol insoluble, water soluble materials. The "unknown" is another compound found on the same chromatograms.
STARCH

RESIDUE AFTER STARCH HYDROLYSIS

ETHANOL INSOL, H₂O SOL. C¹⁴

MALTODEXTRINS

UNKNOWN

ORIGINS
the rapid disappearance of label from these two would suggest a possible role in photosynthesis, they would ordinarily be overlooked in most studies. Trip, Nelson and Krotkov (98) have recently reported that the primary products of $\text{CO}_2$ fixation in many leaves may be associated with the ethanol-insoluble portions of the leaf.

7. **Carbon-14 kinetics in the soybean leaf during steady state labelling**

Kinetic data for the metabolites in soybean leaf during steady state labelling was obtained by feeding a trifoliate leaf in the plexiglass feeding chamber shown in Figure 34. A paper punch was placed in the chamber and could be manipulated through the plastic bag on the side of the chamber. The apparatus in the bottom of the chamber was designed to remove the leaf punch after sampling with a minimum loss of $^{14}\text{CO}_2$. The piece shown below the chamber slides into the opening in the block in the bottom of the chamber. Before a punch is taken, the hole in the sliding piece is aligned with the hole in the top of the block and, after sampling, the punch is dropped into it. By moving the sliding piece a few inches to the right, the punch drops out through the hole in the bottom of the block. A rubber "O" ring around the upper hole increased the effectiveness of the seal between the block and the sliding piece.
Figure 34. Feeding chamber used for sampling a soybean leaf during steady state labelling.
Two hours before feeding, surgery was performed on a soybean plant which was then placed under the experimental lighting conditions (Gro-Lux) in the hood. An 83-liter Plexiglass chamber was included in the system to maintain a chemical steady state. The pattern of sampling during the experiment is shown in Figure 5D. As in the pulse-labelling experiment, only the first six samples could be extracted immediately. Later samples were boiled in 80% aqueous ethanol for about 1 1/2 minutes, cooled, and stored at -20°C until extraction could be completed. The $^{14}$CO$_2$ remaining after the experiment was absorbed in 0.1 N NaOH, precipitated, dried and weighed as described in Section III, B2. Again, more BaCO$_3$ was recovered than expected, although it was only twice as much in this case.

Many of the remarks made in connection with the pulse-labelling experiment (Section III, B5) are also applicable to the data from this experiment, and the comments here will be limited largely to the observations which are peculiar to this experiment. Theoretically (see Section IV, F), if the experiments are comparable in other respects, the curves obtained from this experiment should be the integrals of those obtained in Section III, B5.

a. Carbohydrates  Data for the carbohydrates is shown in Figures 35-40. The sugar phosphate curve showed a sharp drop after the first six samples, which may be due to the fact
Figure 35. Carbon-14 kinetics in sugars during steady state labelling of a soybean leaf
Figure 36. Carbon-14 kinetics in sucrose during steady state labelling of a soybean leaf.
Figure 37. Specific activity of sucrose during steady state labelling of a soybean leaf
Figure 38. Sucrose concentration during the steady state labelling experiment
Figure 39. Carbon-14 kinetics in starch during steady state labelling of a soybean leaf
Figure 40. Carbon-14 kinetics in the residue during steady state labelling of a soybean leaf.
that they were not completely extracted immediately. Bieleski (13) has pointed out some of the difficulties of halting phosphatase action when extracting plant tissues, and it is possible that incomplete deactivation of these enzymes is responsible for the break.

The sucrose specific activity shows a slight lag, then increases fairly rapidly for about the first hour. Although it still continues to increase slowly at later times and appears near isotopic saturation, it only reaches a specific activity which is about 62% of that predicted from the specific activity of the $^{14}$CO$_2$. This is identical to the value found at the end of the first steady state labelling experiment (Section III, B2), and is discussed more thoroughly there. The successful maintenance of a chemical steady state during the experiment is indicated by the analytical data for sucrose, shown in Figure 38.

Again, if it is possible to regard the sucrose as a single compartment being drained by the translocation stream, it should be possible to determine its turnover time by plotting the natural logarithm of the difference of its specific activity and the equilibrium specific activity versus time (53). The slope of the line will be the reciprocal of the turnover time. From the data, however, it is not obvious what the equilibrium specific activity is. If one chooses it to be the upper theoretical limit shown in Figure 37, the line
obtained is straight except for the last four points. If these last points are ignored the slope indicates a turnover time of about 70 minutes. If the equilibrium value is chosen to be the specific activity at the end of the experiment, the last five points are scattered, and the turnover time indicated is about 42 minutes.

Data for the starch and insoluble residue is shown in Figures 39 and 40. Aliquots from the starch hydrolysate were dried in side-arm test tubes under an air stream and chromatographed ascendingly in butanol-propionic acid-water prior to quantitative determination of the glucose. Both the glucose and non-glucose areas were counted for each sample, and it was found that glucose accounted for an average of 92% of the activity in all samples, with possibly slightly more non-glucose $^{14}\text{C}$ appearing at later times. Unfortunately, the amount of glucose per sample was quite erratic, and it was not possible to imply net starch synthesis from the data. The samples contained an average of 147 micrograms of glucose, with extremes of 116 and 207 micrograms. This variation was not felt to be due to analytical errors, since the aliquots analyzed contained about 10-15 micrograms of glucose. A figure for the rate of starch synthesis can be calculated from the rate of $^{14}\text{C}$ accumulation, however, if one assumes that the starch laid down has the same specific activity as the $^{14}\text{CO}_2$ (i.e., the same specific activity as indicated for the
theoretical upper limit for sucrose specific activity in Figure 37). The $^{14}\text{C}$ accumulation occurs at a rate of about $3.25 \times 10^6 \text{ dpm}/3 \text{ hrs}$, or about $1.1 \times 10^6 \text{ dpm hr}^{-1}$. This corresponds to a rate of about $10 \gamma \text{ hr}^{-1}$ of starch per punch ($1.1 \times 10^6 \text{ dpm hr}^{-1}/1.1 \times 10^5 \text{ dpm} \gamma^{-1}$), or $30 \gamma \text{ hr}^{-1} \text{ cm}^{-2}$, and is comparable to the figure given in Section II, B3a.

b. **Lipids** Figure 41 illustrates the kinetics observed for the various lipids.

Following the end of the experiment, the lipids were extracted from the side leaflets of the leaf and separated by thin-layer chromatography. Monogalactosyl diglyceride, digalactosyl diglyceride, and sterol glucoside were hydrolyzed in 1N HCl and the relative amounts of activity in the water soluble and fatty acid (or sterol) portions of the molecule were determined. In all cases, the total amount of activity subjected to hydrolysis was recovered in the sum of the activities in the aqueous and non-aqueous layers of the partitioning solvents. Portions of the aqueous phase were also chromatographed to determine the relative amounts of activity in the water soluble compounds.

The aqueous layer contained 87% of the activity from the monogalactosyl diglyceride. Of this, 92% was accounted for by galactose, 4% by glycerol, and 4% by an unknown which ran ahead of glycerol. For digalactosyl diglyceride, 95% of the activity was in the aqueous phase and, although this chroma-
Figure 41. Carbon-14 kinetics in polar lipids during steady state labelling of a soybean leaf.
togram was lost, the autoradiogram indicated a distribution between galactose, glycerol, and the same unknown compound similar to that obtained for monogalactosyl diglyceride. The hydrolysate of sterol glucoside yielded 98% of the activity in the aqueous layer, and all of this activity appeared as glucose on the autoradiogram.

c. **Amino acids and organic acids** The data for the amino acids and organic acids is shown in Figures 42 and 43. Glycine became labelled in this experiment, but only after relatively long periods of time, and increased slowly.

d. **Total activity** Total activity was calculated by totalling all of the activities present in each compound at each time, and is plotted in Figure 44. As in the pulse-labelling experiment, it is difficult to say from the results whether or not translocation is really occurring.

Assuming a leaf area for the fed leaf of 35 cm², a total $^{14} \text{CO}_2$ fixation rate may be calculated from the graph. The slope of the line indicates the fixation rate per punch, and is $2.42 \times 10^4$ dpm min⁻¹ ($4.0 \times 10^6$ dpm/165 min). For the entire leaf this is $2.54 \times 10^6$ dpm fixed per minute ($2.42 \times 10^4$ dpm x 35 cm² /0.334 cm² per punch).

Another $^{14} \text{CO}_2$ "fixation" rate can be calculated from the data obtained by monitoring the circulating atmosphere, which indicated that 13% of the original activity had been lost at a very nearly linear rate. Since 4.09 mc, or $9.08 \times 10^9$ dpm,
Figure 42. Carbon-14 kinetics in amino acids during steady state labelling of a soybean leaf.
Figure 43. Carbon-14 kinetics in organic acids during steady state labelling of a soybean leaf
Figure 44. Total $^{14}$C per sample during steady state labelling of a soybean leaf
had been fed, this would indicate a total loss of $1.18 \times 10^9$ dpm ($9.08 \times 10^9$ dpm x 0.13), or a rate of loss of $7.0 \times 10^6$ dpm min$^{-1}$ ($1.18 \times 10^9$ dpm/170 min). This is almost three times that calculated above, and indicates that there must be considerable leakage of $^{14}$CO$_2$ from the system. Presumably most of this leaks from the feeding chamber, which is not gas tight, since after generating the $^{14}$CO$_2$ and before feeding, a loss of only about 2% in one hour was indicated by the rate-meter.

8. Translocation in soybean plants following pulse-labelling

Most experimental data available on the kinetics of translocation has been obtained from the transport of photosynthate from steady state or quasi-steady state photosynthetic fixation of $^{14}$CO$_2$. Other than the data from Moorby, Ebert and Evans (70), there is no data available concerning the translocation kinetics following pulse-labelling in soybean. Their data concerns only the gross labelling patterns observed in the stem, however, and does not indicate the kinetics of the different compounds which become labelled. In view of the belief expressed by Clauss, Mortimer and Gorham (24) that the shape of the translocation profile is caused by the removal of tracer from the translocation stream, it was desirable to obtain more thorough data on the non-sucrose activity, especially the insoluble materials. It was felt that the
results of a pulse-labelling experiment would be easier to interpret from this point of view than a steady state labelling experiment would be. The results of this experiment would also provide some independent data on the kinetics in the leaf which could be compared with those in Section III, B5. Finally, by comparing the relative specific activities of sucrose in the leaf and petiole after various periods of time, one should be able to obtain some idea of how much of the sucrose in the petiole is actually in the translocation stream.

Six plants were prepared for feeding as described in Section III, A, and placed under the experimental lighting conditions (Gro-Lux lamps) in the hood two hours before feeding. The plants were fed in a rectangular plexiglass feeding chamber, 5" x 21" x 3/4", which had a removable top and three notches in each side for the petioles. A four-liter flask was placed in the system to maintain a chemical steady state, and a circulation rate of 6000 ml min\(^{-1}\) was maintained by a rotary peristaltic action pump during the three-minute labelling period.

The plant parts were harvested at various intervals after feeding. The times referred to indicate the time elapsed after the labelling period. The root was removed from the soil by dropping the entire contents of the flower pot into a pan of water and gently washing away the soil. The
roots and the leaf were extracted immediately. Stems were sectioned and frozen in liquid nitrogen. The first section from each stem was taken from that portion of the petiole inside the feeding chamber, and the second section came from the petiole outside the chamber. The lengths of each section were recorded, and the data from these sections has been normalized to 2.0 cm. The data from the bottom-most section was also normalized to 2.0 cm.

The lipids and water soluble materials in all of the leaves, all of the petiole sections just outside the feeding chamber, and all of the stem sections from two of the plants, those harvested at 75 and 125 minutes, were separated by thin-layer chromatography or two-dimensional descending chromatography, as appropriate.

The ethanol-insoluble activity from three stems, those harvested at 25, 75 and 125 minutes, was divided into three fractions. The sections were ground with sand and autoclaved, and an aliquot from the supernatant was counted. The remainder of the supernatant was removed, and the residue was incubated with salivary amylase for 24 hours. An aliquot of the resulting hydrolysate was counted. Tests of several samples with I₂-KI for starch were negative. The supernatant was removed and the residue was dried and combusted to CO₂ to determine the remaining insoluble activity. The total ethanol-insoluble activity in the other three stems was also
determined by combustion to CO₂.

The extracted leaf and root tissues were placed in a mortar with a few grams of sand and enough acetone to keep the tissue moist and pulverized to a fine powder. This powder was dried and weighed, and a weighed sample was combusted to determine total activity in the sample. The combustions were duplicated for each sample, and those for the leaves agreed within 5%. The duplicate root samples, which were not ground as thoroughly as the leaves, showed somewhat more variation, but agreed to within 10%. The error for the roots is of less concern, however, since it represented considerably less activity.

a. Gross distribution of radioactivity between leaf, stem and root

The amounts of activity found in the various plant parts and the total amounts recovered and translocated are shown in Table 5. The general pattern of translocation demonstrated by these figures may be summarized roughly as follows. After a brief lag a considerable amount of activity is transported from the leaf, with more than half of the final amount translocated being exported in less than 50 minutes. Following this the rate of export declines. Unfortunately, the data does not allow any more than this description. The plant harvested at 25 minutes seems to have exported sugar at a rate which is above average for the other plants, and the plant harvested at 100 minutes was apparently exporting at a
Table 5. Gross distribution of $^{14}C$ between the leaves, roots and stems of soybean plants following pulse-labelling

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Leaf</th>
<th>Stem</th>
<th>Root</th>
<th>Total fixed</th>
<th>Total translocated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EtOH-sol. (dpm x $10^{-7}$)</td>
<td>EtOH-insol. (dpm x $10^{-7}$)</td>
<td>EtOH-sol. (dpm x $10^{-7}$)</td>
<td>EtOH-insol. (dpm x $10^{-5}$)</td>
<td>(dpm x $10^{-8}$)</td>
</tr>
<tr>
<td>11</td>
<td>6.62</td>
<td>6.10</td>
<td>0.127</td>
<td>0.454</td>
<td>(not extracted)</td>
</tr>
<tr>
<td>%</td>
<td>51.5</td>
<td>47.5</td>
<td>0.99</td>
<td>0.0354</td>
<td>--</td>
</tr>
<tr>
<td>25</td>
<td>7.54</td>
<td>6.42</td>
<td>1.72</td>
<td>4.01</td>
<td>1.71</td>
</tr>
<tr>
<td>%</td>
<td>47.4</td>
<td>40.4</td>
<td>10.8</td>
<td>0.252</td>
<td>1.08</td>
</tr>
<tr>
<td>50</td>
<td>5.40</td>
<td>7.37</td>
<td>1.27</td>
<td>4.20</td>
<td>6.52</td>
</tr>
<tr>
<td>%</td>
<td>36.6</td>
<td>50.0</td>
<td>8.6</td>
<td>0.285</td>
<td>4.43</td>
</tr>
<tr>
<td>75</td>
<td>4.28</td>
<td>8.36</td>
<td>1.46</td>
<td>6.15</td>
<td>5.12</td>
</tr>
<tr>
<td>%</td>
<td>29.1</td>
<td>56.8</td>
<td>10.0</td>
<td>0.418</td>
<td>3.45</td>
</tr>
<tr>
<td>100</td>
<td>3.23</td>
<td>5.78</td>
<td>0.569</td>
<td>3.72</td>
<td>6.76</td>
</tr>
<tr>
<td>%</td>
<td>31.2</td>
<td>55.9</td>
<td>5.50</td>
<td>0.360</td>
<td>6.54</td>
</tr>
<tr>
<td>125</td>
<td>3.84</td>
<td>7.59</td>
<td>0.985</td>
<td>7.29</td>
<td>15.9</td>
</tr>
<tr>
<td>%</td>
<td>26.9</td>
<td>53.3</td>
<td>6.92</td>
<td>0.512</td>
<td>11.2</td>
</tr>
</tbody>
</table>
below-average rate.

There was very little insoluble activity present in either the stem or the root. After 25 minutes, which is the duration of the exponential shape of the translocation profile in soybean, less than 3% of the activity in the stem was insoluble. The increase in insoluble activity in the leaf is also interesting, since this is in agreement with the observations in Section III, B5, which showed that the amount of radioactivity in the starch increased slightly following pulse-labelling.

b. Distribution of total ethanol-soluble radioactivity in the stems

The distribution of the total ethanol-soluble activity (Figure 45), about 90-95% of which is sucrose, does not show a reversal of the gradient at any time, as might be predicted by the data from the petiole (Figure 21) or the sucrose kinetics in the leaf (Figure 22). The level of labelling in the stem does appear to subside at later times, however. The data for the plant harvested 25 minutes after pulse-labelling is of particular interest, since it shows a fairly linear activity gradient instead of the exponential gradient found after steady state labelling.

Marked irregularities in the profiles occur at the nodes. This was reported previously by Vernon (100), and was evident in all studies on the translocation profile in soybean, including Figures 11, 12, 61, 62 and others which are not
Figure 45. Distribution of ethanol-soluble radioactivity in soybean stems following pulse-labelling of the first trifoliate leaf. Nodes are indicated by "n". The bar on the abscissa indicates a common node.
reported. The breaks are characterized by an accumulation of activity at the nodes and, especially in the case of the petiole, by a different level of activity in the adjacent internode sections. This is attributed to a change in the cross-sectional area of the translocation stream in passing from one internode (or petiole) to the next internode, with the consequence that different volumes of translocate are being included in successive stem sections. The "accumulation" at the nodes is probably due to the meandering of the phloem in that area. A corollary of this hypothesis is that the velocity of the translocation stream must change inversely with the change in its cross-sectional area.

An estimate of the change in cross-sectional area may be made by comparing the level of activity in the second petiole section to that in the sections taken from the succeeding internode. The first petiole section is not included in this comparison because it was within the feeding chamber and so contained radioactivity from $^{14}$CO$_2$ fixation. The average ratio obtained is 1:1.8. It is instructive to compare this figure with the differences in the cross-sectional areas of the individual sieve cells in the petiole and stem obtained in Section II, B. Using these measurements, the ratio of the cross-sectional area of sieve cells in the petiole to that of the stem sieve cells is 1:1.63, which is in good agreement with that obtained from the profiles. It should be pointed
out that a change in cross-sectional area need not necessarily be accomplished by an increase in the diameter of the sieve tubes since it could just as easily occur by increasing the number of sieve tubes. The former explanation, however, apparently is a sufficient one.

A velocity of translocation can be obtained from this experiment by extrapolating the curves for the 11 and 25 minute stems to the abscissa, and is about 1.4 cm min\(^{-1}\). Since most of the movement occurs in the stem, this velocity is most applicable to the rate of movement there, while in the petiole the velocity should be closer to 1.8 x 1.4 cm min\(^{-1}\), or about 2.5 cm min\(^{-1}\).

c. Distribution of ethanol-insoluble radioactivity in the stems The distribution of ethanol-insoluble radioactivity in the stems is shown in Figures 46-49. Some trends are evident in the data, although it does not present a simple pattern. In those sections below the node of the fed leaf, the amount of activity present seems to be primarily a reflection of the instantaneous amount of soluble activity present in the section. This would indicate that if radioactivity were incorporated into the insoluble materials from the soluble ones, the incorporation must be reversible rather than irreversible. An opposite inference may be drawn from the insoluble material in the first petiole section and in the section which includes the node of the fed leaf. In these
Figure 46. Distribution of ethanol-insoluble radioactivity in soybean stems following pulse-labelling of the first trifoliate leaf. Nodes are indicated by "n". The bar on the abscissa indicates a common node.
Figure 47. Distribution of ethanol-insoluble, water-soluble radioactivity in soybean stems following pulse-labelling of the first trifoliate leaf. Nodes are indicated by "n". The bar on the abscissa indicates a common node.
Figure 48. Distribution of $^{14}$C-starch in soybean stems following pulse-labelling of the first trifoliate leaf. Nodes are indicated by "n". The bar on the abscissa indicates a common node.
Figure 49. Distribution of radioactivity in the insoluble residue in soybean stems following pulse-labelling of the first trifoliate leaf. Nodes are indicated by "n". The bar on the abscissa indicates a common node.
cases there seems to be a steady and considerable increase in the amount of insoluble activity. Curiously enough, the insoluble activity in the intervening petiole section presents an entirely different picture, except perhaps for the plant harvested after 125 minutes.

Although the data in Figures 47-49 for the ethanol-insoluble activity from three stems has been divided into three separate categories of water-soluble, starch and residual activity, it is not at all clear whether this is, in fact, a meaningful separation. Most of the remarks made concerning the insoluble activity as a whole can be applied equally well to each of the three sub-fractions. The primary advantage of the separation is the demonstration of the very low amounts of activity present in starch, since it is potentially an important reservoir for translocate which has been lost from the phloem, and considerable numbers of starch granules are found associated with the vascular bundles in soybean. The possibility exists, furthermore, that the small amounts found actually represented non-starch activity which was solubilized during the incubation period with salivary amylase. The general features of the distribution, however, resemble that which one would expect from an irreversible incorporation of activity into starch, and do not strongly resemble the distribution of the other two fractions.

Some of the water-soluble extract from the 75-minute stem
was hydrolyzed in 1N HCl at 100°C for three hours in an attempt to identify the general nature of the radioactivity. The hydrolysate was dried in a vacuum desiccator over sodium hydroxide pellets. The resulting dried hydrolysate was dark brown, suggesting the presence of degradation products from the action of the acid on carbohydrates. Some of the hydrolysate was chromatographed descendingly in ethyl acetate-pyridine-water, autoradiographed and sprayed with ninhydrin. Much of the radioactivity remained at the origin, but about half of it moved varying distances in the solvent and was associated with ninhydrin-positive areas.

Another aliquot of the water soluble activity was incubated with amyloglucosidase, and an aliquot of this was also chromatographed in ethyl acetate-pyridine-water. No glucose appeared on the autoradiogram, and most of the activity remained at the origin. About one-third of the activity appeared as sucrose, apparently from incomplete extraction of the stem sections, but it can be seen from the total ethanol soluble activity that the unextracted sucrose only accounted for about half of one per cent of the total sucrose.

Vernon's (100) data on the total insoluble activity present in soybean stems following translocation is quite similar to that reported here. Perchloric acid extraction of the stems removed most of the activity, but very little of
this activity was recovered in the starch precipitated as the iodine complex from this extract. He concluded, however, that most of the perchloric acid soluble activity was actually starch, but that much of the newly formed starch would be preferably degraded by the acid, which explained the lack of activity in the precipitated starch. This explanation was an extrapolation from work performed with leaf starch, and was probably applicable in that case. However, it appears from the present data that most of the ethanol-insoluble material in the stem is actually not starch.

d. Distribution of non-sucrose ethanol-soluble radioactivity in the stems harvested at 75 and 125 minutes. The distribution of the ethanol-soluble radioactivity, except for sucrose, is shown for the 75-minute stem in Figures 50 and 51, and for the 125-minute stem in Figures 52 and 53. Some other compounds, notably alanine, also appeared on the autoradiograms in minor amounts, especially in the stem sections closest to the leaf, but did not usually account for as much activity as these compounds. It is difficult to make many generalizations about their distributions, but some features are of interest. In general, the amount of activity in these compounds reflects the total activity present in each section, including the discontinuities at the nodes. The relative amounts of some of the compounds as compared to the leaves (see Figures 55-58) are different. In particular, the amount
Figure 50. Distribution of some ethanol-soluble compounds in a soybean stem 75 minutes after pulse-labelling the first trifoliate leaf. The bars along the abscissa indicate nodes.
Figure 51. Distribution of some ethanol-soluble compounds in a soybean stem 75 minutes after pulse-labelling the first trifoliate leaf. The bars along the abscissa indicate nodes.
Figure 52. Distribution of some ethanol-soluble compounds in a soybean stem 125 minutes after pulse-labelling the first trifoliate leaf. The bars along the abscissa indicate nodes.
Figure 53. Distribution of some ethanol-soluble compounds in a soybean stem 125 minutes after pulse-labelling the first trifoliate leaf. The bars along the abscissa indicate nodes.
of malate is less, and the amount of glucose relative to fructose is the opposite of the situation in the leaf. In many sections there is twice as much fructose as glucose. This, and the irregularity of their distribution, would suggest that they are probably not translocated. The unknown α occupies a relatively more conspicuous place in the stem than in the leaf. The fact that radioactive asparagine was present in such small quantities is of interest since large amounts were shown to be present by spraying one of the chromatograms with ninhydrin. In many cases, asparagine and glucose overlapped considerably because of the large asparagine spot.

Among the lipids, sterol glucoside was always labelled much more strongly than the others. In those sections closest to the leaf, some of the others, especially the galactolipids, were also radioactive, but there was virtually no radioactivity in other lipids past the fifth or sixth sections. Although it was difficult to be certain, it appeared that esterified sterol glucoside also was very lightly labelled in most sections.

The per cent of total activity in the ethanol-soluble compounds from the second petiole sections is plotted in Figure 54. Again, it is difficult to discern definite trends, but in spite of the fact that the total activity in these sections increases quite rapidly and then declines (see Figure 45), the fraction of the total activity represented by each
Figure 54. The relative amounts of radioactivity in non-sucrose compounds in the petioles from pulse-labelled soybean leaves.
compound remains fairly constant. Possible exceptions to this are asparagine, which was not found at the shortest time, but increased steadily afterwards except for the last time, and malate, which shows a consistent increase.

e. Carbon-14 kinetics in the leaves The data for the ethanol-soluble compounds from the leaves is shown in Figures 55-58. These graphs are presented largely for comparison with the data shown in Section III, B5 for samples taken from a single leaf. In order to make the two comparable, the compounds have been plotted as per cent of total activity present in the plant.

The variability between leaves is considerable, and precludes any but a qualitative comparison of these graphs with those of Section III, B5. Although most points of comparison are similar, some are not. Raffinose and maltose show considerably different kinetics, while in the previous experiment they did not. In almost all of the leaves in this experiment, there was more of the unknown \( \beta \) than glucose and fructose, whereas it was not at all comparable to the hexoses before. A similar observation can be made for the relative amounts of sulfolipid and galactolipids in the two experiments. Finally, sterol glucoside does not show the amount of decline that was shown earlier.

It is difficult to assess these differences at present, but they are sufficient to require some caution in interpret-
Figure 55. Carbon-14 kinetics in sugars following pulse-labelling of soybean leaves
Figure 56. Carbon-14 kinetics in polar lipids following pulse-labelling of soybean leaves.
Figure 57. Carbon-14 kinetics in amino acids following pulse-labelling of soybean leaves.
Figure 58. Carbon-14 kinetics in organic acids following pulse-labelling of soybean leaves
ing the results of the kinetics obtained from sampling a single leaflet.

f. Sucrose concentrations and specific activities in the leaves and petioles  The data obtained from the analytical determinations of sucrose in the leaves and second petiole sections are shown in Table 6. The samples of petiole sucrose were determined in duplicate, which agreed to within 5%.

In order to obtain some idea of how much of the sucrose in the petiole was actually contained in the translocation stream, the ratios of the specific activities in the petiole and leaf were calculated and plotted in Figure 59. The vertical bars indicate the error to be expected from a 10% error in the sugar determinations. From the graph it would appear that the specific activity of sucrose in the petiole quickly rises to about two-thirds that in the leaf, and then continues rising much more slowly afterwards. The simplest interpretation of this would be that the sucrose in the petiole is in two compartments, namely the translocation stream, and another pool external to the translocation stream which slowly accumulates sucrose from it.

If two-thirds of the sucrose in the petiole is in the translocation stream, this, together with the data in Table 6, allows a calculation of the rate of sucrose efflux from soybean leaves under these conditions. The average amount of sucrose contained in one centimeter of petiole is 13.0
Table 6. Amounts and specific activities of sucrose in the leaves and petioles of soybean plants following pulse-labelling

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total sucrose&lt;sup&gt;a&lt;/sup&gt; (micrograms)</th>
<th>Specific activity (dpm/microgram)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf, 11&lt;sup&gt;m&lt;/sup&gt;</td>
<td>688</td>
<td>37,000</td>
</tr>
<tr>
<td>25&lt;sup&gt;m&lt;/sup&gt;</td>
<td>918</td>
<td>36,400</td>
</tr>
<tr>
<td>50&lt;sup&gt;m&lt;/sup&gt;</td>
<td>1260</td>
<td>17,600</td>
</tr>
<tr>
<td>75&lt;sup&gt;m&lt;/sup&gt;</td>
<td>1370</td>
<td>16,700</td>
</tr>
<tr>
<td>100&lt;sup&gt;m&lt;/sup&gt;</td>
<td>850</td>
<td>10,500</td>
</tr>
<tr>
<td>125&lt;sup&gt;m&lt;/sup&gt;</td>
<td>824</td>
<td>11,600</td>
</tr>
<tr>
<td>Average</td>
<td>984</td>
<td>---</td>
</tr>
<tr>
<td>Petiole, 11&lt;sup&gt;m&lt;/sup&gt;</td>
<td>9.9</td>
<td>7,380</td>
</tr>
<tr>
<td>25&lt;sup&gt;m&lt;/sup&gt;</td>
<td>10.6</td>
<td>26,100</td>
</tr>
<tr>
<td>50&lt;sup&gt;m&lt;/sup&gt;</td>
<td>17.7</td>
<td>13,600</td>
</tr>
<tr>
<td>75&lt;sup&gt;m&lt;/sup&gt;</td>
<td>17.1</td>
<td>12,000</td>
</tr>
<tr>
<td>100&lt;sup&gt;m&lt;/sup&gt;</td>
<td>9.9</td>
<td>9,570</td>
</tr>
<tr>
<td>125&lt;sup&gt;m&lt;/sup&gt;</td>
<td>13.0</td>
<td>12,000</td>
</tr>
<tr>
<td>Average</td>
<td>13.0</td>
<td>---</td>
</tr>
</tbody>
</table>

<sup>a</sup>Reported as micrograms per centimeter of petiole length, for the petioles.
Figure 59. Relative specific activities of sucrose in the leaf and petiole following pulse-labelling of soybean leaves
micrograms, and in the translocation stream, two-thirds of this or 8.7 micrograms. From Section III, B8b, this sucrose will be moving with a velocity of about 2.5 cm min$^{-1}$, which gives an efflux rate of sucrose from the leaf of 21.7 γ min$^{-1}$ (2.5 cm min$^{-1}$ x 8.7 γ cm$^{-1}$). Using this figure, an approximate turnover time for sucrose in the leaf may be calculated from the average sucrose content of 984 micrograms, if it is assumed that the only drain on sucrose is due to translocation, and that it can be treated as a single pool. The value which results from this calculation is 45.3 minutes (984 γ/21.7 γ min$^{-1}$).

Although the points for both total and specific activity for the leaf sucrose are somewhat scattered, one can obtain a rough value for its turnover time as in Section III, B5a by plotting the natural logarithms of their values against time. The points from the specific activity gives a value of about 80 minutes, while those from total activity (as per cent of total $^{14}$C in the plant) give about 100 minutes. Although these are certainly only approximations, they are considerably longer than the turnover time obtained from the rate of sucrose efflux from the leaf.
9. The effect on translocation of excising various plant parts

a. Excision of both leaf and root The question of whether significant movement of tracer would occur in a completely excised stem was of importance for two reasons. In several experiments it was not possible to section and freeze a stem immediately. The question was also of interest from a theoretical point of view, since it would be expected that if the mechanism for translocation were located in the stem, some movement of material might occur after the stem and root were excised. This would be true of the streaming mechanisms proposed by Thaine (96) and Canny (19), and perhaps of some other mechanisms as well.

Six plants were prepared for feeding in the manner described in Section II, A1, except the side leaflets were excised, and only the center leaflet was fed. The stems were wrapped in Saran Wrap to prevent desiccation after excision of the roots. The leaflets were fed simultaneously in a rectangular plexiglass feeding chamber, 24" x 3" x 3/4", which had a removable top and notches along one side for the petioles. A twenty-liter bell jar was included in the system to maintain a steady state.

The leaflets were fed for 20 minutes, and all of the stems were immediately excised from the leaves by severing the petiole just outside the feeding chamber, and from the root,
just above the ground.

Two stems were sectioned and frozen immediately. The cut ends of the other stems were covered with stopcock grease. Forty-five minutes after the end of the feeding period, two more stems were harvested, and 90 minutes after the end of the feeding period, the final two stems were harvested. The extract from each of the top stem sections was subjected to two-dimensional ascending chromatography to check for possible metabolism of the activity during the period following excision.

The results of this experiment are plotted in Figure 60. It is apparent that there was virtually no re-distribution of tracer during this time. Vernon (100) had made similar observations following ringing of the soybean stem with hot wax, but only two plants were used in his experiment, and cutting the stem would seem to be a less drastic method of interrupting the phloem.

The chromatograms of the stem sections all appeared quite similar, and did not indicate any extensive metabolism after excision.

b. Excision of leaf or root Since translocation does not occur when both the leaf and root are removed, it was of interest to see what the effect of removing just one of these parts was. For this purpose, six plants were prepared for feeding in the same manner as for the previous experiment.
The following treatments were carried out:

Plant #1 - No treatment, normal translocation.  
Harvested after 25 minutes of feeding.

Plant #2 - Root excised immediately before feeding.  
Harvested after 30 minutes of feeding.

Plant #3 - Same as #2, except harvested after 25 minutes of feeding.

Plant #4 - Same as #2, except harvested after 100 minutes of feeding.

Plant #5 - Leaf only excised after 30 minutes of feeding. Harvested after 40 minutes more.

Plant #6 - Same as #5, except the cut end of the petiole was kept under a 15% sucrose solution until harvesting.

The results are shown in Figure 61. It is apparent that translocation into the stem can occur even when the root and all other parts of the plant have been removed, although it occurs at a reduced rate. Furthermore, when the stem is defoliated, there still appears to be some movement of translocate down the stem if the root is still attached. It is not clear whether administering sucrose to the petiole can accelerate the latter movement, since that stem apparently contained more activity to begin with in comparison to the one which was not fed sucrose.
Figure 60. The effect on translocation of excising both the leaf and the root
Figure 61. The effect on translocation of excising the leaf or the root
(See page 154 for treatments)
IV. THEORETICAL CONSIDERATIONS OF TRANSLOCATION

A. Mathematical Models for the Effects of Leaf Size, Shape, and Source Pool Kinetics on the Efflux of Radioactive Translocate from Leaves

As indicated in previous sections, it seemed quite likely even at the start of this research that factors operating within the leaf were largely responsible for the kinetics observed in the stem. In order to provide a theoretical framework to guide the course of experimentation and to interpret these and published results, some simple models for tracer efflux from leaves were constructed.

Particular emphasis will be placed on the assumptions made for the models, since the equations are only shorthand statements of these assumptions. Much of the discussion will be oriented towards translocation in soybean, since more information on tracer kinetics is available from experiments with that plant.

More detailed derivations of the equations to be presented are found in Appendix C, 1-3.

---

1 The writer is indebted to Dr. R. W. Fahien for his advice and interest concerning these models.
1. Model for a linear, parallel-veined leaf

The geometry for this model is shown in Figure 62. The translocation stream is assumed to be continuous in the leaf, and is bordered by a source pool which supplies the translocate. The assumption of a continuous translocation stream rather than discrete veins is a mathematical necessity, but should not detract from the accuracy of the final solution if we consider leaf areas which are large in comparison to the distance between veinlets. The "source pool" is not necessarily contained in the photosynthetic cells, but probably can be considered most accurately as representing the contents of the cells immediately surrounding the sieve tubes. This would include the phloem parenchyma and bundle sheath cells.

In order to solve the equations for the isotopic unsteady state, it is necessary to have information concerning the variation of the cross-sectional area of the translocation stream within the leaf. If it is assumed that all areas of the leaf are exporting translocate at an equal rate, then it is clear that the cross-sectional area of the translocation stream must increase with distance down the leaf, or either the density or the velocity of the translocate must increase. It is readily apparent from examination of a leaf that the cross-sectional area of the translocation stream increases. For this, and for other reasons to be mentioned, the density and velocity have been assumed to be constant throughout the
Figure 62. Model for a linear leaf
translocation stream.

With these assumptions, the depth, \( z \), of the translocation stream may be expressed as a function of \( x \), the distance from the petiole, by the equation

\[
z = \frac{(x - L)n_0}{\rho V},
\]

where \( L \) = the length of the leaf, [\( = \) cm]

\( n_0 \) = flux of translocate into the translocation stream from the source pool; a constant, [\( = \) gm cm\(^{-2}\) min\(^{-1}\)]

\( \rho \) = density of translocate within the translocation stream; a constant, [\( = \) gm cm\(^{-3}\)]

\( V \) = velocity of the translocation stream; a constant, [\( = \) cm min\(^{-1}\)] (Note that \( V \) will be a negative quantity due to the orientation of the coordinates.)

This equation states that the cross-sectional area of the translocation stream is proportional to the leaf area which has exported translocate to it. This was verified experimentally for soybean (see Figure 4).

Before proceeding further, the validity of these assumptions should be commented on. The assumption that all areas of the leaf contribute equal amounts of translocate (i.e., \( n_0 \) constant) is probably the least troublesome. In both monocotyledons and dicotyledons, it would seem likely that the similarity of leaf anatomy throughout any given leaf, except

\(^1\) Read [\( = \)] as "has units of".
in the vicinity of the major veins, would make this assumption a logical necessity under uniform environmental conditions. The question of a constant density of translocate can only be referred to observations made in the stem, where the density changes but little with distance down the stem (114).

The assumption of a constant velocity is an important point for this model and, since the loss of translocate from the translocation stream may be considered as a case where the velocity of that material simply becomes zero, these two assumptions will be considered together. Some of the best data available on these questions undoubtedly comes from the experiments by Moorby, Ebert and Evans (70). Following labelling of soybean with \(^{14}\text{CO}_2\), they monitored several points along the stem. After a time delay which was proportional to the distance of each point from the source, the amount of activity plotted against time for each point was very much the same for all of the positions monitored. The assumption of a single velocity of translocation with a virtually negligible rate of loss of activity to the surrounding tissues offers a sufficient explanation for this, and is the interpretation given by the authors (37).

Although their curves are, in fact, somewhat different at the different points, it is interesting to note that the differences may be explained in almost all cases by assuming differences in the cross-sectional area of the translocation
stream. For example, if the areas of the translocation stream at two points were different by a factor of two, and if the bulk rate of flow past the points were the same, the rise in radioactivity with time at the one point would occur at twice the rate, and reach twice the total value as at the second point. Changes in the cross-sectional area of the translocation stream in soybean seem to be quite likely in view of the "breaks" in the translocation profile commonly found at or near the nodes (Figures 11, 12, and 45). The shapes of the profiles (Figure 45) also indicate the possibility that the change in cross-sectional area may extend a short distance above and below the node. Under the condition of a constant bulk rate of flow, a change in velocity proportional to the change in area should occur, but presently available data does not appear to be sufficiently accurate to detect this change.

Other experiments showing the progression of translocation profiles down the conduction system provide essentially the same picture as that shown by Moorby, Ebert and Evans (70). This has been demonstrated for sugar beet (71) and for soybean under steady state feeding conditions (Figures 11 and 12).

A somewhat similar and equally important line of evidence supporting the concept that there is no velocity gradient in translocation comes from experiments by Geiger with sugar beets (41). He has shown that the kinetics of sucrose
labelling in a pulse-labelled leaf almost exactly matches that of the rate of arrival of translocate at a sink in the same plant. Since sucrose is the main compound translocated, its level of labelling in the leaf would be expected to reflect the rate at which labelled sucrose is translocated from the leaf. As the rates of export and import match, the velocity gradient during transit must be negligible.

An important point which needs to be considered concerns the undeniable observations that there is, in fact, removal of labelled materials from the translocation stream. Canny (17, 21) has shown that radioactive carbon dioxide appears when labelled translocate moves through the stem. It is also well known that a number of other compounds become labelled (e.g., 24, 100), and autoradiographs have shown that appreciable amounts of radioactivity are found outside the phloem, especially after long periods of translocation (58, 79, 106). Clauss, Mortimer and Gorham (24) have expressed the belief that this removal accounts for the exponential profile found in soybean. It must be remembered, however, that in comparison with the total amount of label which has passed through, the amount which has been removed is quite small. The amount removed is not at all sufficient to account for the exponential shape of the translocation profile, especially at short times when the exponential shape is observed. Canny's experiments with aphid stylets (18) demonstrate that the profile may
very well be contained in the sieve tubes themselves.

We are now in a position to consider the isotopic unsteady state resulting from the introduction of radioactive tracer into the source pool. If the mass fraction of labelled translocate in the source pool is \( \rho_{RL}/\rho_{L} \), where \( \rho_{RL} \) and \( \rho_{L} \) are the densities of labelled translocate and total translocate in the source pool, respectively, and there is no isotopic effect, then \( \rho_{RL}/\rho_{L} = n_{Ro}/n_{O} \), where \( n_{Ro} \) and \( n_{O} \) are the fluxes of labelled and total translocate, respectively, into the translocation stream. Solving for \( n_{Ro} \),

\[
\frac{n_{Ro}}{n_{O}} = \frac{\rho_{RL}}{\rho_{L}} \tag{2}
\]

Taking a mass balance on the isotope over an incremental volume of the translocation stream, \( yz\Delta x \), and substituting Equation 1 for \( z \), we obtain the partial differential equation for this model,

\[
\frac{\partial \rho_{R}}{\partial t} = \frac{n_{Ro}\rho V}{(x - L)n_{O}} - \frac{V}{(x - L)} \frac{\partial [\rho_{R}(x - L)\Delta x]}{\partial x} \tag{3}
\]

where \( \rho_{R} \) = the density of radioactive translocate in the translocation stream; \([=] \text{gm cm}^{-3}\)

In deriving Equation 3, mass transport by diffusion has been ignored.

The solution to Equation 3 may be found by using Laplace transforms, and is
\( P(u) = \frac{P \cdot u}{n \cdot (x - L)} \left[ \int_0^t n_R \cdot (u) \, du - U(t - \frac{x - L}{V}) \int_0^{t - \frac{x - L}{V}} P(u) \, du \right] \)  

where \( u \) is a dummy variable of integration, and \( U(t - \frac{x - L}{V}) \), the unit step function is defined as:

\[
U(t - a) = \begin{cases} 
0, & t < a \\
1, & t \geq a 
\end{cases}
\]

In this particular case, \( a = \frac{(x - L)}{V} \). Since we are interested in the efflux of tracer from the leaf (at \( x = 0 \)), this becomes

\[
\rho_{RP} = \frac{-P \cdot V}{n \cdot L} \left[ \int_0^t n_R \cdot (u) \, du - U(t + \frac{L}{V}) \int_0^{t + \frac{L}{V}} n_R \cdot (u) \, du \right] 
\]

where \( \rho_{RP} = \rho_R(0, t) \).

Since the equation has been derived for any function of \( n_R \), which is dependent only on time, the effects of leaf shape and size can be considered independently of the factors which affect the source pool kinetics. This is a great advantage in considering the many possibly factors which might affect the rate of tracer efflux from a leaf, and is a consequence of the assumption that there is no loss of translocate from the translocation stream. Factors affecting the source pool kinetics will be considered in Section IV, B.

The assumptions made in deriving this equation are
emphasized because the model is only as valid as the assumptions, and because much of the work presented in this thesis was aimed at determining their validity, or in establishing an experimental value for $n_Ro$. In order to provide a complete and convenient reference to these assumptions, they are listed in Table 7. Similar assumptions were made in deriving the other two models.

Table 7. Assumptions made in deriving the equations for a linear leaf model

<table>
<thead>
<tr>
<th>Assumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chemical steady state</td>
</tr>
<tr>
<td>2. $\rho = constant$</td>
</tr>
<tr>
<td>3. No isotope effect</td>
</tr>
<tr>
<td>4. Neglect diffusion</td>
</tr>
<tr>
<td>5. $n_o = constant$</td>
</tr>
<tr>
<td>6. $v_x = V(constant); v_y = v_z = 0$</td>
</tr>
<tr>
<td>7. No removal of translocate from the translocation stream</td>
</tr>
</tbody>
</table>

2. **Model for a rectangular leaf**

The model for a rectangular leaf, shown in Figure 63, consists essentially of two linear leaf models at right angles to a central channel (analogous to a midrib). The contribution of tracer to the central channel by each of the two "side leaves" is given by Equation 5, which can be used in deriving
Figure 63. Model for a rectangular leaf

Figure 64. Model for a peltate leaf
the partial differential equation which describes the tracer kinetics in the central channel. In principle this may be done for a leaf of any shape, but in practice the resulting equations for any but a rectangular shape do not have a ready solution.

The partial differential equation which describes this model is

$$\frac{\partial \rho_R}{\partial t} = \frac{\rho V^2}{n_0 W (L - y)} \left[ \int_0^t n_{Ro}(u) du - U(t + \frac{W}{V}) \int_0^t \frac{W}{V} n_{Ro}(u) du \right]$$

$$- V \frac{\partial \rho_R}{\partial y} - \frac{\rho_R V}{(y - L)}$$

where $W =$ the distance from the central channel to the edge of the leaf; a constant, [cm]

$L =$ the length of the leaf; a constant, [cm]

$y =$ distance along the central channel, measured from the petiole, [cm]

$V =$ velocity of the translocation stream, assumed to be the same as that in the "side leaves". (Note that $V$ will be a negative quantity, due to the orientation of the coordinates), [cm min$^{-1}$]

The general solution is
\[ p_R = \frac{\rho v^2}{n_0 W(L - y)} \left[ \int_0^t \int_0^u n_{RO}(m) dmdu - U(t + \frac{W}{V}) \int_0^t \int_0^u n_{RO}(m) dmdu \right. \\
- U(t + \frac{L - y}{V}) \int_0^u \int_0^u n_{RO}(m) dmdu \right] \tag{7} \\
+ U(t + \frac{L + W - y}{V}) \int_0^t \int_0^u n_{RO}(m) dmdu \]

Evaluated at \( y = 0 \), this becomes

\[ p_{RP} = \frac{\rho v^2}{n_0 WL} \left[ \int_0^t \int_0^u n_{RO}(m) dmdu - U(t + \frac{W}{V}) \int_0^t \int_0^u n_{RO}(m) dmdu \right. \\
- U(t + \frac{L}{V}) \int_0^u \int_0^u n_{RO}(m) dmdu \right] \tag{8} \\
+ U(t + \frac{L + W}{V}) \int_0^t \int_0^u n_{RO}(m) dmdu \]

3. **Model for a peltate leaf**

This model (Figure 64) is similar to that for the linear leaf except that the equations are derived in a cylindrical coordinate system. The partial differential equation that describes this model is
\[
\frac{\partial \rho_R}{\partial t} = \frac{2\rho V n_{Ro}}{(r^2 - W^2)n_o} - \nu \frac{\partial \rho_R (r^2 - W^2)}{(r^2 - W^2)\partial r}
\]  

(9)

where \( W \) = the radius of the leaf, \( [=] \) cm

\( r \) = distance from the center of the leaf, \( [=] \) cm

The general solution is

\[
\rho_R = \frac{2\rho V^2}{n_o(r^2 - W^2)} \left\{ \left[ \frac{r}{V} \int_0^t n_{Ro}(u) du - \int_0^t \int_0^u n_{Ro}(m) dmdu \right] 
- U(t + \frac{W-r}{V}) \left[ \frac{W}{V} \int_0^t + \frac{W-r}{V} n_{Ro}(m) du \right] 
- \left[ \int_0^t + \frac{W-r}{V} \int_0^u n_{Ro}(m) dmdu \right] \right\}
\]  

(10)

Evaluated at \( r = 0 \), this becomes

\[
\rho_{RP} = \frac{2\rho V^2}{n_o W^2} \left\{ \int_0^t \int_0^u n_{Ro}(m) dmdu 
- U(t + \frac{W}{V}) \left[ \int_0^t + \frac{W}{V} \int_0^u n_{Ro}(m) dmdu - \frac{W}{V} \int_0^t + \frac{W}{V} n_{Ro}(u) du \right] \right\}
\]  

(11)
4. **Comparison of the effects of leaf size and shape**

With these equations for the effects of leaf size and shape on the efflux of tracer from leaves, we may now compare their effects for the different models. For this purpose the following values are chosen:

- **Leaf area**: 24 cm$^2$
- $V = -1.0$ cm min$^{-1}$
- $n_o = \text{constant (unknown) for all leaves}$
- **Linear leaf**: $L = 10$ cm
- **Rectangular leaf**: $L = 6$ cm, $W = 2$ cm
- **Peltate leaf**: $W = 2.8$ cm

These values are roughly similar to those found for several experimental plants. Since all of the models have the same leaf area, $n_o$ and $V$, the cross-sectional areas of the translocation stream at the petiole will be the same and it will suffice for our purposes to compare $\rho_R$'s or a quantity proportional to it.

The first comparison will be made for the case where $n_0 = \kappa t$. For the sake of simplicity $\kappa$ will be chosen to be unity, and therefore simply becomes a conversion factor to keep the equation dimensionally correct (i.e., $\kappa = 1$ gm cm$^{-2}$ min$^{-2}$). By substituting the above values into Equations 5, 8 and 11, the following solutions are obtained:
Linear leaf:

\[
\frac{n_0 \rho_R}{\kappa \rho} = \begin{cases} 
\frac{t^2}{20}, & t < 10 \\
t - 5, & t \geq 10 
\end{cases}
\]

Rectangular leaf:

\[
\frac{n_0 \rho_R}{\kappa \rho} = \frac{1}{72} \left[ t^3 - U(t-2)(t-2)^3 - U(t-6)(t-6)^3 ight. \\
\left. - U(t-8)(t-8)^3 \right]
\]

For \( t \geq 8 \), this simplifies to

\[
\frac{n_0 \rho_R}{\kappa \rho} = t - 4
\]

Peltate leaf:

\[
\frac{n_0 \rho_R}{\kappa \rho} = \begin{cases} 
\frac{t^3}{23.52}, & t < 2.8 \\
t - 1.87, & t \geq 2.8 
\end{cases}
\]

The curves for these equations have been plotted in Figure 65. It is readily apparent, both from the equations and from the graph, that there is little effect of the leaf shape on the rates of tracer efflux. After a brief period of time the rate of efflux largely reflects the kinetics of the source pool. Due to the fact that it takes a finite period of time for translocate to pass from its point of origin to the petiole, there is an exponential rise during the first few
Figure 65. Theoretical curves for the tracer efflux from linear, rectangular and peltate leaf models when leaf shape is the only variable. \( n_{RO} = \kappa t \) (\( n_{RO} \) is not drawn to the same scale as the efflux curves).

LEAF AREA = 24 cm²

V = -1.0 cm min⁻¹
minutes. The duration of this rise is directly related to the time it takes for translocate originating at the farthest part of the leaf to reach the petiole. This time is an important parameter in all of the models, and might be referred to as the "kinetic size" of the leaf. With the source pool kinetics assumed it is apparent that the kinetic size of the soybean leaf is not enough to account for the twenty-minute exponential rise in the rate of tracer efflux from the leaf during steady state feeding experiments. This is especially true when one considers that the actual velocity is probably more than twice as great as that assumed for the present model (Section III, B8b).

In Figures 66 and 67, efflux curves for \( n_{Ro} = \kappa (1 - e^{-0.08t}) \) and \( n_{Ro} = \kappa t (1 - e^{-0.08t}) \) are plotted. Again, it is seen that the effects of leaf size and shape are minor, and after short times the efflux curves resemble those for the source pool.

A more instructive exercise is to study the effect of increasing the kinetic size of the models by decreasing the velocity. By keeping the other parameters for the models the same as before and assuming a velocity of \(-0.1 \text{ cm min}^{-1}\), the effects of both leaf size and shape are quite marked. Figure 68 shows efflux curves for \( n_{Ro} = \kappa t \). Similar effects would be noted by increasing the leaf area since this, too, would increase the kinetic size of the models. Instances of experi-
Figure 66. Theoretical curves for the tracer efflux from linear, rectangular and peltate leaf models when leaf shape is the only variable.

\[ n_{Ro} = \kappa(1 - e^{-0.08t}) \]

(\( n_{Ro} \) is not drawn on the same scale as the efflux curves)
Figure 67. Theoretical curves for the tracer efflux from linear, rectangular and peltate leaf models when leaf shape is the only variable.

\[ n_{RO} = \kappa t (1 - e^{-0.08t}) \]

LEAF AREA = 24 cm²

\[ V = -1.0 \text{ cm} \text{ min}^{-1} \]

\[ \rho_{R} \frac{n_{0}}{\kappa} \]

\[ \frac{\rho}{\rho} \]

\[ \text{TIME (MIN)} \]

\[ 0 \]

\[ 10 \]

\[ 20 \]

\[ 30 \]

\[ 40 \]

\[ 50 \]
Figure 68. Theoretical curves for the tracer efflux from linear, rectangular and peltate leaf models when leaf shape is the only variable. The effect of increasing the kinetic sizes of the models, $n_{Ro} = xt$. ($n_{Ro}$ is not drawn on the same scale as the efflux curves).
mental data which might be markedly affected by the kinetic size of the leaf come from sugar beet (71), which may have a relatively large leaf, and in willow (18), where the velocity is quite low. These cases will be discussed in Section IV, D.

In summary, the models for the effects of leaf size and shape on the rate of tracer efflux from leaves show that the effects depend largely on the time it takes for tracer to reach the petiole from the farthest part of the leaf. This time is referred to as the "kinetic size" of the leaf.

B. Source Pool Kinetics

It was pointed out earlier that the source pool for translocate is most accurately regarded as being contained in the phloem parenchyma and bundle sheath cells. The question of source pool kinetics is, then, a question of the tracer kinetics in those cells, or at least in a compartment within the cells.

Factors which might be expected to influence the source pool kinetics were mentioned in the Introduction. They will be discussed more extensively here, in an attempt to construct a tentative model or models which might provide a reasonable description of the source pool kinetics. This will entail considerable speculation, but it seems worth while to cover this ground simply because the subject has not been considered systematically from this point of view before.
The simplest model for the source pool would be a single pool of sucrose which is drained by translocation at a constant rate, replenished by photosynthesis at the same rate, and which does not participate to any significant extent in exchange reactions with other compounds. This model was applied with a great deal of success by Geiger (41) to the kinetics of translocation in sugar beet. It was modified slightly to assume a sucrose pool that increased slowly with time, rather than being in a steady state.

Whether this model is adequate to describe the source pool kinetics in soybean is questionable. In the first place, it seems that if the paraveinal mesophyll actually represents a non-photosynthetic compartment, it will almost certainly modify the kinetics found in the veins. In addition, the specific activity of the sucrose never reached that of the carbon dioxide in the steady state labelling experiments. Finally, the discrepancy in the turnover times found by measuring the decay of labelled sucrose and by calculating it from the efflux rate suggests that the model for soybean may be more complex.

The size of the sugar phosphate pools will undoubtedly have an effect on the source pool kinetics, although it will be a brief one. The sucrose in soybean takes about six minutes to reach a maximum specific activity following pulse labelling. Presumably this is due to loss of activity from
pools of phosphorylated photosynthetic intermediates, primarily the hexose phosphates. In sugar beet the corresponding time is about ten minutes (15). The assumption is being made here, of course, that the phosphorylated sugars are not themselves transported directly to the veins. Although Kursanov (61) has proposed that the phosphorylated sugars produced by photosynthesis are the form in which sugars move to the veins, and it is possible that sugars may be re-phosphorylated before translocation, it would seem from the kinetics of translocation in sugar beet and soybean that the kinetics of those pools would be determined primarily by the labelling in the free sucrose.

As a matter of theory, at least, the high energy content of the sucrose bond would allow it to participate in a large number of transglucosylation reactions (43). If this were to occur, it would seem that the potentially most important reactions would be the transformation of sucrose into starch and/or maltodextrins. Since the bond energies are similar, it is equally possible that starch could be converted to sucrose, and a net exchange of carbon would occur. Porter (80) has emphasized the commonly observed parallels between sucrose and starch metabolism, and Chan and Bird (23) have demonstrated that a carbon exchange can occur between the two in tobacco leaves, even during periods of net starch synthesis. De Fekete and Cardini (33) have outlined a series of
fully reversible reactions to account for the transfer of glucose from sucrose to the starch granule of sweet corn.

In soybean it seems quite likely from the pulse-labelling experiment that there was a net loss of radioactivity from sucrose to starch. This occurred in spite of the low light intensity employed, which was comparable to the lowest used by Chan and Bird, and, of their light treatments, gave the fastest loss of radioactivity from starch. Under their conditions, however, there was also a net loss of starch at this light intensity, while in the soybean leaf there appeared to be a net starch synthesis (see Sections III, B3a and III, B7a). Chan and Bird found that when there was rapid starch synthesis there was little exchange with sucrose, or even some net gain of activity by the starch.

Since the present state of knowledge concerning the metabolic interrelationships of starch and sucrose is characterized by so many uncertainties, it is difficult to propose a kinetic model for exchange between the two. It will simply be pointed out that if there were a chemical equilibrium between starch and sucrose which resulted in a net loss of activity from sucrose to starch, one would expect that the turnover time measured by the decline in radioactivity in the sucrose pool would be less than that measured by comparing the rate of transport from the leaf to the size of the sucrose pool. If the exchange resulted in a net loss of activity by
the starch to sucrose, an opposite finding would be expected. It is interesting to note that the apparent half-life of sucrose in tobacco leaves, where exchange has been shown to occur, is about three hours (58), whereas in soybean and sugar beet (41) where there is no loss of activity from the starch, it is considerably less. Unfortunately, this does not explain the discrepancy between the turnover times calculated in Section III, B8. In fact, using this line of reasoning makes the discrepancy even greater.

The rate of movement of translocate from its site of synthesis to the veins also might affect the source pool kinetics. Evans, Ebert and Moorby (37) have suggested that the initial exponential rise in the soybean profile during steady state labelling is due to varying diffusion times of sucrose to the sieve tubes. Vernon (100) had previously pointed out that an exponential curve might arise if diffusion played a role in the overall transport of material from the leaf.

Since protoplasmic streaming and diffusion would be expected to keep the cell contents fairly well mixed, except for possible intracellular compartmentation, it would seem that the only diffusion-limited steps would occur during passage of photosynthate through the cell walls. At the most, it would have to pass through only one palisade parenchyma cell and one paraveinal mesophyll cell (in soybean) before
reaching the border parenchyma. Passage through only a few more cells would take it to the sieve tubes. An estimate of the total distance over which diffusion might occur would therefore include the thickness of about a half-dozen cell walls, or, if it is assumed that the walls are 1 μ thick, about $5 \times 10^{-4}$ cm. The time to attain a steady state for the diffusion of tracer across this distance after an incremental change in tracer concentration would be approximately $b^2/D$, where $D =$ the diffusion coefficient for sucrose and $b =$ the distance over which diffusion is taking place (14). For diffusion of sucrose in water, $D = 5.2 \times 10^{-6}$ cm$^2$ sec$^{-1}$ (65), and $b^2/D$ would be about 0.05 sec. Under these conditions, then, diffusion would not be a rate-limiting step, and it appears that the diffusion coefficient could be decreased almost a thousand-fold before the effects of diffusion would be noticeable.

It is possible that the structure of the cytoplasm through which the sugar must diffuse might result in a much smaller diffusion coefficient. It would have to be large enough, however, to account for the total efflux of translocate from the leaf. An approximation of a limiting value for the diffusion coefficient necessary to account for this efflux may be made in the following manner: From Section III, B8f, if the leaf has an area of 35 cm$^2$, an efflux rate of about $10^{-8}$ gm cm$^2$ leaf area sec$^{-1}$ can be calculated. If it is
assumed that the palisade parenchyma produces most of the translocate, and that it passes into the paraveinal mesophyll, then the transport rate into the latter cells will also be about $10^{-8}$ gm cm$^{-2}$ leaf area sec$^{-1}$. Diffusion will not be occurring through this entire area, but will probably be limited to the plasmodesmata. If the area available for diffusion is estimated to be 0.1 cm$^2$, then the diffusion flux will be about $10^{-7}$ gm cm$^{-2}$ sec$^{-1}$. From Section III, B8f, the total amount of sucrose in a soybean leaf is about 1 mg. Since the fresh weight is about 0.5 gm, the overall sucrose concentration would be about $2 \times 10^{-3}$ gm cm$^{-3}$. This concentration will be taken as an approximation of the maximum concentration difference to be expected in the leaf. In a chemical steady state, the diffusion flux, $j$, will be related to the concentration gradient and $D$ by the equation

$$j = D \frac{\Delta \rho}{\Delta x}$$

If $j = 10^{-7}$ gm cm$^{-2}$ sec$^{-1}$, and $\Delta x = 10^{-4}$ cm and $\Delta \rho = 2 \times 10^{-3}$ gm cm$^{-3}$, then $D = 5 \times 10^{-9}$ cm$^2$ sec$^{-1}$. This is about one one-thousandth of the diffusion coefficient of sucrose in water and, as pointed out earlier, a value this low might have some observable effect on the kinetics of translocation. It seems quite unlikely, however, that the aggregate area of the plasmodesmata would represent 10% of the leaf area. Adjustments of that quantity towards a more realistic value would
increase the calculated value of \( D \). It would still seem unlikely, then, that diffusion would be a rate-limiting step in the transport of sucrose to the veins. One might yet object that a value of \( \Delta p \) in the cytoplasm may not be so readily assigned from the overall concentration of sucrose in the leaf.

Intracellular compartmentation of sucrose in the leaf could have a considerable effect on the kinetics of the source pool. Arisz (3) has presented considerable evidence in support of his theory of symplastic transport in parenchyma cells. Since this theory is very likely applicable to the transport of sucrose to the veins, this immediately raises the question of just how much exchange may be expected to occur between the sucrose in the chloroplasts, cytoplasm and vacuoles. There is virtually no information in the literature concerning this point, but it may be inferred from Geiger's (41) success in treating the sucrose as a single pool that compartmentalization of this nature is not of great importance. It should be pointed out in this connection that "compartmentation" is a relative matter and, although the diffusion of sucrose between the cytoplasm and vacuole obviously might be hindered by the tonoplast, "compartmentation" would not noticeably affect the translocation kinetics unless it took more than about two or three minutes for exchange between the two to reach an equilibrium.
Tolbert (97) has pointed out that considerable amounts of hexoses may possibly be produced from glycolate during photosynthesis. Since he proposes that glycolate has a special function in the transport of carbon from the chloroplast, the relative roles of hexose synthesis via glycolate and the photosynthetic carbon cycle are of special interest in considering the possibilities for compartmentation of sucrose in the cell. Heber and Willenbrink (50), however, did not find remarkable amounts of either glycolate or phosphoglycolate on their chromatograms. They did not mention whether any glyoxylate was found. They also present evidence that the sugar phosphates are the form in which reduced carbon is transported from the chloroplast. Their conclusions concerning the site of sucrose synthesis are different from those of Stocking, Williams and Ongun (92), however, who carried out similar investigations. While the former authors felt that sucrose was probably synthesized in the cytoplasm, the latter investigators found that sucrose was labelled first in the chloroplasts, and only appeared in the cytoplasm after two minutes of photosynthesis.

Non-photosynthetic tissues of the leaf offer another possibility for compartmentation of sucrose. The epidermal tissues of a leaf commonly occupy more than twenty per cent of the thickness of mesophytic leaves (111). The actual cellular volume represented is closer to thirty per cent of
the total cellular volume, however, since these tissues have no intercellular spaces (111). On the basis of their volume it seems quite possible that they could contain about one-third of the sucrose in a leaf. Yemm and Willis (112) have shown that the sucrose level in the lower epidermis is at least comparable to that in the mesophyll, and may even be higher. They also showed that the diurnal changes of sucrose in the two tissues did not necessarily occur in a similar manner, or even in the same direction. It may be pointed out, furthermore, that the mesophyll produces the sugar which is ultimately translocated, and that this sugar very likely simply moves down the palisade cells into the spongy parenchyma and from there into the veins (44). If this concept is followed completely, then, with respect to translocation, the epidermal tissues represent more or less stagnant pools of sucrose. As an explanation for the low values of the final specific activities obtained in the two pulse-labelling experiments, it is tempting to speculate that this is due to a lack of exchange of newly synthesized sucrose with that in the epidermal layers. It could be pointed out, too, that during steady state feeding, Geiger (41) found that the level of $^{14}$C-sucrose increased rapidly at first, reaching a fairly constant level after about eighty minutes, but still continued to rise slowly. Although he attributes this to an increase in the total pool size, it could also be interpreted
as a slowly saturating sucrose pool. He does present some analytical data, however, to substantiate his viewpoint, although it apparently consisted of only one measurement. It is possible, of course, that there is a rapid exchange of sucrose between the epidermal tissues and mesophyll, and that the epidermal layers may play some role in the transport of sucrose to the veins since they, too, are horizontally oriented tissues. The low specific activity of the sucrose could also be caused by refixation of respiratory CO₂ (Section V).

Another tissue which must be considered as representing a possible non-photosynthetic compartment is the border parenchyma, which usually has fewer chloroplasts than the mesophyll cells (35). Armacost (4) has emphasized the relatively large volume in the leaf occupied by the border parenchyma and has speculated on its possible function as a temporary storage site for photosynthate. The storage material would presumably occur in a soluble form, since he found practically no starch in the border parenchyma of fifteen dicotyledonous plants. In soybean the paraveinal mesophyll appears cytologically related to the border parenchyma. Since the border parenchyma and paraveinal mesophyll have small chloroplasts, it would seem that they represent essentially non-photosynthetic compartments through which photosynthate must pass on its way to the sieve tubes. As
such, they could have marked effects on the source pool kinetics.

It is apparent from the foregoing discussion that in most cases only the broadest generalizations may be made about the manner in which the factors mentioned might affect the source pool kinetics. In proposing a model for the source pool kinetics, however, the presence of the phosphorylated intermediates and the border parenchyma must almost certainly be considered. Although the effects of sucrose exchange with starch cannot be adequately considered from a theoretical viewpoint, experimental data is available on the overall kinetics of the sucrose specific activity which can be used as a gross description of the sucrose kinetics. Although the question of the effect of sucrose in the epidermis cannot be resolved, that pool probably behaves according to one of two extremes. On the basis of Geiger's data (41), it might be considered as a pool which exchanges rapidly with the remaining sucrose in the leaf, or, as pointed out earlier, it may represent a pool which exchanges only very slowly with other sucrose pools. In either case, if most of the remaining sucrose in the leaf exchanged fairly readily, the overall kinetics of the $^{14}$C-sucrose would strongly resemble the kinetics expected of a single pool. Since it is considered advisable to test the simplest cases first and more complicated ones as their consideration is
necessitated by the experimental data, intracellular compartmentation will be ignored. This decision is supported by Geiger's data on sugar beet.

The model which will be adopted tentatively to describe the source pool kinetics in soybean will consist simply of two compartments. The first compartment, which contains most of the sucrose, will represent a photosynthetic compartment and the second will represent the paraveinal mesophyll and border parenchyma. Labelled sucrose is produced in the photosynthetic compartment and passes through the second compartment, which is assumed to be non-photosynthetic, on its way to the veins. There is no transport of sucrose in the reverse direction. On the basis of the relative volumes of the tissues in a soybean leaf, the turnover time of the second compartment will be taken to be roughly 15% of the first. Since the sugar phosphates cause only a brief lag in the maximum labelling of sucrose, they will not be considered.

The equations describing such a model are derived in Appendix D. When turnover times of 77 and 11 minutes are assigned to the photosynthetic and non-photosynthetic compartments, respectively, the curves shown in Figure 69 describe the kinetics for the model. The curve for the smaller compartment which is taken to represent the source pool kinetics, is almost identical to that shown for sterol glucoside in Figure 27.
Figure 69. Solutions to a two-compartment model for source pool kinetics in soybean. \( R_1 \) = radioactivity in the photosynthetic compartment, \( R_2 \) = radioactivity in the paraveinal mesophyll.
C. Carbon-14 Kinetics in the Soybean Stem and Petiole

Under the conditions assumed in Table 7 for the leaf models, the equation describing the distribution of tracer in the stem as a function of time and distance is quite simple (Appendix E). If the distance, $x$, is measured from the point where the translocation stream leaves the leaf, then the equation describing the density, $\rho_R$, of the radioactive translocate will be

$$\rho_R = U(t - \frac{x}{V}) \rho_{RF}(t - \frac{x}{V}),$$  \hspace{1cm} (12)$$

where $\rho_{RF}(t)$ is the density of the radioactive translocate as it leaves the leaf, and $U(t - \frac{x}{V})$ is the unit step function (Section IV, A1).

A graph of $\rho_R$ versus $x$ will be the mirror image of $\rho_{RF}(t)$, except that the origin will be at $x = V/t$, and will be "stretched" or "compressed" along the $x$-axis, depending on whether $V$ is greater or less than 1 cm min$^{-1}$. In applying Equation 12, however, it should be remembered that changes in the cross-sectional area of the translocation stream will be accompanied by an inverse change in the velocity. The value of $V$ for movement through the petiole will be different from that in the stem, and may also be different from that in the leaf.

Although it is felt that Equation 12 offers a fairly
accurate description of the kinetics to be found in the translocation stream, it cannot be applied after long periods of time to the kinetics in the stem as a whole because of leakage from the sieve tubes. This leakage is insufficient to affect greatly the kinetics in the translocation stream over the distances commonly included in tracer work (see Figures 11 and 12), but over relatively long periods, the amount accumulated outside of the sieve tubes could reach levels which might be comparable even to the amount of tracer within the sieve tubes. Since both pools are included when a stem section is extracted, this would cause some distortion of the profile in comparison to that caused by the distribution of radioactivity in the translocation stream itself.

The $^{14}C$-kinetics in the soybean petiole will be approximated here by a two-compartment model. The first compartment represents the translocation stream, and its kinetics will be assumed to be determined entirely by the tracer efflux from the leaf. The second compartment represents the surrounding tissues in the petiole, and receives sucrose from the first in a reversible manner. The system is assumed to be in a steady state. In solving the equations, however, backflow of tracer from the second compartment to the first will be ignored, since the slow turnover time to be assigned to the second compartment will make this tracer contribution negligible in comparison with that coming from the leaf.
In order to simplify the mathematics involved, the effect of leaf size will be ignored, since its kinetic size is so small, and the equation describing the kinetics in the source pool (Equation 43, Appendix D) will be applied to the translocation stream in this model. From the data on relative specific activities in the leaf and petiole (Figure 59), the amount of sucrose in the translocation stream will be taken to be twice that in the surrounding tissues. Since the kinetics in the translocation stream are determined by the kinetics in the paraveinal mesophyll, the solution for the sucrose outside the translocation stream will simply be that for a third compartment (Equation 48, Appendix D). Trial calculations showed that the ratio of sucrose specific activity in the leaf to that in the petiole quickly became more than unity unless the turnover time of the sucrose pool outside the translocation stream were much greater than that within the translocation pool. Accordingly, the turnover time of the source pool was chosen to be twice that of the latter. Curves describing the kinetics in the translocating, stationary and total sucrose pools are shown in Figure 70, where they are compared with the data obtained by monitoring the petiole after pulse labelling the leaf (Section III, B4). Although the curves for the soybean petiole and total sucrose are certainly not identical, they are reasonably close considering the approximate nature of the solution for the model.
Figure 70. Solutions to a three-compartment model for the $^{14}C$ kinetics in a soybean petiole following pulse-labelling ($T_1 = 77$ minutes)
A better fit for the first part of the curve could be expected if the effects of phosphorylated compounds and leaf size were included in the model. The activity peak in the theoretical model definitely comes later than that for the petiole, however, and drops more slowly than for the petiole. In spite of this, the ratio of the sucrose specific activity in the translocation stream after 120 minutes (which may be taken to be the same as that in the leaf, since the activity levels are changing slowly then) to the specific activity of the total petiole sucrose, is about 1.0. This is in agreement with the data in Figure 59.

Since there was some discrepancy in the turnover times obtained in Section III, B8f for the leaf sucrose, it is of interest to examine some curves obtained for this model using shorter turnover times. Figure 71 illustrates the kinetics in a model when the turnover time in the photosynthetic compartment is 50 minutes, and that in the paraveinal mesophyll is 9 minutes. Again, the curve for total sucrose is fairly similar to the kinetics in the petiole. The ratio of the specific activity in the translocation stream to that of the sucrose as a whole is about 1.3, however, a little more than expected from Figure 59.

From Figures 70 and 71, it seems certain that by manipulating the turnover times of the compartments and including the effects of leaf size and phosphorylated intermediates, one
Figure 71. Solutions to a three-compartment model for the $^{14}$C kinetics in a soybean petiole following pulse-labelling ($T_1 = 50$ minutes).
or more combinations of the parameters could be found which would match the observed kinetics in the petiole quite well. Such an exercise would seem to be pointless, however, in the absence of further experimental data. The primary purpose of the foregoing discussion and illustrations has simply been to point out the plausibility of some relatively simple explanations for the tracer kinetics observed in the stem. The presence of the paraveinal mesophyll and the kinetics of sterol glucoside in the leaf are considered to be key observations in support of the assumptions made for the model.

In the stem, it appears both from Figure 45 and from Moorby, Ebert, and Evans' (70) work that the kinetics there may be somewhat different than in the petiole. In particular, the decline of radioactivity seems to be much slower. In considering the model proposed for the petiole, it seems possible that this might be due mostly to a larger sucrose pool outside the sieve tubes, which would result in a greater accumulation of labelled sucrose there.

D. Application of the Models to Willow and Sugar Beet

Some data in the literature offer opportunities to test the models presented in this thesis. The first to be considered will be Canny's studies on translocation in willow (18). In these experiments he allowed willow leaves to
assimilate $^{14}$CO$_2$ for 4-30 minutes and measured both the rate of appearance of $^{14}$C-exudate from aphid stylets and the distribution of total activity along the stem. Both curves were quite similar, as would be predicted from Equation 12. Canny estimated the velocity of translocation in his experiments to be about 2 cm hr$^{-1}$. This is quite low in comparison to that for most other species, and, since the size of the leaves in the willow species he used was about 10-25 cm long (40), would imply that their "kinetic sizes" were in the order of several hours.

Much of the data which is needed for a full comparison of his results with these models is missing. Most important, there is no indication of what the kinetics of sucrose in the fed leaf might have been. In addition, the velocity within the leaf itself must be uncertain, in view of the considerations pointed out in Section III, B8b. However, the fact that he fed the leaves for only about 20 minutes, but the rate of tracer efflux apparently kept increasing for 2-4 hours, is a strong indication that the large kinetic size of the leaf might well play an important part in determining the kinetics in his experiments.

Although Canny fed his leaves for periods of 4-30 minutes, if the labelling of the source pool increases for about the same time, and then decreases exponentially as if the leaf sucrose were a single pool, the low velocity of the
translocation stream will have the effect of making his conditions fairly close to a pulse labelling. This may account for the similarity in his data from different experiments, even though different labelling times were used. A linear leaf model will be used to account for the leaf size and shape since this is the leaf shape found in the species he employed. It will be assumed that the leaf sucrose behaves as a single pool, and that the source pool kinetics can be described by the equation

\[ n_{Ro} = xe^{-\frac{kt}{S}} \]

In order to compare curves of the broadest applicability, the equations describing the tracer efflux are reduced to a dimensionless form. (See Appendix C5). These curves are plotted in Figure 72, and show the dimensionless efflux rate \( \rho_R/\rho_{Ro} \) plotted against dimensionless time, \( kt/S \), for leaves of several kinetic sizes. The continuous curve represents the efflux from a linear leaf of infinite length. It reaches 1.0 at infinite time. The curves which break away from it represent curves for leaves of finite length, and branch away from the first curve when \( t = L/V \), where \( L \) is the length of the leaf. In Canny's data, the slope of the curve before the break is greater than that after, which would predict that the break would come somewhere around \( kt/S < 0.8 \). A prediction of the turnover time to be found in \textit{Salix} can be made from
Figure 72. Solutions to a linear leaf model in terms of dimensionless parameters, assuming a one-compartment model for the source pool.
this since, from the duration of the increase in activity after feeding, the kinetic size (i.e., $t = L/V$) of the leaves must be 2-4 hours. Using this, $S/k > 5$ hrs. This seems to be a remarkably long turnover time for the sucrose pool, and it should be worth while to investigate whether this is, in fact, true. It seems possible, at least, that the low velocity of translocation may result in a longer turnover time in the leaf. Canny points out that the flux of sucrose through the phloem in *Salix* must be considerably less than in most plants with higher velocities of translocation.

Mortimer's (71) recently published experiments on translocation in sugar beet petioles offers another case which can be compared to the mathematical models. In his experiments, sugar beet leaves, about 16 cm long by about 10 cm wide, were pulse-labelled for about one minute and the plants were harvested and analyzed after various times. He presents data for a series of translocation profiles, from which the curve in Figure 73 was reconstructed. This was done by the use of Equation 12, and was quite simple in this case because the velocity was about 1 cm min$^{-1}$. Choosing the exact origin for this curve is difficult, since it will be almost exactly at the point where the first activity is detected. It is possible that this curve should be shifted to the left by up to five minutes. In view of the lag that Mortimer found for the appearance of $^{14}$C in the mid-rib, however, the
Figure 73. Solutions to a rectangular leaf model for the rate of tracer efflux from sugar beet leaves, and comparison to published data
position chosen is probably fairly accurate.

Mortimer's data for the kinetics of sucrose in the leaf indicates that it is still increasing in activity even after 10 minutes, which would presumably indicate a fairly large sugar phosphate pool. Accordingly, a two-compartment model has been adopted to describe the kinetics of sucrose in the photosynthetic pool. The turnover time in the sugar phosphate pool is assumed to be 2.85 minutes. To account for the effects of leaf size and shape, the equation for a rectangular leaf model (Equation 8), with \( L = 16.0 \text{ cm} \) and \( W = 5.0 \text{ cm} \), is applied. The velocity is assumed to be \( 1.0 \text{ cm min}^{-1} \).

Since Mortimer does not provide data for the kinetics of the leaf sucrose after 10 minutes, it is necessary to assume a function which will describe the kinetics after that time. Geiger's (41) data on sugar beet indicates a turnover time of about 40 minutes, and this will be assumed to apply to the sucrose pool in the leaf.

Three curves for the rate of tracer efflux from the leaf have been calculated. The source pool kinetics in the first model are described by the equation for two compartments (Equation 43, Appendix D), the first compartment representing the sugar phosphates and the second the sucrose pool, which is assumed to reflect the source pool kinetics directly. This model, referred to as Model 1 in Figure 73, is felt to meet the simplest possible requirements for an adequate model for
tracer efflux from sugar beet leaves, at least under these experimental conditions.

Model 2 is identical to Model 1 except that the presence of a third compartment has been assumed in order to simulate the effect of a non-photosynthetic sucrose pool in the border parenchyma. The turnover time of this pool is assumed to be about 3.6 minutes and its kinetics will be described by Equation 45. The kinetics for the tracer efflux from Model 2 are also shown in Figure 73.

The third model is similar to Model 2, but represents the effect of doubling the turnover time in the sucrose pools. The turnover time of the sugar phosphate pool remains the same, at 2.85 minutes, and those for the sucrose pools are increased to about 82 and 7 minutes.

It is apparent from Figure 73 that even Model 1, which is considered to represent the minimal realistic one, results in an efflux curve which is at least a reasonable resemblance of the translocation profiles obtained by Mortimer. If the sucrose pool in the border parenchyma is sufficiently large, it will effect an even closer fit of the efflux curve and the actual data (Model 2). Although the curves diverge at longer times from the experimental data, a better fit would be attained with a longer turnover time of sucrose in the leaf (Model 3). Furthermore, if the possibility of sucrose loss from the sieve tubes were considered (see Figures 70 and 71),
a quite close fit could probably be achieved.

E. An Estimate of the Pressure Drop in Soybean Sieve Tubes

A sufficient number of measurements were made in this work to make some approximate calculations of the expected pressure drop in soybean sieve tubes if it is assumed that bulk flow, as described by Münch (72), is responsible for the transport of assimilates. The applicability of the Hagen-Poiseuille equation for capillary flow will be assumed, which is tantamount to making the following assumptions (14):

1. Incompressible fluid
2. Newtonian fluid
3. Velocity at the wall is zero
4. Laminar flow
5. The distance to attain a parabolic velocity is negligible. This "entrance length" will be on the order of \( L_e = 0.035 \frac{\rho v D^2}{\mu} \), where \( D \) is the capillary diameter, and \( \mu \) is the viscosity. If \( \rho = 1 \text{ gm cm}^{-3} \), \( V = 10^{-1} \text{ cm sec}^{-1} \), \( D = 5 \times 10^{-5} \text{ cm} \) and \( \mu = 1.7 \times 10^{-2} \text{ gm cm}^{-1} \), the entrance length for flow through a sieve tube pore will be about \( 5 \times 10^{-10} \text{ cm} \). (The values used for this calculation may be found in other parts of this Section.)
6. The fluid behaves as a continuum. This should be valid unless the diameter of the capillary approaches the mean free path of the molecule. Pappenheimer (77) reviews experimental evidence that the Hagen-Poiseuille equation applies to flow of water through capillaries down to 65 Å in diameter. He also presents theoretical support for its application down to a diameter of 20 Å.

From Section III, B8f, the amount of sucrose contained in the sieve tubes in a one-centimeter length of petiole is about 8.7 Y. The measurements in Section II, B indicate that this is contained in about $5.2 \times 10^7 \mu^3$ of sieve tubes ($35 \text{ cm}^2 \text{ leaf area} \times 10^4 \mu \times 148 \mu^2 \text{ sieve tube/cm}^2 \text{ leaf area}$). These figures give an approximate sucrose concentration in the sieve tubes of about $0.17 \text{ gm cm}^{-3}$ ($8.7 \times 10^{-6} \text{ gm}/5.2 \times 10^{-5} \text{ cm}^3$), or about a 17% sucrose solution. Although somewhat high, this is in reasonable agreement with values reported for the exudate from phloem (28).

In order to calculate the pressure drop across a single sieve plate, it is necessary to know the number of pores through which the solution flows and the bulk flow rate. The latter can be calculated from the assumed volume rate of flow in the petiole, and is about $1.3 \times 10^{-4} \text{ cm}^3 \text{ min}^{-1}$ ($5.2 \times 10^{-5} \text{ cm}^3 \times 2.5 \text{ cm min}^{-1}$). The number of pores must be calculated
from the number of sieve elements present in a cross-section of the petiole. From the measurements in Section II, B, the area of a sieve tube is about 22.1 \( \mu^2 \), and that of all the sieve tubes is \( 5.2 \times 10^3 \mu^2 \). The number of pores, then is 50 pores \( \times 5.2 \times 10^3 \mu^2 / 22.1 \mu^2 = 1.18 \times 10^4 \) pores. The bulk rate of flow through one pore will be \((1.3 \times 10^{-4} \text{ cm}^3 \text{ min}^{-1} / 1.18 \times 10^4) = 1.1 \times 10^{-8} \text{ cm}^3 \text{ min}^{-1} \) or, converting to seconds, about \( 2 \times 10^{-10} \text{ cm}^3 \text{ sec}^{-1} \).

The Hagen-Poiseuille equation states that

\[
Q = \frac{nAPR^4}{8\mu L}
\]

where \( Q = \text{volume flow rate,} \ [=] \text{ cm}^3 \text{ sec}^{-1} \)
\( \Delta P = \text{pressure difference between the ends of a tube of length} \ L, [\text{=}] \text{ gm cm}^{-1} \text{ sec}^{-2} \)
\( R = \text{radius of the tube,} \ [\text{=}] \text{ cm} \)
\( \mu = \text{viscosity,} \ [\text{=}] \text{ gm cm}^{-1} \text{ sec}^{-1} \)

The values to be used in solving for \( \Delta P \) are:

\( Q = 2 \times 10^{-10} \text{ cm}^3 \text{ sec}^{-1} \)
\( R = 2.5 \times 10^{-5} \text{ cm} \) (Section II, B)
\( \mu = 1.7 \times 10^{-2} \text{ gm cm}^{-1} \text{ sec}^{-1} \); this is the viscosity of a 20\% sucrose solution at 25\degree C (52)
\( L = 1.5 \times 10^{-4} \text{ cm} \) (Section II, B)

Solving for \( \Delta P \),
\[
\Delta P = 3.37 \times 10^3 \text{ gm cm}^{-1} \text{ sec}^{-2}
\]

or \( 3.4 \times 10^{-3} \) atmospheres
Since the sieve cells are about 125 $\mu$, there will be about 80 sieve plates per centimeter, and the pressure drop will be about $2.8 \times 10^{-1}$ atmospheres per centimeter. In the stem, where the cross-sectional area of the translocation stream increases, the pressure gradient would be proportionately less.

Since the osmotic potential of a 17% sucrose solution would be of the order of 10 atmospheres, it seems possible under the assumptions made here that there could be a sufficient pressure gradient generated to cause transport at the observed rates in a 30 cm soybean stem.

There are a number of reasons to suspect that the calculations are misleading, however. As pointed out earlier, it is not at all certain that the sieve pore opening is actually 0.5 $\mu$ in diameter, since this figure quite likely includes the callose cylinder. Since the bulk rate of flow is a function of the fourth power of the radius, even just halving the pore diameter would increase the pressure drop sixteenfold. Secondly, electron micrographs have consistently shown that the pores in the sieve plate are traversed by many fibrous strands (38, 51, 60, 113), which would have the effect of creating a huge drag on any solution flowing past them, at least if they are considered to be stationary. We are thus brought squarely against the most outstanding dilemma presently concerning studies of translocation: although many
features of phloem transport are accounted for by Münch's mass flow hypothesis, it seems that when conventional concepts of viscosity are applied the structure of the sieve plate precludes the operation of that mechanism. It seems that either some unconventional concept of viscosity (25) must be applied to sieve tube transport, or another model must be considered, possibly similar to that proposed by Spanner (90), which also calls for bulk flow. If an unconventional concept of viscosity is proposed, it almost certainly must provide for "slippage" at the capillary walls (i.e., eliminate condition 3, p. 206), since it does not seem likely that any of the other conditions implied by the Hagen-Poiseuille equation could be modified sufficiently to account for the necessary flow rate.

F. The Relationship between Pulse Labelling and Steady State Labelling

If the differential equations describing the $^{14}$C-kinetics in a photosynthesizing leaf are linear, with constant coefficients, a relatively simple relationship should exist between pulse and steady state labelling experiments. (For a derivation of this relationship, see (53). Also, see (86).) Specifically, the kinetic curves obtained from the former type of experiment should be the first derivatives of those
obtained from the latter, if the same conditions apply to both experiments. This relationship was used by Evans, Ebert and Moorby (37) in interpreting their data, and is used in the present work as a basis for a qualitative comparison of these two types of experiments.

Strictly speaking, a compartmental system is "pulse-labelled" if only one compartment is labelled at zero time, and no more tracer is added to the system. This is virtually impossible to achieve, of course, but if the period during which the label is introduced is short in comparison to the turnover time of the compartment of interest, this will provide a good approximation of a "pulse" label. In the present case, the photosynthetic intermediates could not be said to be pulse-labelled, but the sucrose pool, for example, will be.

A simple illustration of the relationship may be given by considering a single compartment in which the rates of influx and efflux are equal. If it is pulse-labelled, the solution will be

\[ a_1(t) = a(0)e^{-\frac{kt}{S}} \]  

(13)

where the \( a \)'s are specific activities and \( S/k \) is the turnover time of the compartment. The solution for steady state labelling is

\[ a_2(t) = a_{(in)}(1 - e^{-\frac{kt}{S}}), \]  

(14)
where \( a_{(in)} \) is the (constant) specific activity of the entering isotope. The first derivative of the latter equation is

\[
a_2'(t) = \frac{ka_{(in)}}{S} e^{-\frac{kt}{S}}
\]

Unless the specific activities used in the two experiments are deliberately chosen to meet the mathematical requirements, the relationship between curves such as (13) and (15) will be one of proportionality rather than equality.

The principal difficulty in applying the theorem is the question of whether the linear differential equations actually have constant coefficients. Although it is possible that a similar relationship may hold for at least some cases of equations with variable coefficients, the writer is not aware of more general theorems.

In the derivation of differential equations, variable coefficients commonly arise when the processes involved are non-linear. In the present situation, which supposedly involves mass transport, chemical and physical-chemical reactions, variable coefficients would be expected to arise primarily if these processes were concentration dependent. Although this might be the case in a chemical unsteady state, where concentrations are changing, it would not seem to apply when an isotope is introduced to a system already at a chemical steady state unless there were an isotope effect. It
seems reasonable, therefore, to expect that the differential equations describing the distribution of isotope will have constant coefficients.
V. DISCUSSION

The models proposed in Sections IV, Al-3 are the simplest possible for tracer efflux from leaves. In essence, they simply state that the translocate is introduced into the translocation stream and proceeds from its point of introduction at a constant velocity without further interaction with the rest of the leaf.

For the source pool kinetics, the simplest model would seem to be a single pool with equal influx and efflux. Although this is apparently a good approximation in some cases, it will not be when there are large pools of phosphorylated intermediates. In the case of soybean, the paravinal mesophyll also probably modifies the kinetics in the source pool. The simplest way of accounting for these effects is a sequential compartmental model through which there is a unidirectional flow of sugar. Although such a model is on less certain ground than the model for tracer efflux from leaves, it accounts fairly well for the apparent source pool kinetics in soybean leaves and, when substituted into the equation for a rectangular leaf, for tracer efflux from sugar leaves.

The kinetics exhibited by sterol glucoside offer some tangible evidence that there is at least some carbohydrate pool within the leaf which exhibits the kinetics to be
expected of the source pool. The possibility that sterol glucoside (and esterified sterol glucoside?) is associated in some unique way with translocation is supported by the observation that among the lipids in the stem, it alone becomes radioactive during the translocation of radioactive sucrose. The observation that only the glucose moiety of this lipid is radioactive also makes it attractive to speculate that it may be acting as a carrier in transporting hexoses across a membrane, possibly from the source pool into the translocation stream. If sterol glucoside acts as a carrier for sucrose, the energy of the glycosidic bond could be largely preserved. In that case, the transport of fructose would have to be accounted for either separately or by conversion to sterol glucoside. The latter process would involve an "unnecessary" expenditure of energy, although it might be speculated that such an energy expenditure is necessary for the accumulation of sucrose against a concentration gradient. Some evidence against the possible role of sterol glucoside as an indirect sucrose carrier may be implied from the experiments of Hatch and Glasziou (48) which demonstrated that when fructosyl-\(^{14}\)C-sucrose was administered to sugar cane leaves it was translocated without a redistribution of the \(^{14}\)C between the hexose moieties of sucrose. This would indicate that the hexoses do not pass through a common intermediate during translocation.
In addition to sterol glucoside, the kinetic curves of some of the other compounds are also interesting. In fact, during the first twenty minutes or so, when the rate of tracer efflux from the leaf is changing most rapidly, the kinetic curves for many individual compounds are also changing quite rapidly. The galactolipids are among these, and during the first twenty minutes the kinetics of monogalactosyl diglyceride, especially, resemble that of sterol glucoside. Before the data on $^{14}$C-accumulation in the petiole was obtained (Figures 21 and 45), the kinetic curves for the galactolipids were taken as a possible indication of a role in translocation, since they strongly resembled the curves obtained by Moorby et al. (70). This was quite puzzling, since they are known to be almost entirely confined to the chloroplasts (108). One might still wonder from their initial similarity to the curve for sterol glucoside, whether there is any relationship to that lipid. The specific activities are certainly different, however, since there is so much galactolipid in the leaf in comparison to sterol glucoside (see Figure 8). In respect to specific activity, the galactolipids appear to behave more like glucose and fructose, except that they become labelled sooner.

The kinetic curve for unknown $\beta$ is of some interest in relation to translocation, since it is at least roughly what
one might expect of the source pool. In the pulse-labelling experiment, it increased rapidly for almost twenty minutes and slowly declined afterwards. Virtually no radioactive \( \beta \) was recovered in the stem, however, and its possible role in translocation is difficult to assess because its chemical identity is unknown. Furthermore, it would seem to reach a maximum too soon and decay too slowly to be associated with the source pool.

The relationship between the kinetic curves obtained from the steady state and pulse-labelling experiments (Sections III, B5 and III, B7) is, in most cases, at least roughly that expected from the theoretical considerations discussed in Section IV, F. Many of the differences found are probably attributable to variations between individual plants, such as different pool sizes, metabolic rates, etc. The curves for unknown \( \delta \), glucose and possibly fructose are not so easily reconciled by such hypothetical differences, however. Although it is possible that this may indicate a real deviation from the supposed theoretical relationship between the two types of feedings, the points for the hexoses in the steady state labelling were fairly scattered, and their precise kinetics are ambiguous. Geiger's data (41) for glucose and fructose from pulse-labelling and steady state labelling experiments can be interpreted fairly well by the assumption that the curves from the pulse-labelling experiment are the
first derivatives of those from the steady state labelling experiment.

The assumption of a constant velocity for the leaf models has some important implications concerning possible translocation mechanisms. (Observations supporting this assumption were reviewed in Section IV, A1). The most obvious implication in this respect concerns "activated diffusion" types of transport mechanisms, if by "activated diffusion" is meant a mechanism which can be described by diffusion equations with a high apparent diffusion coefficient. In general, the diffusion equations will yield curves which will change in shape as the translocation profile progresses down the stem (e.g., 22). Additional evidence against an "active diffusion" mechanism comes from experiments by Canny (18) and Zimmermann (114) which show that peaks of radioactivity or sugars, respectively, can progress down a stem. If a diffusion type mechanism were responsible for translocation, rapid spreading of these peaks would be expected. It should be emphasized that these remarks are intended to apply only to activated diffusion, and not to "active transport" mechanisms in general. If the term "active diffusion" is to convey any meaning, it seems that it must refer to a mechanism which results in a high apparent diffusion coefficient, and which therefore can be described by diffusion equations.

The description of the translocation velocity by a
constant is consistent with bulk flow mechanisms, even though a radial velocity gradient would be expected from them. Taylor (94) has shown that in very small capillaries, the effect of a velocity profile is hardly noticeable because of mixing by diffusion.

With regard to the assumptions made for the model, it should be pointed out that, in addition to the experiment with barley (Section III, B1b), there are at least two cases of experimental data in the literature which certainly cannot be accounted for by the models as they are stated here. Clauss et al. (24) found that when a primary leaf of soybean was fed for ten minutes, the amount of radioactivity in a 4 cm stem segment below the primary node kept increasing at a roughly linear rate for at least two hours. This was in spite of the fact that the amount of $^{14}$C-sucrose in the fed leaf decreased after fifteen minutes. These results are difficult to assess, however, because they are completely different from those obtained in the present work, and in the experiments by Moorby et al. (70), who also worked with soybean.

A somewhat similar observation was recorded by Swanson and El Shishiny (93), who found that it took 6-12 hours after photosynthetic labelling of a grape leaf for radioactivity to appear at the node of the fed leaf. After this, translocation proceeded at a velocity of about 1 cm min$^{-1}$. 
The experiments with excised stems (Section III, B9) also have some important implications concerning the possible mechanism of translocation. Since no movement occurs in a completely excised stem, it seems unlikely that a protoplasmic streaming mechanism can be involved. In fact, the operation of any mechanism involving the sieve tubes themselves as the site of the motivation force might be questioned. It is possible, however, that the catenated nature of the translocation process might render such a mechanism inoperable in a completely excised stem.

The movement of translocate into the stem after excision of the root is quite similar to results obtained by Hartt and Kortschak (45) in similar but more extensive experiments with detached sugar cane leaves. In their experiments, prolonged translocation resulted in a positive sucrose gradient from the tip to the base of the leaf. They point out the absence of a "sink" in such a system and the difficulty in reconciling the results with the turgor requirements of Münch's bulk flow mechanism. The observation that excision of the leaf apparently almost completely stops translocation would also be difficult to account for by Münch's model, since in this case a sink (i.e., the root) is still present as well as a region of high osmotic pressure. It is possible that the turgor pressure might be reduced by exudation from the petiole, but when the stems or petioles of other soybean plants were cut
exudation occurred only for the first few seconds, and then ceased.

Probably the most serious question in interpreting many of the experimental results presented here is whether or not translocation actually is taking place from a leaf after several punches have been taken. Although there is no data which can be cited as unequivocally demonstrating that translocation continues under these conditions, most of the results are felt to be in agreement with that view. It is possible, however, that it may be occurring at a diminished rate. The most direct support comes from the demonstration that when a leaf is excised, the sucrose content increases. One would expect that if translocation were also halted by the sampling procedure, that a similar occurrence would be observed. There was no such indication of this in any of the experiments except in the first steady state labelling experiment, where it is quite possible that the accumulation was due to some other effect, particularly the experimental lighting conditions. The data for the amount of daily variation in the sucrose pool (Figures 74 and 75) also indicates that translocation is probably occurring. Hartt et al. (46) used a similar procedure in sampling sugar cane leaves, and their data showed considerable translocation of radioactivity from the leaf after pulse-labelling. The loss from soybean leaves is not as definite, however.
The discrepancies in the turnover times calculated from different experiments also present some difficulties in evaluating the data. The value indicated by the $^{14}$C-sucrose kinetics is about 80-90 minutes (Sections III, B5a; III, B7a and III, B8f) but when the turnover time is calculated from the apparent rate of sucrose efflux from the leaf and the pool size in the leaf (Section III, B8f), about half this value is obtained. The difference could probably be explained if sucrose participated in an exchange reaction with a polysaccharide which resulted in a new gain of activity by the sucrose. It was shown, however, that if such an exchange occurs with starch, the net gain in activity is by starch rather than the sucrose. It would seem that either there is considerably less radioactivity in the translocation stream in the petiole than was proposed, or that the actual velocity was less than calculated. The latter consideration is a possibility since the anatomical irregularities in the translocation path under study (i.e., the soybean petiole and stem) make an interpretation of the translocation velocity somewhat involved. Even so, the uncertainties would not seem to be great enough to account for the differences in turnover times.

The failure of the sucrose specific activity in the steady state labelling experiments to reach that of the fed $^{14}$CO$_2$ was surprising, and not readily explained. The most probable cause would seem to be compartmentation of some type
within the leaf, and the possibility that the epidermal layers represent a more or less stagnant sucrose pool was discussed earlier (Section IV, B). Another, but more indirect, type of compartmentation could explain the low specific activity of the sucrose. The light intensity in these experiments was fairly low, and the photosynthetic rate may have been close to the compensation point. Weigl et al. (107) have shown that in barley, respiratory carbon dioxide is produced largely from endogenous substrate, and recently fixed carbon is not readily respired unless the plant is placed in the dark immediately after $^{14}\text{CO}_2$ assimilation. It seems quite reasonable to suppose that the plant would re-fix much of its respiratory carbon dioxide, and if this were of a relatively low specific activity, the specific activity of the photosynthate would be lower than expected, especially under low light conditions. Some calculations were made, therefore, in order to obtain some idea of the possible relative rates of photosynthesis and respiration in the present experiments.

According to James (55), a representative figure for the respiratory rate of leaves would be about $2\text{ mm}^3\text{ CO}_2$ per hour per milligram of dry weight. From Figure 19, the approximate extracted dry weight of a soybean leaf would be about 65 mg. An approximate figure for the dry weight before extraction might be about 70 mg. This can be compared to the fresh weight of about 600 mg. The expected rate of respiration,
therefore, would be about 140 mm$^3$ CO$_2$ per hour per leaf or about 6.2 x 10$^{-3}$ millimoles of carbon per hour per leaf. A figure of about 8.3 x 10$^{-3}$ millimoles of carbon per hour per (soybean) leaf can be calculated from Weigl et al.'s (107) data for barley.

The photosynthetic rate in a steady state labelling experiment can be calculated from the data for the $^{14}C$-fixation rate per punch in Section III, B7d to be, in terms of radioactivity, 1.55 x 10$^8$ dpm per hour per leaf. The specific activity of the Ba$^{14}CO_3$ recovered from that experiment was 3.3 x 10$^9$ dpm per millimole. The photosynthetic fixation rate, then, is about 4.7 x 10$^{-2}$ millimoles of carbon per hour per leaf. This is about six to eight times the respiration rate calculated above and indicates that the illumination is not as close to the compensation point as might be expected from the light meter reading. It seems quite probable, however, that the spectral qualities of the Gro-Lux lamps would sustain a higher photosynthetic rate than the light intensity indicated by the meter reading.

With the relative rates of photosynthesis and respiration calculated above, even if it is assumed that all of the respired CO$_2$ were refixed and that it was completely unlabelled, this would only cause the specific activity of the sucrose to be lowered by about 13-17% below that of the $^{14}CO_2$. The sucrose specific activity in the experiments reported here was almost 40% below that of the $^{14}CO_2$. 
VI. SUMMARY

The kinetics of translocation in plants was studied from experimental and theoretical viewpoints. Evidence is presented to support the view that the main characteristics of the translocation profile are generated within the leaf itself, and not by processes operating within the stem. Equations are derived to account for the effects of leaf size, shape and source pool kinetics on the rate of tracer efflux from leaves. In these models, the effects of leaf size and shape extend over a time period which is roughly equivalent to the time taken for translocate to travel from the farthest point in the leaf to the petiole. The expression for the source pool kinetics appears in the equations as an unspecified function of time. The assumption of a single velocity of translocation, with virtually negligible loss of translocate from the translocation stream, are key assumptions for the models.

The kinetics exhibited by water soluble compounds, lipids, starch and insoluble materials were followed for three hours in single soybean leaves after pulse-labelling or during steady state labelling. The specific activity of the sucrose for each of the sample times was determined. After pulse-labelling, the sucrose specific activity increases sharply for about five minutes, then decreases with an
apparent turnover time of about 90 minutes. During steady state labelling, the sucrose specific activity increases for about 100 minutes and, although it virtually stops increasing after this time, its specific activity is only 62% of the fed $^{14}C_{O_2}$.

A lipid identified as a sterol glucoside exhibited kinetics in the pulse-labelling experiment which were quite similar to the kinetics of $^{14}C$-accumulation in the petiole of a pulse-labelled soybean leaf. Its activity increased for about 20 minutes, then decreased at about the same relative rate as sucrose. This was also the only lipid which became labelled to a significant extent during the passage of radioactive translocate through the stem. Its kinetics are almost entirely due to the glucose moiety, since after three hours of steady state labelling, the glucose moiety contained 98% of the activity. Another sterol glucoside, probably an esterified sterol glucoside, exhibited qualitatively similar kinetics in the leaf, but could not be counted due to poor separation.

The kinetics of sucrose in the source pool (i.e., in the cells immediately surrounding the phloem) are probably modified by a unique layer of mesophyll cells which form a two-dimensional network between the veins in the plane of the phloem. A two-compartment model was adapted to describe the source pool kinetics in soybean, and was fairly successful in
accounting for the shape of the translocation profile. The kinetics of the theoretical source pool were quite similar to those for sterol glucoside.

The translocation profiles and distribution of $^{14}C$ in compounds in the leaves and stems of soybean plants was determined for times up to two hours after pulse-labelling the first trifoliate leaf. After 50 minutes, the distribution of radioactivity along the stem was fairly uniform. Irregularities in the profiles occurred at the nodes, and were attributed to changes in the cross-sectional area of the translocation stream. This was particularly noticeable in passing from the petiole to the stem, and is reflected by a change in the average diameters of the sieve tubes in the petiole and stem.

By plotting the relative specific activities of sucrose in the leaf and petiole versus time, it was concluded that about two-thirds of the sucrose in the petiole is in the translocation stream. Using the velocity of translocation, a rate of sucrose efflux from the leaves was calculated and, from this, the turnover time of sucrose in the leaf was calculated to be about 45 minutes. No satisfactory explanation could be found for the discrepancy between this figure and that calculated by the disappearance of radioactivity from the leaf sucrose after pulse-labelling.

From measurements made of the phloem, if it was assumed
that the pores in the sieve plates were entirely open and that bulk flow occurred, the expected pressure gradient could be calculated to be 0.28 atmospheres per centimeter, in the petiole.
VII. LITERATURE CITED


VIII. APPENDIX A. LIST OF SYMBOLS

The notation followed has been adopted almost entirely from "Transport Phenomena", by Bird, Stewart and Lightfoot (14), and from "Basic Principles of the Tracer Method" by Sheppard (86). Between these two texts, a flexible system of notation is provided which can be applied to a wide variety of transport and tracer problems.

The dimensions of each quantity are indicated after its definition in terms of mass (M), length (L) and time (t). Symbols which appear only once are not listed.

Arabic letters

\[ A = \text{area, } L^2 \]
\[ a = \text{specific activity (}= \text{R/S}) , \text{ dpm } M^{-1} \]
\[ D = \text{diameter, } L \]
\[ D = \text{diffusion coefficient, } L^2 t^{-1} \]
\[ k = \text{rate of transport between compartments, } Mt^{-1} \]
\[ L = \text{length of a leaf, } L \]
\[ m = \text{dummy variable of integration} \]
\[ n = \text{chemical mass flux, } ML^{-2}t^{-1} \]
\[ n_o = \text{chemical mass flux into the translocation stream from the source pool, } ML^{-2}t^{-1} \]
\[ n_{Ho} = \text{isotopic mass flux into the translocation stream from the source pool, } ML^{-2}t^{-1} \]
\( n_x, n_r, \text{ etc.} = \text{chemical mass flux in the } x-\text{direction, } r-\text{direction, etc., } \text{ML}^{-2}\text{t}^{-1} \)

\( n_{Rx}, n_{Rr}, \text{ etc.} = \text{isotopic mass flux in the } x-\text{direction, } r-\text{direction, etc., } \text{ML}^{-2}\text{t}^{-1} \)

\( p(x,s) = \text{Laplace transform, with respect to } t, \text{ of } \rho_R(x,t) \)

\( R = \text{radioactivity, dpm} \)

\( r = \text{radius, L} \)

\( S = \text{Total (chemical) amount of substance in a compartment, M} \)

\( T = \text{turnover time of a compartment (} = S/k\text{), t} \)

\( t = \text{time, t} \)

\( u = \text{dummy variable of integration} \)

\( V = \text{velocity of translocation, Lt}^{-1} \)

\( W = \text{radius of a peltate leaf model, or the half-width of a rectangular leaf model, L} \)

\( x = \text{distance along the } x\text{-axis, L} \)

\( y = \text{distance along the } y\text{-axis, L} \)

\( z = \text{distance along the } z\text{-axis, L} \)

**Greek letters**

\( \pi = 3.14159... \)

\( \rho = \text{chemical density, ML}^{-3} \)

\( \rho_R = \text{isotopic density, ML}^{-3} \)
\( \rho_{RP} = \) isotopic density as the translocation exists from the leaf, ML\(^{-3}\)

\( \tau = \) dimensionless time
IX. APPENDIX B. EXACT VALUES FOR THE TURNOVER TIMES USED TO DESCRIBE COMPARTMENTAL MODELS

Since the equations for compartmental models (Appendix D) involve the reciprocals to the turnover times rather than the turnover time itself (i.e., $T^{-1}$ instead of $T$), some of the values given for the turnover times in the text are only approximate. The values for $T^{-1}$, which were actually used in computing the curves, are given below.

Table 8. Values for $T^{-1}$ for compartmental models

<table>
<thead>
<tr>
<th>Figure</th>
<th>Values for $T^{-1}$, given in order of the compartments</th>
</tr>
</thead>
<tbody>
<tr>
<td>69</td>
<td>0.013, 0.09</td>
</tr>
<tr>
<td>70</td>
<td>0.013, 0.09, 0.0065</td>
</tr>
<tr>
<td>71</td>
<td>0.02, 0.11, 0.01</td>
</tr>
<tr>
<td>73, Model 1</td>
<td>0.35, 0.024</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.35, 0.024, 0.28</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.35, 0.012, 0.14</td>
</tr>
</tbody>
</table>
X. APPENDIX G. DERIVATION OF THE EQUATIONS DESCRIBING THE EFFECTS OF LEAF SIZE, SHAPE AND SOURCE POOL KINETICS ON THE RATE OF TRACER EFFLUX FROM LEAVES

The approach followed in constructing these models largely follows the principles outlined by Bird, Stewart and Lightfoot (14), and that text should be consulted for particulars. From a practical point of view, the most important advantage of such an approach is that, in the course of constructing a model to describe a system, one can categorically state the assumptions implied by the final equations for the model. The applicability of the model can be judged largely on the basis of the assumptions, and experimental work can be directed at testing their validity.

A. Equations for a Linear Leaf

The geometry for the linear leaf model is shown in Figure 62. In following the derivations, the reader should keep in mind that vector quantities, such as velocity and flux, are defined as being positive if they occur in a positive direction. Many of the actual values for these quantities will be negative in the present models, due to the orientation of the coordinates.

If we consider an incremental volume in the translocation
stream, \( z\Delta x\Delta y \), a mass balance for tracer, \( R \), entering and leaving this volume may be stated as

\[
\text{Rate of accumulation of } R = \{(\text{Flow of } R \text{ in}) - (\text{Flow of } R \text{ out})\} + \{\text{Transport of } R \text{ from the source pool}\}
\]

If it is assumed that no \( R \) enters from the \( y \)-direction, then Equation 16 may be written as

\[
\frac{\Delta \rho_R}{\Delta t} (z\Delta x\Delta y) = Ay(zn_{Rx}|_x - zn_{Rx}|_x + \Delta x)
\]

\[+ n_{R0} \Delta x \Delta y \]

where \( \rho_R = \text{density of } R, = \text{gm cm}^{-3} \)

\( n_{Rx} = \text{flux of } R \text{ in the } x\text{-direction (read } n_{Rx}|_x \text{ as "}\text{\( n_{Rx} \) evaluated at } x\text{")}, = \text{gm cm}^{-2} \text{ min}^{-1} \)

\( n_{R0} = \text{flux of } R \text{ from the source pool}, = \text{gm cm}^{-2} \text{ min}^{-1} \)

Dividing by \( z\Delta x\Delta y \) and taking the limit as \( \Delta x \) and \( \Delta t \) approach zero,

\[
\frac{\partial \rho_R}{\partial t} = \frac{n_{R0}}{z} - \frac{\partial (zn_{Rx})}{\partial x}
\]

We may provide an expression for \( z \) by considering the geometrical necessities imposed on the translocation stream by the other conditions to be assumed for the model. If \( n_0 \) is
the total flux of labelled and unlabelled translocate into
the translocation stream, and it is constant over the entire
leaf, the following expression is obtained by taking a mass
balance over some length, \( L - x \), of the leaf:

\[
\Delta y(x - L)n_o = n_x z \Delta y
\]

where \( n_x \) is the flux of translocate at \( x \). If the velocity of
the translocate is a constant, \( V \), and diffusion is neglected,
then

\[
z = \frac{(x - L)n_o}{\rho V}
\]

Substituting Equation 1 into Equation 18,

\[
\frac{\partial \rho_R}{\partial t} = \frac{\rho V n_o}{(x - L)n_o} - \frac{\partial[n_{Rx}(x - L)]}{(x - L)\partial x}
\]

If diffusion of \( R \) is neglected, \( n_{Rx} = \rho_R V \), and

\[
\frac{\partial \rho_R}{\partial t} = \frac{\rho V n_o}{(x - L)n_o} - \frac{V}{(x - L)} \frac{\partial[\rho_R(x - L)]}{\partial x}
\]

Expanding the last term, this becomes

\[
\frac{\partial \rho_R}{\partial t} = \frac{\rho V n_o}{(x - L)n_o} - V \frac{\partial \rho_R}{\partial x} - \frac{V \rho_R}{(x - L)}
\]

Equation 20 can be solved readily by using Laplace
transforms. The transformed equation is
\[ s \ p(x,s) = \frac{pV}{(x - L)n_o} \ n_{Ro}(s) - V \ \frac{d p(x,s)}{dx} \ - \frac{V \ p(x,s)}{x - W} \]  

(21)

where \( p(x,s) = \) Laplace transform, with respect to \( t \), of \( \rho_{R}(x,t) \)

\[ n_{Ro}(s) = \] Laplace transform, with respect to \( t \), of \( n_{Ro} (= n_{Ro}(t)) \)

Solving for \( p(x,s) \)

\[ p(x,s) = \frac{pVn_{Ro}(s)}{n_o s(x - L)} + \frac{C(s)}{(x - W)} \ e^{-\frac{sx}{V}} \]  

(22)

where \( C(s) \) is a constant of integration. We may solve for \( C(s) \) by applying the boundary condition \( n_{Ro}(L,t) = 0 \). Equation 22 becomes

\[ p(x,s) = \frac{pVn_{Ro}(s)}{n_o s(x - L)} \left[ 1 - e^{-\frac{s(W - x)}{V}} \right] \]  

(23)

The inverse Laplace transform of this is Equation 4, Section IV, A1.

B. Equations for a Rectangular Leaf

The assumptions made at each step will not be pointed out in this case, since they are completely analogous to those made for the linear leaf model.

If the cross-sectional area of the central channel is \( A \), then taking a mass balance similar to Equation 16,
\[ \frac{\Delta \rho_R}{\Delta t}(A \Delta y) = \left( n_{Ry}^A \Big|_y - n_{Ry}^A \Big|_{y + \Delta y} \right) + V \rho_{RC} \frac{n_{wO}^W}{\rho V} \Delta y \quad (24) \]

where \( \rho_{RC} \) = density of isotopic sugar contributed to the central channel by the side leaves

\[ \frac{n_{wO}^W}{\rho V} = \text{depth of the translocation stream from the "side leaf" (from Equation 1)} \]

By dividing through by \( A \Delta y \) and taking the limit as \( \Delta y \) and \( \Delta t \) approach zero, this becomes

\[ \frac{\partial \rho_R}{\partial t} = \frac{\rho_{RC} n_{wO}^W}{\rho A} - \frac{\partial (n_{Ry}^A)}{A \partial y} \quad (25) \]

As before, an expression for \( A \) may be obtained by taking a mass balance on the total translocate over some length, \( y - L \), of the leaf.

\[ A = \frac{W(y - L)n_{wO}}{\rho V} \quad (26) \]

Substituting this into Equation 25, we obtain

\[ \frac{\partial \rho_R}{\partial t} = \frac{\rho_{RC} V}{(y - L)} - V \frac{\partial [\rho_R(y - L)]}{(y - L) \partial y} \quad (27) \]

By expanding the last term, this becomes

\[ \frac{\partial \rho_R}{\partial t} = \frac{\rho_{RC} V}{(y - L)} - V \frac{\partial \rho_R}{\partial y} - \frac{\rho_R V}{(y - L)} \quad (28) \]

The expression for \( \rho_{RC} \) may be obtained from Equation 5 by
replacing L with W. Equation 28 then becomes identical to Equation 6, Section IV, A2. Taking Laplace transforms,

\[ sp(x,s) = \frac{-\rho V^2}{n_0 W(y - L)} \frac{n_{R_0}(s)}{s} \left(1 - e^{\frac{SW}{V}} \right) - V \frac{dp(x,s)}{dx} - \frac{\rho V}{y - L} \] (29)

Solving for \( p(x,s) \) and applying the boundary condition \( \rho_R(L,t) = 0 \),

\[ p(x,s) = \frac{-\rho V^2}{n_0 W(y - L)} \frac{n_{R_0}(s)}{s^2} \left(1 - e^{\frac{SW}{V}} \right) \left(1 - e^{\frac{S(L - y)}{V}} \right) \] (30)

The inverse transform of Equation 30 is Equation 7, Section IV, A2.

C. Equations for a Peltate Leaf

A mass balance on \( R \) over an incremental area of the translocation stream, \( 2\pi rz \Delta r \), yields

\[ \frac{\Delta \rho_R(2\pi rz \Delta r)}{\Delta t} = 2\pi (n_{Rr}rz) \bigg|_r - (n_{Rr}rz) \bigg|_{r + \Delta r} + n_{R_0} 2\pi rz \Delta r \] (31)

Dividing by \( 2\pi rz \Delta r \) and taking the limit as \( \Delta r \) and \( \Delta t \) approach zero,

\[ \frac{\partial \rho_R}{\partial t} = \frac{n_{R_0}}{z} - \frac{\partial (n_{Rr}rz)}{rz \partial r} \] (32)

An expression for \( z \) may be obtained as in previous
sections by considering a mass balance for all of the translocate, which states that

\[ z = \frac{(r^2 - w^2)n_o}{2 \rho V} \]  

(33)

Substituting this into Equation 32, we get Equation 9, Section IV, A3. By expanding the last term,

\[ \frac{\partial \rho_R}{\partial t} = \frac{2rV \rho_R n_{Ro}}{(r^2 - w^2)n_o} - \nu \frac{\partial \rho_R}{\partial r} - \frac{2rV \rho_R}{(r^2 - w^2)} \]  

(34)

Taking Laplace transforms, this becomes

\[ s p(r,s) = \frac{2rV \rho_R n_{Ro}(s)}{(r^2 - w^2)n_o} - \frac{2rV p(r,s)}{(r^2 - w^2)} - \nu \frac{d p(r,s)}{dr} \]  

(35)

Solving for \( p(x,s) \) and applying the boundary condition 
\( \rho_R(W,t) = 0 \),

\[ p(x,s) = \frac{2rV^2}{(r^2 - w^2)} \frac{n_{Ro}(s)}{s^2} \left[ \frac{s(r - W)}{V} - 1 - e^{\frac{s(r - W)}{V}} (\frac{sW}{V} - 1) \right] \]  

(36)

The inverse Laplace transform of this is Equation 10, Section IV, A3.
Most of the kinetic curves presented for the leaf models were calculated by programming the equations on a digital computer, the IBM 7074. The basic Fortran programs are shown below. Specific values have not been assigned to $V$, $L$, $W$ or the integrals of $n_{Ro}$. As written here, the values which will actually be printed for "RHO" must be multiplied by the factor outside the brackets to obtain the actual value for $\rho_R$ (e.g., in the case of a linear leaf, by $-V/n_o L$). The following notation has been employed in the programs:

$$\int \int n_{Ro} = \int_0^t \int_0^u n_{Ro}(m) \, dm \, du$$

$$\int n_{Ro} = \int_0^t n_{Ro}(u) \, du$$

where $u$ and $m$ are dummy variables of integration.

1. **Program for a linear leaf model**

```fortran
1 WRITE (2,2)
2 FORMAT (IH, 22X, 4HTIME, 22X, 3HRHO)
   T = 0.0
3 RHO = GNCTN(T) - U(T - L/V)*GNCTN(T - L/V)
   WRITE (2,4) T, RHO
4 FORMAT (IH, F26.1, F30.3)
5 IF (T-200.0) 6,7,7
6 T = T+1.0
   GO TO 3
7 END
```
FUNCTION U(Y)
    IF(Y) 1, 2, 2
1  U = 0.0
   GO TO 3
2  U = 1.0
3  RETURN
END

FUNCTION GNCNTN(X)
    GNCNTN = \int n_{Ro}
    RETURN
END

2. Program for a rectangular leaf model

1  WRITE (2, 2)
2  FORMAT (IH, 22X, 4HTIME, 22X, 3HRHO)
   T = 0.0
3  RHO = GNCNTN(T) + U(T + \frac{L + W}{V})*GNCNTN(T + \frac{L + W}{V})
   1 - U(T + \frac{L}{V})*GNCNTN(T + \frac{L}{V}) - U(T + \frac{W}{V})*GNCNTN(T + \frac{W}{V})
   WRITE (2, 4) T, RHO
4  FORMAT (IH, F26.1, F30.8)
5  IF (T < 200.0) 6, 7, 7
6  T = T + 1.0
   GO TO 3
7  END

FUNCTION U(Y)
    IF(Y) 1, 2, 2
1  U = 0.0
   GO TO 3
2  U = 1.0
3  RETURN
END

FUNCTION GNCNTN(X)
    GNCNTN = \int n_{Ro}
    RETURN
END
3. Program for a peltate leaf model

1 WRITE (2,2)
2 FORMAT (1H, 22X, 4HTIME, 22X, 3HRHO)
   T = 0.0
3 RHO = FNCTN(T) - U(T + W/V)*FNCTN(T + W/V)
   + W*GNCTN(T + W/V)
   WRITE (2,4) T, RHO
4 FORMAT (1H, F26.1, F30.8)
5 IF (T - 200.0) 6,7,7
6 T = T + 1.0
7 GO TO 3
8 END
9
10 FUNCTION U(I)
11 IF(I) 1,2,2
12 U = 0.0
13 GO TO 3
14 U = 1.0
15 RETURN
16 END
17
18 FUNCTION FNCTN (X)
19 FNCTN = \int \int n_{Ro}
20 RETURN
21 END
22
23 FUNCTION GNCTN(Z)
24 GNCTN = \int n_{Ro}
25 RETURN
26 END

E. Derivation of Dimensionless Equations for a Linear Leaf

If the sucrose in a leaf is assumed to behave as a single pool, then the kinetics of the source pool following a pulse-labelling will be

\[ n_{Ro} = n_{Ro}(0)e^{-\frac{kt}{S}} \]
where \( n_{R_0}(0) = n_{R_0} \) when \( t = 0 \)

\[ S/k = \text{turnover time of the sucrose pool} \]

If \( \tau = kt/S \) and \( \tau_L = kL/SV \), then Equation 4, Section IV, A1

becomes

\[
\rho_R = \frac{-\rho V n_{R_0}(0)}{n_0 L} \left[ (1 - e^{-\tau}) - U(\tau + \tau_L)(1 - e^{-(\tau + \tau_L)}) \right]
\]

(37)

If \( \rho_{R\infty} \) is defined as \( \lim_{\tau, \tau_L \to \infty} \rho_R \), then

\[
\rho_{R\infty} = \frac{-\rho V n_{R_0}(0)}{n_0 L}
\]

(38)

and, dividing Equation 37 by Equation 38,

\[
\frac{\rho_R}{\rho_{R\infty}} = (1 - e^{-\tau}) - U(\tau + \tau_L)(1 - e^{-(\tau + \tau_L)})
\]

(39)
The compartmental models considered in this thesis have all consisted of sequential models with irreversible flow through the compartments, except for the model for \(^{14}\text{C}\) kinetics in the petiole. In that case, however, the solution can be approximated by a similar model by neglecting the contribution of tracer leaking back into the translocation stream from the stationary sucrose pool.

The solutions are given for the kinetics following pulse-labelling of the first compartment. (For the general procedure followed in deriving these equations, see (86).) The solution for the first compartment is well known:

\[
R_1 = R_0 e^{-kt/S_1} \tag{40}
\]

where \(R_1\) = dpm in the first compartment

\(R_0\) = dpm in the first compartment at \(t = 0\)

\(k\) = transport rate from the compartment, \([=] \text{gm min}^{-1}\)

\(S_1\) = compartment size, \([=] \text{gm}\)

The differential equation for the second compartment is

\[
\frac{dR_2}{dt} = k(R_1/S_1 - R_2/S_2) \tag{41}
\]

Substituting Equation 40 for \(R_1\),
If T is defined as the turnover time, S/k, then the solution to Equation 42 is

\[ R_2 = \frac{R_0 S_2}{S_1 - S_2} \left[ e^{-t/T_1} - e^{-t/T_2} \right] \] (43)

For a third compartment, similar procedures may be followed to derive the differential equation for \( R_3 \), which is

\[ \frac{dR_3}{dt} + \frac{k}{S_3} R_3 = \frac{k R_0}{S_1 - S_2} \left[ e^{-t/T_1} - e^{-t/T_2} \right] \] (44)

The solution to Equation 44 is

\[ R_3 = \frac{R_0}{T_1 - T_2} \left[ \frac{(e^{-t/T_1} - e^{-t/T_3})}{T_3^{-1} - T_1^{-1}} - \frac{(e^{-t/T_2} - e^{-t/T_3})}{T_3^{-1} - T_2^{-1}} \right] \] (45)

For the model for \(^{14}\)C kinetics in the petiole, the differential equation for the stationary sucrose pool will be

\[ \frac{dR_4}{dt} = k_4 (R_2/S_2 - R_4/S_4) \] (46)

where \( k_4 \) = transport rate from the translocating pool to the stationary pool and vice versa, \([\text{gm min}^{-1}]\)

\[ R_4 = \text{dpm in the stationary sucrose pool} \]

\[ R_2 = \text{dpm in the translocating sucrose pool} \]
\[ S_4 = \text{size of the stationary sucrose pool, } [=] \text{ gm} \]

\[ S_2 = \text{size of the translocating sucrose pool, } [=] \text{ gm} \]

If it is assumed that the kinetics in the translocation stream are determined by that of the source pool in the leaf, which in turn is given by Equation 43, then the translocation stream kinetics will be proportional to Equation 43. Equation 46 then becomes

\[
\frac{dR_4}{dt} + \frac{k_4 R_4}{S_4} = \frac{C k_4 R_0}{S_I - S_{II}} \left[ e^{-t/T_I} - e^{-t/T_{II}} \right]
\]

(47)

where \( C \) = a proportionality constant

\[ T_I = \text{turnover time in the photosynthetic sucrose pool, } [=] \text{ min} \]

\[ T_{II} = \text{turnover time in the source pool, } [=] \text{ min} \]

This is almost identical to Equation 44 and has a similar solution.

\[
R_4 = \frac{C k_4 R_0}{S_I - S_{II}} \left[ \frac{e^{-t/T_I} - e^{-t/T_4}}{T_4^{-1} - T_I^{-1}} - \frac{e^{-t/T_{II}} - e^{-t/T_4}}{T_4^{-1} - T_{II}^{-1}} \right]
\]

(48)
If the conditions listed in Table 7 are applied to the movement of tracer in the stem, the equations describing this movement may be readily derived. In this model, the origin of the x-axis will be at the point where the petiole leaves the leaf, and will extend in the positive direction from the leaf. Taking a mass balance on the tracer, \( R \), over an incremental volume, \( A\Delta x \), of the translocation stream,

\[
\frac{\Delta p_R}{\Delta t} \left| A \Delta x \right| = AV \rho \left| x \right| - AV \rho \left| x + \Delta x \right|
\]  

(49)

Dividing by \( A\Delta x \) and taking the limit as \( \Delta x \) and \( \Delta t \) approach zero,

\[
\frac{\partial p_R}{\partial t} = -V \frac{\partial p_R}{\partial x}
\]  

(50)

Taking Laplace transforms, and applying the initial condition \( p_R(x,0) = 0 \),

\[
s p(x,s) = -V \frac{dp(x,s)}{dx}
\]  

(51)

where \( p(x,s) \) is the Laplace transform, with respect to \( t \), of \( p_R \) \( (= p_R(x,t)) \). Solving for \( p(x,s) \),
\[ p(x,s) = e^{-sx/V}c(s) \] (52)

where \( c(s) \) is a constant of integration. The inverse Laplace transform of this is

\[ p_R = U(t - \frac{x}{v})c(t - \frac{x}{v}) \] (53)

If \( p_R(0,t) = \rho_{R_P}(t) \), where \( \rho_{R_P}(t) \) is the density of \( R \) entering the translocation stream at the petiole, then Equation 53 becomes

\[ p_R = U(t - \frac{x}{v})\rho_{R_P}(x - \frac{t}{v}) \] (Eq. 12, Section IV, C)
XIII. ACKNOWLEDGMENT

The writer is indebted to Dr. S. Aronoff for his encouragement in pursuing a theoretical approach to translocation, for the freedom granted during the work, and for the opportunity of attending a summer course in Botanical Histochemistry offered at the Berkeley campus of the University of California in 1963.

A number of persons have assisted materially in particular phases of the work. This has been acknowledged in the appropriate sections of the thesis, and it is a pleasure to reiterate appreciation for their contributions. In addition, the writer extends his thanks to Mr. John M. Dear for many discussions and assistance in carrying out some of the experiments.

To his wife, Rita, he is indebted for patience and helpfulness throughout his work.

Finally, the writer extends appreciation to the National Institutes of Health for a pre-doctoral fellowship during the last two years of work. Part of the investigation was also supported by a National Science Foundation grant, GB 114, to Dr. S. Aronoff.