A STUDY OF TRANSLOCATION IN CEREAL LEAVES

USING Cs-137 AND C-14

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by

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ABSTRACT

In the absence of natural carbohydrates a number of radioactive tracers undergo translocation in young rye leaves but only when external sugars are simultaneously applied. Sucrose and lactose are among such transport-activating sugars.

The distribution of tracers down the leaf length when applied at the leaf apex shows the common exponential fall-off pattern with the distance, while the semi-logarithmic curves display upward concavity. When tracers are applied in media of low osmotic potential there is an absorption of water by the leaf with consequent flow of tracer downwards in the xylem; this unwanted effect can be overcome by raising the osmotic potential of the milieu.

On a plausible model the negative slope of the semi-logarithmic plot is simply related to the transport velocity and to the rate of lateral leakage out of the conducting channels. The slope of these curves is a function of the concentration of applied sugars, and varies as the 5th or 6th root of the latter. The slope is steeper for chloride than it is for potassium; while the slope for caesium is less steep than that for potassium.
When lactose is administered more is absorbed by the leaves normally illuminated than by those emptied of their natural photosynthates by prior dark treatment, thus suggesting an active uptake. New and promising microextraction and colorometric methods are introduced with some initial success.

In general the investigation supports a theory of mass-flow in the sieve tubes. Observations such as the relationship between sieve tube concentration and velocity, a faster lateral leakage for potassium than for caesium and a faster cation mobility compared with that for anions suggest a preference for the electrokinetic theory against the pressure-flow hypothesis.
PART ONE

INTRODUCTION

MATERIALS AND METHODS
The significance of a knowledge of the phenomenon of translocation in plant physiology is very fundamental. Apart from its practical usefulness (to those concerned with growth regulators, herbicides and systemic pesticides etc., many of which have to be translocated to different regions of the plant to prove effective) the importance of translocation to the plant can be visualised from the fact that it constitutes an essential integrating (and co-ordinating) mechanism between such localised and varied processes as photosynthesis, mineral uptake, mobilisation of reserves and biosynthesis of particular compounds (such as vitamins).

Interest in the subject is heightened all the more when it is realised how little agreement there still is over the question of the mechanisms involved; in fact, in spite of all we know about translocation, the impression given on this point is still one of widespread disagreement.

A historical account or a review is not intended here but a few points of comparative interest about some prominent theories that still occupy the field and may continue to do so for a long time, might not be entirely outside the scope of this work.

One theory which has stood the test of time and which in spite of valid criticisms still seems to continue in one form or another is the well-known pressure-flow hypothesis of Munch,
i.e. that under the influence of a pressure gradient originating in a gradient of osmotic potential, itself a result of source and sink activity, the movement of carbohydrates results throughout the plant body.

The objection to this pressure-flow concept are well known: the high rates of flow observed in phloem in spite of the presence apparently of cytoplasmic barriers at the sieve-plates; the adverse effects of narcotics and poisoning on translocation; the influence of temperature on the rate; the failure to give any adequate account of such persistent anatomical features as companion cells and abundant sieve-plates; the requirements for most, if not all, of the primary energy supply to be expended at the ends rather than along the column; and perhaps also the phenomenon of bidirectional transport; all of these afford formidable obstacles to a successful defence of the pressure-flow theory.

Yet on the other hand there are some well known lines of evidence which support the theory. Exudation from cut phloem demonstrates the feasibility of bulk-flow in the sieve tubes, as also does the phenomenon of aphid stylet exudation. Further, Dixon's and later Ziegler's work with thermo-electric methods indicated the actual existence of a flow in the intact phloem, an osmotic-potential gradient has been observed in many cases (Dixon (2), Mason & Phyllis (10), Zimmerman (29)) down the trunks of woody plants; and a model of Munch's mechanism has been set up with glass apparatus (1) and also with single long nitella internodel cells (8).
Another theory still being canvassed is based on the phenomenon of protoplasmic steaming. As recently advanced by Thaine ( ), this involves not cyclotaxis but rather linear steaming through the sieve-plates of "transcellular strands" of cytoplasm. The objections to this theory lie in the problematical nature of the observations; the absence of supporting electron microscope evidence; but chiefly the difficulty of meeting the demands for measured rates of transport and the probable high energy requirements involved in the mechanism.

On the other hand the steaming hypothesis is highly physiological, fitting in with the known sensitivity to poisons, narcotics and temperature; steaming in general is an established phenomenon in protoplasmic systems and in particular it does now seem to exist in mature sieve tubes, though some workers deny the observations, e.g. Esau et al ( ).

Mason and Maskell's ( ) well known suggestion of some sort of "activated diffusion" involved in the transport process was not qualified with any clear outlines of the actual mechanism envisaged, nor has it been subsequently clarified. Further, the idea of transport on interfaces put forward by Van den Honert ( ) and more recently by Van Overbeek ( ) need hardly detain us either, since it fails to account for the sheer magnitude of assimilate transport.

Finally, of recent years a theory has been put forward based on electro-kinetic effects.

As stated in detail by Spanner in 1958 ( ) the electro-osmotic theory incorporates the general idea of a bulk flow along
the sieve tube column but eventually under the influence of active electro-osmotic forces involved at each sieve plate. The theory provides for the magnitude of force required for the flow of translocates effectively across the sieve plates, being far beyond the scope of those which can be met by the pressure-flow theory of Munch.

The theory assumes a more physiological outlook not only in transforming the cytoplasmic barriers at the sieve pores (a formidable problem for Munch's theory) to its advantage by explaining these as elements interacting with gradients of electrical potential, but also in bringing in the concept of positive feedback (associated with the circulation of K ions).

Another distinguishing feature has been that an attractive explanation has been put forward for the constant close association of such cytologically differing cells as companion cells and the sieve cells. Further, the theory utilizes metabolic energy along the whole length of the conducting columns and not merely at the ends as the pressure flow theory largely does. It accounts for the key role of sugar in energising the mechanism (Munch's theory possibly does this too). Finally, much of the evidence that has been adduced for the latter theory will also support the electro kinetic one, since both are in fact theories in which mass flow occurs according to a similar pattern, the difference lying in the origin of its driving force.

The aim of the present work therefore has been mainly to obtain evidence in favour of one or the other of the current
theories of translocation (within, of course, the limits of experimental facilities that were available).

Most of the studies carried out have been on the leaf of Rye (Secale cereale) the significance of such a choice and other relevant considerations being discussed in detail elsewhere in this work. The objectives of the investigations may be summarised as: (1) Establishing whether the movement of tracer in cereal leaves was dependent on available mobile sugars. (2) Investigating the nature of such a dependence with regards to (a) the concentration of the applied sugars and (b) the chemical nature of the sugar, (c) the nature of the tracer ion. (3) And finally obtaining evidence in favour of one or the other of the current theories of translocation.

In the course of the work it became necessary to develop suitable methods for assaying the activity in short lengths of cereal leaf, both when the tracer was a strong $^3\beta$-emitter and when it was a weak one. Further it was required to extract sugars quantitively from similar leaf samples (about 30 mg in fresh weight). For this purpose a new pattern of micro-extractor operating on the Soxhlet principle was constructed and this also is described; while the extracted sugars were chromatographed and assayed in a single home-made photo electric colorimeter.
MATERIALS AND METHODS.
MATERIALS AND METHODS

The experimental plant material was grown continuously inside a chamber maintained at 20°C (68°F) with light arrangements giving diurnal periods of light and darkness (13 hours of light and 11 hours of darkness) and receiving a total light intensity of about 300 ft.-candles at the leaf level, from five 80 watt mercury-vapour lamps and twelve 80 watt fluorescent warm white tubes (there being no regulation for controlling humidity, however). In the greater part of this work seeds were germinated in fine vermiculite held in 2" x 2½" polystyrene pots nourished with Hoagland's nutrient solution though some sand-culturing was also carried out in early work.

The principal object of the experiments being a tracing of the translocation pattern down a linear plant organ the essential technique consisted of treating the tip of an intact plant with solutions of the sugar under investigation containing small quantities of suitable radioactive tracers. Following a given lapse of time the subsequent distribution of tracer down the length was determined with the help of suitable counting instruments.

The details of the radioactive assay techniques will be discussed later. The first problem however was to find a good subject and a suitable method of ensuring an effective contact between the radioactive tracer and the plant surface.

Accordingly in the preliminary stages of the work a number
of different plants were tried and several methods of applying tracer tested out.

**THE SPOT TECHNIQUE**

One of the first techniques employed was the spotting of a drop of tracer (about 0.5 μCi) on the primary leaves of young soyabean plants.

Soyabean was selected because its relatively delicate texture (compared with such plants as French bean) ensures better radioactive assay should soft β isotopes be employed. Further a good deal of work on it in connection with translocation has already been carried out. It was the intention to analyse the tracer distribution down the petioles alone in order to avoid complications arising from anastomoses of the vascular anatomy in the stem, but as mentioned later this proved difficult.

The bean plants were grown in sand cultures and when of age the selected ones were transplanted to a trough containing Hoaglands' culture solution (see appendix) with a perforated plastic tray fixed horizontally over the surface of the solution and holding the plants in a vertical manner with their roots bathed in the solution. The shoots were supported upright (above the plastic plate) with the help of circular wire clips. These clips were in turn clasped at a convenient level on 1/8" x 10" dural rods that were fixed vertically in brass screws with hollow ends. A diagram illustrating the arrangements is given in fig. (1).

These arrangements completed the primary leaves were spread
Fig. 1 Method of supporting soybean plants and applying tracer spot. The polythene bowl contained Hoagland's solution. For further description see text.
across over the span of the circular wire coils; sometimes they were made to adhere to the coil with the help of a little lanolin grease applied to their lower surfaces. The tracer was deposited on the middle of the upper leaf surface with the help of an Agla micro syringe (with a piece of fine polythene tubing of about 1mm bore fixed to the end of the syringe needle to avoid as far as possible a direct contamination of the syringe itself) and the drop was quickly covered with a circular disc of very thin gauge polythene film of diameter 6mm, to define the area of contact and to prevent evaporation. The plants were then left for a desired period (about sixteen to twenty hours) under light or dark conditions inside the chamber prior to their harvesting and assay.

In spite of careful manipulations however this method of tracer administration proved rather unsatisfactory. The risk of tracer spilling was considerable, while the evaporation in spite of the polythene discs was too rapid. This is important since tracer absorption is very slow once the drop has dried up, and it is therefore of advantage to prevent this for as long as possible.

In view of the fact that this procedure with soyabean did not prove as satisfactory as had been hoped, and still more in view of the realisation that the stems (as opposed to the petioles which had been first envisaged) had a vascular anatomy \(^1\) which

\(^1\) The tracer will first move down the phloem to the cotyledonary nodes where the traces fuse. It will then split and follow two paths, one upwards towards the growing tip and one downwards to the roots. Clearly this pattern complicates precise analysis.
would have rendered precise analysis of the results dubious, soyabean was discarded and attention turned to cereals. Accordingly young leaves of cereals like wheat, barley and rye were given a trial with suitable modifications in the technique. Marked improvements were achieved in the results. Subsequent work was carried out on rye (Secale-cereale) variety Charles supplied by Messrs Carters. Later on when this variety became unobtainable, Petkus summer rye was used. It may be mentioned here that rye was chosen owing to its rapid germination and vigorous growth, though on account of its being cross-fertilised it may not have been genetically quite so uniform as some other cereals, such as barley.

THE RING TECHNIQUE

Owing mainly to the narrow shape and the delicate form of the young cereal lamina the previous tracer spot method was thought unsuitable and a ring technique was tried. Small polythene rings (0.8cm by 0.4cm internally and 0.4cm high) were obtained by partially flattening polythene tubing of about 0.6cm bore between two metal strips for about 30 minutes at 80°C and then cooling it suddenly in cold water; the flattened tubing being then cut into the requisite lengths with a razor blade. Towards the proximal end of the leaf but avoiding the narrow extremity and supported over the wire clip (as shown in the diagram - fig. ) the ring was fixed on the upper surface of the leaf in a longitudinal posture, using a trace of lanolin grease. The tracer drop was then carefully lodged within the ring walls which constrained it
very effectively while the ring was covered with a lanolined square piece of polythene film to reduce evaporation.

A few experiments carried out employing the above technique of tracer-contact gave better results. However it was felt that the technique could be still further improved and the ring method was consequently abandoned.

THE LEAF-TIP IMMERSION TECHNIQUE

Tracer administration technique finally adopted was as following:

Rye seedlings growing in the plastic pots were selected for size and uniformity. Plastic covers holding a length of polythene tubing 0.6cm in bore and a dural rod 1/8" diameter by 10" length were fixed on each plastic-pot as shown in the fig. (2). The leaf tip was then carefully bent and inserted into the cavity of the reservoir and usually it remained there satisfactorily, but any flicking-out tendency on the part of the leaf tip was effectively prevented by clamping another of the light aluminium clips, bent in a concave fashion, over the convexed part of the leaf.

Tracer administration was next carried out, by depositing the measured drop on the bottom of the reservoir with the syringe shown. The reservoir was then swiftly filled up with the required sugar solution using an ordinary dropper.

It might be mentioned in the connection that the presence of the inserted leaf did not interfere in any way with the manipulations described above and the safe introduction of the end of the Agla micro-syringe without damage to the leaf was possible due to the fine terminal nozzle of polythene described earlier.
Fig 2. Experimental arrangements:  A, Plant in poly styrene pot; B, Detail of polythene reservoir; C, Tracer applicator; D, Leaf section ready for radioactive alloy.
MAKING THE POLYTHENE RESERVOIRS

About 3 cm long tablets were cut from polythene tubing of about 5mm bore and 0.8mm wall and a dozen at a time were clamped between two steel strips (measuring 10" by 3/4" by 1/4" thickness) held together by means of two 1/4" bolts tightened to flatten the tubing till the cavity was between 0.15mm and 2mm wide, while a constant width was ensured by placing steel washers between the strips. The steel press was then left in an oven at 85° to 95°C for about 30 minutes when on being plunged into cool water the tablets "set" to a permanent flat shape.

Without releasing them from the press one end of these flattened tablets was sealed off by touching them with a hot smooth steel knife blade while the opposite end which protruded about 0.5cm out of the steel press and remained circular was enlarged a little in circumference to a bell shape by inserting and rotating the conical end of a hot brass rod into it, subsequently cooling in water.

The resultant small reservoirs were released from the press and after giving them a test for leakage by attaching a rubber tube and blowing they were ready for use. The shape and size of these reservoirs easily allowed the insertion of about 2.3cm of young cereal leaf tips without damage.

The reservoir technique described proved the best of those tried and as a result it was used throughout the subsequent work.
THE HARVESTING

After allowing the requisite period of translocation under light or dark conditions the plants were ready for harvesting.

In the case of soyabean plants the treated leaf was cut off at the base of the lamina and discarded. The petiole was then quickly severed with a sharp scalpel at its lower end and placed horizontally on a piece of filter paper.

It was cut into segments with a multiple cutter made up of a parallel assembly of stainless steel razor blades separated by perspex blocks 3/4" (aprx.2cms) thick. The pieces were then immediately labelled with serial order numbers, with the help of a fine brush and Indian ink and transferred to polystyrene pill boxes for gamma assay or (in the case of later experiments with cereal leaves) encased in cellophane for beta-assays as described below.

SAMPLE PREPARATION TECHNIQUES

In the greater part of this work where $\beta$-assay was preferred the active harvested leaf pieces were spread out on a narrow strip of sellotape and covered with a strip of thin non-waterproof cellophane of equal size forming a sealed packet (fig. 2 D). These small packets of embedded leaf were then cut out and trimmed to a size of about 2.8cm by 0.5cm as shown. In this form they could be inserted easily and counted with high efficiency in a especially designed well-type plastic phosphor described later.

The arrangement described was very satisfactory for Cs-137.
However for the soft $\beta$-emission from C-14 the case is clearly different and precautions have to be taken to make the specimen absorption as small as possible. The procedure used was to introduce the flattened dried leaf segment into a length of lay-flat tubing formed (commercially) from very thin (gauge 25 = 0.00635 mm thick) Melinex$^1$ film. The tubing was 7.5 mm wide and introduction of the leaf was facilitated by first threading it with a length of stout cotton to open the cavity. The tubing was then cut and sealed at one end in one operation with an electrically heated wire. The small bag containing the leaf segment was placed between two thin flat rectangles of plastic phosphor as described later. The specimen package is shown in fig. 2D and the method ensured a good geometry for counting and a low background.

1. Messrs I.C.I. Ltd. The tubing was fabricated by Messrs Secol Ltd, Shetford, Norfolk.
THE RADIOACTIVE ASSAY

Radioactive assay work was mainly carried out with a Universal scintillation counter. Preliminary calibrations for optimum operation voltages and amplifier gains were performed in detail. This was done by squaring the net sample count rate and dividing it by the background rate. This function was plotted against the E.H.T. for various values of the discriminator and amplifier settings, and those settings chosen which made it a maximum. The values selected were:

- E.H.T. 1250 volts
- Amplifier gain X1000
- Discriminator 24 volts

These values were adopted uniformly for all the subsequent assay work.

PHOSPHORS:

(A) CRYSTAL PHOSPHOR A 1" sodium iodide crystal-phosphor was used in the earlier experimental work for \( \gamma \)-counting of the Cs-137. The material was laid in 1" x 1" polystyrene pill boxes with polythene covers. A thin paper disc was first placed at the bottom of these containers to avoid contaminating them and they were then assayed immediately over the one inch diameter sodium iodide crystal inside the Panax Castle.

The usual background count rate under these conditions was about 540 counts per 400 seconds (or 8.5 per minute) and since most of the net sample count rates did not greatly exceed this value counting even for fairly prolonged periods gave only a
moderate accuracy, about 6% for a total count over 400 seconds.

The geometrical efficiency was obviously rather low due to the external mounting of the source in relation to the phosphor, and further the background was high owing to the fairly big volume of the crystal.

In order to overcome these drawbacks, a -assay was tried using a carefully designed plastic phosphor.

(B) WELL-TYPE PLASTIC PHOSPHOR Since the -particles from caesium-137 are of moderate penetrating power possessing a maximum energy of about 0.51MeV and a maximum range equalling 160mg per cm$^2$ while the dry weight of the experimental plant material involved (rye) is roughly about 1mg per cm$^2$ and of the encasing cellophane and sellotape to 9mg/cm$^2$ it follows that a good fraction of the -particles will emerge and will require a thickness of plastic phosphor of the order of 153mg/cm$^2$ or 1.5mm to intercept them completely. The thickness will also serve fairly well for the Cl-36 and also for K-42 used later in the work, and it is desirable that it should not be exceeded in order to keep the cosmic ray background low.

Accordingly the NE102 plastic phosphor was shaped into a hollow cylinder (fig. 3) of 1.5mm wall thickness which was mounted in a vertical manner in a buoy-shaped piece of polyvinyltoluene light piping to act as a light guide. This was stood in a pool of silicone oil in contact with the photomultipher inside the castle.

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NE102 plastic phosphor is manufactured by Messrs Nuclear Enterprises Ltd., Edinburgh. Its density is 1.032 gm/cm$^3$. 

Fig. 3 Arrangement of Ne102 plastic phosphor for counting leaf sections mounted as in fig. D.
Another modification in the use of plastic phosphors was devised when counting soft $\beta$-particles from lactose C-14. The soft $\beta$-particles possess a maximum energy around 0.155 MeV and a penetration range of about $28.5 \text{ mg/cm}^2$. The usual cellophane envelope would therefore be a little on the heavy side and a search was made for something lighter. Fine gauge melinex film was chosen and fabricated as already described. The package was assayed by enclosing between two pieces of plastic phosphor sheet 0.75mm thick x 8mm x 24mm. These were pressed into close contact with the specimen by means of a small rubber band (cut from valve tubing) at the lower end and a draper’s miniature bulldog clip at the upper end. The complete assembly was vertically lowered into a pool of silicone oil filling the central cavity of the light guide (fig. 3) from which the plastic phosphor well had been removed. The melinex protected the specimen from the oil (uptake of which would have increased self-absorption) and the whole formed an arrangement with good geometry and detection and fairly low background.

**COMPARATIVE EFFICIENCES:**

An understanding of the comparative advantages of $\beta$-assay over that for the $\gamma$-assay may be gained from the following typical figures for a low level sample counted for 100 seconds.
1 inch NaI crystal phosphor

| Table I. |
|-----------------|-----------------|
| Photomultiplier voltage | 1250 v. |
| Amplifier gain | x 1000 |
| Discriminator voltage | 24 v. |
| Background count | 160 cps |
| Gross count with sample | 225 cps |
| Nett ditto | 65 cps |
| Statistical accuracy | 23% |
| Figure of merit \(\frac{\text{sample count}}{\text{background}}\)^2 | 26 |

Plastic phosphor

| Table I. |
|-----------------|-----------------|
| Photomultiplier voltage | 1250 v. |
| Amplifier gain | x 1000 |
| Discriminator voltage | 24 v. |
| Background count | 49 cps |
| Gross count with sample | 677 cps |
| Nett ditto | 628 cps |
| Statistical accuracy | 4.2% |
| Figure of merit \(\frac{\text{sample count}}{\text{background}}\)^2 | 8040 |

It is apparent from the above that the ratio of sample counts to the background counts is approximately thirty times higher in case of the plastic phosphor for the low level case quoted, and the improvement in the figure of merit (sample count^2/background) very considerably higher (about 3000). There would of course be less difference for higher levels.
SPECIFICATIONS OF TRACER AND SUGAR SOLUTIONS

CAESIUM-137

About 4mC of high activity (25c/gm Cs) caesium-137 as CsCl obtained from Amersham Radio-chemical Centre were delivered as solution in IN-HCl. The solution was evaporated to dryness to get rid of the acid and re-dissolved in 1ml of distilled water. This was treated as the primary stock solution. Suitable quantities of Cs-137 (i.e. about 0.025ml giving a total of about 100μC) were withdrawn from time to time and further diluted with 2ml of 1% Lissapol (a non-ionic detergent from Messrs I.C.I. Ltd.). The resulting secondary stock solutions of about 2.025 ml were sufficient to inoculate about 200 plant replicates at the rate of about 0.5μC per plant, a dose maintained more or less throughout the work. In the earlier part of this work, as already stated the requisite tracer quantity was mixed with pure sugar solutions of varying strengths inside the polythene reservoirs.

It was observed however that the level of the solution when osmotically dilute used to go down considerably during the course of the experiment. It was inferred that water was being absorbed from the reservoirs by the immersed leaves as a consequence of their suction potential. Consequently a series of visual observations on the falling of water level in the reservoirs with and without immersed leaf tips were carried out. These clearly indicated a marked fall in the levels only in reservoirs in which leaf tips were immersed. This seems to rule
out the possibility of mere evaporation. Further it was also observed that the shrinkage in volume became less as the sugar concentration in the reservoirs was increased and that when the sugar concentration reached 1 molar the shrinkage was negligible. This confirmed that uptake of water was involved, and this conclusion was further strengthened when the pattern of the tracer distribution was investigated and it appeared that some movement had occurred down the system (see further below). Since the object of the investigation was phloem movement it was clearly desirable to prevent this water uptake, and following the clue above it was decided to make the tracer solution always up to an osmolarity of one using an inert material like mannitol. It was first established that mannitol was inert and then the procedure was consistently adopted of supplementing the sugar concentration with mannitol to the desired degree, with one change. The mannitol was replaced with an equal mixture of mannitol and sorbitol. Mannitol has not an adequate solubility, and in the first trials some crystallization occurred. Sorbitol shares, at least in the case of rye, the inertness (as far as translocation is concerned) of mannitol, though apparently this is not so with the apple - (1.0). Results demonstrating the effects of inert solution addition are presented later.

CHLORINE - 36:

Radioactive chlorine was obtained from Amersham as sodium chloride solution of activity about 640 μC per gm Cl. Twenty microcuries were supplied in about 1 ml solution. In order to
apply 0.5 μC per plant it was necessary to make up the sugar solutions to about 1.2 M strength so that on addition of the tracer the final strength would be about one molar. Apart from this the procedure was identical with that used for caesium-137.

CAESIUM-137 WITH POTASSIUM-42

In experiments designed to compare the behaviour of two different isotopes it is obviously desirable to apply the two together, and in the case of Cs-137 and K-42 this is possible since the short half life of the potassium (12.5 hours as compared to 33 years for Cs-137) makes the separate assay of the isotopes a simple matter.

Potassium-42 was obtained from the Radio-chemical Centre in the form of potassium chloride containing about 1 mC in 10 ml. The specific activity was about 20 mC per gm of potassium. As a rough guide it was assumed that the sample had decayed to about 500 μC when received, and that when harvested this would have fallen to about 100 μC. Hence for the amount per plant to be 1 μC at the time of harvesting the volume of the original stock which would be required per dose becomes

$$\frac{1 \mu C}{100} \times 10 \text{ ml} = 0.1 \text{ ml}$$

On this basis the potassium-caesium mixture was made up as follows:

- 2 ml K-42 solution as received from Amersham
- 0.005 ml of Cs-137 stock, containing 20 μC
- 2 ml Distilled water containing 0.1% Lissapol
- 1.4 gm solid sucrose.
This gives a total molarity of approximately one. The reservoirs were filled directly with this mixture, using a disposable polystyrene syringe. The 0.2 ml (approx.) administered per plant contains about 1 μC each of potassium and caesium.

This amount was chosen rather than the previous one of 0.5 so that the first scaler measurements (see below) could be completed sooner, in view of the short half life of potassium $^{42}$. The separation of the potassium and caesium was performed as follows:

Assays were made with the plastic phosphor well, previously described for caesium (though this is not quite the ideal for the more energetic $\beta$-particles of K-42.) The first measurement was made as soon as possible after harvesting. (Count $n_1$ at time $t_1$). A second measurement on the same sample was made after the lapse of a suitable period ($n_2$ at time $t_2$). The period ($t_2 - t_1$) was chosen to be a fair multiple of the half-life (see later table). The decay characteristics of K-42 were expressed graphically on semi-logarithmic paper by plotting the coefficient $\beta$ against the time in half lives, where $\beta$ is the ratio of the initial activity to the activity at some later instant (thus $\beta > 1$).

For a number of half lives $\eta$, the relation to be used is

$$\beta = e^{\frac{-\eta}{\lambda}} = e^{0.693\eta}$$

Two curves were plotted with different time scales (see figs 24 and ...) for the two corrections to be described.

The calculations were performed as follows:
If $n'_k$ is the potassium count at time $t_1$, and $n_{cs}$ the caesium count (virtually independent of time) then we can write

\[ n_1 = n'_k + n_{cs} \]  
\[ n_2 = \frac{n'_k}{\beta} + n_{cs} \]  

Subtracting these equations give

\[ n'_k (1 - \frac{1}{\beta}) = n_1 - n_2 \]

or

\[ n'_k = \left( \frac{\beta}{\beta-1} \right) (n_1 - n_2) \]  

(3)

and substituting this value for $n'_k$ in (2)

\[ n_{cs} = n_2 - \frac{(n_1 - n_2)}{\left( \beta - 1 \right)} \]  

(4)

As a check on the calculations these values of $n_{cs}$ were substituted in (1).

The value $n'_k$ (which refers to time $t_1$) has now to be corrected to an instant the same for all samples (say $t_0$). This was taken at the instant $t_1$ for the first segment assayed and the correction was made by using the factor $\beta$ (not very different from unity) read from the second decay curve (fig. 24). The final corrected value is referred to in the results as $n_k$.

**Lactose C-14**

Radioactive lactose was employed to study the lactose gradient in the semi logarithmic plots in comparison with those for the sucrose activated caesium transport. It was especially intended to find out whether lactose moved as lactose or only after conversion to other sugars, e.g. sucrose; and also to make measurements on its gradient when moving. However, the experiments partly miscarried and less information than expected was obtained, though a different approach using inactive lactose and
chromatography was more successful.

DILUTION

Lactose C-14 was directly mixed with varying lactose concentrations made for external applications to the plants, to which 0.5% detergent (Lissapol) had already been added. In order to obtain a strength of about one μCi per plant (instead of the usual 0.5 μC) the 50 μCi of Lactose C-14 (from Amersham) were dissolved in 2.50 ml of 5% Isopropanol to permit dispensing but prevent bacterial action. Out of this stock solution 0.6 ml equalling about 12 μCi were withdrawn for the first experiment with radioactive Lactose. The rest of the 30 μCi (equalling about 1.9 ml) were used in the succeeding experiment. The alcohol content of the solution was removed by evaporation under an infra red lamp followed by dilution with the requisite quantities of distilled water, before adding the prepared solutions of the inactive lactose. The resulting mixtures were about 0.00043 M in tracer lactose.
EXTRACTION AND CHROMATOGRAPHY

In connection with two problems - determination of the fate of lactose and of sugar gradients - it was necessary to employ extraction and chromatography techniques. Unfortunately time and conditions did not allow these techniques to be exploited to the full. They will however be briefly discussed.

EXTRACTION OF SUGARS

In view of the necessarily limited scale of experiments and the relatively minute quantities of sugars involved successful recovery of sugars from the experimental plant materials required. The development of a new extractor, which will be described shortly. Zimmermann's micro extraction technique for small samples (also see fig. ) was first tested. It works well but is delicate and a little cumbersome. Further apparatus to this design would have been constructed had not an alternative suggested itself.

DEVELOPMENT OF A NEW SOXHLET MICRO EXTRACTOR

The micro extractor developed works on the soxhlet principle and can be constructed with ordinary laboratory-workshop resources. It met requirements in an ideal manner.

The central position in this extractor is that of a small metallic condenser formed from a 14" length of pure annealed and highly polished nickel tubing of external diameter 1/16" and bore 1/32". The apparatus is illustrated in figs (425). Description is hardly necessary, but the following are the main points of interest. The chimney A is cut from a 32mm × 180mm...
Fig. 4  A micro extractor working on the Soxhlet principles. For description see text.
The Soxhlet microextractor set up in quadruplicates.
pyrex boiling tube and acts to conserve heat. The boiler C is made from a standard 18mm x 180mm pyrex test tube, and is blown to a slightly bulbous form at the base. The specimen tube D, 12mm x 75mm pyrex, has a 2mm hole drilled through the side about 3 cm from the bottom, the drilling being done with a copper tube moistened with turpentine carborundum paste. This hole takes the nickel siphon J. The member D is held concentrically in C with a vapour-tight silicone rubber ring (easily made from silicone paste SR500) and a split ring B of stainless steel wire (gauge 16). The nickel condenser F fits closely inside the tube D and is provided with an internal glass baffle. It is supplied with mains water via the two 1.5mm bore nylon tubes G. The polythene cap H prevents rapid convective heat loss and holds the assembly central in the chimney A. The heater M is composed of about 14" of a gauge 18 Nichrome wire enrowned with fine braided glass fibre and soldered L (with Easy-flow silver brazing alloy) to two supporters K of stainless steel wire (16 gauge). The heater runs off a 4 V transformer and is controlled by a simple rheostat consisting of about 3" of gauge 18 nichrome wire bent into a U and with a moveable spring clip shorting opposite sides. This arrangement enables any power between 20 and 40 watts to be provided.

The working of the extractor is quite simple. About 2 - 3ml of solvent is placed in the bulb of C (80% ethanol was usually employed) and the leaf segments in the tube D. The vapour from the gently boiling liquid passes through the lateral hole in the later and condenses on the metal spiral from which it drips
back into D. The level builds up till it is a few mm from the hole, when it suddenly begins to siphon over. The action is fairly regular and complete and bumping presents little difficulty. The cycle commonly takes 3–5 minutes, varying with the heater power.

When extraction is judged complete (typically about 1/2 to 3/4 hour) the extract can be concentrated by removing the siphon and allowing solvent to collect in the upper tube D. If necessary the closed end of a boiling tube such as A can be used as a water bath and the dismantled tube C placed in it to secure further evaporation. This avoids overheating the residue, and the process can be made very rapid by inserting a rubber or plastic tube into the mouth of C (fig. 6) and attaching it to an air exhaust manifold. The apparatus was found very suitable for extracting samples of leaf tissue consisting of two or three of the 2cm segments previously mentioned.

CONSTRUCTIONAL DETAILS

(A) The nickel condensers were formed by bending 1/4" lengths of soft metal tubing double and winding them around a brass mandril on which two deep square-sectioned screw threads had been cut. The nickel was previously buffed to a high polish. The same nickel tubing (from Messrs Fine Tubes Ltd.) was used for the siphon.

(B) The silicone rubber gaskets were formed from two triangular-section rings stuck back to back with the same silicone paste
Fig. 6  Method of evaporating extract to small bulk.
For description see text.
(SR300, from Messrs Esco Ltd.). The rings were made by filling a suitably shaped groove turned in the end face of a polythene rod with the paste and allowing it to dry overnight.
CHROMATOGRAPHY

Ascending paper chromatography was carried out in an apparatus constructed in the department and to a well-known design. It consisted of an aluminium frame carrying five 10" x 10" square papers held parallel to each other and with their lower edges dipping into solvent contained in an aluminium tray. (One of the papers as marked out for spotting is attached). The upper edges were clamped between wads of absorbent paper held between small brass angles by spring clips. This ensured a travel for the solvent front much greater than the 10" depth of the paper. The paper assembly and the tray were placed in a rectangular iron frame aquarium tank covered with a sheet of plate glass. During operation this was sealed to the tank with self adhesive polythene tape.

To help in saturation of the internal atmosphere the glass sides of the tank were covered internally with filter paper moistened first, before closing, with the solvent being used. This precaution was found very necessary to secure regular results.

OPERATING DETAILS

(A) SOLVENT: The solvent used was isopropanol-water in the ratio of 4:1. This solvent is not very sensitive to temperature, and unfortunately facilities were not available for the close temperature control of the tank (19 - 24°C range was experienced). Further isopropanol-water separates lactose very satisfactorily from glucose and sucrose.
(B) SPOTTING Normally Whatman's No.1 was used. Spots of 0.5 ml were placed on the paper 3 cm from the lower edge. These were dried rapidly using a novel form of drier (fig. 7) made from a ceramic element of a type employed to prevent shop windows from misting. This has a loading of 40 watts per foot and it proved a very convenient and quick drier when numbers of papers were being prepared in quick succession. The drying of the last spots on the sheet was further speeded by directing a small jet of air from a Dymax pump on to the upper surface.

The papers were dried after running (usually overnight) by removing the aluminium frame intact and placing it over a screen of perforated brass supported above a 1 kw boiling ring. By fixing metal plates against the exposed sides of the aluminium frame with its paper squares a natural chimney was formed and drying of the papers was satisfactory and rapid.

(C) LOCAL REAGENTS The sugar spots were developed by one of two reagents.

**BENZIDINE** Freshly prepared benzidine mixture (for details see appendix) was diluted with acetone just before spraying when desired results with nearly all the sugars were obtained on heating at 100°C for a few minutes. The reagent was employed only qualitatively.

**TETRAZOLIUM** For quantitative colorometric determinations the method of Wallenfel's (for details see appendix) was used. Tetrazolium chloride reacts with nearly all reducing sugars forming a formazan which gives a pink deposit on the paper readily soluble in pyridine acidified with HCl, a quality utilized in
Fig. 7 Apparatus used for drying the samples spotted on the chromatography paper. Ceramic element has a loading of 40 watts per foot and is of the shop-window type.
subsequent estimation.

DEVELOPMENT WITH WALLENFEL'S REAGENT

Tetrazolim reagent is a very sensitive reagent and a rather delicate development procedure was required. After spraying the chromatograms were quickly placed between two pieces of polythene nettings (see fig. specimen) of similar size, and the whole was then placed inside a moistened (containing droplets of distilled water) 12" x 16" polythene bag, whose opened end was folded back and clamped with the help of large clips. These bags were left 8 to 12 minutes in an oven at 40°C, when the desired results were obtained.

(D) INVERTASE REACTION

However Wallenfel's tetrazolim reagent had the drawback of not reacting with sucrose and some other non-reducing sugars. To assay sucrose using the reagent therefore a further step was necessary, the use of Invertase to hydrolyse the sucrose on the chromatograph paper subsequent to running. Invertase analytical (made by Difco Labs. Michigan, U.S.A.) was employed; 10 mg of the dehydrated invertase was added to 10 ml distilled water. One ml from this concentrated invertase solution was again diluted with 30 ml of distilled water, this solution was used directly as follows.

The paper was spotted with the leaf extract and also with reference spots of glucose and sucrose (0.5 μl of 5% and 10% solutions respectively). After running and drying as usual it was sprayed lightly with the invertase and allowed to dry slowly at room temperature (about 40 mins). It was then sprayed with
the Wallenfel's reagent and the usual post-spraying procedure for this (described above) was followed. The sucrose spot was rendered visible at its correct position.

COLORIMETER ASSAY

A simple photocell colorimeter was constructed to help estimate the sugar-formozan compounds formed on the developed chromatograms. This was based on a 2" EEL Selenium barrier-layer cell, whose output was measured by a 450 Ohm Cambridge spot-galvanometer with a shunt sensitivity control. The cell faced vertically, and above it was a sliding tray with a circular hole into which fitted the flat base of a cut-down 1/4" glass specimen tube. The chromatogram spot to be assayed was cut out with a special 1.2" diam. cork-borer and placed flat in the base of the specimen tube. It was then covered with a fixed volume (2.5 ml) of acidified pyridine (90 mls of Pyridine with 10 ml of 10% Hydrochloride acid) and the upper ground edge of the tube was sealed with a trace of vaseline and square of thin glass. The purpose of the pyridine was to dissolve the formozan and render the specimen more uniform in colour and more transparent.

The photocell faced a 1.5 volts 0.11A bulb suspended from the top of the housing. This was in series with a single dry cell and a press switch. Before assaying a series of samples the galvanometer control was adjusted so that the galvanometer reading was full scale with a tube containing pyridine and "background" filter paper disc in position (this was cut from the blank part of a developed chromatogram). The instrument was calibrated by
Fig. 8. The photometer used for direct assay of the formozan spots produced by Wallerfet's tetracycline. A specimen cell holding the paper disc in acidified pyridine is visible.
Calibration curve for photometer measuring Lactose treated with Wallenfelah's tetrazolium reagent. The galvanometer deflection moves asymptotically with increasing sugar not to zero but to a value of about 39; consequently to obtain a straight line the deficit $= (100 \times D)$ is scaled up by calculating $= (. \infty)$

The quantities plotted are $= (100$ To use the curve, the measured deficit must first be multiplied by $100/51$ to obtain.
running under working conditions a series of spots containing known amounts of sugar; and all subsequent estimates were based on the calibration curve obtained. Sensitivity was improved by incorporating a green gelatine filter. The instrument is shown in the photograph attached (fig. 8)
PART TWO

CHROMATOGRAPHIC STUDIES
Results of Chromatographic Studies

While the results to be reported from the use of the sugar extraction and chromatographic techniques previously described, are very meagre (owing to unfortunate hindrances to the work), they will nevertheless be briefly set out in the present section, since a few points of interest emerge.

The objectives of this part of the investigation were two-fold. Firstly, to investigate the fate of 'foreign' sugars effective in promoting translocation and to find out whether they were converted in the process into any of the usual transport sugars — sucrose, starchyose, raffinose and so on. This, it was felt, might throw some light on the question as to why, broadly speaking, only sugars of the sucrose family normally fill this role. Secondly, to investigate how the concentration of the activating sugar varies down the axis, and to correlate this as far as possible with the results of the tracer studies, which give some idea as to the variation of the transport velocity.

It would obviously be interesting to know whether it is sugar level which parallels velocity, or sugar gradient. Unfortunately little information resulted on this point.

Fate of lactose externally administered

On this point some definite evidence was obtained. Fig. which is a tracing from a chromatogram run in isopropanol-water and developed in benzidine reagent, relates to the sugars present in normal illuminated rye leaves. It is one of a number and clearly shows that lactose and D-xylose are absent. Almost certainly glucose and sucrose are both present.
Fig. 47 Ascending chromatogram No. 47 of 3 July, 1963 showing sugar extracted from a normal undarkened leaf of ten day old barley against marker spots of lactose, glucose, sucrose and d-xylose. Chromatogram run for 18 hours and developed in benzidine.

Note: Pattern with rye essentially the same.
Fig. 11 Ascending chromatogram No. 38 of 12 August, 1963 showing sugars present in extract of young rye leaves darkened for 48 hours and then treated for 36 hours with 10% lactose on tip. Run in isopropanol and developed with benzidine.
Fig. 12. Ascending chromatogram No. 65 of 5 August 1963 showing sugars present in extract of young rye leaves darkened for 72 hours and then treated for 48 hours with 5% Lactose on tip. Run in iso propanol and developed with benzidine.
Fig. 11 shows the results of extracting rye leaves from plants given the following treatment: two plants were kept in the dark for 48 hours to empty their leaves of mobile carbohydrate, then given 10% (= 0.28 M) lactose in the usual way for 36 hours in the dark. It is clear that lactose has entered the leaf and been translocated intact in quantity. However, sucrose also appears to be present, though whether this is 'native' or formed from lactose is uncertain. Unfortunately, a fully satisfactory control experiment to determine the natural content of the leaves emptied by darkening remains to be done. This would have thrown light on the origin of the sucrose. Incidentally glucose (& fructose) are also probably present.

Fig. 12 reports a similar experiment. The leaf apices were given 5% lactose, and calibrating spots for lactose are shown. The rye lactose spot was nearest in size to the smallest calibration spot (0.5 µl of 5% lactose). In assaying the leaves the lamina and sheath were extracted and run separately. Lactose was absent from the sheath, and this is fairly good evidence that the sucrose found is 'native' and not derived from the lactose. However, the evidence is not conclusive, and it is difficult to see how 'native' sucrose could be present in such large amount in leaves kept dark previously and subsequently to administration for so long. The point obviously needs further investigation.

An interesting point not illustrated by these two chromatograms is that more lactose seems to be absorbed by the leaf when it has not been subjected to a previous lengthy dark
period (i.e. when native mobile carbohydrates are present) than when it has received such treatment. This result is opposite to what would have been expected had lactose uptake been a matter of simple movement down a concentration gradient. It agrees rather with the suggestion (see Jyyung & Wittwer, 1964) that it is metabolically energised.

Gradient of lactose promoting translocation

The second point on which information of a preliminary nature was obtained concerned the gradient of externally-applied sugar during translocation. Lactose was the only sugar investigated, and it was first necessary to calibrate the photoelectric colorimeter for it. This was done by running known quantities of lactose chromatographically in a manner identical with that adopted for extracts, developing them by the standard method and assaying them in the colorimeter. Since the light used was not monochromatic infinite density of the formazan spot did not correspond to zero galvanometer reading, but to a value of about 39. In other words the maximum absorption had a measure of $(100 - 39) = 61$ rather than 100. This means that a straight line calibration curve (see fig. 9) could be obtained by plotting the amount of lactose against the logarithmic of a hypothetical deflection calculated as indicated.

The results of experiment 49 of 15 October, 1963, are given in Table 5 and plotted in fig. 13. They indicate that there is a gradient of lactose present in the leaf (though this must not be taken as the gradient of total mobile sugar since the chromatograms already presented show that sucrose was also present).
### Table 5

Galvanometer readings \((\theta')\) of photometer

<table>
<thead>
<tr>
<th>Replicate sets</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Mean Value</th>
<th>(\theta')</th>
<th>(\delta)</th>
<th>Lactose per 8cm of leaf</th>
<th>(\mu g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf segments</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>76</td>
<td>69</td>
<td>78</td>
<td>70</td>
<td>73.2</td>
<td>26.8</td>
<td>43.9</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>77</td>
<td>73</td>
<td>81</td>
<td>73</td>
<td>76.0</td>
<td>24.0</td>
<td>39.3</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>88</td>
<td>80</td>
<td>80</td>
<td>72</td>
<td>80.0</td>
<td>20.0</td>
<td>32.8</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>84</td>
<td>78</td>
<td>83</td>
<td>75</td>
<td>80.0</td>
<td>20.0</td>
<td>32.8</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>85</td>
<td>80</td>
<td>80</td>
<td>70</td>
<td>78.8</td>
<td>21.2</td>
<td>34.7</td>
<td>34</td>
<td></td>
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<tr>
<td>6</td>
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<td>80</td>
<td>81</td>
<td>81</td>
<td>81.8</td>
<td>18.2</td>
<td>29.8</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>7</td>
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<td>81</td>
<td>80</td>
<td>81</td>
<td>80.8</td>
<td>19.2</td>
<td>31.5</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

Table 5 refers to experiment 49 of 15 October, 1963.

Sixteen 15-day old rye seedlings were selected for size and treated as four replicate sets of four. The plants were kept for 24 hours in the dark. The tips were then treated with 0.25 M lactose in the usual way for a further period of 24 hours in the dark. After harvesting, the laminas below the wetted segments were cut into seven 2-cm lengths and each group of four corresponding segments was extracted with 80% ethanol in the Soxhlet micro extractor. The lactose extracted was estimated after chromatography using Wallenfel's tetrazolium reagent.

For further discussion see text.
Fig 13. Plot refers to Experiment 49 of 15th October, 1933. Showing the distribution of lactose down the leaf of rye. For programme of treatment see Table 5 (p. 52) and for further details see text.
Fig. 14 Data of fig. plotted semilogarithmically. This figure should be compared with fig...
It is difficult to say whether the distribution pattern should be regarded as showing an exponential decrease. The proximal values are too low, but if this point be overlooked the agreement with the comparable results obtained with radioactive lactose (fig. 10) is not too bad. Neither set of results is based on extensive enough data for any reliable conclusions to be drawn from them. Their principal value is to indicate the feasibility of the method for obtaining gradients of mobile foreign carbohydrate in the leaf; however before such information can be made relevant to the problem of translocation it will have to be supported by data on the gradients of other mobile sugars present, and on the distribution of total sugars between conducting tissue and ground tissue. It should not be difficult however to secure this.

Conclusions

From the moderate amount of work done with extraction and quantitative chromatography the following conclusions may be drawn:

1) Leaves given 24 hours or more treatment in the dark contain a much lower level of sucrose.

2) This reduced level does not however lead to a more rapid uptake of externally-applied lactose, but rather the converse. This suggests metabolic uptake.

3) Externally-applied lactose to dark-treated leaves promotes translocation and the lactose passes down the leaf as lactose. However it is possible that some of it at least becomes chemically altered to other sugars.
4) Lactose applied in this fashion can be extracted and assayed and reveals a fall-off in concentration distribution from its point of application which appears to suggest an exponential law.

4) It has proved feasible to extract with a new micro-extractor and assay with Wallenfel's tetrazolium reagent the quantities of sugar in samples of young rye leaves of the order of 8cm or less in aggregate length. The line of approach appears a promising one, but further work is needed to exploit it.
PART THREE

TRACER STUDIES.
RESULTS

The results have been generally plotted on semi logarithmic paper showing the movement of tracer along the leaf length (petiole length in the case of soyabean) by plotting distance as abscissa and the count rate as ordinate while curves for each replicate have been displaced horizontally and often lined-in in different styles for clarity.

In every case the tracer movement is basipetal from left to right. Further the leaf section nearest the application point is marked with a solid circle while some points which seem to be suspect for valid reasons have been marked with a solid star and are eliminated in the final analysis; the respective justification for this has been provided later in the text.

In order to provide a comparative reference of the statistical accuracy of the counts the standard deviations are presented on the right side and this routine has been followed more or less throughout the results. Thus the S.D. attributable to the logarithmic results is

\[ \sqrt{\left( \frac{\alpha + \beta}{\alpha} \right) / \alpha} \]

This was arrived at as follows.

If the background count for the specified time is \( \beta \) and the sample count (corrected for the background) is \( \alpha \) then on account of the Poisson distribution involved the sample count \( \alpha \) is subject to the variance \( \alpha + \beta \). It can thus be written

In the logarithmic form this becomes:
\[ \ln (\text{sample count}) = \ln \left\{ \alpha + \sqrt{\frac{\alpha + \beta}{\alpha}} \right\}. \]

\[ = \ln \alpha + \ln \left\{ \frac{(\alpha + \beta)}{\alpha} \right\}. \]

\[ = \ln \alpha \pm \sqrt{\frac{\alpha + \beta}{\alpha}}. \]

provided the factor is not too large. This fraction is the standard duration of the semi log plot and is easily found when \( \beta \) is known.

SOME PRELIMINARY INVESTIGATIONS

Some earlier trials carried out to find a suitable plant subject and technique (and using material and methods subsequently discarded) is reported separately in the Appendix.

INFLUENCE OF APPLIED SUGAR ON TRANSPORT IN RYE

The plots in fig. record a later but still exploratory experiment to test whether like many other translocation indicators (e.g. 2, 4-D etc.) Cs-137 could be made to move through the agency of externally applied sugars. It involved rye plants given a 24 hour prior dark treatment.

Half of them were supplied with tracer and 1-molar sucrose, the other half being given distilled water in place of sugar. The curves show a comparative picture of the subsequent 24 hours translocation in darkness, and it is obvious at once that the externally applied sugar has been effective.

One puzzling result, however, to which further reference is made later concerns the fact that there was an undoubted though small movement in the "no-sucrose" treatment. The
FIG. 15 Experiment 54 of March 1964. Movement of S5-I3 down the leaf of rye plants in dark for 24 hours previous to tracer application and for 30 hrs subsequently. Dose 0.5uc per leaf. Solid spots indicates section nearest tracer reservoir. Curves represent averages of results plotted on fig.

FIG. 15a Experiment 55 of April 1964. Details as in fig.
Fig. 16 Data of fig. plotted semilogarithmically. Curves displaced horizontally by varying amounts for clarity.
characteristics of this are particularly the low activity involved, and the very level nature of the semi logarithmic lines.

The preceding observations encouraged a more detailed and systematic exploitation of the phenomenon and the experimental techniques having been finalised earlier the way was open to proceed. The immediate object was to investigate the extent to which the level of carbohydrate influences translocation and was met through varying the concentrations of the externally applied sugars.

THE PHLOEM LIMITED NATURE OF THE TRANSPORT

However an important point remained to be settled. This was whether the tracer movement observed was in fact taking place in the phloem. To some extent this has already been settled since in the leaves deprived of both photosynthetic sugar and artificially provided sugar very little tracer movement occurs and this strongly suggests the mediation of the phloem. However the point was further established by following up a clue afforded by the preceding observation that in the absence of any applied sugar darkened plants nevertheless translocate a small amount of tracer which shows very little fall-off in its distribution pattern.

Figures 15 and 16 show the results of an experiment in which the effects of applied sugar, mannitol and plain water on tracer movement were investigated. The salient features of the curves in fig. 15 (which represents the averages of the replicates of fig. 15) are firstly the markedly favourable effect of sucrose;
secondly, the general similarity of the curves for water and mannitol, with however, a very sharp lowering in the level portion when mannitol is given; and thirdly (and this shows up still better in fig 16) the increased downward slope of the level part when mannitol replaces plain water. The results can be readily understood if it is visualised that two transport processes are at work; a movement in the phloem which is appreciable only in the presence of sucrose, and a faster movement in the xylem which is appreciable only when the suction potential of the leaf is unopposed by the osmotic potential of the tracer milieu. The faster movement in the xylem implies on the basis of the model to be introduced shortly, a slower exponential fall-off in the level lower down the leaf, and correspondingly with mannitol present the xylem intake of water is slower and the exponential fall-off in the level part of the mannitol curve is greater than in case of the water curve. Thus the result seems to be wholly consistent with the view that both the phloem and the xylem are involved, and that the influence of the latter can almost be nullified by adding to the tracer milieu sufficient inert solute to bring the molarity to one.

It might be added that as much as the tracer moves appreciably in the first two segments in the 'no sucrose' series it may be moving in phloem under the influence of extremely low quantities of endogenous sugars, or it might be undergoing transport by some other means, such as cyclosis and diffusion in mesophyll cells.
Fig. 17 Results of fig., averaged and plotted on linear scales.
Fig. 18. Experiment 67 of 26 April 1964. Movement of Cs-137, down the leaf of rye. Tracer applied as in sucrose solution of strength stated and with mannitol-sorbitol. Other details as in Experiment 67 of 26 April 1964. Standard deviations of the counts are shown to the right. The figures at the same activity level for Cs-137, K-47, and O-15-80 are omitted from final analysis.
Accordingly as already described the standard practice of employing the mannitol-sorbitol mixture in the tracer milieu was maintained throughout the subsequent work and as fig. 10 typically demonstrates the influence of the mixture, in contrast with that of plain water, seems clearly to be to establish a predominantly phloem transport.

EFFECT OF VARYING THE SUCROSE CONCENTRATION

The problem of whether the transport was phloem-limited or not being settled to a fair degree of certainty the work with varying concentrations of applied sugar was carried out in a series of similar experiments.

Fig. 11 represents the results of an experiment (No. 56 of 26.4.64) with sucrose concentrations of zero, 0.04M, 0.1M, 0.25M and one molar sucrose plotted semilogarithmically while the same results (averaged) can be seen plotted linearly in fig. 12. Fig. 19 shows a similar experiment in which the sucrose concentrations were taken down to the lower level of 0.01 molar.

It can be seen clearly from both of these experiments that there is agreement in the shape of the curves with the interpretations given in connection with the earlier comparative set of experiments with water, sucrose and mannitol. A gradual decrease in the downward slope of the curves with increasing sucrose concentration in the tracer milieu is indicated by both experiments. This strongly suggests phloem transport. Further, a comparison of the linear plots (figs. 15 and 17) when water and 0.04M sucrose respectively were used indicates that in the lower
Fig. 19 Experiment 61 of 17 July 1964. Movement of \( \text{^{32}}\text{P}\) down the leaf of rye. Tracer applied in sucrose solution of strength stated and with aggregate molarity raised to one with mannitol-sorbitol. Other details as in fig. Starred results omitted from final analysis.
Fig. 18: Experiments of 5 June 1964. Movement of Cs-137 down the leaf of rye. Tracer applied in sucrose solution of strength stated and with aggregate molarity raised to one with mannitol-sorbitol. Other details as in fig. Starred results omitted from final analysis.
Fig. 19 Experiment 58 of 7th May 1964 — Movement of CaO137 down the leaf of rye. Tracer applied in a sucrose solution of strength stated and with an exaggerated molarity raised to one with mannitol — sorbitol. Other details as in fig.
segments of the leaf the plain water mediates a greater trans- 
port of the tracer than the sugar - a clear suggestion that in 
the former case (owing to the absence of osmotically-active 
but otherwise inert solute) xylem transport was involved.

Another experiment in the series is shown in fig.18a. 
This was carried out as a further check on the preceding 
experiments to establish the position of the 0.5M curve in 
relation to the others. (This concentration, included in the 
design of experiment 58 of 7.5.64 (fig.19) had been accident- 
ally replaced by 0.05M. The starred point in this experiment 
is considered aberrant and is eliminated in the final analysis. 
It was probably due to contamination of the segment adjacent to 
the reservoir - see further below). A further experiment (No.60 
of 5.6.64) is reported in fig.18. This was designed to provide 
further coverage in what appeared to be an interesting concentra-
tion range.

It would obviously have been desirable to have run all 
these experiments on a single occasion but the cramped facilities 
in space, and the absence of technical help did not allow this. 
However, there is a very fair degree of agreement between the 
results of similar treatments on different occasions, and in the 
analysis the experimental results shown, with a few others, 
have been pooled.

ABERRANT RESULTS

The reasons for omitting the results marked with a solid 
star were as follows.
In figs. 1B and 19a the single points are fairly obviously aberrant and can easily be accounted for by contamination of the uppermost segments from the reservoir or by splashing from the applicator (introducing the tracer was an operation requiring considerable care). The two lines for "0.1 M sucrose" in fig. 13b are more difficult to account for. Both on account of their steep slope and because of the very low levels they reach in the segments further down the leaf they fall markedly out of place in the total series, and it can only be conjectured, this is due to some local fault in the technique. The complete lines have been ignored in the analysis. Finally, to anticipate, the starred point in fig. 2D is obviously subject to great uncertainty as to its abscissa, owing to the uncertainty associated with the concentration of the endogenous sugars of the leaf; certainly these cannot be assumed to be quite zero.

THE MATHEMATICAL MODEL

The results of the preceding experiments will be analysed on the basis of a sample model first proposed by Horwitz (1958) and discussed in more detail by Spanner and Prebble (1962). This model (see fig. 15) supposes that a bulk flow is occurring in the conducting elements at a constant mean velocity \( \nu \). The conducting strands which have a cross-section \( A \) are in contact with the ground tissue over a perimeter \( s \), and the tracer which is being carried passively along in the flow leaks out irreversibly from the conducting elements into the ground tissue at a rate per unit length of axis given by \( ksc \), where
is the concentration in the conducting elements and \( v \) is a coefficient with the dimensions of velocity. The bulk flow incidentally, may be actuated passively by a Munch mechanism, or actively by an electrokinetic one. The postulate of lateral leakage imposes an exponential fall-off pattern on the tracer activity down the axis (cf. fig.12) and the semilogarithmic plot (cf. fig.11) becomes approximately a straight line with a negative slope of provided the activities are measured not too close to the radio active "front".

In the light of this model the following preliminary points may be made. As remarked above the curves obtained when the tracer is applied in pure water (figs. 15 and 16) seem to reveal two distinct phenomena: near the tracer reservoir the slope of the semi-logarithmic plot is very steep, while further down it becomes almost horizontal. These can now be understood as arising from a very slow movement in the phloem (which during the course of experiment is not able to carry the tracer very far) and a much more rapid movement in the xylem. The relative magnitudes of the velocities influence the slopes inversely as indicated by the expression \( k^2 / A^2 \) (though the other factors in this, for example \( k \), may also play a part). Further, for a given rate of entry of tracer into the leaf the levels of concentration attained in the conducting tracts (phloem xylem) will also be inversely as the velocities. Both these points are reflected in results shown in figs. 15 and 16. As already stated further support is given to the interpretation by
recalling that raising the osmotic potential of the tracer medium by inert salutes (mannitol and sorbitol) lowers the horizontal part of the curves virtually to zero (fig. 5) and this again can be readily understood if this part reflects a movement in the xylem.

COMPARATIVE RESULTS WITH AN ANION AND A NON-ELECTROLYTE

It was observed in the work of Spanner and Prebble (1962) with Nymphoides that the semi-logarithmic plots yield curves which are concave upwards, a fact which as they point out seems difficult to account for in terms of any reasonable mathematical model. A very prevalent tendency of upward concavity in the present results is therefore all the more interesting. Since Cs-137 is present as an ion it will leak out laterally at a rate dependent on both concentration and transverse electrical potential gradients. Since the concentration difference is the simple function which defines most nearly the rate of transport of a salute in thermodynamically passive movement at low levels of concentration a potential difference does not influence the mass rate of movement of ions very markedly; at higher levels it does. This follows since the equivalence (so far as transport of a univalent ion is concerned) of a concentration difference \((C_1 - C_2)\) and an electrical potential difference \(\Delta \psi\) is given by the well-known equation

\[
RT \ln \frac{C_1}{C_2} = F \Delta \psi
\]

where \(F\) is the faraday. This indicates that at low levels
Fig. 20. Experiment 56 of 8th October 1964 Movement of lactose 1-14 down the leaf of rye. Dose luc per plant, the tracer was applied in inactive lactose of the strength indicated and the solution was made up to an aggregate molarity of one with mannitol-sorbitol. Other appropriate details as in fig.
Fig. 21 Experiments 34 and 65 of 15 and 23 Sept., Movement of $^{31}$I down the leaf of rye. Results are the averages of the numbers of replicates shown in the brackets. Tracer applied in sucrose solution of the strength stated and with aggregate molarity raised to one with mannitol - sorbitol. Other details as in the fig.
it takes a very high potential difference to 'balance' a given low concentration differential. Since therefore in the present experiments the tracer is present in exceedingly small amounts\(^1\) neglecting potential gradients (which the model in fact does) as a factor in lateral leakage does not seem very important as a possible explanation of the upward concavity. However to investigate the issue on more direct lines it was decided to employ an anion (chlorine 36) in place of a cation. Fig.\(3d\) shows the results of two experiments in which NaCl-36 was employed\(^2\) in place of Cs-137 in virtually identical conditions.

It is apparent that the pattern of movement is very similar and upward concavity is still very evident. Further when a non-electrolyte (lactose-C\(^{14}\)) was employed as tracer (unfortunately only a very little was available) the curvature of the semi-logarithmic plot was also the same (fig.\(3c\)). Some further account of the issue is given in the following section.

**COMPARISON OF K-42 WITH Cs-137**

A further comparison of interest which will be mentioned before the analysis is carried further is illustrated by an exploratory experiment in which Cs-137 and K-42 were applied simultaneously. As mentioned earlier the separate assay of these two tracers is easy owing to the difference in their

---

1. The specific activity of the Cs-137 was 25 C per g Cs which is relatively very high.

2. About 0.5mc per plant was given at specific activity 500mc per g.Cl. This activity is fairly low, so that a fair concentration of total chloride was present (contra Caesium).
Fig. 22. Experiment 68 of Nov. 1964. Simultaneous movement of K-42 and Cs-137 down the leaf of rye. Tracers applied together in one molar sucrose solution. Corresponding pairs start at same ordinate. For duration of treatment see Fig. ___, and for details of tracers see text. (The related curves for K-42 and Cs-137 are shown in the next figure.)
Fig. 28 Results of fig. reported to show initial points for K-42 and Cs-137 superimposed for each replicate. For discussion see text.
<table>
<thead>
<tr>
<th>Replicate No.</th>
<th>Counts per 100 secs.</th>
<th>Lapse ((t_1 - t_2))</th>
<th>Decay factor (\beta)</th>
<th>Difference (n'_k = \frac{(n_k - n'_k)}{\beta} (\frac{\beta - 1}{\beta - 1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 24019</td>
<td>12.30 pm 7443 12.46 pm</td>
<td>48.3 3.86 14.5 16579 17803 1227 6216</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 7213</td>
<td>12.34 599 12.48 599</td>
<td>48.2 3.85 14.4 5431 5536 405 1377</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 2715</td>
<td>12.37 565 12.51 565</td>
<td>48.2 3.85 14.4 1756 1887 131 828</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 1915</td>
<td>12.45 482 12.58 482</td>
<td>48.1 3.85 14.4 766 825 57 287</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 1400</td>
<td>12.46 344 12.56 344</td>
<td>48.1 3.85 14.4 693 687 48 301</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 1112</td>
<td>12.50 344 12.56 344</td>
<td>48.1 3.85 14.4 693 687 48 301</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 988</td>
<td>12.53 344 1.01 344</td>
<td>48.1 3.85 14.4 693 687 48 301</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2**

EXPERIMENT 68 OF 18th NOVEMBER, 1964
<table>
<thead>
<tr>
<th>Number of Replicate</th>
<th>( n'_k )</th>
<th>Clock Time</th>
<th>Lapse from instant of first count for each replicate in half lives</th>
<th>Decay Factor ( \beta ) ( [\beta = \beta_0 e^{-34 \times \lambda_k}] )</th>
<th>( n_k = \beta n'_k ) (corrected to initial instant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17803</td>
<td>12.30 pm</td>
<td>Zero</td>
<td>1.000</td>
<td>17803</td>
<td></td>
</tr>
<tr>
<td>5836</td>
<td>12.34 &quot;</td>
<td>0.005</td>
<td>1.003</td>
<td>5834</td>
<td></td>
</tr>
<tr>
<td>1887</td>
<td>12.37 &quot;</td>
<td>0.009</td>
<td>1.006</td>
<td>1898</td>
<td></td>
</tr>
<tr>
<td>1451</td>
<td>12.43 &quot;</td>
<td>0.017</td>
<td>1.012</td>
<td>1468</td>
<td></td>
</tr>
<tr>
<td>1063</td>
<td>12.46 &quot;</td>
<td>0.021</td>
<td>1.015</td>
<td>1079</td>
<td></td>
</tr>
<tr>
<td>825</td>
<td>12.50 &quot;</td>
<td>0.026</td>
<td>1.018</td>
<td>840</td>
<td></td>
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<tr>
<td>687</td>
<td>12.53 &quot;</td>
<td>0.030</td>
<td>1.021</td>
<td>701</td>
<td></td>
</tr>
<tr>
<td>8846</td>
<td>12.56 pm</td>
<td>Zero</td>
<td>1.000</td>
<td>8864</td>
<td></td>
</tr>
<tr>
<td>4337</td>
<td>12.59 &quot;</td>
<td>0.004</td>
<td>1.003</td>
<td>4350</td>
<td></td>
</tr>
<tr>
<td>2227</td>
<td>1.01 &quot;</td>
<td>0.006</td>
<td>1.004</td>
<td>2236</td>
<td></td>
</tr>
<tr>
<td>1688</td>
<td>1.03 &quot;</td>
<td>0.009</td>
<td>1.006</td>
<td>1698</td>
<td></td>
</tr>
<tr>
<td>1042</td>
<td>1.06</td>
<td>0.013</td>
<td>1.009</td>
<td>1052</td>
<td></td>
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<td>794</td>
<td>1.09</td>
<td>0.017</td>
<td>1.012</td>
<td>804</td>
<td></td>
</tr>
<tr>
<td>628</td>
<td>1.11</td>
<td>0.020</td>
<td>1.014</td>
<td>637</td>
<td></td>
</tr>
<tr>
<td>9223</td>
<td>1.15 pm</td>
<td>Zero</td>
<td>1.000</td>
<td>9223</td>
<td></td>
</tr>
<tr>
<td>3341</td>
<td>1.19 &quot;</td>
<td>0.005</td>
<td>1.003</td>
<td>3351</td>
<td></td>
</tr>
<tr>
<td>1515</td>
<td>1.21 &quot;</td>
<td>0.008</td>
<td>1.006</td>
<td>1525</td>
<td></td>
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<tr>
<td>891</td>
<td>1.24 &quot;</td>
<td>0.012</td>
<td>1.008</td>
<td>898</td>
<td></td>
</tr>
<tr>
<td>517</td>
<td>1.26 &quot;</td>
<td>0.014</td>
<td>1.010</td>
<td>522</td>
<td></td>
</tr>
<tr>
<td>310</td>
<td>1.29 &quot;</td>
<td>0.017</td>
<td>1.012</td>
<td>314</td>
<td></td>
</tr>
<tr>
<td>17491</td>
<td>1.32 pm</td>
<td>Zero</td>
<td>1.000</td>
<td>17491</td>
<td></td>
</tr>
<tr>
<td>6538</td>
<td>1.35 &quot;</td>
<td>0.004</td>
<td>1.003</td>
<td>6598</td>
<td></td>
</tr>
<tr>
<td>4120</td>
<td>1.38 &quot;</td>
<td>0.008</td>
<td>1.006</td>
<td>4145</td>
<td></td>
</tr>
<tr>
<td>2506</td>
<td>1.41 &quot;</td>
<td>0.012</td>
<td>1.008</td>
<td>2526</td>
<td></td>
</tr>
<tr>
<td>1740</td>
<td>1.43 &quot;</td>
<td>0.014</td>
<td>1.010</td>
<td>1757</td>
<td></td>
</tr>
<tr>
<td>1309</td>
<td>1.46 &quot;</td>
<td>0.018</td>
<td>1.012</td>
<td>1325</td>
<td></td>
</tr>
<tr>
<td>778</td>
<td>1.49 &quot;</td>
<td>0.022</td>
<td>1.015</td>
<td>790</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 24 Curve for reading off the decay factor when using isotopes of short half-life.
<table>
<thead>
<tr>
<th>Value</th>
<th>( \lambda )</th>
<th>( \lambda + \Delta \lambda )</th>
<th>( \lambda - \Delta \lambda )</th>
<th>( \Delta \lambda )</th>
<th>( n )</th>
<th>( l )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.57</td>
<td>( \frac{3.09}{17.49} )</td>
<td>( \frac{2.55}{12.4} )</td>
<td>( \frac{3.78}{6.69} )</td>
<td>0.44</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>0.59</td>
<td>( \frac{3.38}{29.4} )</td>
<td>( \frac{2.97}{16.3} )</td>
<td>( \frac{2.07}{33.6} )</td>
<td>0.33</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>0.69</td>
<td>( \frac{2.63}{13.9} )</td>
<td>( \frac{1.94}{8.86} )</td>
<td>( \frac{2.69}{18.75} )</td>
<td>0.67</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>0.27</td>
<td>( \frac{3.27}{17.80} )</td>
<td>( \frac{3.02}{18.03} )</td>
<td>( \frac{0.6}{62.16} )</td>
<td>0.05</td>
<td>7</td>
<td>1</td>
</tr>
</tbody>
</table>
Statistical Analysis of Results of K-42 and Cs-137 Experiment

The analysis is made by the method of paired comparisons, using the t-test on account of the small sample number. The differences in ln-r from the last column of Table 4 are compared with zero. It should be noted that ln r is proportional to the slope of the semi-logarithmic plot; however, the third value has to be multiplied by the factor 7/6 to make it comparable with the others.

<table>
<thead>
<tr>
<th>Replicate Number</th>
<th>Measure of difference in slopes (K42-Cs137)</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.21</td>
<td>0.0441</td>
</tr>
<tr>
<td>2</td>
<td>0.69</td>
<td>0.4761</td>
</tr>
<tr>
<td>3</td>
<td>0.59 x 7/6 = 0.69</td>
<td>0.4761</td>
</tr>
<tr>
<td>4</td>
<td>0.57</td>
<td>0.3249</td>
</tr>
<tr>
<td></td>
<td>2.16</td>
<td>1.3212</td>
</tr>
</tbody>
</table>

\[
\bar{\chi} = \frac{1}{n} \sum \chi = 0.540
\]

\[
S^2 = \frac{1}{3} \left\{ 1.3212 - \frac{1}{4} \times 2.16^2 \right\}
= \frac{0.1548}{3} = 0.0516
\]

\[
S/\sqrt{n} = 0.0258
\]

\[
t = \frac{\bar{\chi} - \mu}{S/\sqrt{n}} = \frac{0.540 - 0.000}{0.0258} = 20.9
\]

With 3 degrees of freedom the value of t is considerably in excess of that required for significance at even the 0.1% level (i.e. 12.92). Consequently there can be no doubt that the slope for K-42 is significantly greater than that for Cs-137.
half-lives. Details of the calculation are given in the table 2 and the results are shown plotted semi-logarithmically in fig. 12. Four replicates were employed, and visual comparison between the results for potassium and caesium is rendered easier by displacing the respective curves vertically till the initial points of corresponding pairs coincide. This makes it fairly obvious that the slope of the semi-logarithmic line for potassium is steeper than that for caesium. The point is summarised statistically in table 4, using mean slopes calculated from the first and last points on the curves of fig. 21.

EVALUATION OF THE SEMI-LOGARITHMIC SLOPES

When the results reported in figs. 16 to 19 are considered together it is apparent that there is a relationship between the slope of the curves and the concentration of the applied sugar. In analysing this relationship several practical problems arise. Firstly, it is desirable to base any calculation of the slopes on as great a length of leaf as possible to overcome statistical variability. Secondly, the expression \( \frac{K_s}{A_N} \) derived from the model refers most accurately to the slopes in a steady-state region well behind the "front". However, in the case of the low sucrose curves phloem transport had not proceeded very far and the slope had therefore to be evaluated rather near the front. Thirdly, slopes should obviously relate to portions of different leaves as morphologically comparable as possible. To some extent these desiderata are in conflict. The procedure adopted had
Fig 25 Analysis of results. Starred points omitted from regression analysis. For discussion see text.
Fig. 26 Analysis of the slopes of the semi-logarithmic plots for Cs-137 and Cl-36. Standard deviations of the individual results are indicated. Standard errors of the mean are respectively $\frac{1}{\sqrt{12}}, \frac{1}{\sqrt{3}}, \frac{1}{\sqrt{5}}, \frac{1}{\sqrt{2}}$, $\frac{1}{\sqrt{6}}, \frac{1}{\sqrt{3}}, \frac{1}{\sqrt{2}}$, $\frac{1}{\sqrt{3}}, \frac{1}{\sqrt{2}}$, $\frac{1}{\sqrt{3}}$, of those shown for Cs-137 and of those shown for Cl-36, reading from left to right. For further details see text. $\star \frac{1}{\sqrt{2}}, \frac{1}{\sqrt{5}}, \frac{1}{\sqrt{6}}, \frac{1}{\sqrt{5}}$. 

- caesium 137
- chloride 36
therefore to be a compromise and this was arrived at as follows. In the case of the "zero-sucrose" curves the slope was evaluated from the first two points only, the vertical distance between these being read off in units of the horizontal scale. In other words, the slope was in effect calculated from the expression

\[ \text{slope} = \frac{1}{\ln 2} \cdot \ln \left( \frac{A_v}{K_s} \right) \]  
(ratio of activities)

so that the unit of slope corresponds to a factor of two as between the activities of adjacent sections. In the case of other treatments the slopes were calculated by similarly measuring the vertical distance between the first and the third points and dividing by two.

Fig. 26 shows the results obtained from this analysis. The numerical values of the slopes are plotted relative to the left hand scale and their reciprocals relative to the right hand one. The reciprocal of the slope (i.e. according to the model \( \frac{A_v}{K_s} \)) reflects directly the velocity \( (v) \) of the bulk flow, and the result suggests that it might be related to the concentration of the applied sugar by an equation of the form

Reciprocal of slope = constant \( \frac{1}{v} \) (concentration)

If this is so the value of the index \( \frac{1}{v} \) can be found by plotting the logarithms of the slope against that of the concentration. This has been done in fig. 25, and it will be seen that a reasonably straight line results. A regression line has been calculated statistically in the usual way and the numerical value of the slope works out as \( \frac{1}{5.6} \) taking the quantity \( \frac{A_v}{K_s} \) as substantially constant. The significance of these results will be discussed in the next section.
DISCUSSION

A model which represents the leaf as an absorbing and translocating system is shown in Fig. 27. Assuming a concentration in the external milieu of $C_1$, imagine sugar to penetrate the epidermis and reach the mesophyll where under steady conditions its concentration reaches a level $C_2$. Next, sugar is "loaded" from the mesophyll into the sieve tubes where its concentration is $C_3$ at the point of basipetal exit from the immersed leaf-tip, the corresponding linear velocity of the translocation stream being then $v$. As the uptake of the sugar by the leaf may be an active process (Jyung and Wittwer, 1964) it would seem reasonable to write its net rate of entry into the leaf in the form $\alpha C_1^{\gamma y y^{m_1}}$ ($C_2$ being assumed low) and to equate this expression under steady conditions to the rate of removal from the terminal wetted segment (say $\beta v C_3$) in the conducting strands. Thus we have

$$\alpha C_1^{\gamma y y^{m_1}} = \beta v C_3$$

(2)

where $\alpha$ and $\beta$ are constants.

Further as a very tentative move we might assume that the velocity in the sieve tubes is similarly related to their sugar concentration by an equation of the form

$$v = \gamma C_3^{\gamma y y^{m_2}}$$

(3)

Elimination of the unknown $C_3$ between (2) and (3) gives

$$v = \delta . C_1^{\gamma y y^{m_1 (m_2 + 1)}}$$

(4)

where $\delta$ is a new coefficient.
Model of the leaf as a translocating system. For description see text.
To see the meaning of this equation suppose first that the rate of uptake of sugar into the leaf is a simple linear function of the applied concentration, i.e. write \( \mathcal{M}_1 = 1 \). Further, suppose that \( \nu \) is simply proportional to the sieve tube concentration \( C \) (a state of affairs which in the present context might be considered reasonably consistent with Munch's hypothesis). Writing \( \mathcal{M}_2 \) also equal to unity we have

\[
\nu = S \sqrt{C_1}
\]

which shows how \( \nu \) varies with \( C_1 \) in this simplest case.

(The relationship is like that of fig.26 (upper curve) but the curve does not flatten off so quickly).

However, if we wish to take leaf absorption as rising less steeply with applied concentration than linearly we may write \( \mathcal{M}_1 = 2 \).

This gives

\[
\nu = S C_1^{\frac{1}{4}}
\]

or if velocity in turn increases only with the square root of the sieve-tube concentration (i.e. if \( \mathcal{M}_2 = 2 \)) then

\[
\nu = S C_1^{\frac{1}{6}}
\]

Thus the final index is rather sensitive to the indices of the

1. Thus if we assume that the sugar concentration falls to a low level (at \( X \), near the roots say) which is fairly constant as between experiments, the sugar gradient down the leaf will be roughly proportional to the sugar level in the leaf. Munch's theory depends much on the sugar gradient.
individual stages.

The index obtained in the present study ($1/\sqrt{3.6}$) is rather close to that of equation (7), but this agreement has to be interpreted rather cautiously since the experimental value refers to the quantity $A_U/K_S$ and so reflects also possible changes in the leakage coefficient $K$ (which as relating to an ion movement may well depend on sugar level), and less likely, changes in $A$ and $S$ also. However, it would seem very probable that both sugar uptake and dependence of translocation velocity of sieve tube sugar concentration involve indices ($1/\theta_1$ and $1/\theta_2$) both significantly less than unity. It would be unwise however to infer too much from the present data about the form of the interesting relationship between translocation velocity and the concentration of sugar in the translocation stream, and some more work will be needed to provide firm evidence for or against either the Munch or the electrokinetic theories.

The data obtained for Cl-36 movement provide the possibility of a comparison between anion and cation transport. If the slopes of the semi-logarithmic plots are evaluated exactly as in the case of caesium and inserted in the graph of the latter it is found that they lie consistently above the caesium points (see fig.26). This result can be interpreted tentatively as indicating either a slower rate of transport for chloride or a more rapid rate of lateral leakage. If the sieve plate pores are filled with cytoplasmic slime having ultramicroscopic
channels and a negative colloid charge (conformably to the electrokinetic theory) a lower velocity for the anions would be expected. On the Munch hypothesis however such a result (i.e. lowered velocity of anions) would hardly be anticipated since either the pores would have to be postulated as open and so too wide to exert an electrical retardation on anions, or the cytoplasm would have to be regarded as moving with the stream. Either way cations would not be at an advantage relative to anions. However, it is at least possible that chloride has a higher rate of lateral leakage than caesium (or potassium), so the evidence in favour of electrically potent pores is not conclusive. Further comparative work needs to be carried out to follow up this preliminary evidence.

The evidence from the experiments with caesium and potassium moving simultaneously (see fig.12) seems to show that potassium has a more rapid rate of lateral 'leakage' than caesium (since it seems hardly likely that the translocatory velocities of the two would be markedly different). When it is recalled that the sieve-tube sap is naturally fairly rich in potassium\textsuperscript{1} and that the tracer potassium would have to compete with this native potassium if leakage is a non-linear process, the suggestion that potassium 'leaks' faster than caesium is important. It would fit in well with the requirements of the electrokinetic theory of transport. However the conclusion must be very tentative for a number of reasons. Caesium may also have to compete with native potassium in the lateral

\textsuperscript{1} The potassium in the tracer miHeu was also much higher than the caesium owing to the much lower specific activity. (see earlier.)
leakage process, and if so this would weaken the argument. It is in fact believed to compete with potassium in certain cases, as for instance in barley roots (Epstein and Hagen, 1952). Again this is an interesting line to be followed up.

The experiment with lactose C-14 was unfortunately not on a large enough scale to be very significant, and the results appear rather inconsistent among themselves. However, they do agree with the others in indicating an upward concavity of the semi-logarithmic curves. The most likely explanation of this feature still seems to be (see Spanner and Prebble, 1962) that the velocity \( U \) rises down the conducting tracts. This would be expected on a basis of progressive intake of water and approximate constancy of cross section; both of which postulates would seem to be reasonable in the present case (i.e. the cereal leaves). The slope of the lactose curves gives the impression of being rather less than that of the caesium ones, suggesting a lower rate of leakage from the sieve tubes. However, there is certainly not enough evidence here to assert this; moreover the 'activating' sugar was itself lactose instead of sucrose, so that the sets of data are not really comparable.

**CONCLUSION**

In conclusion, it may be remarked that the present work establishes the following points:

(i) When natural carbohydrate is absent tracers such as
Caesium-137, potassium-42, chloride-36 and lactose C-14 move only in the leaves of rye when sugars are supplied artificially at the same time. This phenomenon is of course widely known in other contexts.

(ii) Sucrose and lactose are among the sugars which can activate transport in this way when externally applied.

(iii) The distribution of tracer down the leaf when applied apically shows the common approximately exponential fall-off pattern. The semi-logarithmic curves are universally concave upwards.

(iv) The slope of these curves is a function of the concentration of applied sugar, falling as the latter is raised.

(v) The slope is appreciably steeper for chloride than for caesium. For potassium it is a little steeper than for caesium.

(vi) Where tracer is applied in a medium of low osmotic potential there is an absorption of water by the leaf apex with consequent movement of tracer downwards in the xylem. This can be avoided by raising the osmotic potential of the milieu.

Further, while they cannot be regarded as definitely established, since among other things they represent interpretations based on a specialised though plausible model, the following points seem probably to be the case:

(i) The velocity of translocation is a function of the applied sugar concentration, and so of the concentration in the sieve tubes.
(ii) It appears to depend on some fractional power of the latter concentration.

(iii) It is greater for cations such as caesium and potassium than for comparable anions such as chloride.

(iv) Potassium seems to move laterally out of the sieve tubes a little faster than caesium, and probably faster still than lactose.

(v) The velocity of the translocation stream increases down the leaf (even under conditions of terminal sugar application).

So far as the present work has a bearing on the controversy over the mechanism of phloem transport it would seem to lend considerable support to a theory of mass flow, and to show a modest preference for the electrokinetic theory. This preference arises from the suggestions which it raises as to the functional relationship between sieve tube sugar concentration and velocity, the likelihood that cations move faster than anions and that potassium 'leaks' transversely faster than caesium. However, all these suggestions are very tentative.
PART FOUR

APPENDICES

ACKNOWLEDGEMENTS AND REFERENCES
APPENDIX I.

A few examples of typical early work carried out with plants and by methods which were later discarded is reported separately in this appendix. Where not otherwise stated the conditions of growing and harvesting were similar to those used for the rye experiments already described. However, most often the plants were maintained throughout in light and were given no prior dark treatment.

The experiment shown in fig. was carried out with young soyabean seedlings using the spot method of tracer application (already described in detail in the chapter of methods). As can be seen from the plot on the semilogarithmic paper, there has been uptake of the tracer and a fair amount of transport from the leaves, and in a pattern which can very roughly be described as subject to an exponential fall-off; the lines appear straight in tendency.

However, considering the initial dose of tracer given externally (approximately 0.5 μc) a higher intake by the leaves was clearly desirable.

The details of the use of a polythene ring technique for tracer-contact had already been described (see page ) and fig. here represents some results for the same employing young wheat leaves (the results shown are, as with the soyabean, replicates).

As is apparent from the figure, compared with the results in the preceding (soyabean-spotting) experiment there has been a
Fig. 28 Movement of Cs-137 (0.5 uc per plant) down the stem of young soybean plants using spot technique. (The segments were segments of stem and not leaf blade as implied). For further details see text.
**Fig. 29** Movement of Cs-137 (0.5uc per plant) down the leaf of wheat using ring technique. For further details see text.
considerable improvement in the evenness of the pattern with the new plant material, but the replication is poor.

As an alternative method of holding the tracer in contact with a defined area of the leaf the expedient was tried by applying it on a flimsy strip of lens paper 5mm x 5mm. It can be seen from the results shown in fig. that the quantity of tracer which has penetrated and moved down the leaves has been almost negligible; also the overall pattern of the activity fall off is anything but exponential. It is obvious that the lens paper strips interfere with the leaf uptake of tracer, possibly by holding the caesium by adsorption. The method was not tried with an anion, which might be less liable to absorption on the cellulose.
Fig. 30 Movement of Cs-137 (0.5 uc per plant) down the leaf of wheat. Tracer drop applied on lens paper. For further details see text.
APPENDIX II.
APPENDIX

CULTURE SOLUTIONS

Arnon & Hoagland's culture solutions which was the one employed consists of two portions containing major and micro-nutrients:

A. MAJOR NUTRIENTS: Quantities per 100 ml of water

- $\text{KNO}_3$ 1.02 g
- $\text{Ca}\,(\text{NO}_3)_2$ 0.492 g
- $\text{NH}_4\,\text{H}_2\text{PO}_4$ 0.230 g
- $\text{MgSO}_4$ (as $\text{MgSO}_4\,7\text{H}_2\text{O}$) 0.49 g

B. MICRO-NUTRIENTS: Quantities per 100 ml of water

- $\text{H}_3\text{BO}_3$ 2.86 mg
- $\text{MnCl}_2\,\text{H}_2\text{O}$ 1.81 mg
- $\text{CuSO}_4\,5\text{H}_2\text{O}$ 0.08 mg
- $\text{ZnSO}_4\,7\text{H}_2\text{O}$ 0.22 mg
- $\text{H}_2\text{MoO}_4\,\text{H}_2\text{O}$ 0.09 mg

STOCK SOLUTIONS

was made by mixing the two portions in ratios of 100 to one and this was then supplied to the plants.

CHROMATOGRAPHIC REAGENTS used for sugars

1. BENZIDINE

(a) Benzidine 1 g in glacial acetic acid 40 ml
- Trichloro-acetic acid 30 ml
- Water 40 ml

(b) Acetone 9 vol
The Benzidine was dissolved in the glacial acetic acid, while the trichloroacetic acid was dissolved in the water and the two solutions were then mixed. The resultant monophasic solution was stable in the refrigerator for a couple of weeks. It was diluted with the acetone immediately before use. Most sugars react on heating to 90-100°C for about 5 to 10 minutes giving yellow to dark brown spots.

2. WALLENFEL'S REAGENT

The spray was freshly prepared by making a 1:1 mixture of 2% aqueous triphenyl tetrazolium chloride and N sodium-hydroxide. The sprayed paper was maintained in a water-saturated atmosphere at 40°C for 20 minutes. Excess reagents were then carefully washed out with water and the paper dried gently at 25°C to 30°C. The sensitivity of the reagent is around 0.5 mg.
REFERENCES


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