PHLOEM TRANSPORT IN THE STOLON OF *SAXIFRAGA SARMENTOSA*

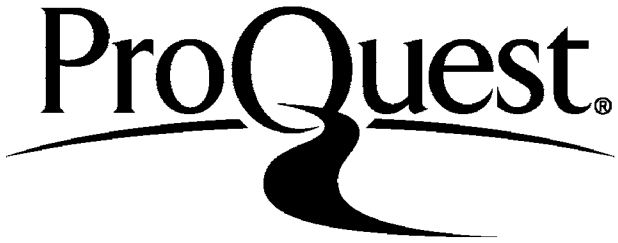
A thesis submitted for the degree of Doctor of Philosophy in the Faculty of Science in the University of London

By

Fayyaz Ahmed Qureshi

Department of Botany,
Bedford College,
Regent's Park,
London NW1 4NS.

1973



Abstract

A study was made of assimilates and ionic tracer movement along the stolon of Saxifraga sarmentosa. When ionic tracers or ^{14}C-sucrose are applied to the translocating stolon they undergo a long-distance transport which is strictly polarised, with a short-distance movement which is symmetrical with respect to the point of application. The distribution along the axis after a suitable length of time shows an accurate exponential fall-off pattern, except with naturally assimilated or applied ^{14}C-sucrose where it is fairly level. In this case the linear velocity of movement was estimated as about 20 cm h^{-1}.

Transport of materials is readily reversible by interchanging the roles of parent and daughter plants as source and sink. The transpiration stream can be similarly reversed. By these means it was confirmed that ^{14}C-assimilates and ^{137}Cs move in the phloem, whereas ^{89}Sr moves in the xylem. The sieve tubes are thus not inherently polarised in this organ.

Inhibitors such as nitrogen, cyanide and DNP applied over lengths of 20-30 cm exert a more or less complete and reversible inhibition of the movement of ^{14}C-assimilates and ^{137}Cs. Cyanide gas is considerably more effective than solution. A variety of approaches, including the use of ^{14}C-cyanide, confirmed that the inhibition was effective in the sieve tubes themselves, and not merely at the terminal sites. Electron microscopic examination showed that callosing in the sieve plates was not involved; however, in all cases, including the normal one, the sieve plate pores appeared blocked with P-protein. Tests with Valinomycin which possesses a particular affinity for potassium, were ineffective. This may have been due to its insolubility in water and to the large size of the molecule.

Comparative studies were carried out by applying two ionic tracers together. These gave consistent and precise results in terms of the slopes of the plots of log activity against distance. Of the tracers used (^{22}Na, ^{42}K, ^{86}Rb, ^{137}Cs and ^{82}Br) the comparison of ^{137}Cs and ^{82}Br was perhaps the most interesting; the anion is transported more or less equal by with the cation, but its lateral leakage appears to be less.

The results of this study provide a well-established case for the following conclusions relevant to the controversy over mechanism:

(1) Phloem transport in the stolon is strictly unidirectional.

(2) Nitrogen, Cyanide and DNP exert a strong effect localised in the sieve tubes themselves (as well as at the terminals); it is reversible.

(3) The inhibition of transport is not due to callose blockage.

(4) Anion transport follows a similar pattern to cation.

These conclusions are very adverse to the Munch hypothesis, and to any diffusion-analogue theory; they favour a theory of active mass flow. The electro-osmotic theory faces the difficulty of anion transport.

Acknowledgements:

I would like to express my sincere gratitude to my supervisor, Professor D.C. Spanner, for his keen interest and encouragement which has been a constant source of inspiration throughout the course of this investigation. I wish to thank Prof. L.J. Audus for the provision of research facilities; Prof. W.F. Widdas of the Physiology Department for permission to use his $\beta - \gamma$ spectrometer (Packard Tricarb); and Dr. P. Pal for his valuable assistance in the preparation of computer programmes used for the analyses of some of the experimental data.

Acknowledgements are also given to Mr. R. Jones for his expert advice and technical assitance in electron-microscopic work; to Miss A. Peacock for printing the micrographs presented in this thesis and the publications; and to Mr. F.J. Funnel, Mr. R. Howes, Mr. S. White and Mr. P. Waite for their assistance in their respective fields. I am grateful to Mr. Rees of the Botanical Supply Unit (University of London) for a constant supply of the plant material throughout this work.

I highly appreciate the financial assistance provided by the British Council in paying my fees, by the University Central Research Fund for purchasing the radio-tracers and by the Edwina Mountbatten Foundation in contributing towards the cost of preparation of this thesis.

Many thanks are also due to my research colleagues, especially to Miss S. Akhtar and Miss M. Zelwer for interesting discussions and for their assistance in typing the first draft of the manuscript.

Finally, I wish to express my gratitude to my parents and family for being so encouraging and helpful during my stay in the United Kingdom.

Contents

Introduction.

Malpighi (1679) was probably the first to realize that the plants do not depend solely for their nutrition on minerals absorbed by the roots but on some sort of nutrients manufactured in the leaves and then transported to the other parts of plants. He could not pinpoint the pathway of this transport and it was generally considered that these materials were translocated in the xylem vessels (the only known transporting channels) along with other minerals and water. In 1837, Hartig discovered sieve elements in the secondary phloem of woody plants and later, in 1860, he related those elements to the moevements of carbohydrates in plants. This idea remained unconfirmed for many years, in fact till the classical investigations in the 1920's of Mason and Maskell. These workers presented experimental data which established the movement of carbohydrates and nitrogenous compounds in the phloem tissue. Soon Munch (1930) proposed that the assimilates in plants move through the sieve tubes by mass flow of solution along a gradient of turgor pressure established in the translocating system. He described leaves or the organs where the carbohydrates where the carbohydrates are synthesised or mobilised as the source region and the part of the plant where such products are consumed as the sink. He considered sucrose as the major translocate. This is assimilated in the leaves, moved into their sieve tubes and thus raises the latter's osmotic pressure. It is moved out of the tubes to be consumed by the growing tissue, thus lowering the osmotic pressure in the conduits. Such conditions create a pressure gradient which initiates a mass movement of sucrose solution from source to sink, and Münch suggested that maintaining a constant pressure gradient between the two terminals in this way would mean a continuous transport. Metabolic energy is only required to "load" and "unload" the sieve tubes at the two terminals and maybe to maintain the

impermeability of the sieve tube walls to sucrose. Münch proposal thus constitutes a physical mechanism and might seem quite adequate for promoting transport in small plants; however, there are difficulties for larger plants. Weatherley and Johnson (1968) calculated from the data than an available total pressure difference of 15 atm. in willow would be adequate to transport over a distance of 25 m assuming open sieve plate pores, but for the longer distances in the tallest trees a pressure difference greater than that acceptable would be required. Such a high pressure has never been reported in ordinary plants, the highest osmotic pressure reported being 20-25 atm. This seems to invalidate the mechanism for taller plants and thus some workers like Kursanov (1963), Weatherley and Johnson (1968),have suggested that some sort of energy consuming mechanism might be in operation at the level of each conducting channel. Such a process may be called 'activated mass flow'. The energy for such a mechanism must come from respiration of the phloem tissue or specifically of the sieve elements and companion cells. The requirement of energy for such a mechanism all along the conduit is not yet finally established, and a lot of work is currently being reported about the effects of anoxia, respiratory inhibitors, temperature, etc., on the transport process. Kursanov (1963) has reviewed this aspect of transport and concluded that transport is an active process and need energy not only at the two terminals of the system but all along the conduit. He discussed in details the experimental data of some workers like Turkina (1954) and Palvinova (1955) which indicate a higher rate of respiration of the phloem tissue and the inhibitory effect of respiratory poisons like KCN and DNP. Duloy et al. (1961) have reported similar results, but they considered that the inhibitory effect is not localised in the phloem tissue but due to the fact that the inhibitors might have entered the xylem, passed to the leaves and affected the process of loading at the source, or moved with the assimilates into the sink and inhibited the "unloading" from the

sieve tubes. Similar criticisms were also put forward by Harel and Reinhold (1966), but Willenbrink (1968) and Ho and Mortimer (1971) considered that such inhibitor as moved to the terminals did not affect the physiological processes there enough to account for the transport inhibition. They were convinced that inhibition of transport due to KCN is mainly due to its effect on the respiration of the conduction channels. Mc Nairn and Currier (1968) associated the effect of metabolic inhibitors with the formation of sieve plate callose rather than with the energy supply from respiration. They suggested that the plant responds to the metabolic inhibitors as it does to heat treatment (Webster 1965), and sieve plate callose is induced blocking the sieve pored and causing the fall in assimilate transport. The riddle of the mechanism of the metabolic inhibition of transport is still to be solved, and needs further investigation under more precise and analytical control.

The idea that assimilates move in the sieve tube under a pressure gradient becomes much more problematical after examination of the sieve elements by electron microscopy. Phloem tissue is a living tissue and composed mainly of sieve elemnts, companion cells, phloem parenchyma and fibers. The conducting elements are long tubular cells joined end to end and thus constitute long tubes with cross walls or sieve plates. The functional sieve tube have the rather modified cytoplasmic organelles (e.g., nucleoli, plastids, mitochondria) in the parietal cytoplasmic layer surrounding a big central lumen. The sieve plates are perforated and at the present it is still a matter of acute controversy whether their pores are empty (Anderson and Cornshaw 1970), filled with cytoplasmic connections (Thaine 1961), or plugged by fibrillar material called P-protein (Mishra and Spanner 1970). The state of functioning sieve pores is an important piece of information which could go a long way towards deciding the mechanism of transport through these tubes. Generally it is accepted that the pores contain fibrils but to an

uncertain degree. If the degree was considerable, a great resistance would be offered to the moving stream and it would need a far higher pressure difference to overcome it. Such an objection could be partially met if the obstructing material in the sieve pores was moving along with the sap flow. But this would mean an accumulation of these cytoplasmic structures at the sink region, requiring some mechanism for its utilisation or disposal. So far it seems difficult to demonstrate or justify belief in the existence of such a phenomenon. This tends to discredit the pressure flow theory as a basic mechanism of transport in plants.

Spanner (1958) considered that P-protein fibrils in the sieve pores may provide a charged matrix through which an electro-osmotic movement may be generated. Thus he suggested that a sieve plate is the site of electro-osmotic forces that would impel the sap through the sieve pore. According to this mechanism, the necessity for an overall hydrostatic gradient down the length of the sieve tube is reduced or conceivably even eliminated completely; there will be a fall in pressure between the upstream and the downstream ends of the sieve element but across the sieve plate where the water is being swept along by the ions a rise in pressure in the downstream direction may even occur. Thus the mass flow in the sieve tubes depends on the functioning of the sieve plates as pumps.

The potential difference is created by the secretion of potassium ions into the sieve tubes (see Fig. 1, subsection iv of section II) on the upstream side of the sieve plate and their escape on the downstream side. Such a mechanism, unlike Münch pressure flow, would be more effective if the sieve pores are closely filled with fibrils. It is a mechanism which requires energy input along the conduit. Like the Münch theory, it is compatible with the findings of workers like Swanson and Whitney (1953), who observed independent and different rates of movements

of ions like ^{32}P, K^{42}, Cs^{137}, in the phloem. But again, like the pressure flow theory, it is unable to explain the reported evidence of simultaneous bidirectional movement in the phloem tissue, that is, in the same sieve tube.

In plants the assimilates are moved both acropetally and basipetally but whether this takes place in separate or the same vascular bundles is a question which it is important to settle. Mass flow types of mechanism can accomodate the evidence if the opposing movements are in separate vascular strands (Biddulph and Cory 1960) or even separate sieve tubes; but then would be unable to explain matters if the movements of the solutes take place in the same sieve tube (Thaine 1961). Some physiologists like Curtis (1935), Thaine (1961), Eschrich (1967), Trip and Gorham (1968), have reported experiments to demonstrate a bidirectional movement in the same phloem bundles and even the same sieve tube. On the contrary, Biddulph and Cory (1960), Peterson and Currier (1971), concluded that the bidirectional movement was in separate phloem bundles at least in short term experiments. Crafts (1971) explains the results of Trip and Gorham on the basis of transitory stages of the maturing leaf which was changing over from sink to source, or as due to extrafascicular bundles in the petiole. Similarly, Eschrich (1967) suggested as possible a homodromous loop path involving oppositely directed streams in adjacent sieve tubes that are in contact through anastomoses, as an alternative mechanism to explain his apparent bidirectional movement of tracers in the phloem. Such explanations emphasise we need further investigation, especially with a plant material which has simpler vascular anatomy and a well defined sink region, if we are to establish finally the existence of bidirectional movement in the same sieve tube.

Thaine (1961) and some other workers who believe in a mechanism based on protoplasmic streaming have supported the existence of such bidirectional transport. They believe that the sieve element is

traversed by cytoplasmic strands and that subcellular particles move inside these strands. Thaine (1962) produced a cinefilm to show particles moving in opposite directions in separate strands but in the same sieve tube. Thaine considered these strands to be very delicate structures, and to run through the sieve pores continuously from one element to the next. Esau et al. (1963) and Parker (1965) have rejected the existence of such strands in the mature sieve elements, and considered that these are lines due to diffraction of light from walls out of focus. Another explanation is that they may be the remnants of the degenerating cytoplasm of the maturing sieve elements, which contain definitely orientated P-protein fibrils. Thaine on the other hand considered that the dispersed fibrils are due to the disruption of the transcellular strands during the preparation of the material for microscopic studies.

Although this mechanism unlike the others could explain the so-called bidirectional transport, it has other difficulties to face as a basic mechanism of transport. The very existence of two-way transport reduces the cross section for transport which would mean that a rather higher rate of velocity of streaming would be required. The energy requirements are apparently too high to be met by the plant for this elaborate mechanism and it is inefficient where long distances are involved.

Plan of the present work.

The investigations to be reported in this thesis were planned to produce more evidence to discriminate between the present day suggestions for the mechanism of phloem transport. The work was divided into three sections as follows:

Section I The study of the transport of ions and natural assimilates along the stolon of Saxifraga sarmentosa under ordinary conditions.

Section II The investigation of the effect on the process of transport of metabolic inhibitors with a view to its more precise localisation.

Section III A comparative study of the transport of two simultaneously-applied tracers.

Section I not only includes the study of the comparative pattern of transport of various alien and naturally-assimilated tracers along the stolon, but also throws light on the question of simultaneous biridectional transport.

Section II is based on studies with nitrogen, cyanide and DNP, and special importance was given to distinguishing the effects of the inhibitor treatment on the conducting channels from those on the terminal components of the translocating system. Electron-microscopic studies were carried out to determine the effects of these inhibitors on sieve plate callose as a possible cause of transport inhibition. Valinomycin, an antibiotic, was also selected for the inhibitor studies because of its special affinities for K-ion which fulfils a special role in the electro-osmotic theory.

Section III was another attempt to provide positive evidence for or against this promising theory which implies a differential transport of anions and cations along the sieve element.

The plant material.

Saxifraga sarmentosa, a common ornamental indoor plant, was chosen for these investigations. The plant is a small herb with one stolon arising from the axil of each mature leaf. Each stolon after growing to a certain length bears a small daughter plant, which could be used for propagation. Attempts were made to explore the factors which cause the stolons to grow long before bearing the daughter plants. Finally the plants were grown in plastic pots under normal greenhouse conditions on a platform and the stolons were allowed to hang vertically into a semi-light-tight enclosure which was kept at high humidity. In this way the stolons could be grown as long as 50-80 cm. Each plant was allowed to bear 3-6 stolons and stolons were carefully exposed to normal light for at least 4-7 days before using them for the experiments. A stolon was more or less uniformly thick, especially the major central portion. It always bore buds in the axil of small scale leaves, which sometimes grew into lateral shoots. These unwanted organs were always very carefully removed at least 3-5 days before the plants were required for the experiments.

The plant has certain advantages over the ones used by other workers. Unlike the petiole and stem systems of plants like Phaseolus, Glycine, Cucurbita, etc., there is less problem of anatomical complexity. The stolon conducts normally in only one direction, i.e., towards the growing daughter plant, which being a very active sink also obviates any recirculation of assimilates. This means that the transport experiments could be run for fairly long times without interference due to lateral transfer, etc. The material also proved to be easily manageable for the process of electron microscopic studies. As a matter of comparison, petioles of Nymphoides, barley and maize were also used in some of the experiments.

Section: I

Single Tracer

Sub-section: (i)

The movement of ^{137}Cs and ^{89}Sr or Unidirectional movement of tracers along the stolon of Saxifraga sarmentosa.

Planta (Berl.) 101, 133–146 (1971)

Unidirectional Movement of Tracers along the Stolon of *Saxifraga sarmentosa*

F. A. Qureshi and D. C. Spanner
Bedford College, London

Received July 16, 1971

Summary. When radioactive tracer is applied locally to the stolon of *Saxifraga* its long-distance movement after 18 hours is found to be strongly polarised; there is in addition a short-distance movement which is unpolarised. With caesium, the long-distance movement is predominantly in the phloem; with strontium in the xylem. These interpretations, *a priori* probable, were confirmed by artifically reversing, separately, the xylem and the phloem currents. With long pieces of excised stolon only the unpolarised short-distance movement is observed. These results constitute evidence against simultaneous bidirectional translocation in the same sieve tube, and are consistent with either the Münch or the electro-osmotic theory.

Introduction

The possibility that phloem tissue can conduct simultaneously in both directions is one which early attracted attention, for not only is it of interest in connection with the recognised requirement for different solutes to move concurrently upwards and downwards (e.g. mineral ions and assimilates respectively); it has also an obvious bearing on the intriguing question of sieve tube transport mechanism. In so far as such bidirectional conduction is shown to be a property of the axis as a whole the point is not crucial for mechanism; it is when the evidence is narrowed down to the single sieve tube that it becomes decisive. This is because the various hypotheses are rather sharply divided on this issue; some, such as the "transcellular strand" theory (Thaine, 1964) and perhaps "activated diffusion", can readily accommodate it, while the mass-flow theories fairly obviously cannot. In the latter category are the pressure-flow theory of Münch, and the potassium or electro-osmotic theory. These two hopeful hypotheses would therefore be excluded if unimpeachable evidence were forthcoming that individual sieve tubes could conduct simultaneously in both directions. Evidence which *prima facie* appears such has recently been provided (Eschrich, 1967; Trip and Gorham, 1968), and Trip and Gorham have in fact reached the firm conclusion that "bidirectional movement of sugars in the same sieve tube has been observed. ... The mass flow theory ... is no longer adequate to explain these observations." However, Peterson

and Currier (1969) in a paper providing a very useful critique of the historical evidence have given an alternative interpretation of Trip and Gorham's results, an interpretation which the present authors feel does real justice to the situation. In fact, the data given by Trip and Gorham possibly justify it to a rather greater extent than Peterson and Currier have remarked. For in their Fig. 1 (Trip and Gorham, 1968) the authors have noted the ^{14}C count at two points on the petiole below the ^{14}C-fed leaf. If the usual exponential fall-off relationship holds for this petiole and for the stem below it then at the point of junction with the older petiole conveying tritiated sugar there would be a ^{14}C count of possibly 500–1 000. Thus if there are sieve tubes carrying tritiated sugar into the ^{14}C-fed leaf from this point, then clearly these tubes could become ilabelled with ^{14}C at this node. This would seem to be a quite credible point of view, and until it is successfully disputed Trip and Gorham's dismissal of a mass-flow mechanism cannot be accepted. Peterson and Currier's presentation of their own positive evidence to the contrary, i.e. in favour of unidirectional flow, seems difficult to gainsay.

The work reported in the present paper is in several respects complementary to that of Peterson and Currier. Whereas they used plant axes which *a priori* would be expected to be conducting in both directions simultaneously, and showed that for short periods of translocation, bundles labelled with tracer above the reservoir did not carry it below, and *vice-versa*, we have used axes which *a priori* would be expected to be conducting unidirectionally. This has meant that experiments could be run for much longer periods, there being no problem arising from lateral transfer at the nodes. To some extent therefore the results meet the objection noted by Peterson and Currier (and partly answered by them) that movement in one direction might be much slower than in the other. Secondly, whereas they used fluorescein as tracer we have used ^{137}Cs, ^{89}Sr and though it is not reported here, ^{14}C-labelled sucrose. This, especially the fact that we have used labelled sucrose, reduces any uncertainty arising from the use of such a non-physiological substance as fluorescein. Finally, we have recorded not the presence or absence of tracer in the bundles, but its quantitative longitudinal distribution along the axis. This enables us to establish the quantitative difference between movement in the two directions, and to distinguish (in a less direct way than Peterson and Currier) between phloem and xylem transport.

Materials and Methods

The experiments to be described were performed on the stolons of *Saxifraga sarmentosa* (Fig. 1). The plants were grown in plastic pots in the greenhouse in the usual way, except that the stolons were allowed to hang vertically into a

Movement of Tracers along the Stolon of *Saxifraga sarmentosa*

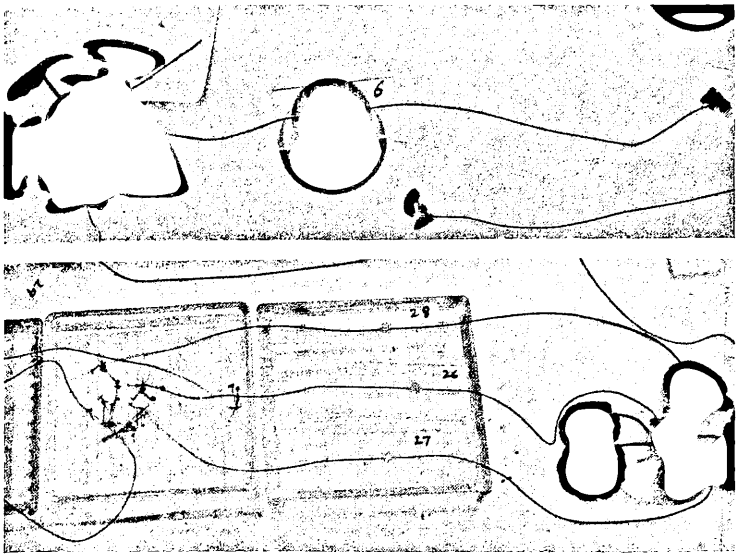


Fig. 1. Experimental set up. The upper photograph shows the long stolon connecting the parent plant (left) with the daughter (right). The tracer is being applied at mid-length (see Fig. 2 for details). The lower photograph shows daughter plants treated to secure a reversed xylem flow towards the parent plant, which is without independent water supply

light-tight enclosure below the platform on which the pots stood. By this means they could be grown very long (50–60 cm) and uniform. Any lateral shoots which developed were carefully removed together with the scale leaves, at least three days before the plants were required. Sometimes, for special purposes described below, the daughter plants were placed with their bases in water; under these conditions they developed abundant roots and could supply water if necessary to the parent plant. In winter the plants were given supplementary light (350 lumens) from low pressure mercury lamps, the photoperiod being 16 hours.

Tracer was applied to the stolon in the manner indicated in Fig. 2. Before placing the stolon in position the upper surface at the chosen point was lightly touched with fine emery paper, and rinsed with distilled water. It was then bedded over a 2 cm length in petroleum jelly as indicated. The reservoir, of transparent PVC tubing, was placed over the stolon and carefully luted with petroleum jelly also. The tracer was added with a small syringe, and the reservoir covered loosely with a glass cover slip. When the experimental period had ended the stolon was at once cut with a sharp razor blade at the inner faces of the transverse slots, the 2 cm length in contact with the reservoir being discarded. The remainder of the stolon was subdivided according to the scheme shown (Fig. 2), the larger 2 cm segments being cut together with a special razor blade assembly, the smaller inner ones individually.

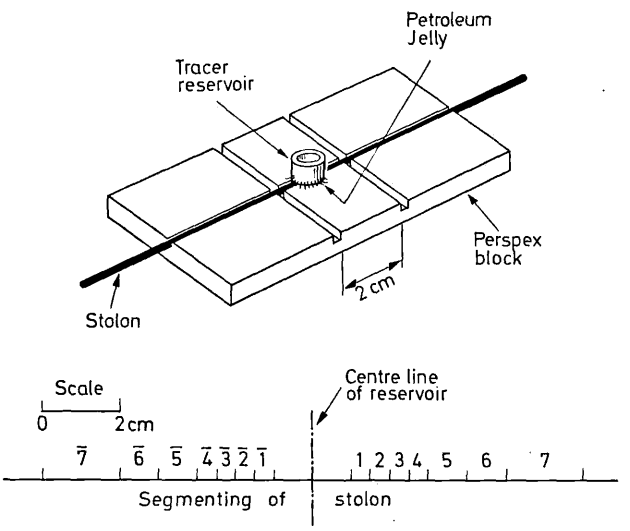


Fig. 2. Method of applying tracer to stolon, and subsequent division of stolon into segments

The segments were prepared for assay of ^{137}Cs and ^{89}Sr by pressing on to self-adhesive tape, drying at 70° C in a vacuum oven, and then sealing with thin melinex film to form a closed envelope. The envelopes were marked, trimmed to size and assayed in a narrow plastic scintillator well as described earlier (Husain and Spanner, 1966). This β-scintillating technique gave good efficiency with a low background. Later, segments were assayed in an automatic counter under an end-window GM tube by first sticking to planchettes with diluted UHU adhesive and then drying in the vacuum oven. On account of the high energy of the β-rays no allowance was deemed necessary for self absorption, especially in view of the uniformity of the stolon.

The tracers, obtained from Amersham, were made up in 0.05 M buffer of pH 7.2 containing 0.1% of the non-ionic detergent Lissapol. Phosphate buffer was used for caesium and veronal for strontium.

Usually 2.5 to 5 μCi were given in 35 to 75 μl; the particular dosages are given in the legends to the figures.

Results

Fig. 3 shows the results of an experiment in which ^{137}Cs was applied at one of three positions on the stolon: near the parent plant, at the centre, and near the daughter plant. The daughter plants were enclosed in black polythene bags for two days before the experiment and during it, the purpose of this treatment being to raise their effectiveness as sinks, and to control transpiration at a low level. The tracer was applied

Movement of Tracers along the Stolon of *Saxifraga sarmentosa*

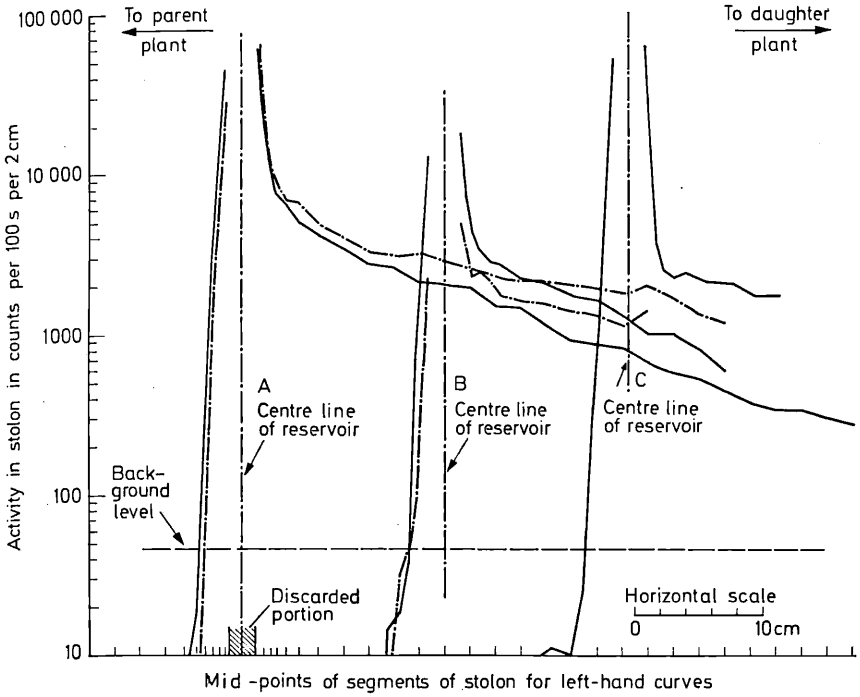


Fig. 3A–C. Distribution of ^{137}Cs along the stolon of *Saxifraga* when applied A, near the parent plant; B at mid-length; C near the daughter plant. Duration of experiment 18 hours. The marks along the horizontal axis refer to case (A); the other curves are displaced laterally by somewhat arbitrary amounts. Zero transpiration of daughter plant. Dose of tracer, 5 μCi

within the time interval 3.00 to 3.15 p.m., and harvesting took place the following morning between 9.00 and 10.30 a.m. The period allowed for translocation was thus about 18 hours, and this was followed in subsequent experiments unless the contrary is indicated in the legends. Temperatures were not closely maintained in this work, but were those of a typical heated greenhouse; they varied between 20 ° and 30° C during the day.

These results make it clear that long distance transport of ^{137}Cs is very polarised in the direction of what, on strong general grounds, we may conclude is that of the assimilate stream. It seems to be independent of the position of the well, i.e. of the age of the tissue. The pattern is that of a typical exponential fall-off down the axis. However,

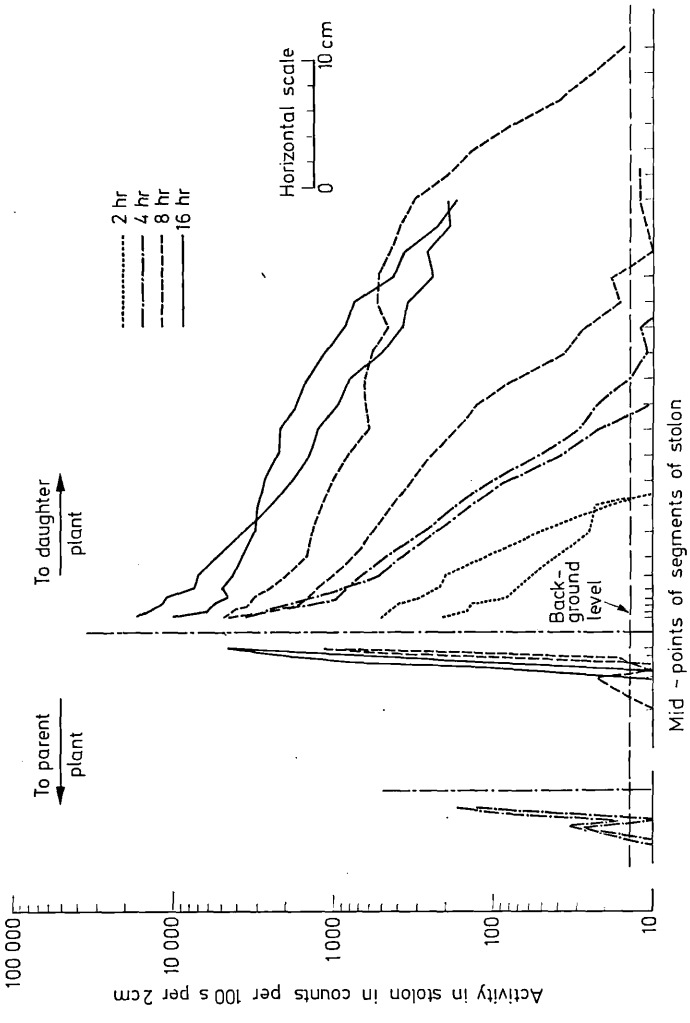


Fig. 4. Distribution of ^{137}Cs along stolon as a function of time. The centre line for curves showing distribution to the left for durations of 4 hours are displaced for clarity; the corresponding counts for 2 hours were not significant. Dose of tracer, 10 μCi

near the tracer well there appears to be a short-distance transport more or less symmetrical in the two directions and falling off much more steeply. This seems to be of a different nature from the long-distance transport, and reaches only as far as segments plus and minus six, i.e. to about 4 cm from the well centre. It is further reported on below.

The time-course of movement was investigated in an experiment whose results are presented in Fig. 4. The treatment here was similar,

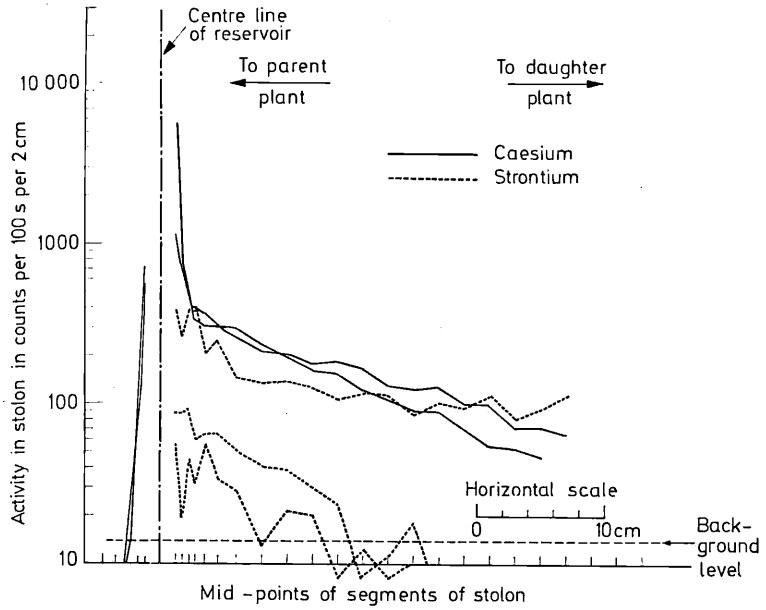


Fig. 5. Comparative distributions of ^{137}Cs and ^{89}Sr along stolon. Strontium movement to the left insignificant. Doses: ^{137}Cs, 5 μCi; ^{89}Sr, 2 μCi

but stolons were treated in duplicate near the parent plant and allowed to translocate for periods of 2, 4, 8 and 16 hours respectively. For reasons discussed in a previous paper (Spanner and Prebble, 1962) it is probably not possible to get a reliable estimate of velocity directly from these curves; certainly the rate at which the profile moves downwards is very low compared to the usually-accepted velocities of the assimilate stream.

The results in Fig. 3 were obtained with xylem movement reduced probably to a very low value; hence there is little likelihood that the long-distance movement was other than in the phloem. However to confirm this, further experiments were designed using several different approaches. In the first of these (Fig. 5), the movement of ^{137}Cs was compared with that of the tracer ^{89}Sr, known from a variety of reports to be almost phloem-immobile (Bukovac *et al.*, 1957; Biddulph *et al.*, 1959; but see Millikan *et al.*, 1969). In this experiment the daughter plants were not enclosed, consequently there was probably an appreciable transpiration stream flowing. Caesium movement was as before; the strontium distribution differed in being probably more variable between

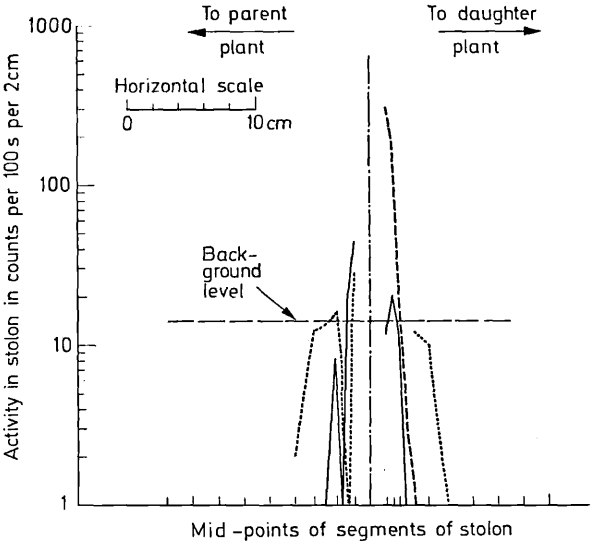


Fig. 6. Movement of ^{89}Sr under conditions of zero transpiration of daughter plant. Dose of ^{89}Sr, 0.1 μCi. Experiment in triplicate, replicates plotted in different styles

replicates and in lacking the rapid symmetrical increase near the tracer well noted before. Toward the parent plant in fact strontium movement was virtually nil. However, this experiment left open the possibility that caesium had moved downwards, as presumably strontium had done, in the xylem; or else that strontium too had moved in the phloem.

To distinguish between these possibilities an experiment was run in which the daughter plants were carefully enclosed in polythene bags (as in Fig. 3). The movement of strontium under these conditions is shown in Fig. 6. It is obvious that transport of tracer was extremely low, and probably unpolarised. This state of affairs is consistent with the accepted view that long-distance movement of strontium has to be in the xylem, since as the earlier experiments show phloem activity was certainly occurring.

Reversed Movement in the Xylem and Phloem

These conclusions were further tested by experiments in which attempts were made to reverse the normal directions of xylem and phloem movement. In the first, the daughter plants were grown with their bases in water for three weeks before the experiment, but deprived

Movement of Tracers along the Stolon of *Saxifraga sarmentosa*

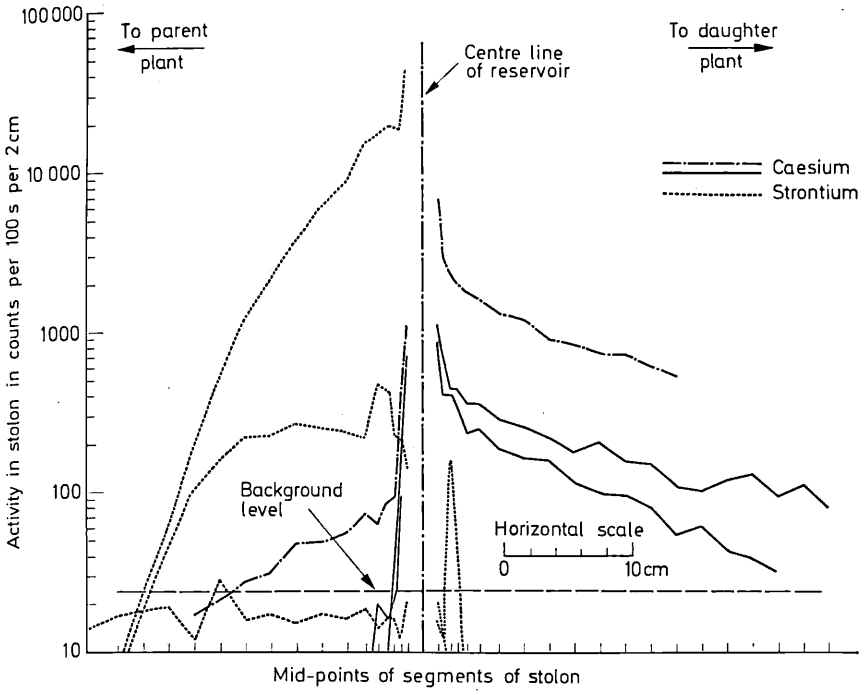


Fig. 7. Comparative distribution of ^{137}Cs and ^{89}Sr along stolon with reversed xylem movement (i.e., daughter to parent). The phloem movement was normal. Doses: ^{89}Sr, 2 μCi; ^{137}Cs, 5 μCi

of light to keep them otherwise dependent. Under these conditions they grew abundant and long roots, and when the parent plants were shaken free of soil six hours before the experiment began the daughter plants proved capable of maintaining them turgid. Caesium and strontium were used as tracers and applied near the centre of the stolon. The experiment was performed in triplicate (Fig. 7). The caesium results were very uniform, and for clarity only the two extreme ones are plotted; but a third caesium result is added from an earlier experiment because possibly owing to the higher dose coupled with high transpiration it shows a long-distance transport towards the parent plant also. Presumably this is in the xylem. The strontium results are less uniform. The long-distance transport is in the direction of the xylem movement and very uncertain in the other direction; the single tall peak on the right hand side may well have been due to contamination. Thus these results confirm the earlier interpretations.

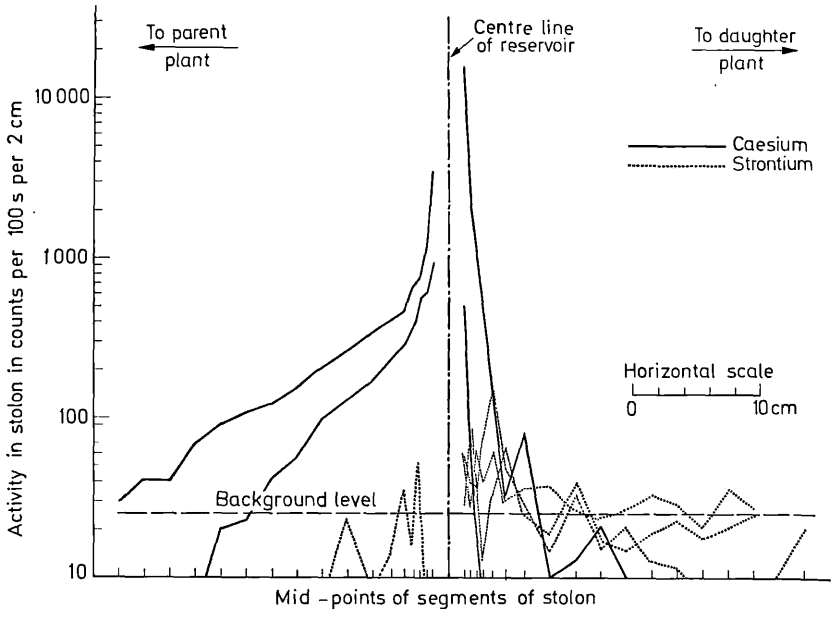


Fig. 8. Comparative distribution of ^{137}Cs and ^{89}Sr along stolon with reversed phloem movement (i.e., daughter to parent). The xylem movement was normal. Doses: ^{137}Cs, 5 μCi; ^{89}Sr, 2 μCi

Reversed phloem movement was sought by growing the parent plants for three weeks in darkened but well-watered containers while the daughter plants grew in good light, but without an independent water supply, and in fact free to transpire. Fig. 8 presents the results. It is apparent that the long-distance caesium movement has been reversed. Only the two extreme results again are given and both show it, though the stolon with the higher activity also indicates a small amount of movement in the direction of the xylem stream (compare Fig. 7). The strontium movement however remains unaffected by the presumed reversal in the assimilate stream. All the activities are low, and only one of the three replicates showed any to the left. These results again corroborate the previous conclusions.

A final experiment was performed to see whether translocation continues to any extent in excised axes, and to throw further light on the symmetrical short-distance transport process. Pieces of healthy stolon about 40 cm long were cut out, smeared at the ends with petroleum jelly, and placed in flat polythene tubing with moist filter paper. The

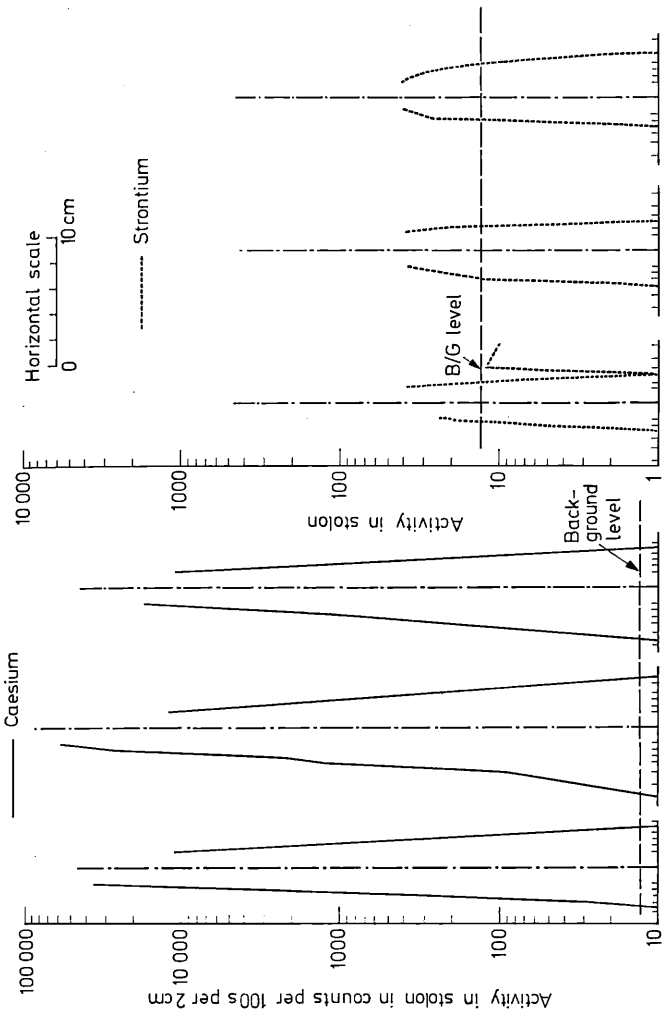


Fig. 9. Comparative distribution of ^{137}Cs and ^{89}Sr in lengths of stolon (approx. 40 cm) excised at both ends. Tracer applied at centre. Doses: ^{137}Cs, 5 μCi; ^{89}Sr, 0.1 μCi. (The low dose of ^{89}Sr was due to decay of stock solution)

centre 4 cm was left uncovered and given tracer as usual. After the customary 18 hours the stolon was harvested. Results are presented in Fig. 9. They suggest that under these conditions long-distance transport of both tracers ceases. Caesium still shows the pronounced unpolarised short-distance effect, and with strontium too any movement appears restricted. At least with this subject therefore excision seems to bring any long-distance processes to an end.

Discussion

This work was undertaken in the hope that it would throw light on the question of simultaneous long-distance bidirectional movement in the phloem. It seems to provide a clear answer that in the stolon of *Saxifraga* at least, such bidirectional transport does not occur. Were the sieve tubes to function by protoplasmic streaming or by some sort of "activated diffusion" the usual assumption is that the direction of transport would be dictated by the gradient of whatever was being translocated. In that case caesium would move outward rapidly in both directions from the tracer reservoir; this clearly does not happen. It moves instead in the direction in which, on strong presumptive grounds, assimilate movement is believed to be taking place. Thus it can be reversed when the normal carbohydrate sink is made to serve as source. Any long-distance movement of caesium in the other direction is usually very much less (say two orders of magnitude at least); where it is more than this (as in Fig. 7 where it is about 7.5%) it can easily be accounted for as movement in the xylem. Such movement could occur either because there is leakage of tracer into a strong through transpiration stream or because the reservoir is supplying water as well as tracer to xylem under tension. This is probably the case in Fig. 7, and accounts not only for the unusual caesium movement to the left, but also for the large strontium movement. Where steps were taken to reduce the transpiration stream strontium showed negligible tendency to move (Fig. 6).

Additional evidence that the caesium was travelling in the phloem came from the concurrent behaviour of strontium. In the presence of a similarly-directed transpiration movement (Fig. 4) this tracer appeared rather similar to caesium; but as soon as either the assimilate stream (Fig. 8) or the transpiration stream (Fig. 7) were artificially reversed the two tracers behaved differently. The former manipulation changed the direction of the caesium, the latter that of the strontium. Thus whatever their mechanism, in this plant the sieve tubes are not morphologically polarised, and this is all the more notable in an organ where such polarisation might be expected, since never in its normal life would it be called upon to conduct in the reverse direction. These considerations tell fairly strongly against any mechanism relying on cytoplasm streaming of any sort, or implying independent movement of solutes each in the direction of its own gradient. On the other hand they lend strong support to theories of mass flow, either the pressure-flow theory of Münch, or the "servo-assisted" theory of electro-osmosis. Unfortunately they do not help us to choose between these alternatives.

About the unpolarised short-distance movement the following remarks can be made. It is apparently more effective for caesium than

for strontium (contrast Fig. 3 with Fig. 6). It is still operative in the excised stolon (Fig. 9) where long-distance movement has stopped. This is interesting on both counts in view of the observations of Thaine (1962) and Bowling (1969) on excised pieces of axis. Two possible mechanisms suggest themselves for this movement. It may be symplastic, by means of protoplasmic streaming in parenchyma cells; or it may be apoplastic, by means of flow in the cell walls under the small pressure head from the reservoir. Both interpretations would be consistent with the greater movement of caesium, which would cross protoplasmic membranes more readily and be less adsorbed by the negatively-charged colloids of the cell walls. It would be interesting to compare the movement of tracer anions under similar circumstances, and experiments in this direction are in progress.

One obvious respect in which our experimental data are incomplete is in the absence of information about the effect of the tracer application on callose formation. It is hoped to rectify this in future reports. Eschrich *et al.* (1965) have reported that 1.5×10^{-4} molar calcium chloride injected into the petioles of *Cucurbita* causes a local deposition of callose. Strontium might be expected to behave similarly. However, the specific activity of the ^{89}Sr used was about 400 $\mu Ci/\mu g$. Thus the solution applied was about 5×10^{-6} M and at this strength would probably be innocuous. There is still the possibility that the buffer used with strontium (veronal) might have had an inhibitory effect on the sieve tubes, but experiments in which caesium was combined with veronal rather than phosphate indicated no distinction between the two buffers. However, the behaviour of callose needs further investigation.

In conclusion, it would seem fair to assert that the present results, obtained on an axis in which phloem transport can clearly be reversed, constitute evidence against the idea that simultaneous bidirectional movement in sieve tubes is possible. To this extent they support a theory of mass flow.

References

Biddulph, O., Cory, R., Biddulph, S.: Translocation of calcium in the bean plant. Plant Physiol. **34**, 512–519 (1959).

Bowling, D. J. F.: Evidence for the electroosmosis theory of transport in the phloem. Biochim. biophys. Acta (Amst.) **183**, 230–232 (1969).

Bukovac, M. J., Wittwer, S. H.: Absorption and mobility of foliar-applied nutrients. Plant Physiol. **32**, 428–435 (1957).

Eschrich, W.: Bidirektionelle Translokation in Siebröhren. Planta (Berl.) **73**, 37–49 (1967).

— Currier, H. B., Yamaguchi, S., McNairn, R. B.: Ein Einfluß verstärkter Callosebildung auf den Stofftransport in Siebröhren. Planta (Berl.) **65**, 49–64 (1965).

Husain, A., Spanner, D. C.: The influence of varying concentrations of applied sugar on the transport of tracers in cereal leaves. Ann. Bot. **30**, 549–561 (1966).

Millikan, C. R., Hanger, B. C.: Movement of foliar-applied ^{45}Ca in Brussels sprouts. Aust. J. biol. Sci. **22**, 545–558 (1969).

Peterson, C. A., Currier, H. B.: An investigation of bidirectional translocation in the phloem. Physiol. Plantarum (Cph.) **22**, 1238–1250 (1969).

Spanner, D. C., Prebble, J. N.: The movement of tracers along the petiole of *Nymphoides peltatum*. I. A preliminary study with ^{137}Cs. J. exp. Bot. **13**, 294–306 (1962).

Thaine, R.: A translocation hypothesis based on the structure of plant cytoplasm. J. exp. Bot. **13**, 152–160 (1962).

— The protoplasmic-streaming theory of phloem transport. J. exp. Bot. **15**, 470–484 (1964).

Trip, P., Gorham, P. R.: Bidirectional translocation of sugars in sieve tubes of squash plants. Plant Physiol. **43**, 877–882 (1968).

F. A. Qureshi
D. C. Spanner
Botany Dept
Bedford College
London NW1 4NS

Sub-section: (ii)

The movement of ^{14}C-assimilates

or

Movement of ^{14}C-sucrose along the stolon of *Saxifraga sarmentosa*.

MOVEMENT OF SUCROSE - ^{14}C ALONG THE STOLON OF *SAXIFRAGA SARMENTOSA*

Summary The characteristics of ^{137}Cs transport along the stolon of *Saxifraga* previously reported have been confirmed for applied sucrose and natural assimilate. Long-distance transport is strictly unidirectional, with a symmetrical short-distance spread from the point of application. Only the latter takes place in a long piece of excised stolon. Transport is readily reversed when the parent plant is darkened and the daughter plantlet allowed to photosynthesise. These findings strongly support a mass-flow mechanism for the stolon. They also confirm the value of ^{137}Cs as a tracer for assimilate movement, though in contrast to assimilate it suffers appreciable lateral leakage. Pulse-labelling of the subtending leaf failed to produce a sharp peak of activity in the stolon. A flattening with time of the ^{14}C profile is considered to be due to differing linear velocities in parallel sieve tubes.

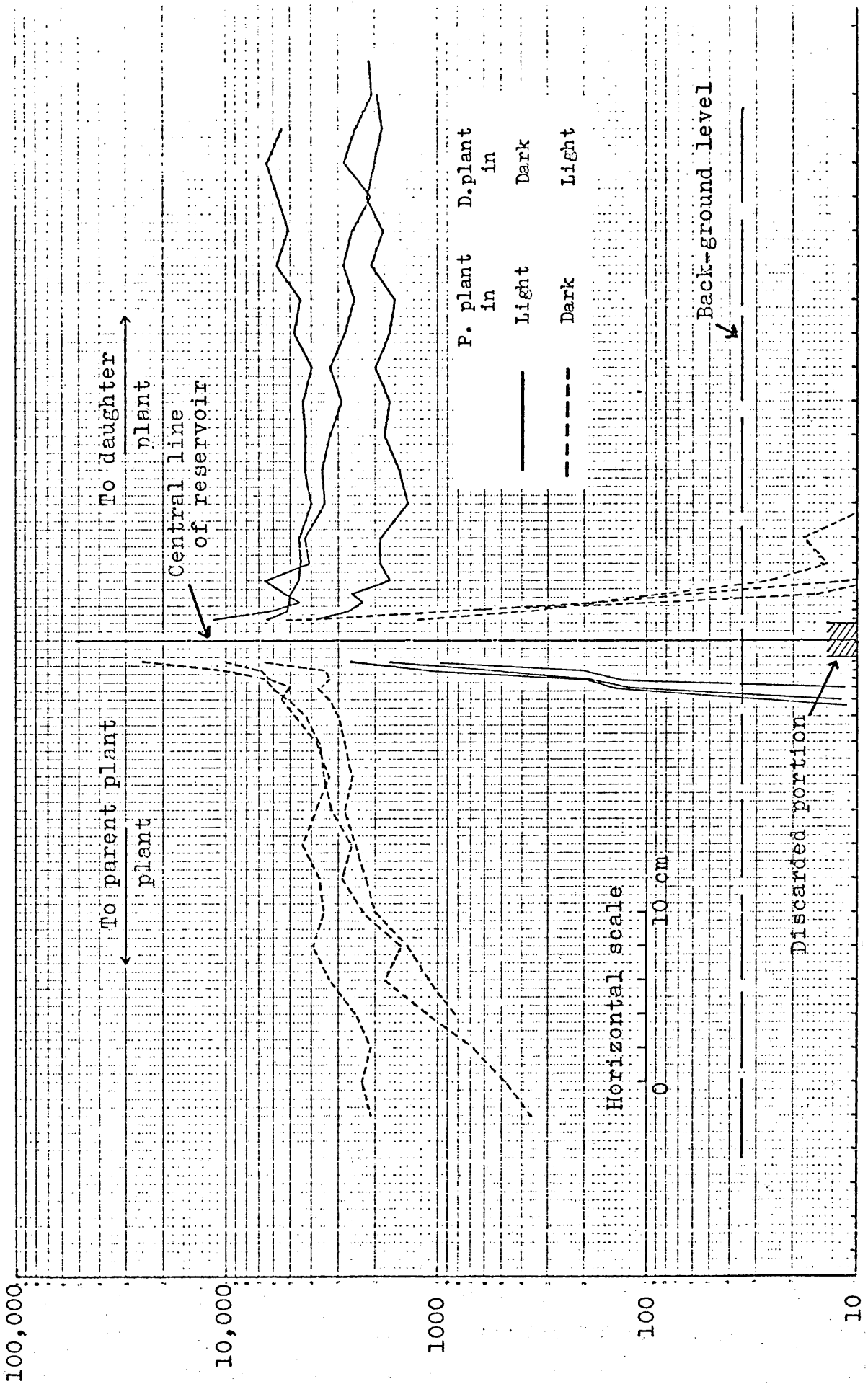
Introduction

In a previous communication (Qureshi and Spanner, 1971) evidence was presented that the movement of foreign tracers (^{137}Cs and ^{89}Sr) down the long thin stolons of Saxifraga sarmentosa is consistent with the view that long-distance phloem transport in this organ is strictly unidirectional; that it can be readily reversed by manipulating the natural relationships of source and sink; and that it ceases in excised pieces of stolon of considerable length (40 cm). These findings were regarded as antagonistic to those theories which imply simultaneous bidirectional movement, and favourable to those embraced by the term 'mass flow'. One weakness of the work described was that it did not relate to natural assimilates, though the use of ^{137}Cs and ^{89}Sr has an interest in itself. However, it was clearly desirable to confirm the findings using labelled carbohydrate, and it was for this purpose that the present work was undertaken.

Materials and Methods

The experimental plants were grown and prepared as described in the earlier paper, and $^{14}CO_2$ was administered as described in a later one (Qureshi and Spanner 1972). In general 30-40 μCi was given and the leaf allowed to assimilate for an hour, after which the leaf chamber was removed. After a further three hours the stolon was cut into 2cm segments by a razor-blade assembly, and the segments assayed in an automatic counter. Further details are given in the legends to the figures. Where sucrose -^{14}C was applied to the stolon about 5 μCi were made up in 0.05 M phosphate buffer, pH 7.2, containing 0.1% of the non-ionic detergent Lissapol. It was applied in the manner used for ^{137}Cs. After 18h the stolons were

Fig 1 Movement of Sucrose-^{14}C applied at midpoint of stolon. Dose of tracer, 5μCi; duration of experiment 18h. Two separate experiments, each in triplicate, are recorded on the same axes: solid lines, normal; dotted lines, daughter plant acting as source, parent plant as sink. The 'normal' curves are remarkably level. In the other case phloem transport has been reversed; movement is slower and the 'front' has not passed out of the axis.


Activity in stolon in counts per 100s per 2cm
100,000
10,000
1000
100
10
To parent plant
plant
To daughter
plant
Central line
of reservoir
P. plant in Light
P. plant in Dark
D.plant in Dark
D.plant in Light
Back-ground level
Horizontal scale
0
10 cm
Discarded portion
Mid-points of segments of stolon

harvested, segmented as in the case of ^{137}Cs, and assayed for ^{14}C.

Two methods were used for assay. In the first, the segments were dried in a vacuum oven at 70-80°C, ground in an agate mortar, and mixed with Cab-O-Sil in 10ml of a xylene-based liquid scintillator. About 3%-5% $^{w}/_{v}$ of Cab-O-Sil gave a satisfactory gel. The vials were thoroughly shaken and left to set in the dark for 24h before counting in a Panax scintillation castle. Later in the work, a Packard Tri-Carb automatic counter became available. For this, fresh segments of the stolon were dropped into 10ml of a 2:1 $^{v}/_{v}$ mixture of conventional toluene-based scintillator and commercial Triton X-100 (Turner, 1968). The vials were well shaken and left for at least 24h before counting in the Packard. A comparison of commercial with specially-purified Triton X-100 had previously indicated little difference in performance, with a great difference in cost. The effect of the natural pigments of the stolon on the count rate had been ascertained as small, and in any case the stolon has a fair degree of uniformity. Drying the stolon first before immersing it in the mixture had been found to reduce the count rate to quite a small fraction ($\frac{1}{2}$ to $\frac{1}{5}$) of what it was using the fresh pieces. Finally, it had been found that the count rate for the samples rose with time until it levelled-out after a period of about 24 - 48h. These observations determined the procedure followed.

Results

Fig.1 shows the results of two experiments in which sucrose-^{14}C was applied in a small reservoir to the centre of the stolon. Each experiment was in triplicate and lasted 18h. In the first series the parent plants were in the light and the daughters in a small black polythene bag kept humid inside with damp filter paper. Transpiration

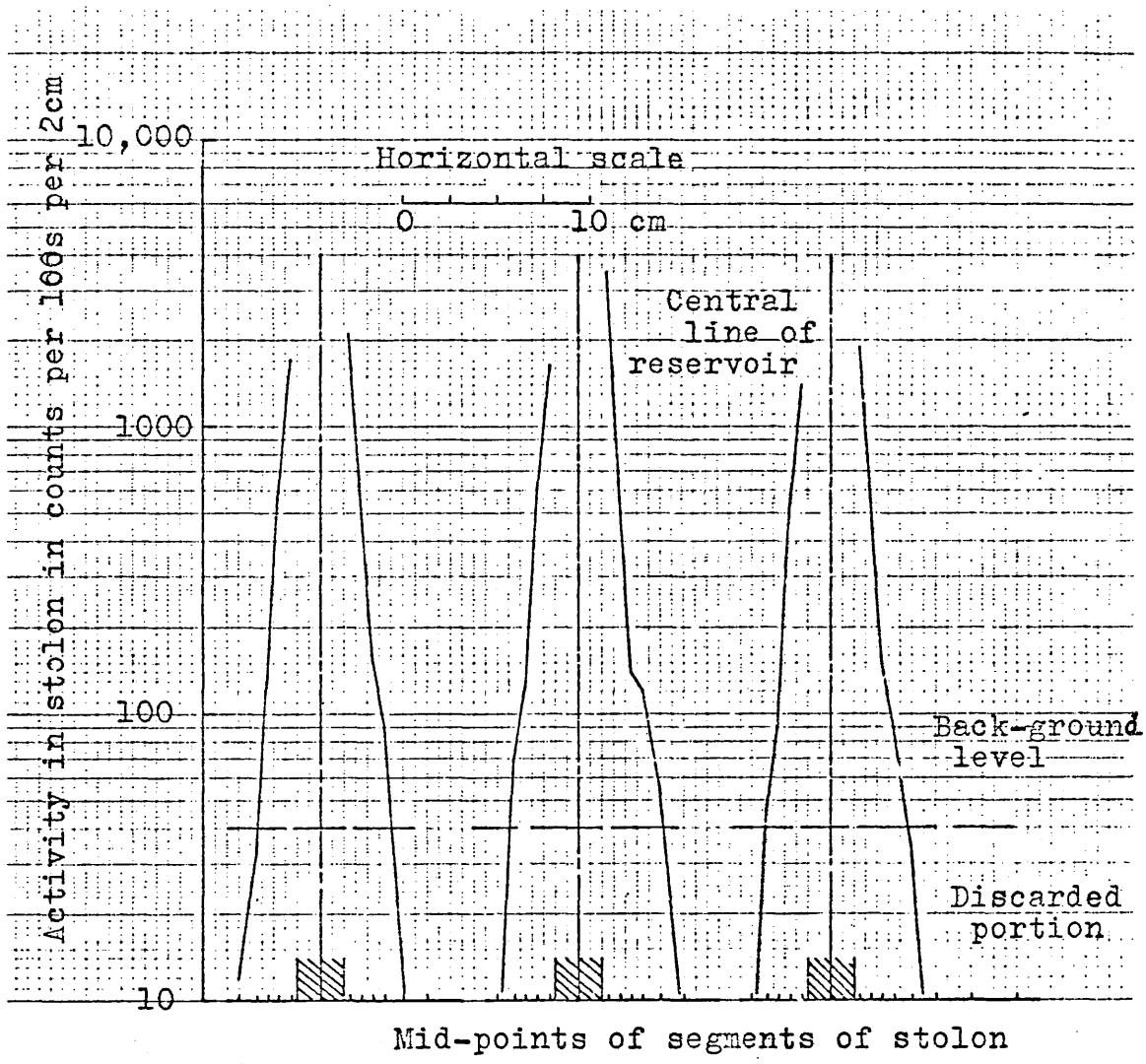


Fig 2 Movement of Sucrose-^{14}C applied at mid-points of pieces of excised stolon 40cm long; duration of experiment 18h; dose, 5 μCi. Only symmetrical short-distance transport has occurred.

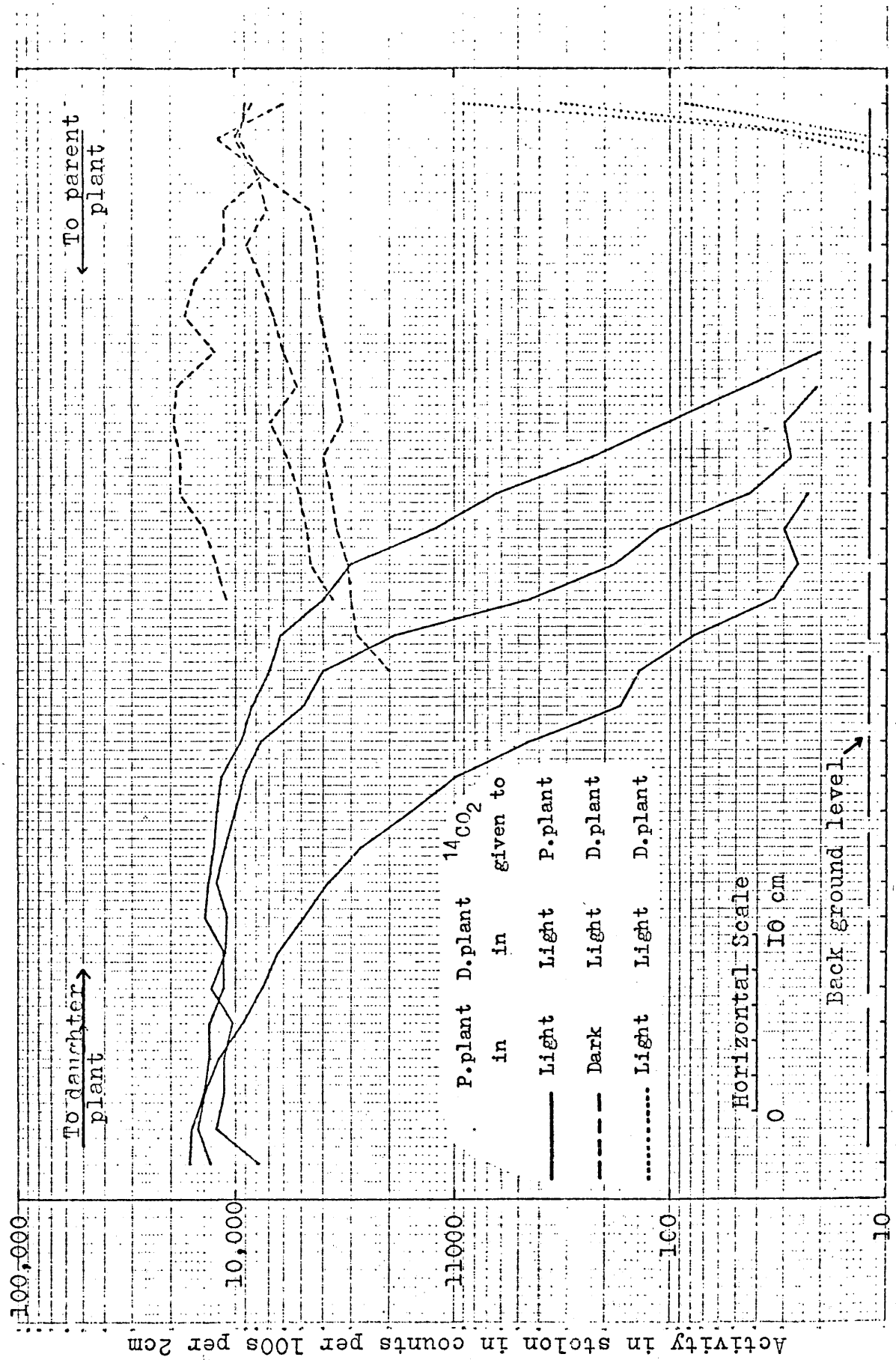
was thus maintained low. Long-distance transport is exclusively to the daugnter plant, with the same symmetrical short-distance spread about the reservoir found earlier (Qureshi & Spanner 1971). In the second series the parent plants were kept well-watered but in the dark for about 3 weeks before and during the experiment; the daughter plants were throughout exposed and in the light. In this case the transport is clearly reversed. Such xylem movement as occurred must have been towards the daughter plant; hence the distribution of tracer can only reflect phloem movement from the daughter plant (serving as source) to the parent plant (serving as sink).

The next experiment, reported in fig.2, was designed to investigate the long-distance transport of sucrose in pieces of excised stolon. The sucrose was applied at the centre of segments about 40cm long enclosed in internally-moistened transparent polythene layflat tubing as described in the earlier paper (1971). The stolon was harvested after 18h. The results indicate clearly that only short-distance movement has taken place, that it is quite symmetrical, and that it is of the same magnitude as the short-distance symmetrical component which can be observed in cases of normal transport (fig 1). As in the case of ^{137}Cs and ^{89}Sr it may represent movement in the parenchyma cells or in the apoplast, probably the latter (ibid).

Naturally-assimilated ^{14}C

Further experiments were carried out to investigate the behaviour of natural assimilate, the movement of which had hitherto been presumed known. In fig 3 the results of three series of experiments are shown. In the first, $^{14}CO_2$ was applied to the leaf of a parent plant in the light for 1 h; after 3h more the stolon was harvested. The daughter plants, which were rather small, were not darkened. Transport has clearly occurred towards them over a

Fig 3 Movement of naturally assimilated ^{14}C down the stolon. Three experiments, each in triplicate, have been superimposed. Duration of feeding $^{14}CO_2$, 1h; subsequent time for translocation, 3h; dose 30-40μCi. Note that there is normally no long-distance transport from the daughter plant(compare with fig 2), but that such transport can readily be obtained by reversing source and sink.


100,000
10,000
1000
100
10
Activity in stolon in counts per 100s per 2cm
To daughter plant
To parent plant
P.plant in Light
D.plant in Light
P.plant in Dark
D.plant in Light
P.plant in Light
D.plant in Light
$^{14}CO_2$ given to
Horizontal Scale
0
10 cm
Back ground level
Mid-points of segments of stolon

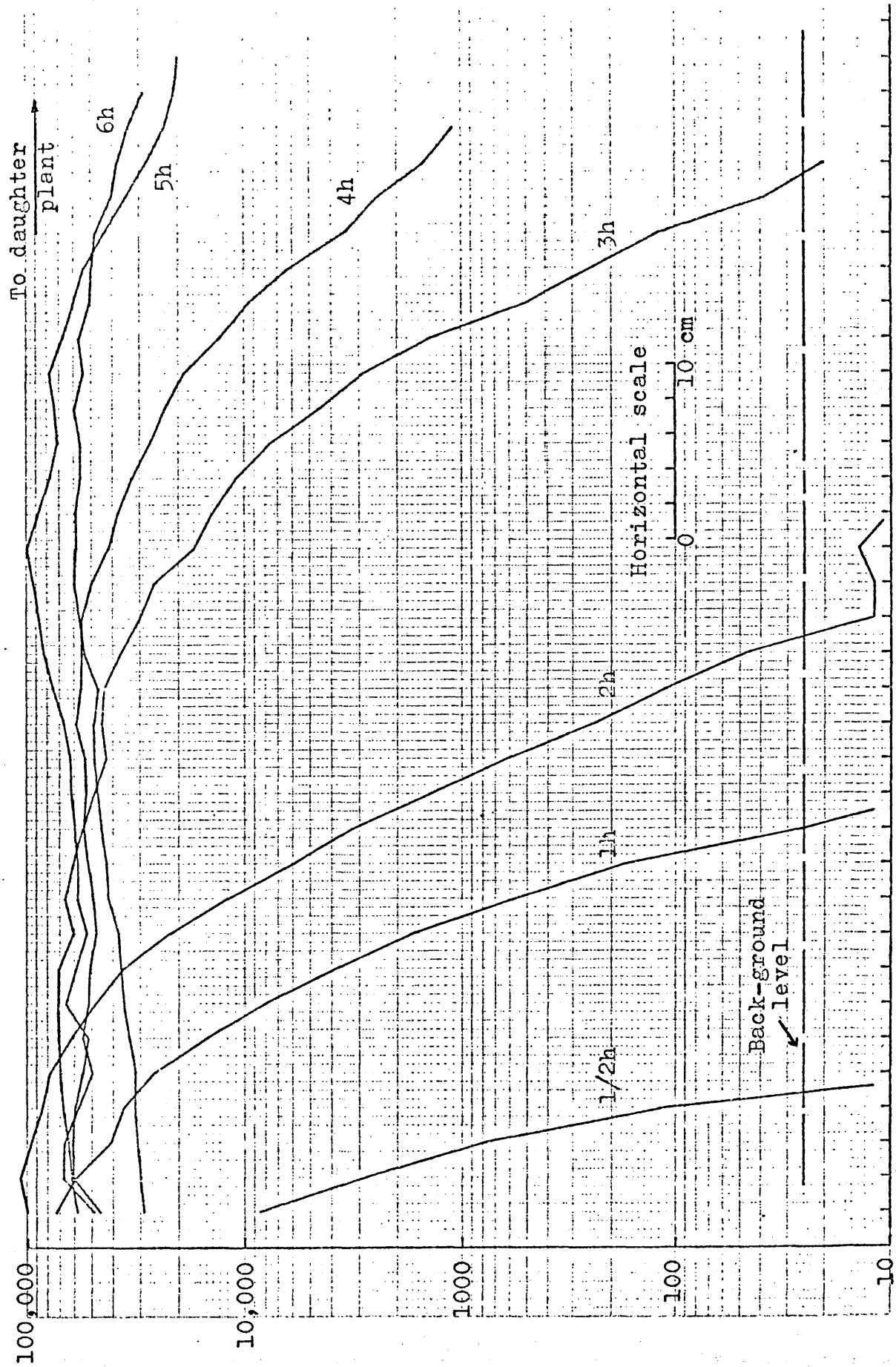
distance of at least 45cm of stolon, to which must be added about 10cm of petiole. In the second series $^{14}CO_2$ was given to the daughter plant, the parent being also in the light. Here, very little tracer is found outside the limits of the feeding chamber, and there is no compelling reason for regarding the little that is as representing transport in the phloem. In the third series the parent plants were darkened and had been in the dark since seven days before the experiment. Radioactive tracer was again given to the daughters. Here it undergoes marked long-distance transport towards the parents. Since the daughter plants had no independent water supply this movement was opposed to the transpiration stream. This reinforces the conclusion, already certain on other grounds, that the distribution of radioactive assimilate represents a phloem transport.

Time course of movement

One striking difference between the pattern of movement of ^{137}Cs down the stolon and that of ^{14}C sugar is that, well behind the front, the semi-logarithmic plot of the former is markedly inclined, whereas that of the latter is virtually horizontal. This can be readily seen by comparing the solid lines in fig 1 with fig 3 of the former paper (Qureshi and Spanner 1971). In both cases the tracers were similarly applied and the duration of transport was the same; further, the reservoirs were probably supplying a fairly steady input of tracer. We have argued that this difference is due to the fact that ^{137}Cs leaks readily out of the sieve tubes, whereas sucrose -^{14}C does not.

With this interpretation short term experiments with $^{14}CO_2$ allowing different times for translocation should show a steady profile of activity moving along the stolon, and from its successive

Fig 4 Time course of movement of naturally assimilated ^{14}C down stolon. The results, in triplicate, have been arithmetically averaged. Note that behind the 'front' the curves show no fall-off. Dose, 30-40μci; duration of feeding, 1h. Times marked are from commencement of feeding.

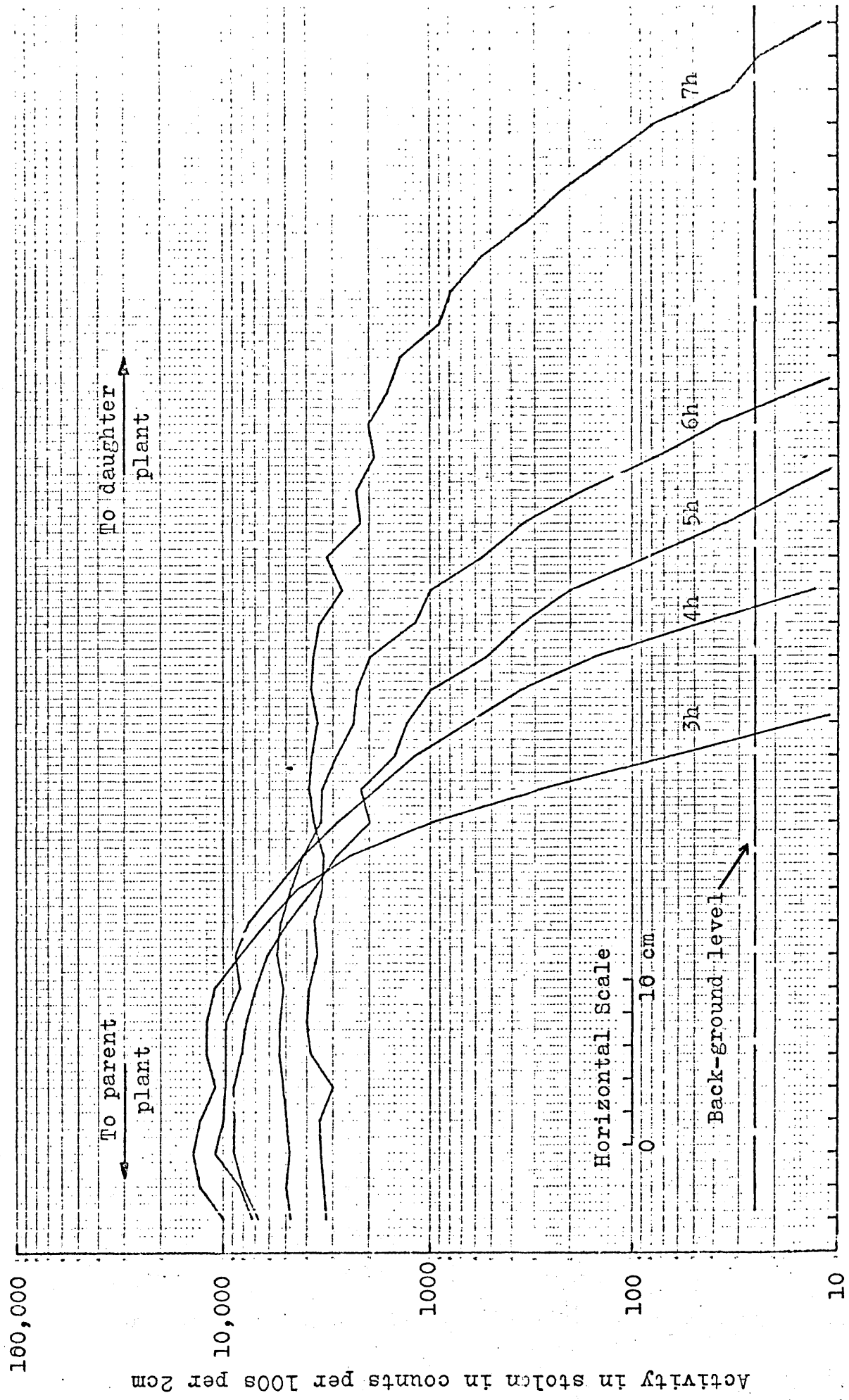

Activity in stolon in counts per 100s per 2cm
100,000
10,000
1000
100
10
To daughter plant
6h
5h
4h
3h
2h
1h
1/2h
Horizontal scale
0
10 cm
Back-ground level
Mid-points of segments of stolon

positions it should be possible to calculate a linear velocity.

Fig 4 reports the results of a series of experiments in which $^{14}CO_2$ was fed to a mature leaf for 1h after which the leaf chamber was removed. The stolon was harvested after periods varying from 30 min to 6h measured from the beginning of the period of feeding. The experiments were in triplicate, and as was almost always the case in this work, the replication was very good. Because of this, and for clarity, the replicate counts have been averaged and the results plotted in fig 4. These indicate the way the profile moves down the stolon. After about 6h the curve of activity has become virtually horizontal. Before that, the 'front' moved down the axis at a linear velocity of about 20cm h^{-1}. It became flatter as it moved, perhaps because some sieve tubes were conducting faster than others. A fall in activity at the proximal end of the stolon is distinctly evident at the longer times (and would be much more so on an arithmetic plot) suggesting that the source was becoming emptied of radioactivity. This observation suggested that it might be worthwhile to see if rapid pulse-labelling of the leaf with $^{14}CO_2$ would result in a recognisable 'hump' in the activity profile down the stolon (compare Geiger & Swanson 1965; Geiger & Batey 1967). If this proved the case, and if the width of the 'hump' was small enough, it might be possible to repeat the process after a suitable interval and record two 'humps' in a single stolon. With no attenuation due to lateral leakage, this would enable a very accurate estimate to be made of the linear velocity, avoiding the uncertainty introduced by having to compare one plant with another.

A series of experiments was accordingly run in duplicate as follows. At 11.00 h, after the plants had been in the light for about 5h, flexible plastic leaf chambers of small internal volume

Fig 5 Movement of natural assimilate after pulse-feeding of subtending leaf. Dose 25-30μc $^{14}CO_2$, duration 3 minutes. After the further times marked the stolons were harvested. The results represent the arithmetical averages of two replicates. They show little evidence of a moving 'hump'.


100,000
10,000
1000
100
10
Activity in stolon in counts per 100s per 2cm
To parent plant
To daughter plant
Horizontal Scale
0
10 cm
Back-ground level
3h
4h
5h
6h
7h
Mid-points of segments of stolon

(a few cm^3) were fitted over the leaves. These contained a small square of filter paper on which had been dried down about 25-30 μCi of $Na_2{}^{14}CO_3$. After sealing the chambers the filter paper was moistened with a hypodermic syringe containing dilute sulphuric acid. Three minutes later the chambers were removed and the leaves allowed to photosynthesise in air. The stolons were allowed to translocate for a further period of 3h or more and were then harvested, segmented and assayed in the usual way. Fig 5 shows the results averaged for the two replicates. The result is unexpected. It is plain that the expected 'hump' is not realised; in fact the curves for the longer times are remarkably flat. One or two other series of experiments, under slightly different conditions, gave substantially the same result. It can only be suggested at this stage that the radioactive assimilate enters a pool, perhaps of polysaccharide, from which it is only slowly released for transport. The matter needs further investigation, but unfortunately it had to be dropped, at least temporarily, at this point.

Discussion

In our first report on the unidirectional transport along the stolon of Saxifraga we discussed work in which the non-physiological tracers ^{137}Cs and ^{89}Sr were used. This established that at least with Cs (Sr does not move in the phloem) simultaneous bidirectional transport in the same sieve tubes does not occur. It demonstrated nevertheless that the direction of transport is easily reversed by interchanging the sources and sinks for assimilate; and that the distribution of introduced ^{137}Cs follows very accurately an exponential fall-off pattern.

The present work fills in some important gaps in this evidence. It confirms that Cs moves in all cases in the direction

of the assimlate stream. It demonstrates that in this axis the pattern of ^{14}C distribution is not one of exponential fall-off, let alone of error function except at the front, where it reflects and is probably dominated by the kinetics of the entry-process into the conducting system. The interpretation in the present case is not complicated by the relationships of leaf traces and cauline bundles which spoils the simplicity of such subjects as Cucurbita or Vicia. We are probably on fairly safe ground therefore in interpreting our results as negating (for Saxifraga at least) a diffusion-analogue mechanism of sieve tube function (Canny, 1971) or any mechanism in fact which permits simultaneous bidirectional movement at a single cellular locus. This conclusion is also borne out by the fact that the activity at the proximal end of the stolon can eventually fall below that lower down, producing a 'hump'. This is a condition the diffusion-analogue theory cannot easily accomodate.

That sucrose does not move out laterally to any great extent from the sieve tubes is suggested by the level nature of the curves often obtained, especially where, as in the solid lines in fig 1, the tracer supply would seem a priori to have had a long-term constancy. The matter is not quite so easy to argue in the case of the $^{14}CO_2$ experiments; but if the lack of a distinct 'hump' in the pulse-labelling experiments is due to the delay imposed on freshly-assimilated carbohydrate by its being sequestered in a reserve form before transport, then even pulse experiments may have resulted in a fairly long-drawn-out and steady supply of radioactive sugars to the transport channel (compare Geiger & Batey, 1967).

The flattening of the 'front' as it moves down the axis has already been commented on as probably reflecting a lack of uniformity in sieve-tube velocities. Zimmermann noted that the idea of a linear velocity in the sieve tubes is a little imprecise,

if only because with streamline flow the velocity varies from a maximum in the centre to zero at the walls. However, the sieve tubes are so narrow that radial diffusion within the lumen is rapid enough to obliterate this variation in velocity as a factor in drawing-out the profile in the front (Taylor, 1953); it cannot, however, offset the effect of differing velocities in parallel sieve tubes, a state of affairs inherently very probable.

The conclusion therefore is that the present work accords well with a theory of mass flow, but not with one of 'activated diffision' on the Canny and Phillips Model (see Canny, 1971).

References

Canny, M.J. Translocation: Mechanisms and kinetics. Ann.Rev.Plant Physiol. 22, 237-260 (1971)

Geiger, D.R., Swanson, C.A.: Sucrose translocation in the sugar beet. Plant.Physiol. 40, 685-690 (1965)

Geiger, D.R., Batey, J.W.: Translocation of ^{14}C sucrose in sugar beet during darkness. Plant Physiol. 42,1743-1749 (1967)

Qureshi, F.A., Spanner, D.C.: Unidirectional movement of tracers along the stolon of Saxifraga sarmentosa. Planta (Berl.) 101, 133-146 (1971)

: The effect of nitrogen on the movement of tracers down the stolon of Saxifraga sarmentosa, with some observations on the influence of light. Planta (Berl.) In the press (1972)

Taylor, G.: Dispersion of soluble matter in solvent flowing slowly through a tube. Proc.Roy.Soc. A 219, 186-203,(1953)

Turner, J.C. : Triton X-100 scintillant for carbon -14 labelled materials. Inter.J.App.Rad. Isotopes. 19, 557-563 (1968)

Zimmermann, M.H.: Translocation velocity and specific mass transfer in the sieve tubes of Fraxinus americana. Planta (Berl.) 84, 272-278 (1969)

Section: II

Inhibitors

Sub-section: (i)

The effect of nitrogen-anoxia

or

The effect of nitrogen on the movement of tracers down the stolon of Saxifraga sarmentosa with some observations on the influence of light.

The Effect of Nitrogen on the Movement of Tracers down the Stolon of Saxifraga sarmentosa, with some Observations on the Influence of Light.

Summary The movement of applied ^{137}Cs and of naturally-assimilated ^{14}C down the long stolon of Saxifraga is strongly inhibited by confining a length of 20 to 30 cm of the stolon in an atmosphere of nitrogen. The inhibition is reversible, normal transport being restored after less then 4h when the stolon is returned to air from 5h in nitrogen. Callose formation does not seem to be involved. There is evidence that local darkness has a similar adverse effect on phloem transport.

These findings are considered antagonistic to the pressure-flow hypothesis, but favourable to the active mass-flow theories.

Introduction

Among the inhibiting agencies whose effect on translocation have been investigated, deprivation of oxygen was one of the earliest. Curtis (1929), following Wortman (1890), waxed the petioles of Phaseolus. He also applied nitrogen under slight pressure to about 5 cm of petiole. There was an effect on the dry weight loss of the leaf, but it was hardly conclusive. In a long paper in 1936 Mason & Phillis concluded that because of

transpiration and photosynthesis this type of experiment was unsuitable; but they reported extensive work on the transport of materials to developing cotton bolls along defoliated laterals given a thick coating of plasticene. The results were very significant, and they concluded that oxygen supply was important in maintaining some "special state of the cytoplasm" necessary for high-speed transport. Willenbrink (1957) and Ullrich (1961) however could find no evidence for any effect of anoxia on the transport of fluorescein through the exposed central petiole bundle of Pelargonium. Ullrich concluded that oxygen "seems to play no part in transport through the sieve tubes", although HCN gas "reliably and reversibly" inhibited it. He suggested that a peroxidase system might be important (cf.Mason & Phillis, 1936). More recently, Geiger & Christy (1971) investigating a rather different aspect of the overall problem have reported that anoxia of the sink region (an immature leaf of Beta) caused a rapid fall in the import rate of labelled assimilates to almost zero.

Thus the question of whether deprivation of oxygen has any effect on the transport channels themselves must be regarded as an open one, and it seemed worthwhile to re-investigate it with the help of tracers and on a system possessing some advantages over those previously used. This paper accordingly reports the results of experiments in which the distribution of tracers and labelled assimilates was followed down the long uniform stolon of Saxifraga sarmentosa part of which was subjected to anoxia with nitrogen.

Materials and Methods

The plants were prepared, and where appropriate, the tracers were applied as described in an earlier paper (Qureshi & Spanner 1971). In order to expose the stolons to nitrogen they were

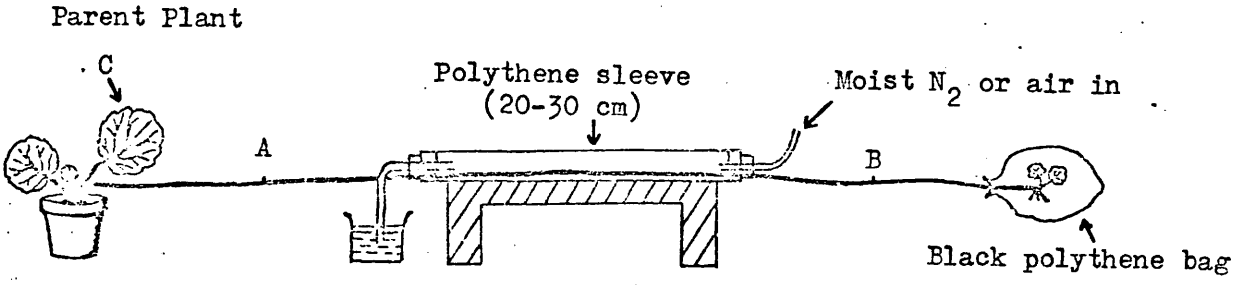


Fig 1 The general experimental arrangement. Where appropriate ^{137}Cs was applied at the point A; sucrose–^{14}C at the point B; and $^{14}CO_2$ was administered to the leaf C. The polythene sleeve and bag contained moistened filter paper. The drawing is approximately to scale.

run through a sleeve 20-30 cm long of black polythene layflat tubing which when opened out formed a tube of about 3 cm diameter. This contained some damp filter paper, and was closed at each end with a rubber bung carrying a central glass tube for inlet or outlet of nitrogen, the outlet tube dipping below water to ensure a slight positive pressure. Peripheral grooves in the bungs accomodated the stolon, which was luted in with petroleum jelly. The daughter plant at the end of the stolon was enclosed in a small black polythene bag containing moist filter paper; this curtailed both its transpiration and its photosynthesis. Fig.1 shows the general disposition. Nitrogen gas of commercial "oxygen-free" quality was bubbled through water before passing into the sleeve. Its oxygen content was stated to be less than 0.5%.

Caesium -137 and Sucrose -^{14}C were made up in 0.05M phosphate buffer, pH 7.2. The quantities applied are stated in the legends. Labelled carbon dioxide was supplied in the form of sodium carbonate dried down on a small piece of indicator paper. This was enclosed with the leaf in a small chamber formed from thin transparent plastic (Polyglaze), and the $^{14}CO_2$ was released by introducing just enough sulphuric acid by hypodermic syringe to flood the paper.

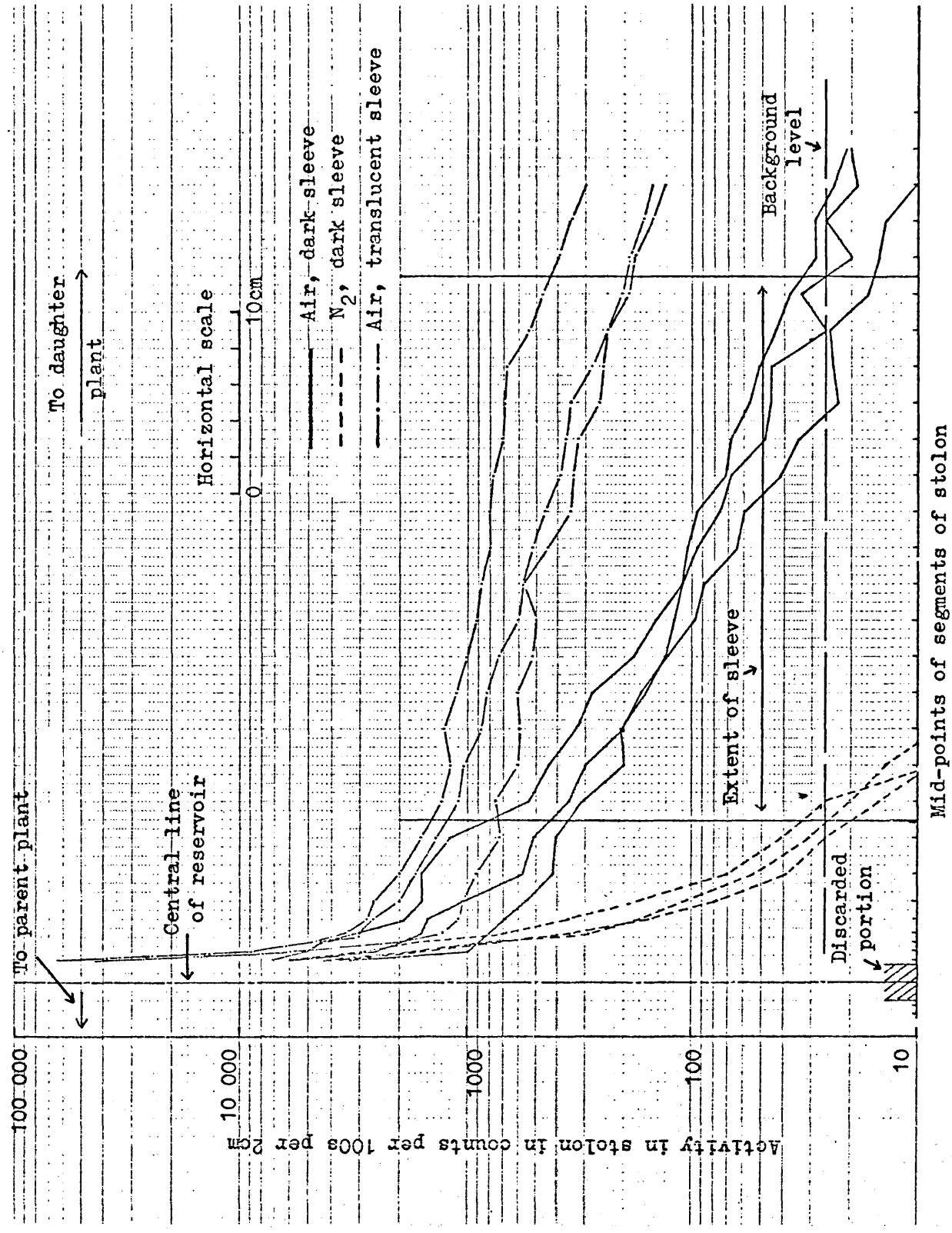
On conclusion of the experiment the stolons were cut up as previously described. They were assayed mainly by automatic β-counting under an end-window counter (^{137}Cs), or in a Packard Tri-Carb counter by liquid scintillation techniques, using Cab-O-Sil or Triton X-100 with a Toluene-based scintillator (^{14}C).

Results

1. Movement of ^{137}Cs under nitrogen

In the experiment reported in fig.2 the effect of nitrogen on ^{137}Cs movement was investigated. Nitrogen flow was started at

Fig 2 Movement of ^{137}Cs under nitrogen and darkness (Jan.1971). Temp. 20-24°C; dose of tracer 5 μCi in 50 μl. Nitrogen treatment started 24h and tracer applied 20h before harvesting.


Activity in stolon in counts per 100s per 2cm
100 000
10 000
1000
100
10
To parent plant
To daughter plant
Central line of reservoir
Horizontal scale
0
10cm
Air, dark sleeve
N_2, dark sleeve
Air, translucent sleeve
Extent of sleeve
Background level
Discarded portion
Mid-points of segments of stolon

time 12.00 and tracer was applied at 16.00 at about 8 cm from the start of the sleeve. The stolon was harvested at 12.00 the next day. During the night the plants were kept under low pressure mercury illumination of 350 lux (this practice was in fact general throughout this work). The experiment was run in triplicate, and as controls the nitrogen was replaced with air for two sets of three stolons, one set being enclosed in the usual black polythene sleeves, while the other was exposed to light in translucent sleeves. On conclusion of the run the stolons were cut up into the sections indicated on the horizontal axis, and counted as described earlier. The replication in all cases is close. It is accordingly obvious that, judging either by the slope of the linear portions of the curves, or by the indication of distance travelled, the nitrogen treatment has had a marked effect on the transport of caesium; further that there is a significant difference between the stolons in darkness and those in light It seems unlikely that this latter effect is due to temperature, though unfortunately no observations were made at the time of the temperature within the tubes. Subsequent measurements have however indicated that the difference was probably less than 1°C.

2. Movement of natural assimilates under nitrogen

In the next experiments the effect of nitrogen on the movement of naturally-assimilated ^{14}C was investigated. In the first set (fig.3), the stolons were enclosed and nitrogen or air flow commenced at about 16.30 and continued till harvesting. The next morning at 9.00, $^{14}CO_2$ was administered to the subtending leaf for an hour. The leaf-chamber was then removed and the plants allowed to translocate for another 3h when the stolons were harvested. The control experiment reported with translucent sleeves passing air was run a year later; its comparability with the others is therefore

Fig 3 Movement of natural assimilates under nitrogen and darkness (April 1971). Dose of $^{14}CO_2$, 40 μCi. Nitrogen treatment started 20½h and $^{14}CO_2$ given 4h before harvesting.

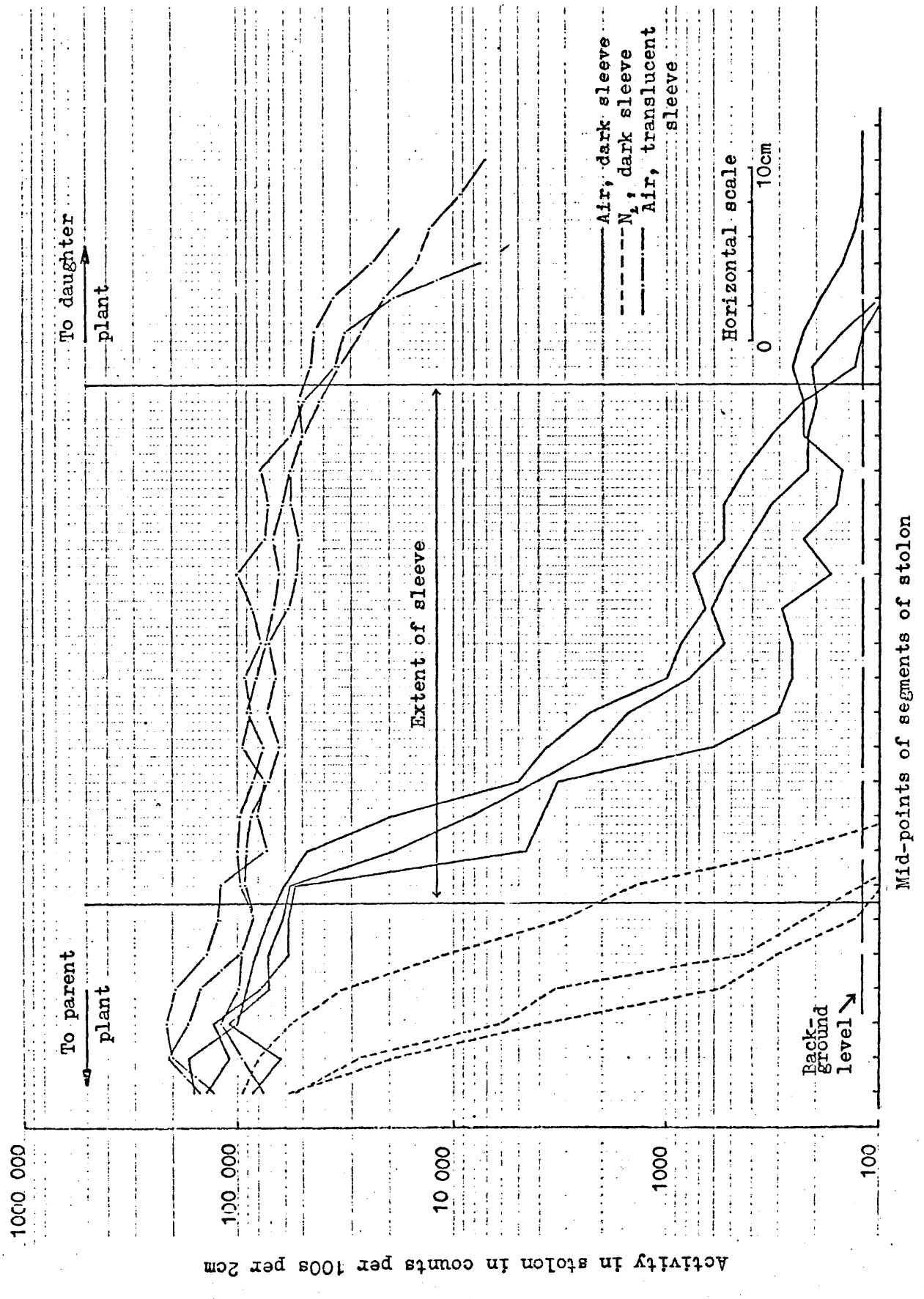

Activity in stolon in counts per 100s per 2cm
1000 000
100 000
10 000
1000
100
To parent plant
To daughter plant
Extent of sleeve
Air, dark sleeve
N₂, dark sleeve
Air, translucent sleeve
Horizontal scale
0
10cm
Back-ground level
Mid-points of segments of stolon

Fig 4 Movement of natural assimilates under nitrogen and darkness (April 1971). Dose of $^{14}CO_2$, 50 μCi. Temp. 25-32°C. Nitrogen treatment started 5h and $^{14}CO_2$ given 4h before harvesting.

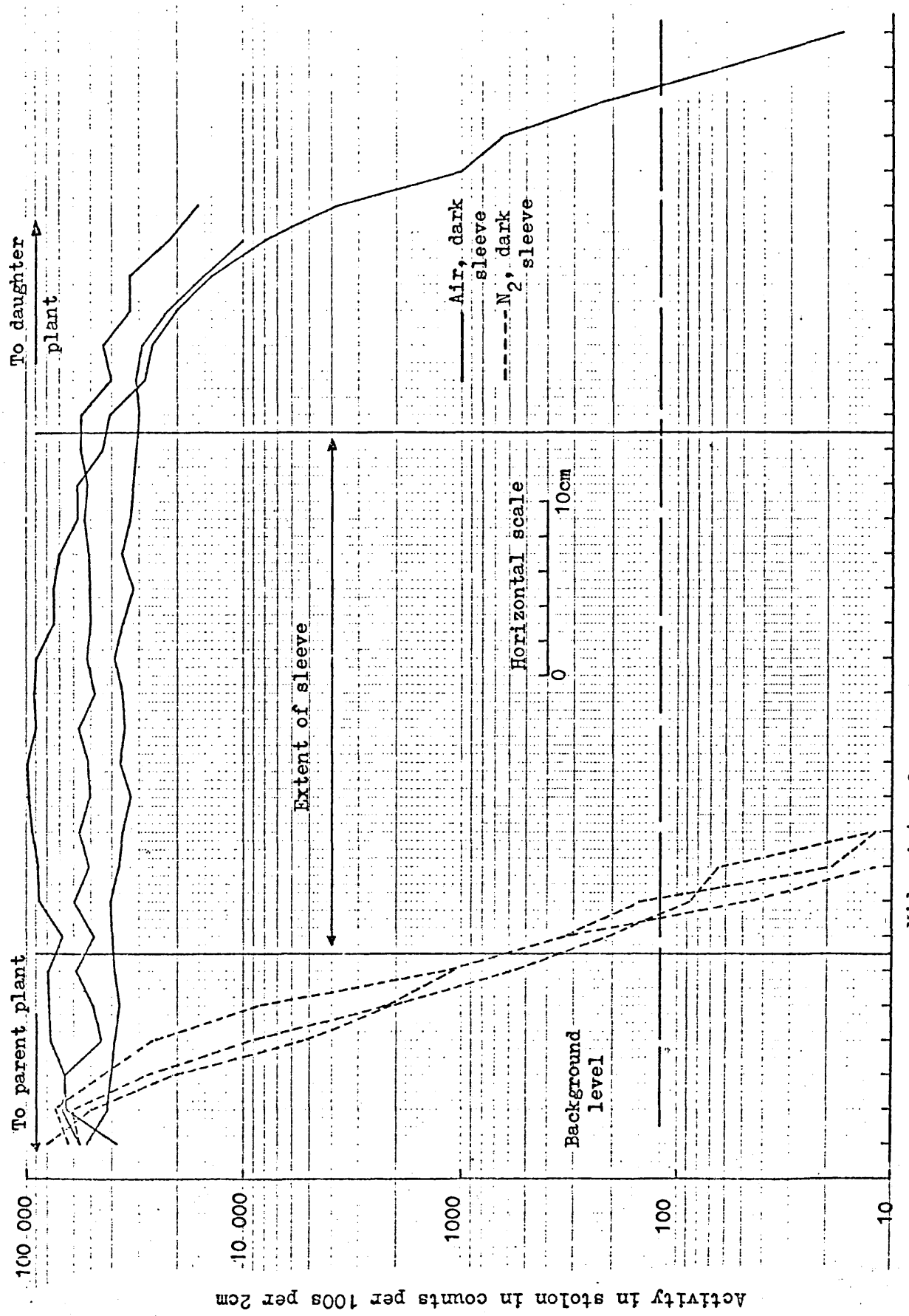
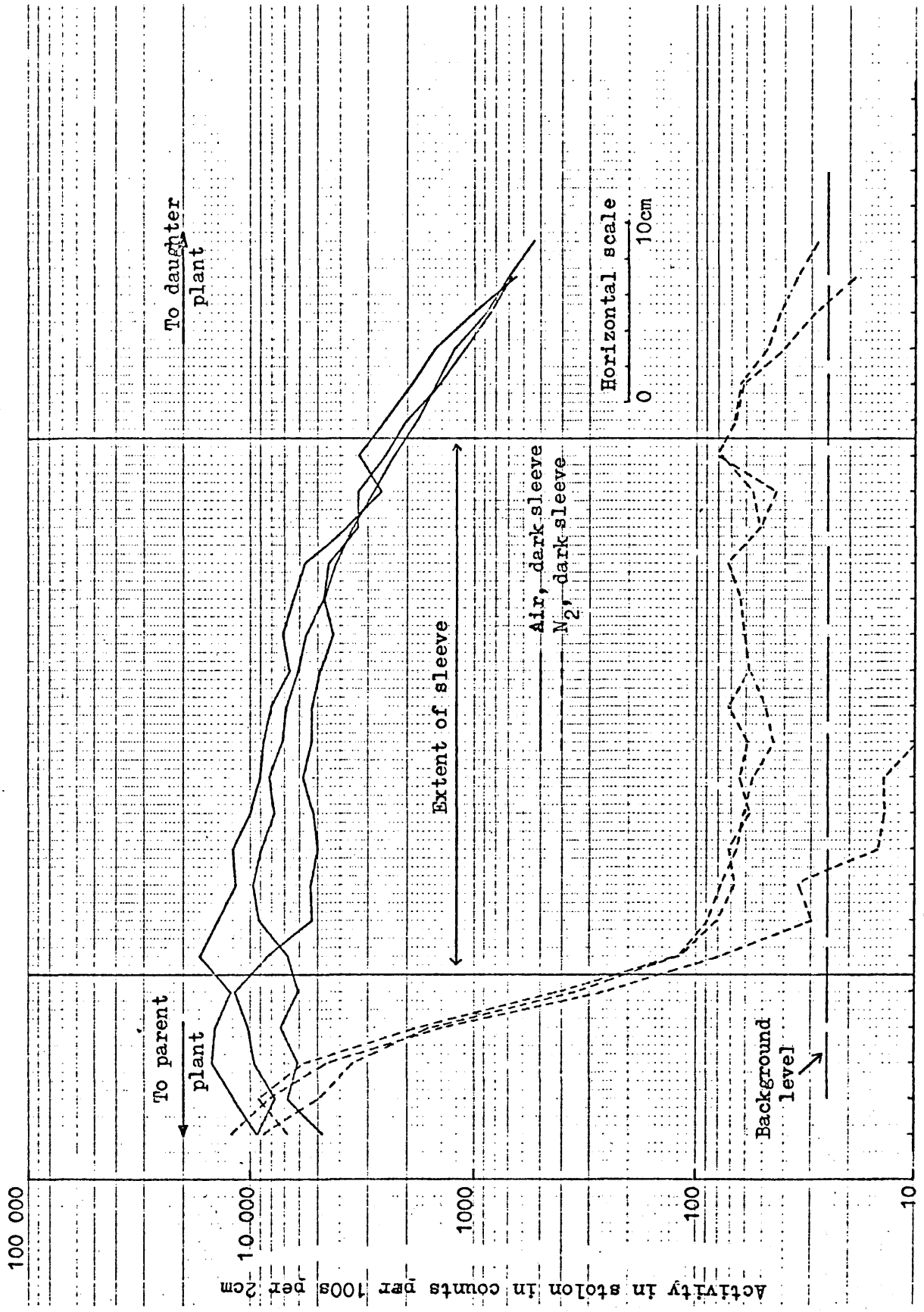

To daughter plant
To parent plant
Extent of sleeve
Air, dark sleeve
N_2, dark sleeve
Horizontal scale
0
10cm
Background level
100 000
10 000
1000
100
10
Activity in stolon in counts per 100s per 2cm
Mid-points of segments of stolon

Fig 5 Movement of natural assimilates under nitrogen and darkness (April 1972). Nitrogen treatment started and $^{14}CO_2$ given simultaneously 4h before harvesting.


Activity in stolon in counts per 100s per 2cm
100 000
10 000
1000
100
10
Mid-points of segments of stolon
To parent plant
To daughter plant
Extent of sleeve
Air, dark sleeve
N_2, dark sleeve
Horizontal scale
0
10cm
Background level

a little open to question. In the second set of experiments (fig.4) the nitrogen treatment was not commenced so early. The sleeves were fitted about 12.00, $^{14}CO_2$ administered from 13.00 to 14.00, and the stolons harvested at 17.00.

It is again obvious that nitrogen treatment has markedly influenced transport. There is a suggestion (fig 3) that darkness has had an adverse effect of its own, though as noticed earlier the control at this point is not very good and the comparison needed repetition under stricter conditions (see below). Further the controls in air in prolonged darkness appear to translocate much less effectively than those given a shorter period of pre-treatment (compare figs 3 & 4).

In a third set of experiments (fig 5) the nitrogen treatment was begun at the same time as $^{14}CO_2$ administration. Four hours later the stolons were harvested. There has been a marked effect (again all the replications agree closely); but a little long-distance transport has taken place, and the impression is that the nitrogen treatment takes some time to become effective.

A fourth set of experiments was designed to test the point raised earlier; does darkness have an effect of its own on translocation even when the stolon is in air? Fig 6 records the results of an experiment in which stolons enclosed in black or translucent sleeves were subjected to a moist air stream for 18h under continuous light. Labelled CO_2 was then given for 1h to the subtending leaf, and after a further 3h the stolons were harvested. Again replication is very good, and the positive effect of light on translocation is evident, confirming the earlier indications (fig 3). In this experiment two additional replicates were run and examined at the close for sieve plate callose; the results, which were negative, are reported more fully below.

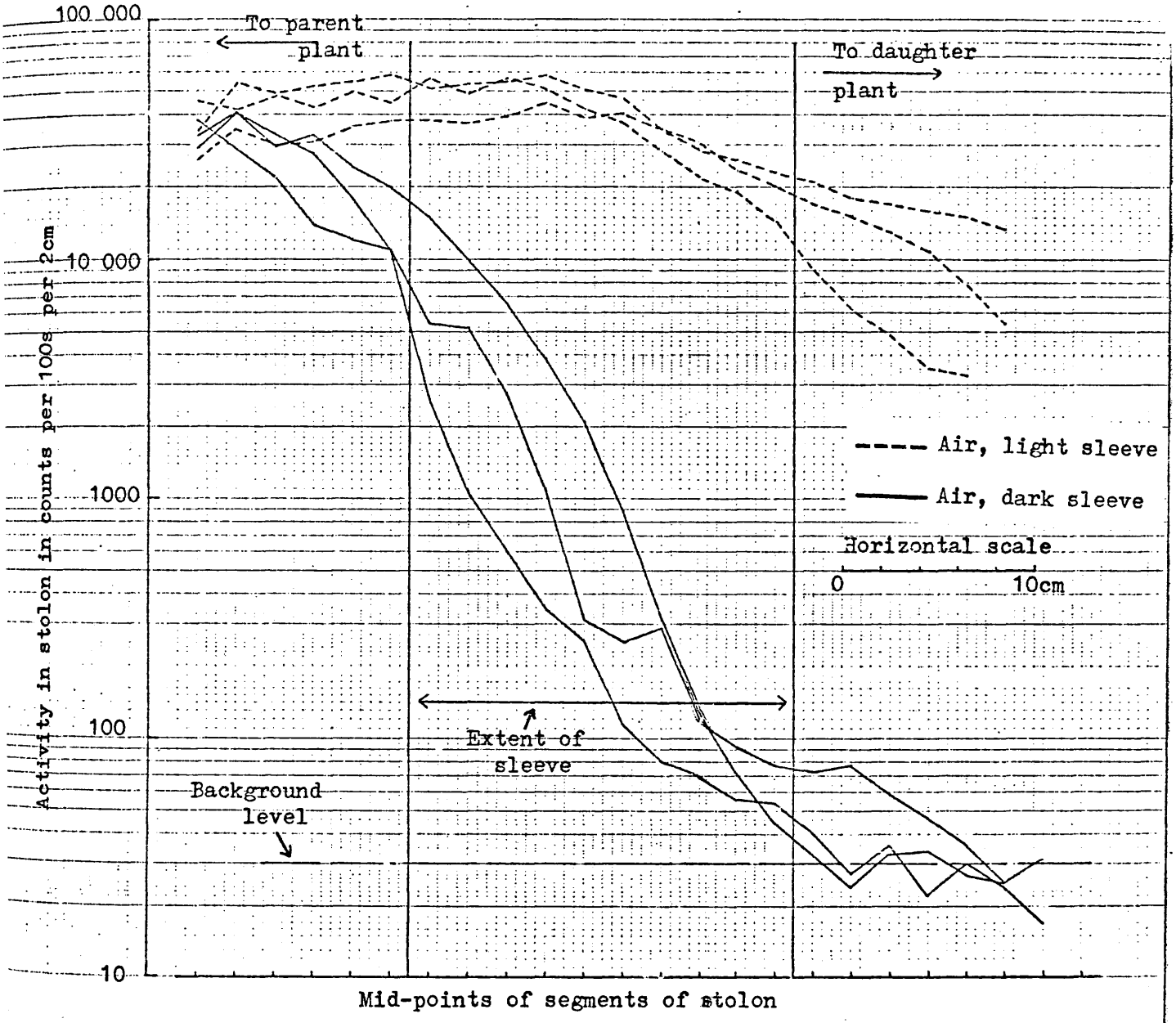


Fig 6 Movement of natural assimilates under light and darkness in air (Feb.1972). Treatment started 22h and $^{14}CO_2$ given 4h before harvesting. (See fig 11 for condition of callose on sieve plates).

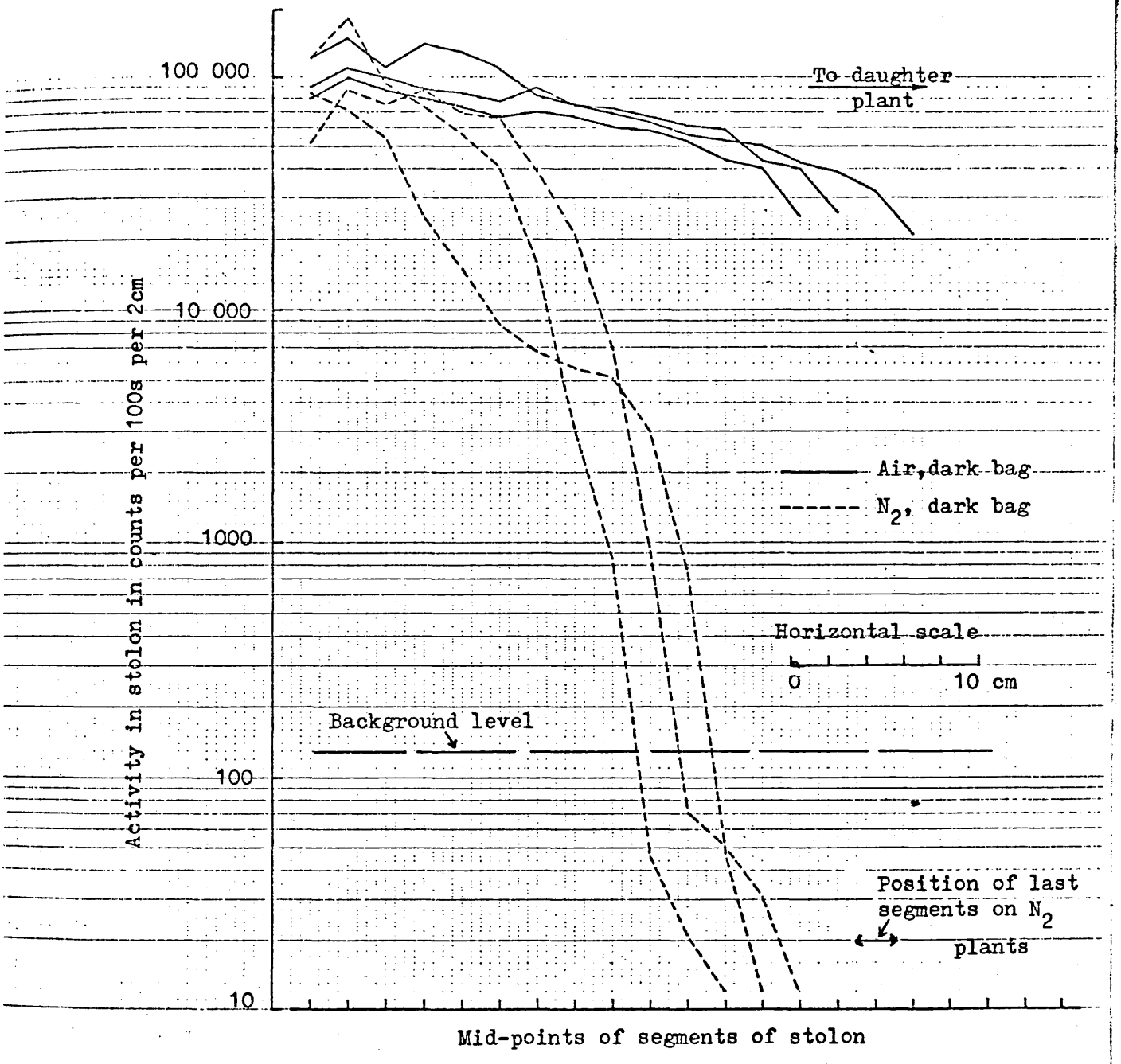


Fig 7 Effect of nitrogen treatment of sink region (daughter plant) on movement of natural assimilates. Treatment started 5h and $^{14}CO_2$ given to subtending leaf 4h before harvesting. The curves end about 1 cm short of the daughter plant. Average fresh weight of daughter plants 0.10g; average activity (controls) 52,100 per 100 s.

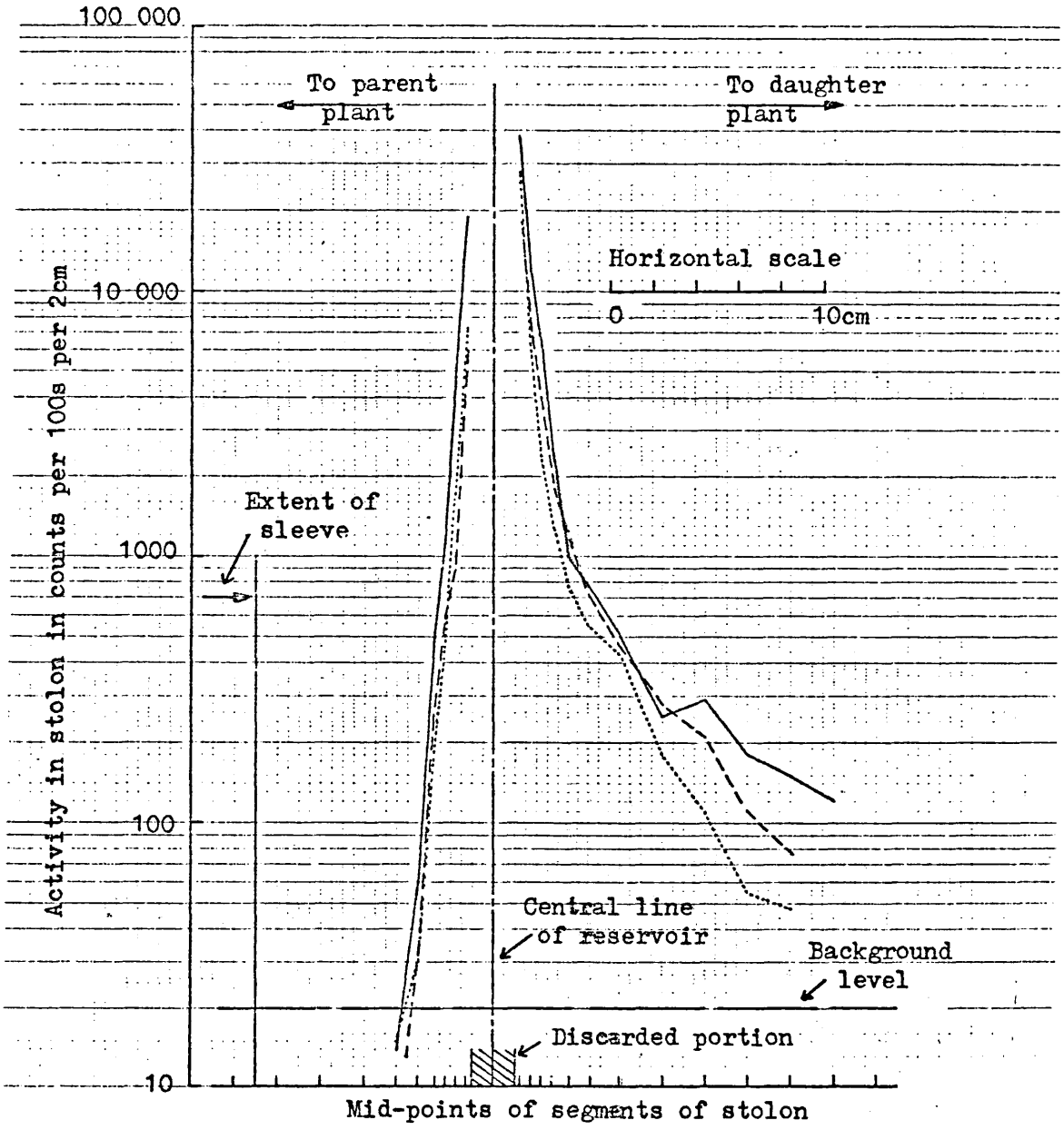


Fig 8 Movement of applied sucrose – ^{14}C along aerial stolon distal to nitrogen-treated region. Dose of sucrose – ^{14}C, 5 μCi in 50 μl, osmolarity about 0.15. Average activity in daughter plants 273 per 100 s.

3. Effect of nitrogen applied to sink

The Saxifraga system lends itself readily to investigation of the effect of oxygen-deprivation of the sink region, and in the following experiments the daughter plant was given nitrogen treatment in a dark sleeve for 1h before $^{14}CO_2$ was administered to the mother leaf subtending the stolon. Four hours later with nitrogen treatment still continuing the stolon was harvested. Fig 7 records the results. Translocation has resulted in a fairly high level of activity for about a third of the distance along the stolon. Then the activity falls rapidly, and the last third of the stolon shows virtually none. Correspondingly, no tracer at all was detectable in the daughter plant, whose sink capacity had apparently been stifled. By contrast, in their Beta system Geiger & Christy (1970) found that transport recovered to about 50% of its original rate after two hours in nitrogen. This situation recalls the differing capacities of species to recover from low temperature treatments (Geiger 1969).

Evidence on a somewhat different matter can be introduced at this point. It concerns whether the portion of stolon beyond a nitrogen sleeve retains the capacity to translocate. This point was investigated by applying to the stolon about half-way between the distal end of a 20 cm dark nitrogen sleeve and the daughter plant a reservoir containing sucrose-^{14}C. This was applied 1h after nitrogen treatment began, and 4h later the stolon was harvested. The results (fig 8) indicate that unidirectional transport had taken place towards the daughter plant which had accumulated an activity about equal to that in 6-8 cm of the adjacent stolon. There is also the normal symmetrical short-distance spread in both directions (Qureshi & Spanner 1971, 1972). On the face of it, therefore, the nitrogen effect does not incapacitate the unsleeved region provided the latter has sugar to transport.

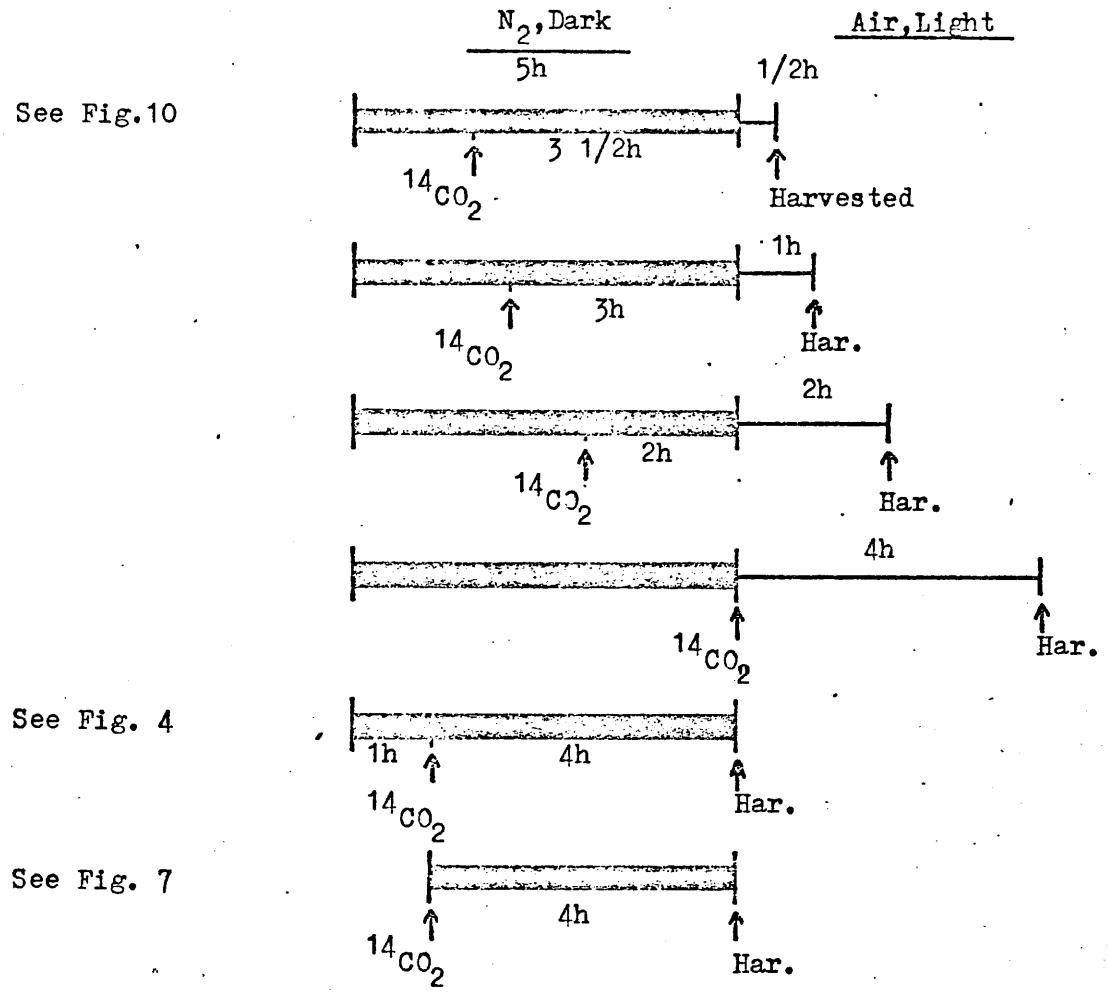
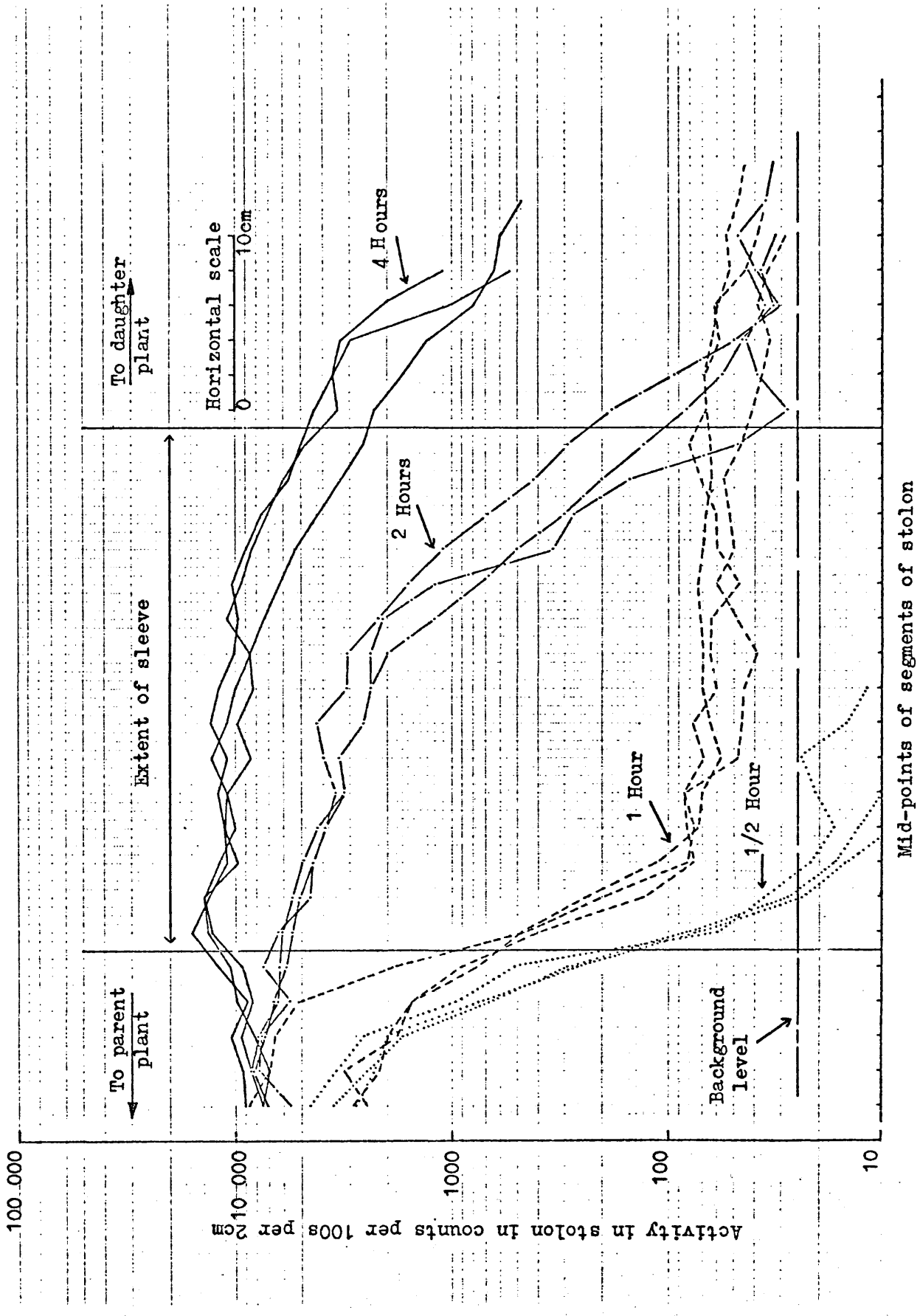


Fig 9. Programme of treatments designed to test reversibility of nitrogen effect.

Fig 10 Reversibility of nitrogen effect on movement of natural assimilates. For programme see fig 9. The durations refer to the period allowed for translocation in light and air after the removal of the dark sleeve. For additional information see figs 4 and 5.


100 000
10 000
1000
100
10
Activity in stolon in counts per 100s per 2cm
To parent plant
Extent of sleeve
To daughter plant
Horizontal scale
0
10cm
4 Hours
2 Hours
1 Hour
1/2 Hour
Background level
Mid-points of segments of stolon

4. Reversibility of nitrogen effect

Finally, experiments were run to ascertain whether the inhibition caused by anoxia was reversible. The stolons were subjected to nitrogen treatment in the dark for 5h and then the sleeves were removed, leaving the axes in light and air. They were harvested after a lapse of a variable period. Radioactive CO_2 was given to the subtending leaf 4h before the stolon was due for harvesting; thus in every case the period available for translocation was the same. The pattern of treatment is set out diagramatically in fig 9, and the results in fig 10. It is clear that recovery is well under way after 2h. By 4h it is complete, since observations made after 8, 12 and 24h showed no further change; they have accordingly been omitted for clarity. Fig 4 provides an idea of the distribution of tracer immediately at the termination of the nitrogen treatment.

Strictly speaking, these results indicate a reversibility of the joint effect of anoxia and darkness. However the control curves in fig 4 suggest that over a period of 5h darkness by itself has negligible effect. Provided therefore there is no strong interaction between darkness and anoxia (a point worth investigating) the present results would seem to justify us in asserting that the anoxia effect is completely reversible within about 3 or 4 hours.

5. State of the sieve plate callose

An obvious suggestion to account for phloem inhibition is to invoke callose formation on the sieve plates (cf.McNairn & Currier, 1968). The importance of this suggestion is that it would weaken the impact of our observations as evidence against the pressure-flow theory. Electron microscope studies were therefore made to see if nitrogen treatment of the stolon promoted callose formation.

Fig 11 State of the sieve plates in stolons given 22h of nitrogen treatment in darkness (1), and 22h in air in darkness (2). There is negligible callose present in either. The results after 5h treatment were similar. Fixation by sudden immersion into acetic alcohol at -20^{0}C for 24h before further processing.

Fig 11 shows a typical sieve plate from a stolon given 22h anoxia in the dark, together with a control; both show negligible callose. That the fixation employed did not actually remove callose was ascertained by fixing segments in glutaraldehyde for 4h at room temperature before plunging them into the cold acetic alcohol. The plates showed the appreciable amounts of callose commonly found with glutaraldehyde treatment. There was a slight suggestion that lengthy dark treatment (22h in a black sleevewith air) caused an increase in sieve plate callose. This effect, if real, would agree with Eschrich's observations (Eschrich, 1965) on Cucurbita.

6. Effect of anoxia on the source leaf

One further point was investigated: the effect of anoxia on the supply leaf subtending the stolon. This leaf was dried and autoradiographed in a number of experiments. On no occasion did the result indicate that nitrogen treatment of the stolon had reacted on the leaf. On the other hand if the leaf itself was confined to nitrogen in the dark before and after a 15 min exposure in the light to $^{14}CO_2$, vein-loading was reduced, as found by Leonard & Glenn (1968). It can be concluded therefore that the nitrogen effects reported in this paper do not arise in the source leaf, but reflect genuine properties of the transport channel.

Discussion

The present work confirms in a rather more precise way the conclusions of Mason & Phillis (1936) on the necessity of oxygen for phloem transport. On the other hand it conflicts with the evidence of Willenbrink (1957) and Ullrich (1961) mentioned earlier. However, Mason & Phillis studied transport into a terminal boll along a defoliated branch, whereas Willenbrink & Ullrich used a petiole and

lamina. Two obvious differences in these systems were the magnitude of the transpiration stream (which might convey oxygen) and the length of the inhibited axis; in both of these respects the Saxifraga system is much closer to Mason's & Phillis's. Both factors would tend to reduce the effect of anoxia in the experiments of the German workers, so that perhaps there is no deep-seated conflict to be resolved.

In interpreting the present results it should be borne in mind that the curves drawn on a semi-logarithmic basis provide two indications: the axial extent of the long distance transport, and the balance between axial transport and lateral leakage. The later is indicated, on a plausible model, by the slope of the linear portion of the curve some distance behind the 'front' (cf.Spanner & Prebble 1962).

In the case of assimilates the lateral leakage appears to be small, since the curves (e.g.fig 4) are remarkably level behind the front. Here therefore the distance the tracer has travelled is probably a fairly direct measure of the linear velocity. In the case of ^{137}Cs lateral leakage undoubtedly occurs, and the curves reflect the influence of anoxia on this as well as on the forward velocity. However, the conclusions to be drawn are hardly different in the case of the two tracers: anoxia has a notable and localised effect on the conducting channels, and this effect is reversible within 3-4 hours. It is not apparently connected with callosing of the sieve plates, nor with sink or source activity.

It is difficult to reconcile these findings with the Münch hypothesis. If anoxia merely weakens the membranes of the sieve tubes considered as passive conduits (as the hypothesis pictures them) then one would expect material to leak out and

accumulate in the weakened region under the thrust of the assimilate stream. This one clearly does not find. One must conclude therefore that the membranes retain substantial integrity. One other possibility that would save the hypothesis is that anoxia might induce a blockage (otherwise of course than by callose). The sieve plate pores of the stolon certainly appear filled with P-protein after anoxia, but so do they always (in our experience) after normal treatment. Further, it would seem most unlikely that a treatment which curtails the supply of energy should cause such a sophisticated response. The present evidence therefore seems to tell fairly strongly against the Münch hypothesis.

Of the other theories in the field the 'diffusion analogue' theory (Canny, 1971) is probably sufficiently ruled out by the evidence that long-distance movement in Saxifraga stolon is unidirectionally polarised throughout, i.e. it does not proceed away from the point of origin in both directions (Qureshi & Spanner, 1971). Two other theories regard the sieve plates as active pumps, dependent on protein contractility (MacRobbie, 1971; Fensom, 1972) or potassium electrosmosis (Spanner, 1958; Spanner & Jones, 1970). It is considerably easier to interpret the present evidence consistently with these, since each envisages the transport channel as the site of energy release. It is not immediately obvious however why transport should occur right up to the boundary of the treated section. It might be argued that in an unbranched non-leaky system in which solution was being pumped, blockage anywhere would produce stoppage everywhere, i.e. throughout the aerobic portion as well as in that under anoxia. Why then does the tracer appear to move in the former up to the beginning of the latter (see, e.g. fig 4)? In answer to this objection one suggestion is that anoxia does not reduce the stream velocity to zero but merely to a low value. The resistance

of the channel, it may be supposed, changes little; but the pumping stations within the sleeve cease to function. This would lower the velocity to an amount dependent on the fraction of pumping stations left operative in the whole length between source and sink. Thus translocation would still proceed but at a fraction of its speed in the control. With this understanding the fact that the front just reaches the start of the sleeved region is fortuitous; if more time had been allowed it might have reached the centre of this region. Indeed, this may well have happened in the middle three curves of fig 3 where the inhibiting influence has been constituted by a long period in darkness. This explanation weakens to a small extent the conclusion that the nitrogen effect is localised, and it certainly needs to be tested by varying the time allowed for transport. Another possibility exists however; the more active sieve tube strands in the aerobic section may impose a reverse flow via lateral sieve areas on the less active strands alongside them. If the latter found an alternative outlet in the vascular anastomosis at the subtending node an 'out-and-back' flow along the basal section of the stolon would be set up which would charge this portion with a level activity of tracer. In view of the fact that it is not difficult to reverse the direction of movement in the phloem of the stolon (Qureshi & Spanner 1971) this possibility seems quite likely. Further work is needed on this point.

That light has an influence of its own is perhaps not surprising in an organ fairly well supplied with chlorenchyma. The conclusion reinforces that derived from study of the nitrogen effects. It is in line with reports from other laboratories (see for instance Hartt & Kortschak, 1967) that light may have effect on translocation other than by the provision of assimilates. The matter needs further investigation.

References

Canny, M.J.: Translocation: Mechanisms and Kinetics. Ann.Rev.Plant Physiol. 22, 237-260 (1971)

Curtis, O.F.: Studies on Solute Translocation in Plants. Experiments indicating that Translocation is dependent on the Activity of Living Cells. Amer.J.Bot. 26, 154-168 (1929)

Eschrich, W.: Physiologie der Siebröhrencallose Planta (Berl.) 65, 280-300 (1965)

Fensom, D.S.: A Theory of Translocation in Phloem of Heracleum by Contractile Protein Microfibrillar Material. Can.J.Bot. 50, 479-497 (1972)

Geiger, D.R.: Chilling and Translocation Inhibition. Ohio J.Sci. 69, 356-366 (1969)

Geiger, D.R., Christy, A.L.: Effect of Sink Region Anoxia on Transportation Rate. Plant Physiol. 47, 172-174 (1971)

Hartt, C.E., Kortschak, H.P.: Translocation of ^{14}C in the Sugarcane Plant during the Day and Night. Plant Physiol. 42, 69-94 (1967)

Leonard, O.A., Glenn, R.K.: Translocation of Assimilates and Phosphate in Detached Bean Leaves. Plant Physiol. 43, 1380-1388 (1968)

MacRobbie, E.A.C.: Phloem Translocation . Facts and Mechanisms: a Comparative Survey. Biol.Rev. 46, 429-481 (1971)

Mason, T.G., Phillis, E.: Further studies on Transport in the Cotton Plant. V Oxygen Supply and the Activation of Diffusion. Ann. Bot. 50, 455-499 (1936)

McNairn, R.B., Currier, H.B.: Translocation Blockage by Sieve Plate Callose.
Planta (Berl.) 82, 369-380 (1968)

Qureshi, F.A., Spanner, D.C.: Unidirectional Movement of Tracers along the Stolon of Saxifraga sarmentosa.
Planta (Berl.) 101, 133-146 (1971)

——— ——— : Movement of Sucrose $-^{14}C$ along the Stolon of Saxifraga sarmentosa (in preparation).

Spanner, D.C.: The Translocation of Sugar in Sieve Tubes.
J.expt.Bot. 9, 332-342 (1958)

——— Jones, R.L.: The Sieve-Tube Wall and its Relation to Translocation.
Planta (Berl.) 92, 64-72 (1970)

——— , Prebble, J.N.: The Movement of Tracers along the Petiole of Nymploides peltatum. I
J.expt.Bot. 13, 294-306 (1962)

Ullrich, W.: Zur Sauerstoffabhangigkit des Transports in den Siebröhren.
Planta 57, 402-429 (1961)

Willenbrink, J.: Uber die Hemmung des Stofftransports in den Siebröhren durch Lokale Inaktivierung Verschiedener Atmungsenzyme.
Planta 48, 269-342 (1957)

Wortman, J.: Quoted in Curtis, O. (1929), (1690).

Sub-section: (ii)

The influence of cyanide

or

Cyanide inhibition of phloem transport along the stolon of *Saxifraga sarmentosa*.

Cyanide Inhibition of Phloem Transport along the Stolon of *Saxifraga sarmentosa*

Summary Long thin stolons of *Saxifraga* were treated with cyanide in both the solution and gaseous forms; the latter was much more effective. Cyanide strongly inhibited the transport of ^{137}Cs and of natural ^{14}C-assimilates. As judged by a variety of approaches, including the use of ^{14}C-cyanide, the inhibition was certainly effective in the sieve tubes themselves. Callose formation did not seem to be promoted. The inhibition was completely reversible. Inhibition was never accompanied by a build-up of tracer in, or before, the treated zone; failure to traverse it was not therefore due to membrane damage and consequent leakage. For these reasons the results are held to favour a mechanism of mass flow invoking active pumping in the sieve tubes.

Introduction

Long-distance transport of assimilates in the phloem is a major activity of plants. Unlike xylem transport, which finds an adequate source of free energy in the physical process of evaporation from the leaves, phloem transport fairly obviously relies on metabolism. What is still undecided is how and where metabolic energy is applied to the transport system. It is more or less agreed that to some extent at least, this is accomplished through the processes of 'loading' at the source and 'unloading' at the sink; and the Münch hypothesis regards these as necessary and sufficient as far as the bringing-to-bear of energy is concerned. However there are many physiologists who view them as palpably inadequate, at least in the case of trees; and accordingly several theories of the transport process regard energy mediation as significant within the conduits themselves. In order to elucidate this question the use of metabolic inhibitors on the conducting channels has been a favourite approach, though it has proved difficult to localise their action sufficiently to rule out any possibility that it was being exerted at the source and sink terminals rather than within the conduit. An earlier paper of this series reported work using nitrogen to promote anoxia, the long stolon of Saxifraga sarmentosa offering the possibility of better localisation than previous objects. The present paper reports a continuation of work on this material using cyanide.

It is unnecessary to summarise previous work with this inhibitor, as this has been done adequately in a recent monograph (Crafts & Crisp, 1971). It is sufficient to recall that several workers (notably Willenbrink, 1957, 1966, 1968; Ullrich, 1961; and Ho & Mortimer, 1971) have regarded a definite action on the conduits

themselves as established; while others (Duloy et al, 1962) have held the view that the cyanide migrates, perhaps in the xylem, to the terminals of the system and exerts its inhibitory effect there. A third possibility which still remains open is that the inhibitory effect on translocation is concerned less with a failure in the energy supply than with a blockage of the channels, perhaps by callose (but see Ullrich, 1963). The situation therefore is that the evidence from studies with cyanide is not yet quite conclusive and an approach which seemed to promise a rather definite answer to the question of mechanism has so far fallen short of expectations. The continuing questions on which the present work set out to throw light were therefore these: does cyanide exert an inhibitory effect locally within the sieve tubes? is the effect reversible? is it connected in any way with callose formation, or with some other blockage phenomenon? These points were investigated by examining the distribution down the long stolon of ^{14}C naturally-assimilated by the subtending leaf or of applied ^{14}C-sucrose and ^{137}Cs. The transpiration stream was reduced as much as possible, and cyanide applied in both the gaseous and solution forms. Wandering of the cyanide was monitored using $K^{14}CN$.

Materials and Methods

Plants of Saxifraga sarmentosa were grown under normal greenhouse conditions and with long stolons as previously described (Qureshi & Spanner 1971) and pruning of laterals was completed at least 3 days before the experiments were done. Where the cyanide was applied as a liquid, the stolon was run in a groove (5 x 3 mm) in a perspex block 20 cm long, submerged in 4 to 5 ml of potassium cyanide made up in 0.05M phosphate buffer

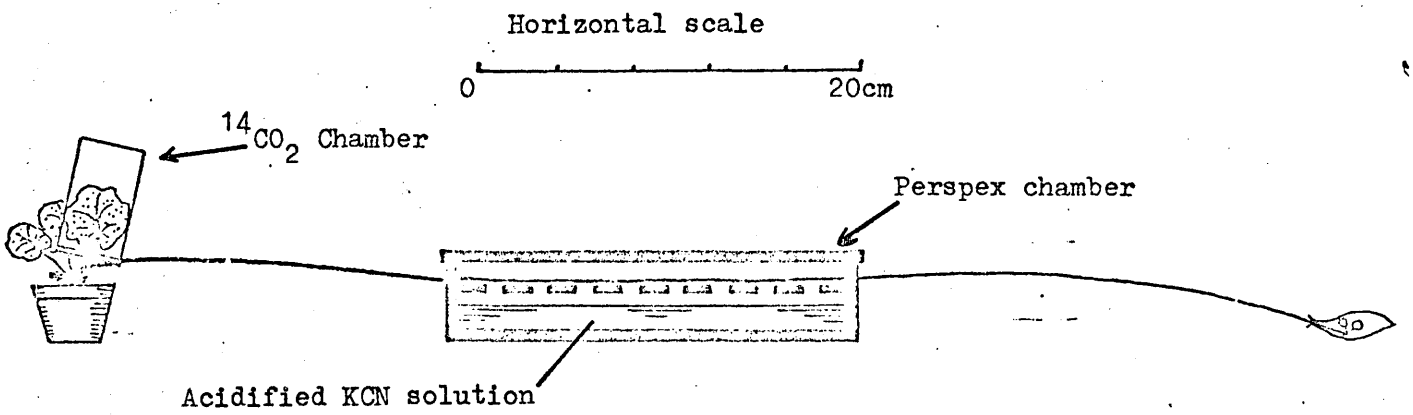


Fig 1 Method of feeding subtending leaf with $^{14}CO_2$ and applying gaseous cyanide to stolon. Approximately to scale.

of pH 7 to 7.2. The 20 cm submerged length represented the central portion of a stolon whose total length was 50-60 cm. Thus it was adequately long to be well-separated from the source and sink regions. Where the cyanide was applied in gaseous form the three replicate stolons were supported on a perforated platform above a reservoir containing 25 ml of cyanide acidified with sulphuric acid (fig 1). In the fume cupboard in which the experiments were done light of 3700 lux was provided by low-pressure mercury lamps during the translocation period.

The methods of applying tracers to the stolon, and $^{14}CO_2$ to the subtending leaf were similar to those previously reported (Qureshi & Spanner, 1972 a,b). Usually 10-15 μCi of ^{137}Cs and 5 μCi of ^{14}C-sucrose in 50 μl of neutral 0.05M phosphate buffer were used, or 25-40 μCi of $^{14}CO_2$ as gas. Particular doses are noted on the legends. Where it was desired to monitor the movement of cyanide about 150 μCi of $K^{14}CN$ were incorporated in the cyanide reservoir along with the inactive cyanide necessary to provide the right concentration.

At the conclusion of the experiments the stolons were harvested, segmented as indicated on the horizontal axes of the figures, and counted by an automatic IDL GM counter (^{137}Cs), or a Packard Tri-Carb (^{14}C) using the Triton-100 x - toluene scintillator previously described (Qureshi & Spanner, 1972b).

The subtending leaves given $^{14}CO_2$ were either heat- or freeze-dried and autoradiographs were made in the conventional manner. For electron-microscopical examination for callose the stolon was quickly immersed in an alcohol: acetic acid (3:1) mixture at -20°C. After at least 24h it was brought to room temperature, segments 2mm long were cut out, washed in tap water for 4-6h, post-fixed in osmium and embedded in TAAB epoxy resin.

Fig 2 Transport of ^{137}Cs down the stolon under the influence of cyanide applied in solution to 2cm of axis. The right-hand curves are displaced horizontally for clarity. Cyanide applied 3h prior to ^{137}Cs; total duration of experiment including pretreatment 22h; continuous light.

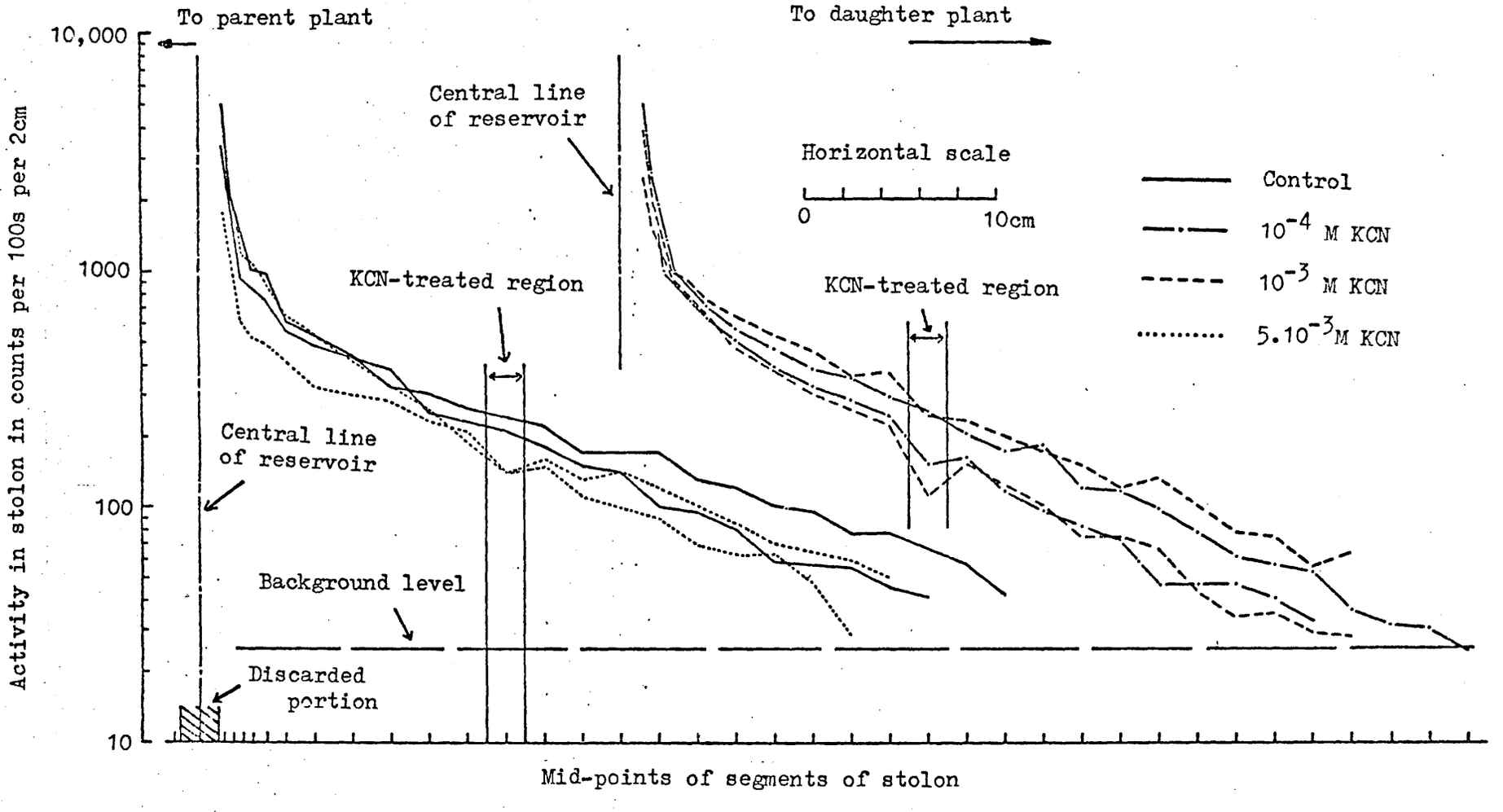

To parent plant
To daughter plant
10,000
1000
100
10
Activity in stolon in counts per 100s per 2cm
Central line of reservoir
Horizontal scale
0
10cm
Control
10^{-4} M KCN
10^{-3} M KCN
5.10^{-3}M KCN
KCN-treated region
KCN-treated region
Central line of reservoir
Background level
Discarded portion
Mid-points of segments of stolon

Fig 3 Transport of ^{137}Cs down the stolon under the influence of cyanide applied in solution to 20cm of axis. Other details as fig 2.

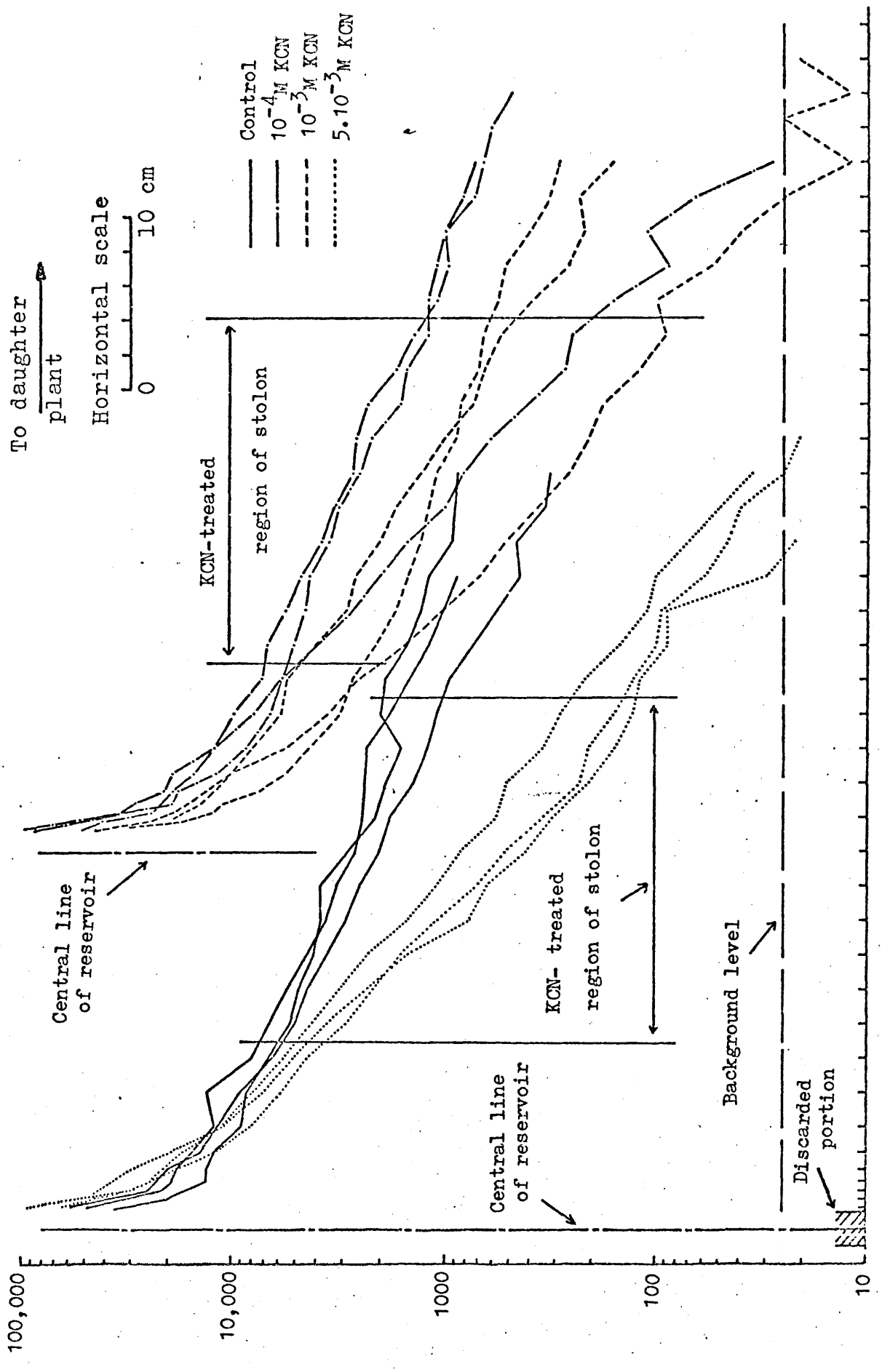
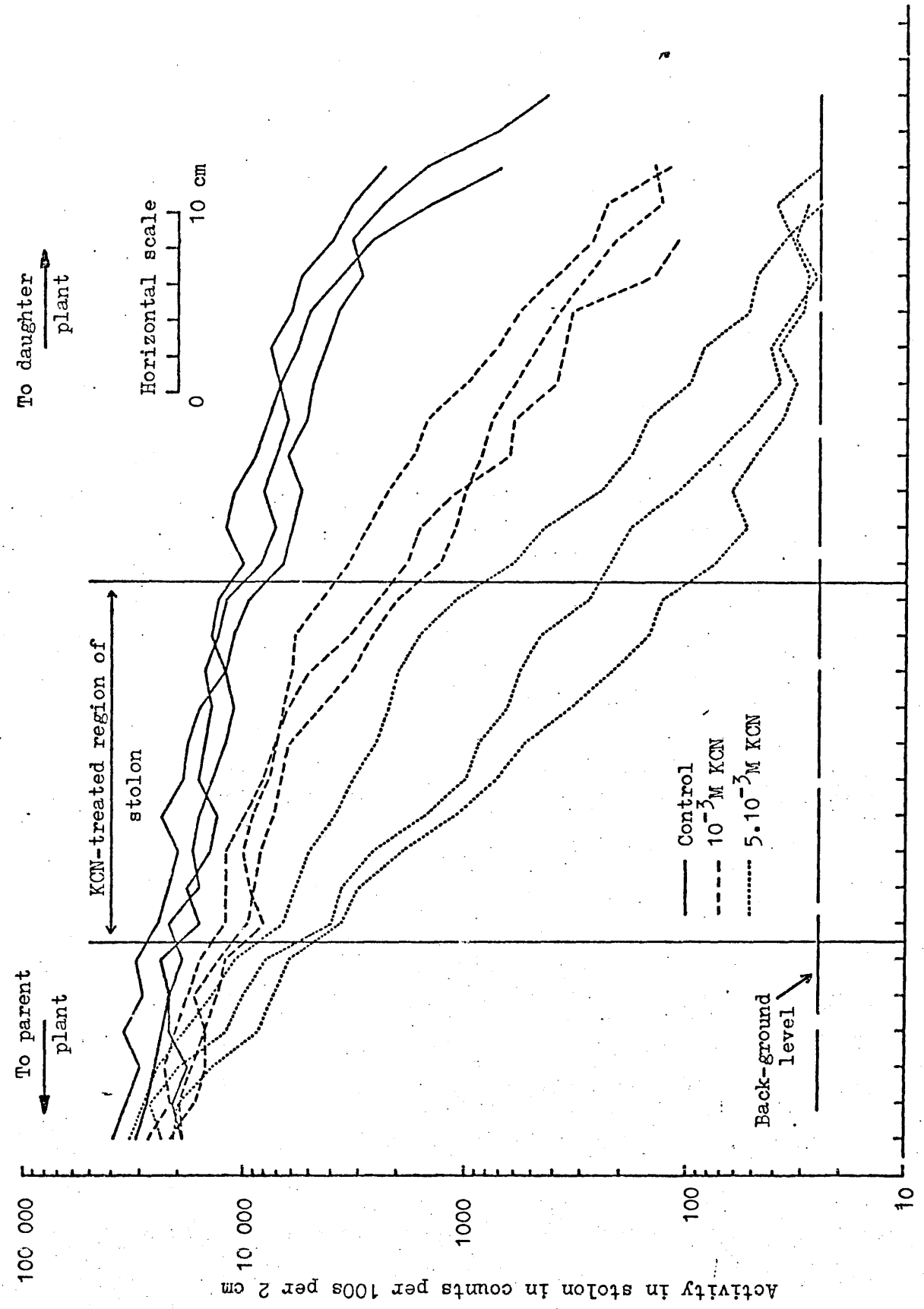

Activity in stolon in counts per 100s per 2cm
100,000
10,000
1000
100
10
Mid-points of segments of stolon
Control
10^{-4}M KCN
10^{-3}M KCN
5.10^{-3}M KCN
Horizontal scale
0
10 cm
To daughter plant
KCN-treated region of stolon
Central line of reservoir
Background level
Discarded portion
KCN- treated region of stolon
Central line of reservoir

Fig 4 Transport of naturally-assimilated ^{14}C down the stolon under the influence of cyanide applied ~~to~~ *in* solution to 20cm of axis. Cyanide applied 3h prior to feeding $^{14}CO_2$ to subtending leaf. Total duration of experiment including pretreatment 7h; continuous light.


Activity in stolon in counts per 100s per 2 cm
100 000
10 000
1000
100
10
To parent plant
To daughter plant
KCN-treated region of stolon
Horizontal scale
0
10 cm
Control
10⁻³M KCN
5.10⁻³M KCN
Back-ground level
Mid-points of segments of stolon

Results

Transport of ^{137}Cs and ^{14}C-assimilate along stolon immersed in KCN solution.

In the earliest experiments only about 2cm of the stolon was subjected to cyanide solution. Three hours later ^{137}Cs was applied, and after a further 19h the stolon was harvested. Fig 2 reports some typical results. It can be seen that the cyanide treatment has had no obvious effect at any of the concentrations used. In the next series the length of submerged stolon was increased to 20cm; otherwise the pattern was as before. The results (fig 3) indicate a definite effect of the treatment, revealed by a steepening of the slope of the semi-logarithmic plot, an effect which increases progressively with concentration. Inasmuch as the slope probably reflects the balance of lateral leakage of tracer and longitudinal transport its interpretation is ambiguous at this point; clearly however, there is a pronounced reduction in movement.

The next experiments repeated those first described except that $^{14}CO_2$ was fed to the subtending leaf as tracer instead of ^{137}Cs being applied to the stolon. Three hours after submersion of the stolon in KCN the leaf was given $^{14}CO_2$ for an hour. A further 3h was allowed for transport in the light and then the stolon was harvested. The results, reported in fig 4, follow basically the same pattern as those with ^{137}Cs. Replication again is very good, and the conclusion unequivocal; cyanide has a pronouced effect, but it falls short of complete stoppage at the highest concentration used (5×10^{-3}M). How high was the concentration <u>within</u> the tissue it is difficult to say; but in view of the fact that the axis was unmutilated there remained the possibility that it was quite low,

Fig 5 Transport of naturally-assimilated ^{14}C down the stolon under the influence of cyanide administered as gas. Cyanide given 3h prior to feeding $^{14}CO_2$ to subtending leaf. Total duration of experiment including pretreatment 7h; continuous light.

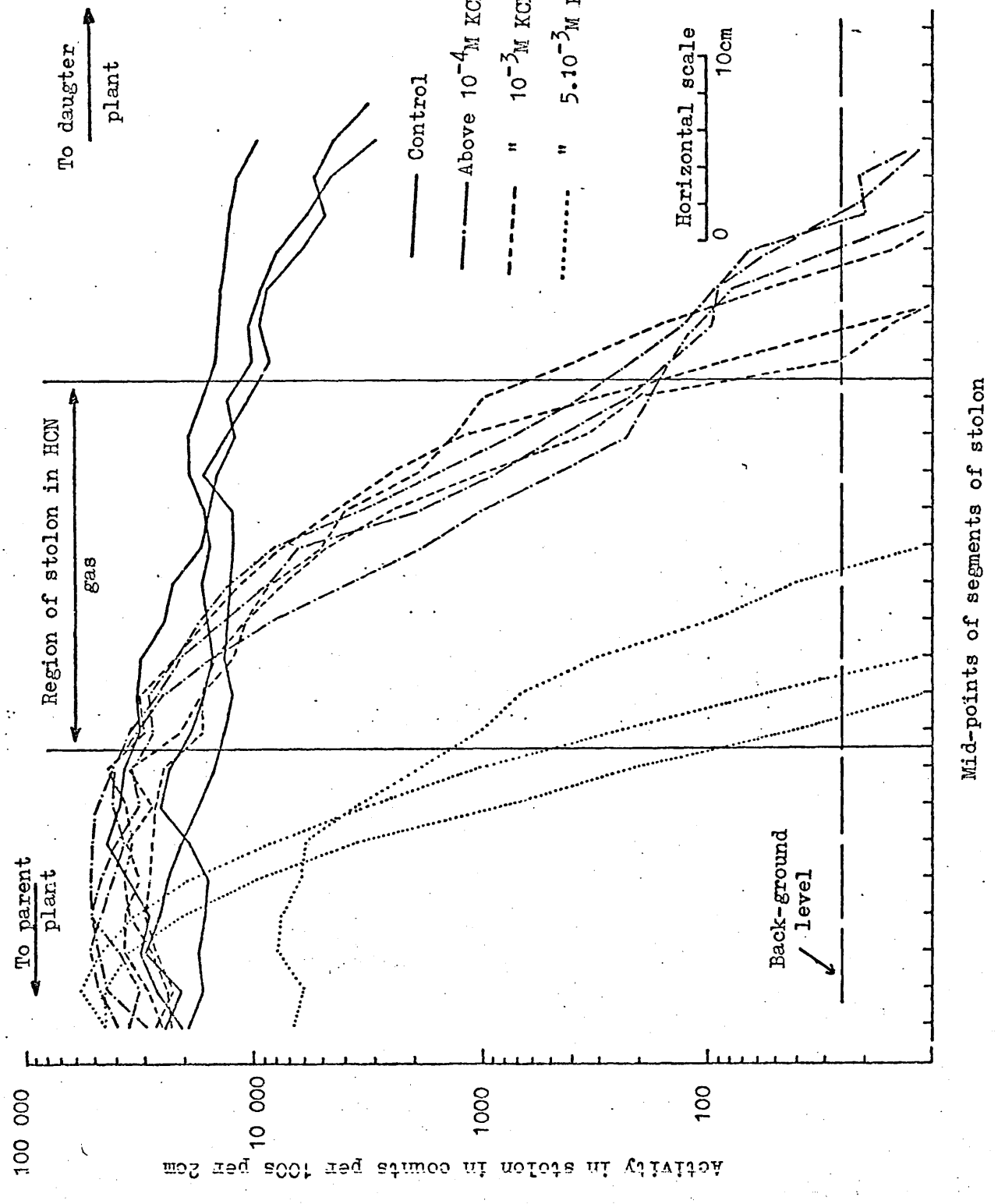
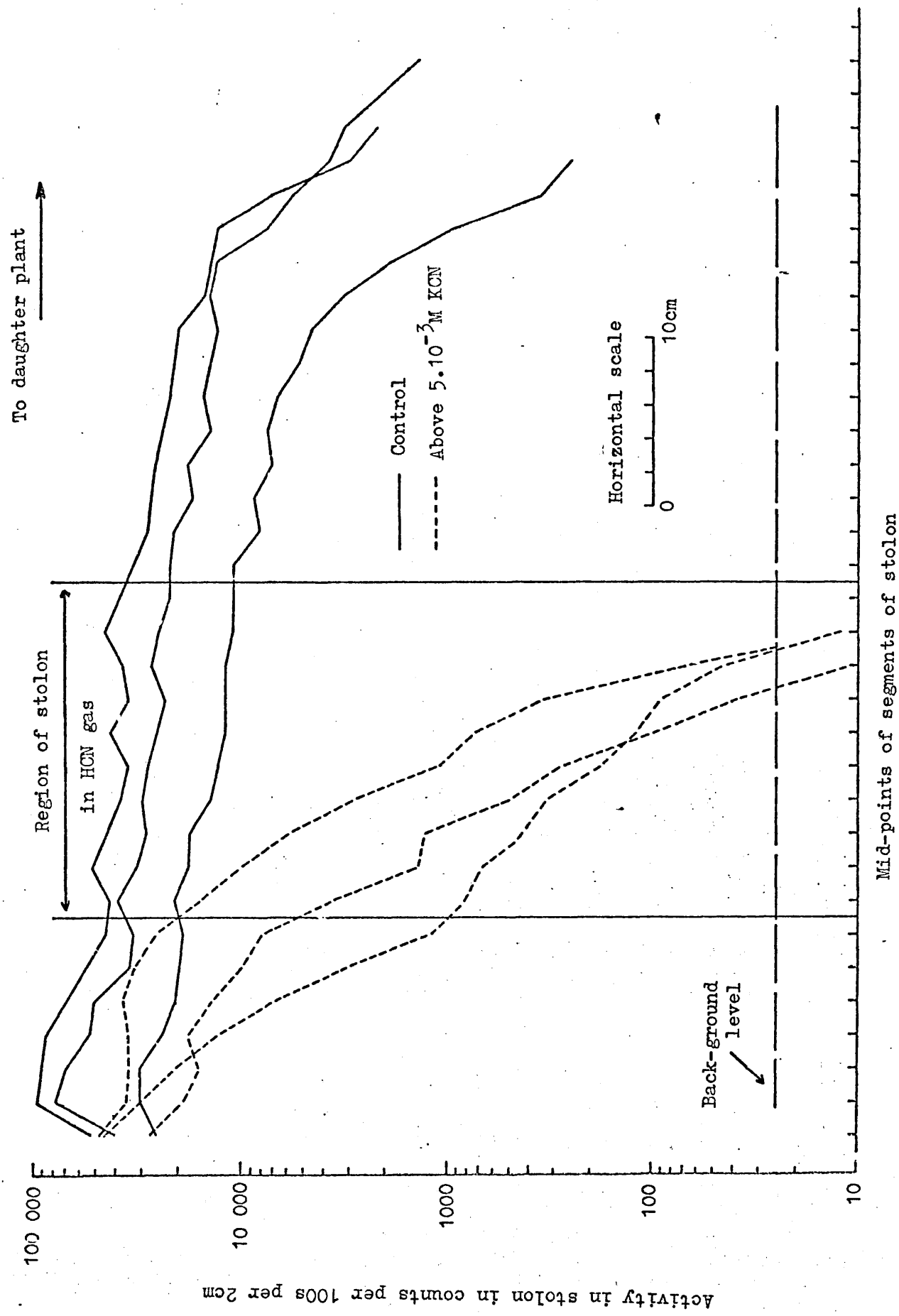

To daughter plant
Control
Above 10^{-4}M KCN
" 10^{-3}M KCN
" 5.10^{-3}M KCN
Horizontal scale
0
10cm
Region of stolon in HCN gas
To parent plant
Back-ground level
100 000
10 000
1000
100
Activity in stolon in counts per 100s per 2cm
Mid-points of segments of stolon

Fig 6 Details as fig 5, but $^{14}CO_2$ given at the same time as cyanide. Total duration of experiment 4h.


To daughter plant
Region of stolon
in HCN gas
Control
Above 5.10⁻³M KCN
Horizontal scale
0
10cm
Back-ground level
100 000
10 000
1000
100
10
Activity in stolon in counts per 100s per 2cm
Mid-points of segments of stolon

and the whole problem needed further investigation.

Use of gaseous HCN on Saxifraga

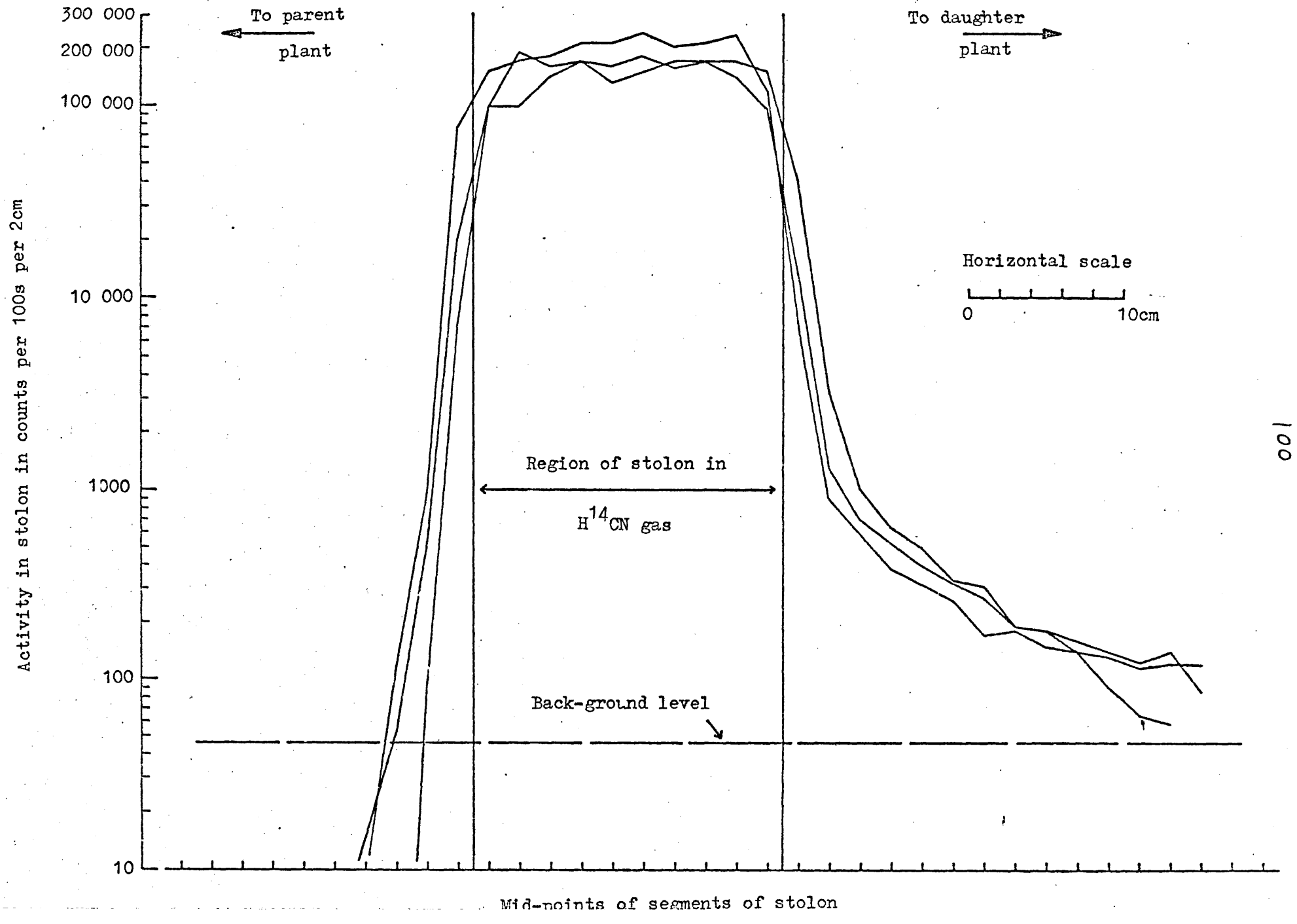
An attempt to treat the stolon of Saxifraga by shaving-off the outer layers to facilitate entry of cyanide proved abortive; so it was decided to pursue the matter by using gaseous HCN after the manner of Willenbrink (1957). The central 20cm of the stolon were accordingly enclosed in the special perspex chambers (fig 1) above the acidified cyanide. After the treatment had been proceeding for 3h, $^{14}CO_2$ was given to the subtending leaf for an hour and 3h later the stolon was harvested. Fig 5 presents the results in triplicate. It is obvious that while the indications are the same as those following the use of cyanide in solution (fig 4), they are very much more pronounced. Inhibitor of 5×10^{-3}M strength leads to virtual exclusion of labelled translocate from the region under treatment. As before, the fall-off towards this region is gradual; there is no build-up of escaped tracer on the threshold, as would be expected if the treatment had merely destroyed the integrity of the sieve-tube membranes.

In order to test how rapidly the cyanide inhibition established itself a further experiment was carried out in which the $^{14}CO_2$ was given at the time cyanide treatment was commenced. The results (fig 6) suggests that the treatment takes quite a short time to become effective.

Spread of cyanide

The difference between the actions of cyanide applied in the liquid and gaseous phases raised an interesting question which challenged investigation. However, the originally-posed problem of the localisation of action remained to be answered first. Accordingly, an experimental series was run in which the inhibitor

Fig 7 Movement of labelled cyanide when administered as gas.
Concentration 5×10^{-3}M; dose of $K^{14}CN$ 150 μCi in 25 ml
Duration of experiment 7h; continuous light.


300 000
200 000
100 000
10 000
1000
100
10
To parent plant
To daughter plant
Horizontal scale
0
10cm
Region of stolon in
$H^{14}CN$ gas
Back-ground level
Activity in stolon in counts per 100s per 2cm
Mid-points of segments of stolon

solution of strength 5×10^{-3}M was enriched with 150 μCi of ^{14}C-cyanide (of specific activity 30-50 mCi per mM). After the usual 7h the experiment was terminated and the stolon assayed; the petiole of the subtending leaf, cut up into 1cm segments, and five 1cm discs from the lamina, were also included. The assay revealed no activity at all in the leaf discs or petiole; the distribution down the stolon is shown in fig 7. Towards the parent plants there has been no long-distance transport at all, the sharply-falling lines representing merely the usual symmetrical short-distance spread consistently found (Qureshi & Spanner, 1971, 1972a, b). Towards the daughter plants (situated immediately beyond the end of the curves) there has been a limited transport; at the position of the plantlets the concentration has fallen to about 1/2000th of its value in the region under treatment. This suggests that interference with the unloading process is hardly likely to be the cause of the cyanide effect. This limited long-distance transport may well be due to phloem movement in the delay-period before cyanide has accumulated to a damaging extent; a priori one would certainly expect some such transport during this phase. Xylem movement is also a possibility, but it is unlikely owing to the enclosure of the plantlets in a moist atmosphere. In any case, however it occurred, it seems reasonable to suppose that the integrity of the unloading processes was unaffected, and the effect of the poison therefore specifically on the sieve-tubes.

Effect of HCN on source and sink

Subtending leaves given $^{14}CO_2$, from HCN-treated and control plants were autoradiographed and compared to see if there was any difference in their distribution of labelled assimilate. The experiments followed the previous pattern. No difference was

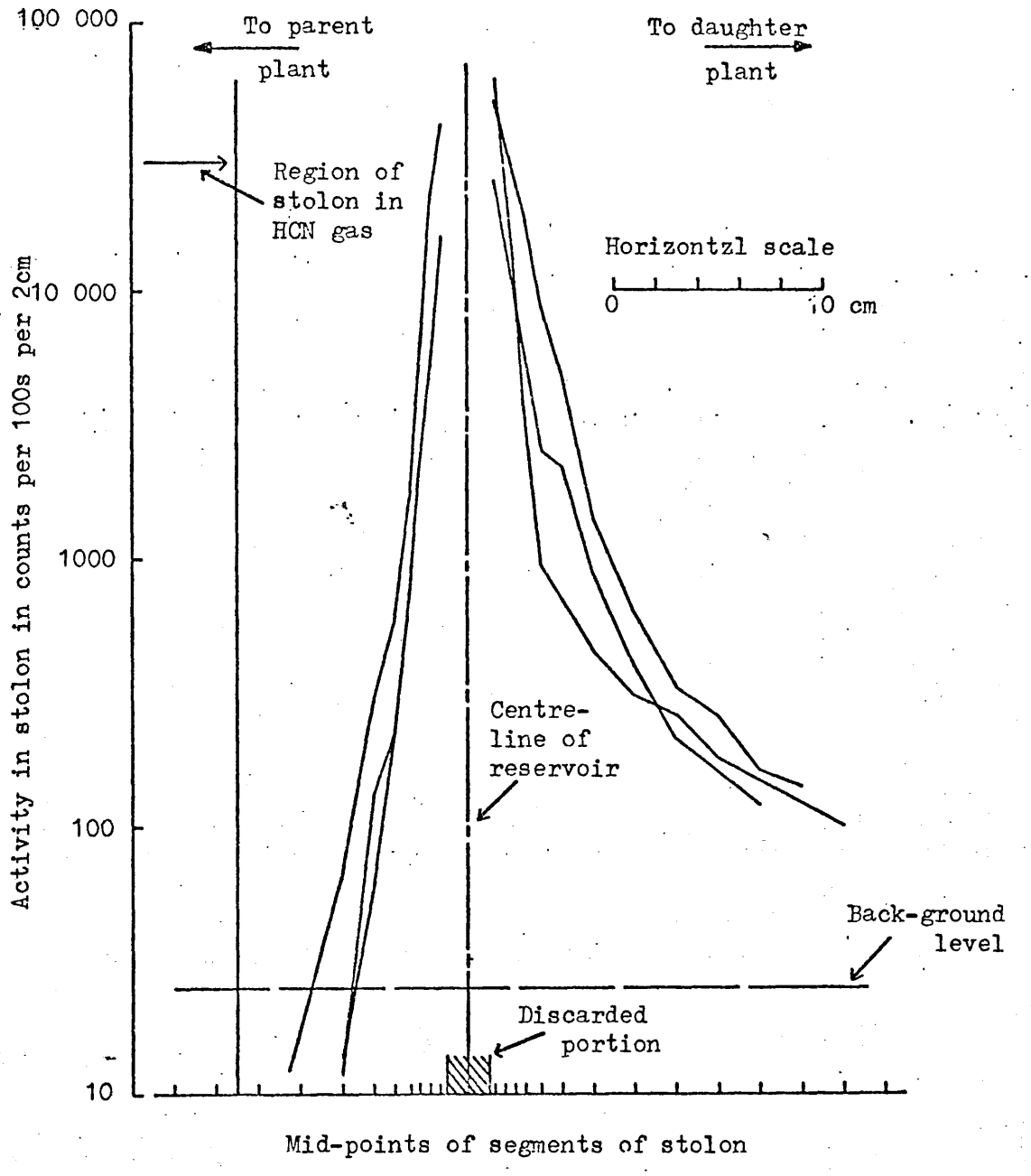


Fig 8 Movement of ^{14}C-sucrose applied to stolon at a point beyond a 20cm zone subjected to gaseous cyanide. Concentration of KCN, 5×10^{-3}M. Cyanide treatment began 3h before sucrose application; total duration of experiment 7h. Continuous light.

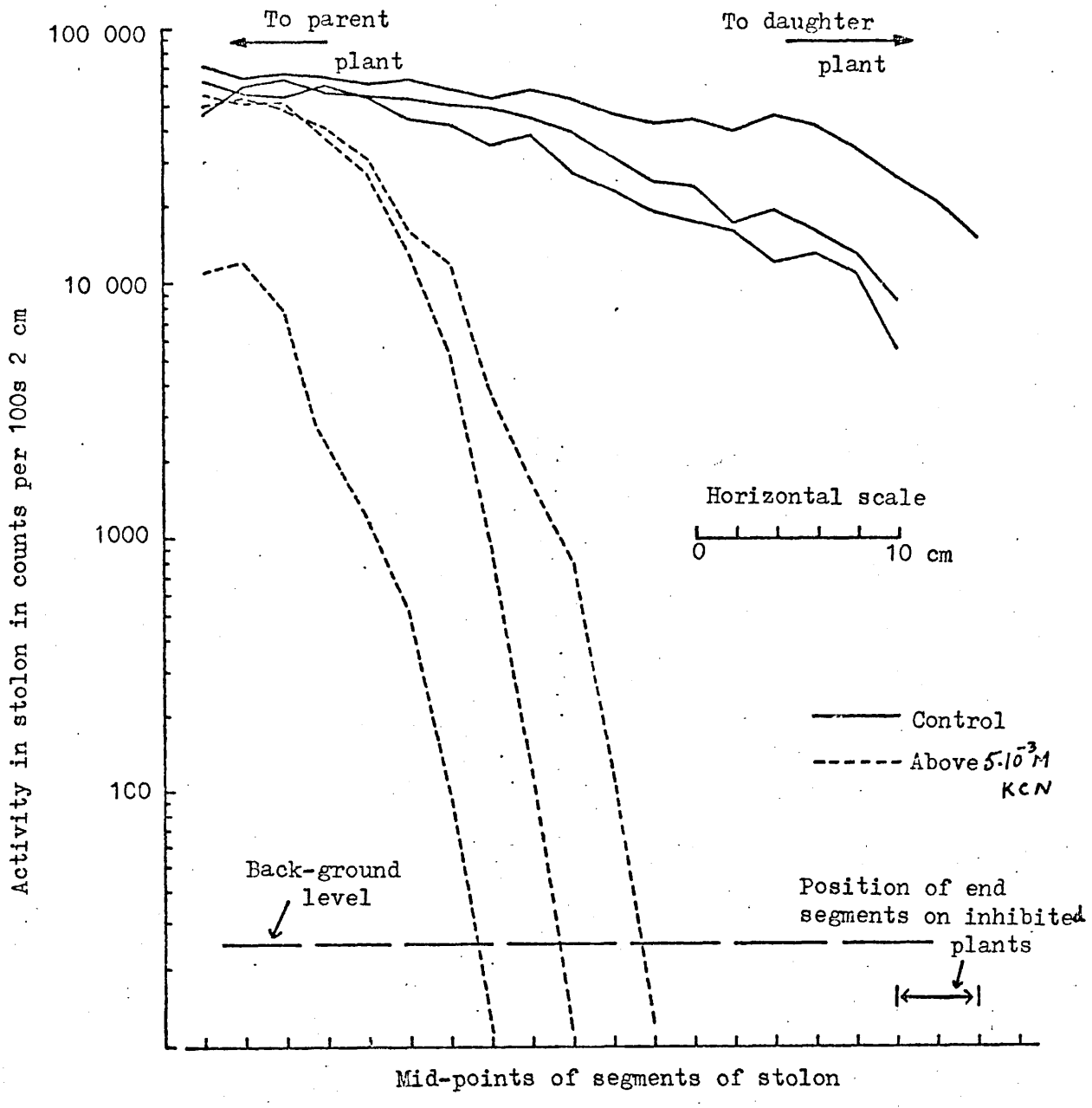


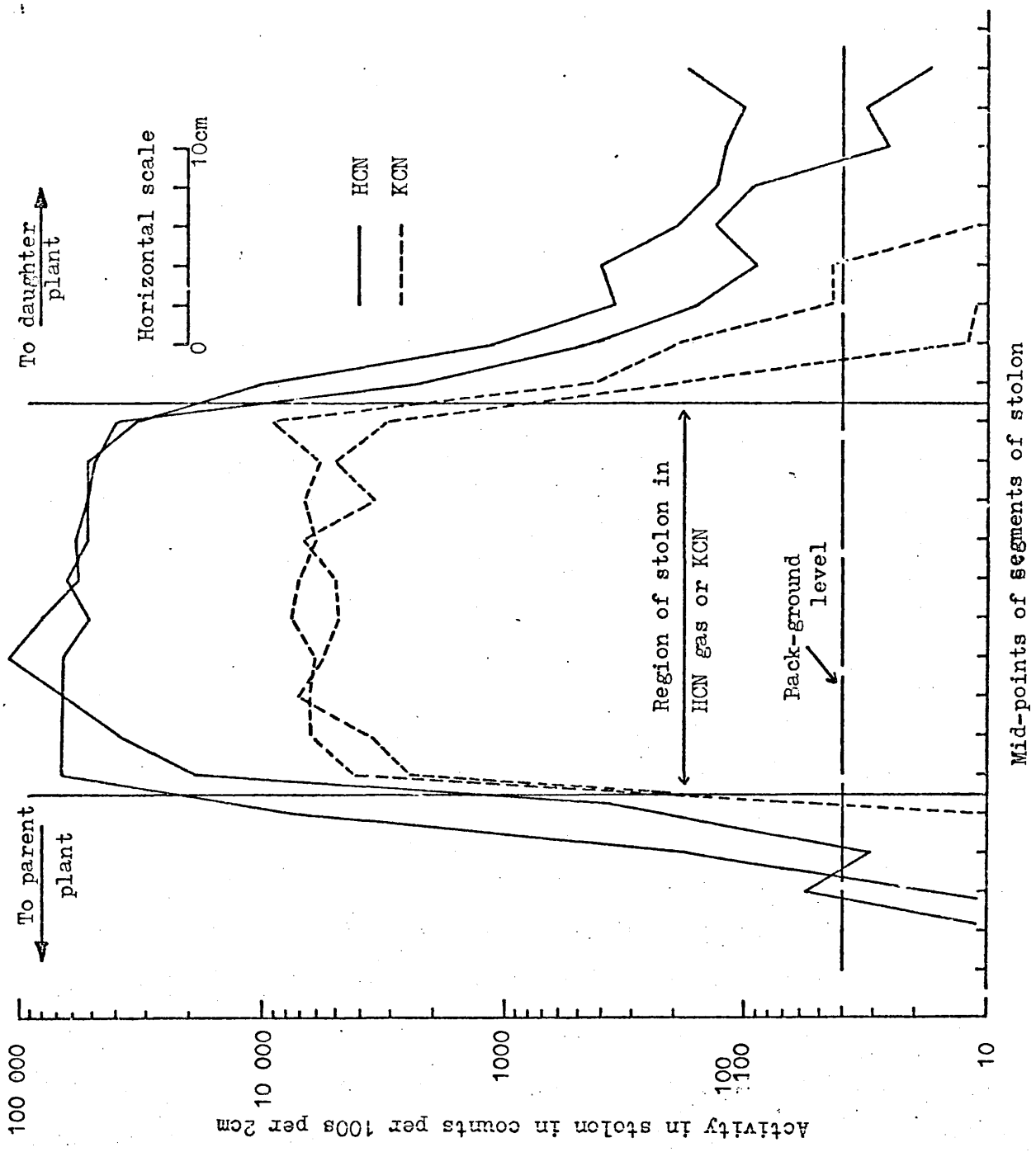
Fig 9 Movement of naturally-assimilated ^{14}C under the influence of cyanide gas administered directly to daughter plants. Strength of KCN 5×10^{-3}M. Cyanide treatment began 3h before $^{14}CO_2$ was given to subtending leaf. Total duration of experiment, including pretreatment, 7h. Continuous light.

apparent; the vein-loading and petiolar activity were identical. This contrasts with the result when a leaf was subjected to HCN for 3 hours, given $^{14}CO_2$ for an hour, and then replaced in HCN. The general level of label was low, and there was little in the veins and petiole.

In order to test the effect of cyanide on the sink region two types of experiment were run. In the first (fig 8) ^{14}C-sucrose was applied to the centre of the section of stolon between the lower end of the treatment zone and the daughter plant. Application was 3h after the initiation of treatment with gaseous cyanide from 5×10^{-3}M solution; 4h later the stolon was harvested. Long distance transport towards the daughter plants has occurred. In view of the short time allowed for absorption and transport of the applied sugar (contrast the 18 h of Qureshi & Spanner, 1972b, fig 1, and compare the same authors, 1971, fig 4) this transport may be regarded as fairly vigorous. As such, it seems to indicate that the translocating system beyond the treated zone (and this includes of course the sink) is still intact.

That the sink region is, nevertheless, susceptible to cyanide is apparent from the results in fig 9. The daughter plants here were themselves exposed to the atmosphere above 5×10^{-3}M cyanide. Three hours later the subtending leaf was given $^{14}CO_2$; and 7h from the start of cyanide treatment (as usual) the stolon was harvested. Transport has been considerably slowed down; nevertheless it has proceeded some distance (the petiole is to be included). In view of the high concentration of HCN to which the daughter plants were here subjected it seems unlikely that the inhibiting action of cyanide (fig 7) can be attributed to its effect on the sink region. Both of the lines of evidence in this section (figs 8,9) support this view.

Fig 10 Uptake of ^{14}C-cyanide by stolons immersed in cyanide solution and enclosed in gas phase above. Strength 5×10^{-3}M, activity 200 μCi per 30ml. Duration of experiment 8h. Stolons not excised.


To daughter plant
Horizontal scale
0
10cm
HCN
KCN
Region of stolon in HCN gas or KCN
Back-ground level
To parent plant
100 000
10 000
1000
100
100
10
Activity in stolon in counts per 100s per 2cm
Mid-points of segments of stolon

Why is gaseous HCN more effective than KCN solution?

An interesting question thrown up by the present work concerns the relative effectiveness of gaseous cyanide and cyanide in solution. This seemed the occasion to investigate it. Accordingly, an experiment was set up in which a comparison was made between stolons exposed in the usual way to the two modes of administration. The cyanide was made up to 5×10^{-3}M strength in 0.05 M phosphate buffer of pH7. To 30ml of this 200 μCi of $K^{14}CN$ was added. Samples of 0.01, 0.03 and 0.05ml were taken from this and assayed in the usual way in the toluene-Triton 100 X Scintillator, a section of inactive stolon being added to improve comparability. Meanwhile, 5ml of the solution was applied to 20cm lengths of two replicate stolons in the manner previously employed; the remaining 25ml was acidified with sulphuric acid in the apparatus shown in fig 1, and two comparable stolons were inserted. After 8h of treatment under normal photosynthesising conditions the stolons were harvested, segmented, weighed fresh and assayed as usual, those subjected to the liquid being first well washed and dried with absorbent tissue. Fig 10 shows the results. It is obvious that the stolons exposed to the gas have absorbed considerably more cyanide than the others, in fact about ten times as much. The measurements of fresh weight, and the assays of the inhibitor solution enable us to make an estimate of the mean concentration of cyanide in the stolons. Table 1 sets out the results, based on a measured water content of 91% of the fresh weight. Taking into account the fact that the stolons in gas were stouter than the others their advantage in terms of concentration absorbed in about five to six times over those exposed to solution. It seems reasonable to suggest that it is this that makes exposure to the gas so much more effective than exposure to solution, though

Table 1

Stolon	Fresh weight per 2cm (g)	Mean count rate per 100s per 2 cm	Mean cyanide concentration in stolon (M)
1) in KCN	0.0214 to 0.0136, mean 0.0175	4724	1.6×10^{-4}
2) solution	0.0138 to 0.0078, mean 0.0102	6540	3.9×10^{-4}
3) in HCN	0.0441 to 0.0194, mean 0.0276	57228	1.3×10^{-3}
4) gas	0.0287 to 0.0179, mean 0.0223	56600	1.6×10^{-3}

The inhibitor solution (5×10^{-3}M) assayed 90,100 counts per 100s per 0.01ml.

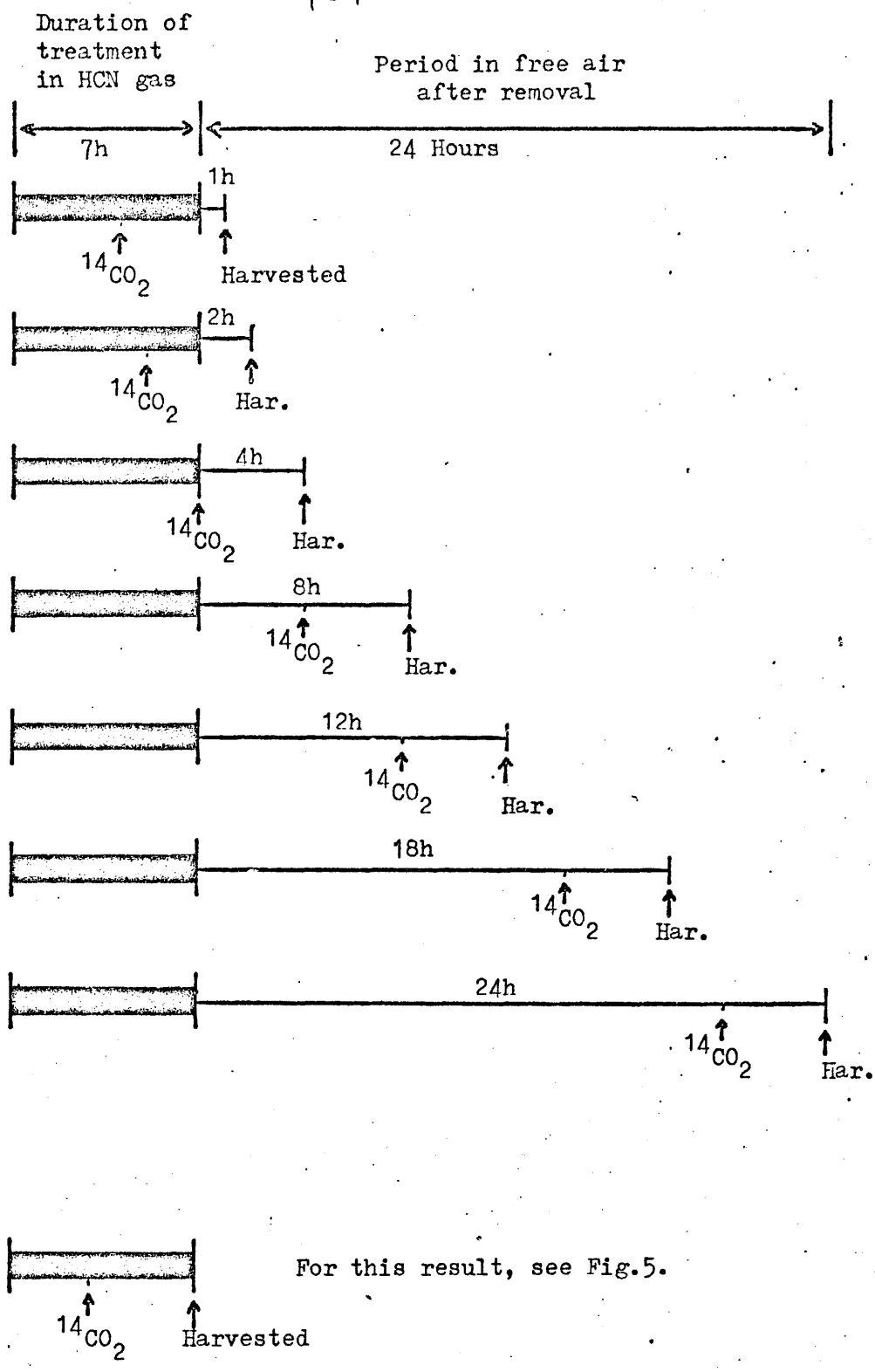


Fig 11 Design of experiment to test reversibility of cyanide inhibition.

Fig 12 Transport of naturally-assimilated ^{14}C down the stolon; recovery after removal of stolon from gaseous cyanide to free air. Treatment above 5×10^{-3}M KCN for 7h. Time allowed for assimilation and transport, 4h. For detailed programme see fig 11. Continuous light.

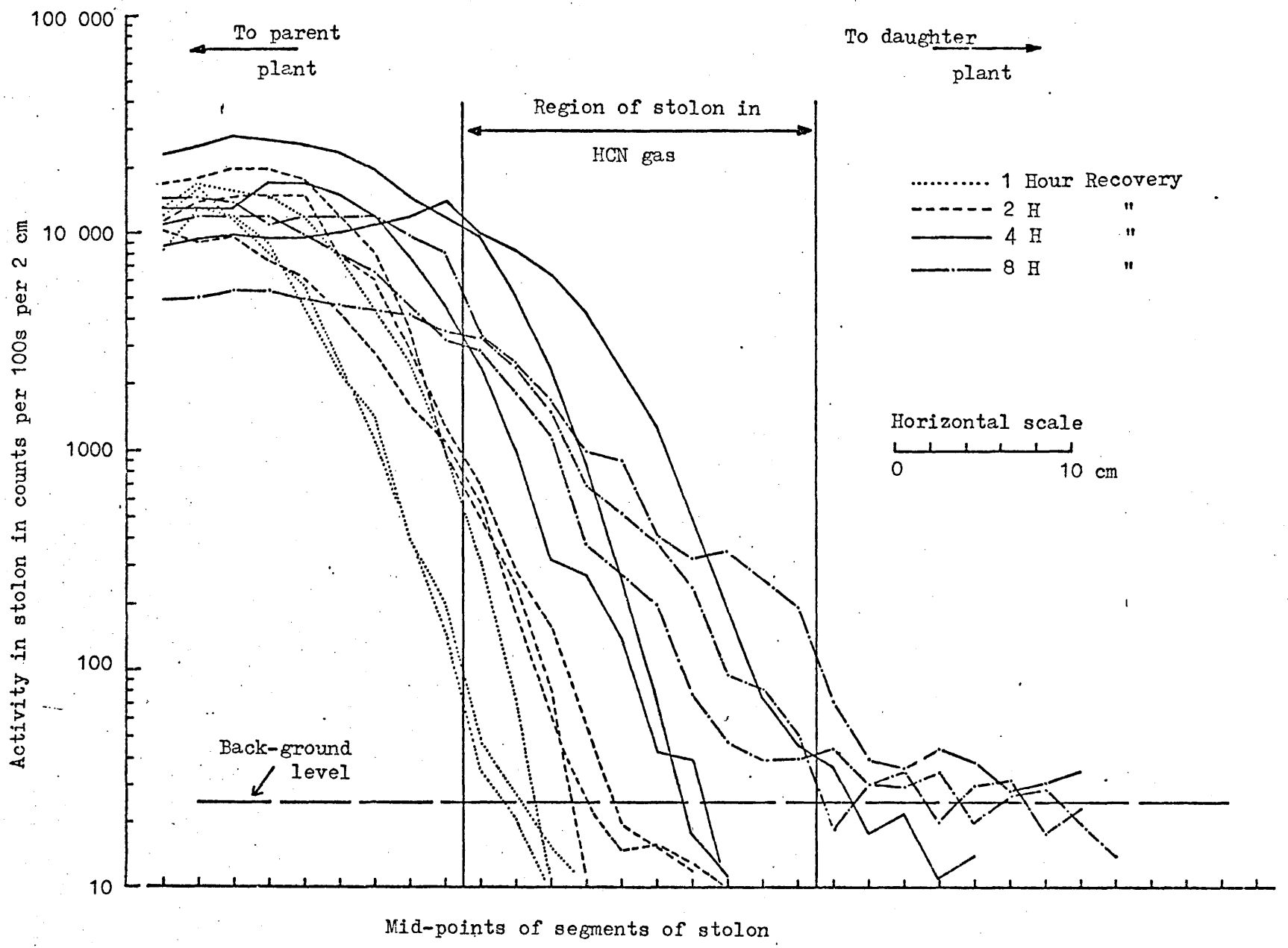
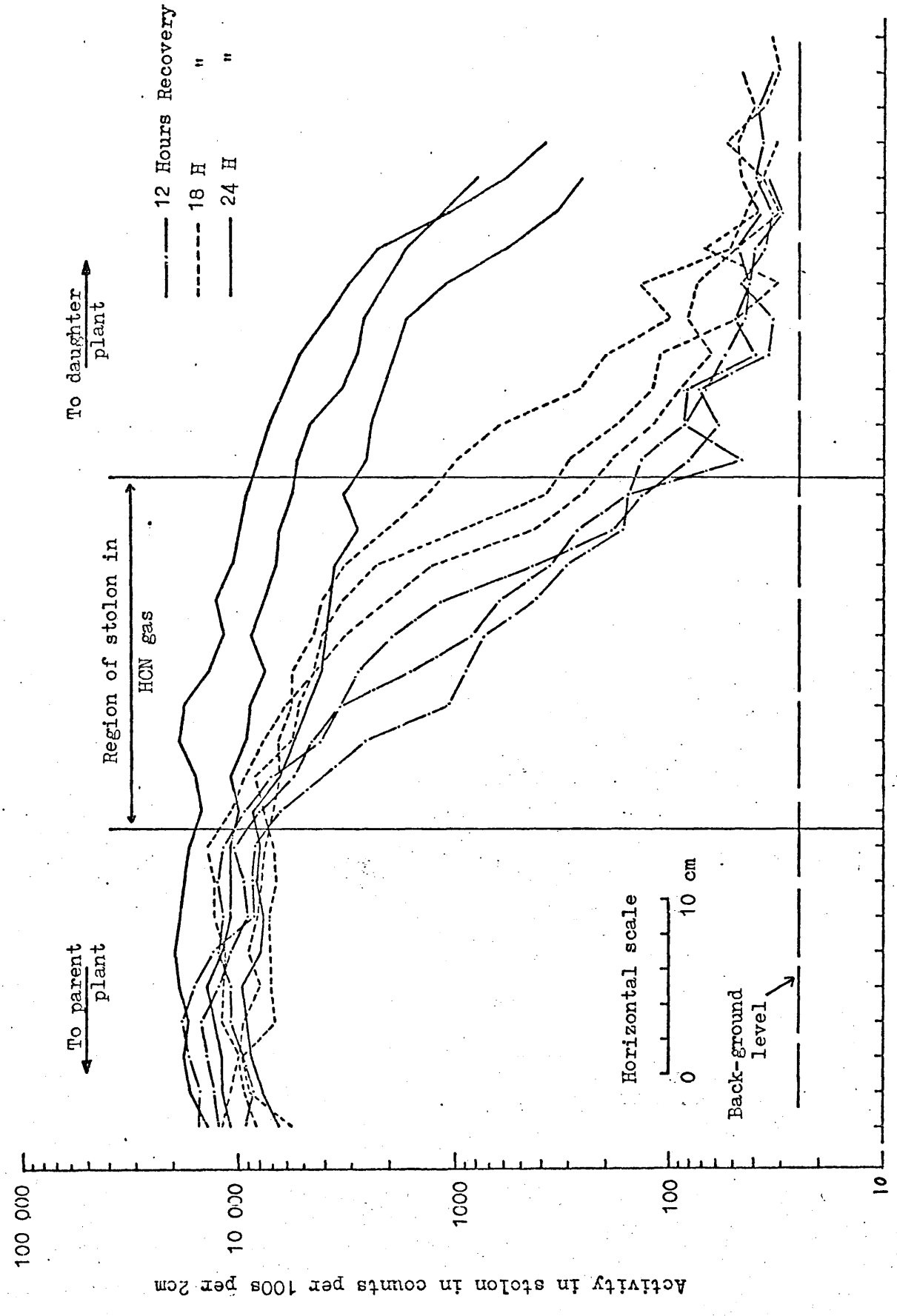

100 000
10 000
1000
100
10
To parent plant
To daughter plant
Region of stolon in HCN gas
1 Hour Recovery
2 H "
4 H "
8 H "
Horizontal scale
0
10 cm
Back-ground level
Activity in stolon in counts per 100s per 2 cm
Mid-points of segments of stolon

Fig 13 As fig 12. The longer recovery times have been plotted separately for clarity.


12 Hours Recovery
18 H "
24 H "
To daughter plant
Region of stolon in HCN gas
To parent plant
Horizontal scale
0
10 cm
Back-ground level
100 000
10 000
1000
100
10
Activity in stolon in counts per 100s per 2cm
Mid-points of segments of stolon

it must be remembered that in the latter case the elements of the phosphate buffer are also involved. The curves in fig 10, incidentally, confirm the small extent to which cyanide travels towards the sink region, though the results of fig 7 represent a more careful assessment.

Is cyanide inhibition reversible?

To answer this important question a set of experiments was designed as shown in fig 11. Stolons were treated in the light with gaseous cyanide for 7h. They were then removed from the chamber to normal light and air. Labelled carbon dioxide was fed to the subtending leaf at various stages in the programme. and 4h later the stolon was harvested. The results are presented in figs 12 and 13, the longer recovery times being separated for the sake of clarity. It is apparent that while recovery commences very soon it is not substantially complete till 24h have elapsed. The results for four hours of translocation may be compared with those of fig 4 in a previous paper (Qureshi & Spanner, 1972b). They should be interpreted in the light of the fact that cyanide does not disappear at once from the liberated stolons; especially would one expect a delay in the case of the sieve tubes whose rather alkaline sap will promote ionisation with its lowered ability to penetrate the plasmalemma. Fig 14 indicates the results of a simple experiment designed to throw light on this point. Stolons were subjected to gaseous labelled cyanide in the usual way. They were then segmented, placed in a desiccator over NaOH solution, and sampled at suitable intervals. The curve shows the escape of cyanide over 24h; the level in this time has fallen to about 1/30th. In the intact stolon it might well have fallen faster due to the recovery of transport.

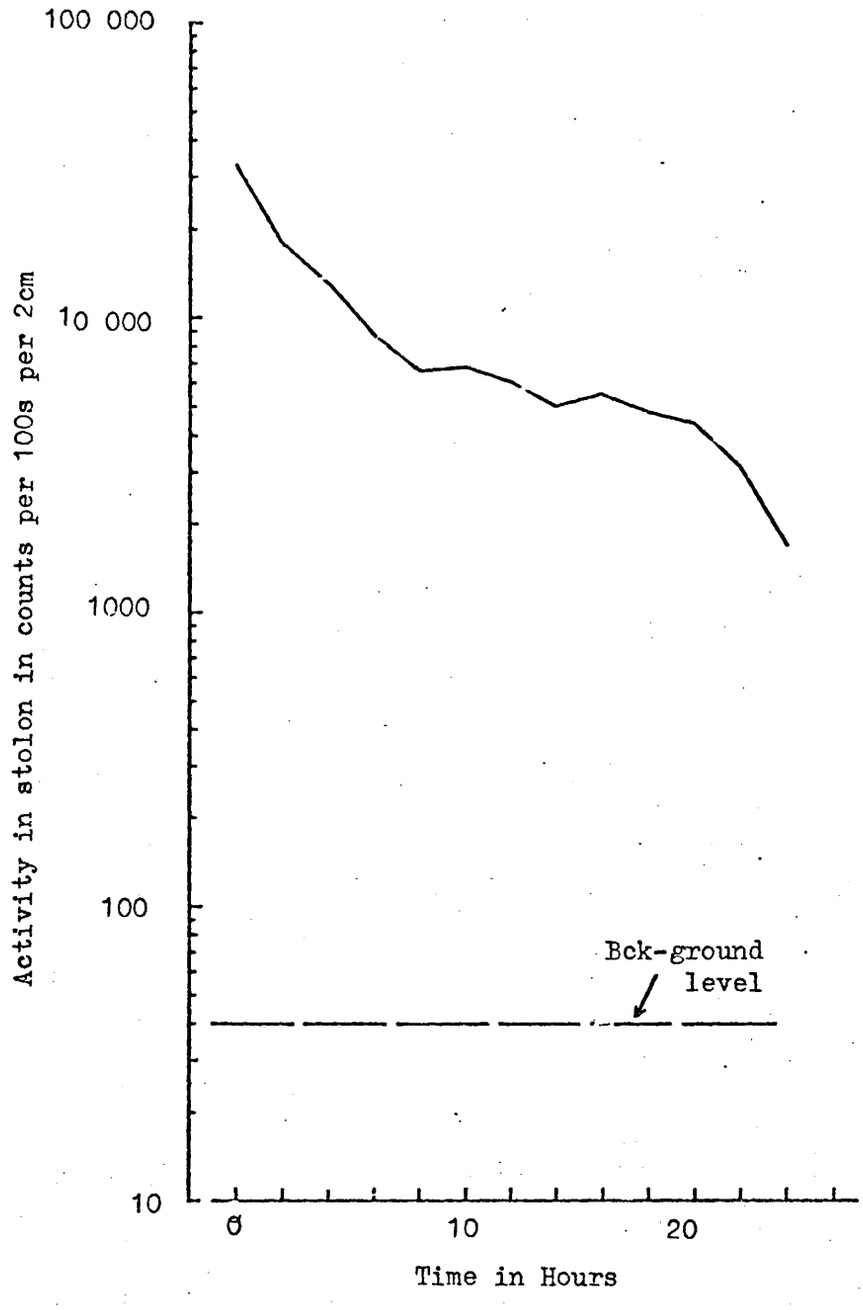


Fig 14 Escape of cyanide from stolon into moist still air. Stolon pretreated with gaseous ^{14}C-cyanide from 5×10^{-3}M solution.

Cyanide treatment and callose

It was clearly desirable to examine the sieve tubes to see if any obvious callose obstruction had been caused by the cyanide, since many poisons like boron and eosin are known to promote callose formation on the sieve-plates (see Crafts & Crisp, 1971). The results in the present examination however were negative; so far as could be ascertained treatment for 7h with the gaseous cyanide from a 5×10^{-3}M solution did not cause any obstruction. This result is in disagreement with the findings of Ullrich (1963) on Pelargonium. However, even so, Ullrich did not consider that callose formation was the cause of the transport inhibition he observed, for recovery of function was not associated with disappearance of callose; moreover, marked callose formation was a quite natural and regular phenomenon in the ageing petioles. It is hoped to subject cyanided stolons to more careful and thorough electron-microscopical examination at some date in the near future; but meanwhile it is considered unlikely that physical obstruction was the cause of the inhibition observed.

Discussion

It is probably true to say that even before the present work the balance of opinion was decidedly in favour of the view that cyanide inhibited phloem transport "reliably and reversibly" as Ullrich put it, and that such inhibition occurred locally in the sieve tubes and not only at the terminals. The results reported here strongly support and amplify this view. They confirm by methods complementary to those used by others several important conclusions. These will be dealt with in turn.

Cyanide suitably applied certainly exerts a marked inhibitory effect. That it is localised in the sieve tubes is consistent with

the difference in behaviour (figs 2 & 3) between stolons of which 2cm and 20cm respectively were treated. In the former case it can be plausibly maintained that the supply of high-energy intermediates to the inhibited region is being adequately kept up by the process of translocation itself. Further, that since transport continues, cyanide is being delivered to the sink terminal fairly rapidly, perhaps as rapidly as in the 20cm case where transport falls drastically (compare the two results for 5×10^{-3}M KCN). If this argument be accepted (and it can be challenged) then it follows that we cannot be dealing with a sink effect, and the inhibition is being exerted on the conduits themselves.

The use of gaseous cyanide instead of solution enabled levels of inhibition considerably higher to be easily achieved. In terms of percentage inhibition of transport through the treated region (a common but not very satisfactory index) the data of fig 5 indicate 100% inhibition at 5×10^{-3}M. Perhaps even more strikingly the same is true when the cyanide had no advantage in time over the labelled CO_2 (fig 6). In this latter case the inhibitor can hardly be held to have moved to the sink terminal and accumulated and acted there with sufficient rapidity to have prevented the arrival of at least a trace of ^{14}C-assimilate; one is therefore forced to the conclusion that its inhibiting action is exercised locally, i.e. in the sieve tubes. This inference is borne out even more directly by the results obtained by using labelled cyanide, for fig 7 shows that the poison moved very little towards the sink terminal. In the case of the solution-applied KCN virtually none reached the daughter plant, yet transport was substantially inhibited (fig 4). In that of gaseous application, where the degree of inhibition was very high (fig 5) the data of Table 1 indicated that the stolon adjacent to the daughter plants acquired a level of cyanide of only about 10^{-6}M.

It can hardly be maintained that this concentration was enough to produce the drastic overall inhibition observed. Any doubt on this score is probably met by the evidence that the portion of stolon beyond the inhibited region is still able to conduct when provided locally with sucrose (fig 8). As remarked earlier, the transport observed is reasonably vigorous in view of the fact that the sucrose has to penetrate the outer tissues, and that the experiment was of short duration. The sink region is certainly very sensitive to cyanide, as fig 9 shows; but the transport of natural assimilate is nevertheless so considerable that action on the sink terminal cannot plausibly explain earlier results. These (e.g.fig 5) show a degree of inhibition at 5×10^{-3}M greater than those of fig 9; yet the cyanide in the latter case was applied directly to the sink whereas in the former it was far away. The results of fig 6 can again be invoked. The cyanide applied to the stolon here has not the 3h advantage in time over the $^{14}CO_2$; yet inhibition is almost as great as in the case where there is this advantage and in addition the cyanide is applied directly to the sink (fig 9). If the sink region is the only locus of inhibition this result seems quite inexplicable.

That cyanide inhibition is reversible is fairly obvious from the data of figs 12 & 13. There is a steady recovery of the system up to 24h after removal of the cyanide; at this point recovery is virtually complete. Cyanide escapes fairly rapidly from the stolon (fig 14); at 2h it has fallen by nearly 50%, and after 24h to about 1/30th. This corresponds well with the recovery data. No measurements of velocity were made on the stolon after recovery, but the fact that the total time allowed for assimilation and transport was held constant at 4h enables a direct comparison to be made with the corresponding curve of fig 4 in a previous paper (Qureshi & Spanner,

1972b) and this indicates substantial normality.

The question of whether cyanide causes the deposition of callose on the sieve plates seems to be answered in the negative. One cannot attribute inhibition, it would seem, to this cause; and a priori it would seem rather unlikely that a metabolic inhibitor of this type would promote such an energy-requiring response. Of course callose formation is not the only conceivable type of blockage, and it is hoped in future work to institute a more thorough ultrastructural study of the effect of cyanide on the stolon to see if there is any other form of obstruction. Meanwhile the most likely interpretation of the present work is that the activity of cyanide is being exercised by an inhibition of metabolism in the region of the conduits. As noted in an earlier paper (Qureshi & Spanner, 1972a) the fact that tracer does not leak out and accumulate at the entry to the blocked region indicates that the inhibitor hasn't merely destroyed the integrity of the sieve tube membranes. For these reasons, therefore we must conclude that the present results are highly inimical to the Münch hypothesis.

References

Crafts, A.S., Crisp, C.E.: Phloem transport in plants
San Francisco: Freeman 1971

Duloy, M., Mercer, F.V., Rathgeber, N.: Studies in translocation III The cyto-physiology of the phloem of Cucurbita pepo. Aust.J.Biol.Sci. 15, 459-467, 1962

Ho, L.C., Mortimer, D.C.: The site of cyanide inhibition of sugar translocation in sugar beet leaf.
Can.J.Bot. 49, 1769-1775 (1971)

Qureshi, F.A., Spanner, D.C.: Unidirectional movement of tracers along the stolon of Saxifraga sarmentosa.
Planta (Berl.) 101, 133-146 (1971)

Qureshi, F.A., Spanner, D.C. : The effect of nitrogen on the movement of tracers down the stolon of Saxifraga sarmentosa with some observations on the influence of light.
Planta (Berl.) In the press. (1972a)

Qureshi, F.A., Spanner, D.C.: Movement of ^{14}C-sucrose along the stolon of Saxifraga sarmentosa.
Planta (Berl.) In the press (1972b)

Ullrich, W.: Zur Sauerstoffabhängigkeit des Transportes in den Siebröhren.
Planta (Berl.) 57, 402-429 (1961)

Ullrich, W.: Über die Bildung von Kallose beieiner Hemmung des Transportes in den Siebröhren.durch Cyanid.
Planta (Berl.) 59, 387-390 (1963)

Willenbrink, J.: Über die Hemmung des Stofftransports in den Siebrohren durch lokale inaktivierung verschiedener Atmungsenzyme.
Planta (Berl.) 48, 269-342 (1957)

Willenbrink, J.: Zur lokalen Hemmung des Assimilat-transports durch Blausaure.
Z.Pflanzenphysiol. 55, 119-130 (1966)

Walenbrink, J.: Einige Beziehungen zwischen Stoffwechsel und Ferntransport im Phloem.
Vortrage Gesamt, Bot. 2, 42-49 (1968)

Sub-section: (iii)

The influence of 2,4 dinitrophenol or The influence of dinitrophenol on phloem transport along the stolon of Saxifraga sarmentosa.

The Influence of Dinitrophenol on Phloem Transport along the Stolon of *Saxifraga sarmentosa*

Summary Dinitrophenol in concentrations of 5×10^{-3} M applied to the centre 30 cm of 60-70 cm stolons of *Saxifraga* produces a strong and reversible inhibition of the phloem transport of ^{137}Cs or ^{14}C-assimilates. There is every reason to believe that this effect is localised in the sieve tubes; callose formation does not occur. This evidence is very difficult to reconcile with the Münch hypothesis; it seems on the contrary to demand a theory of active pumping.

Introduction

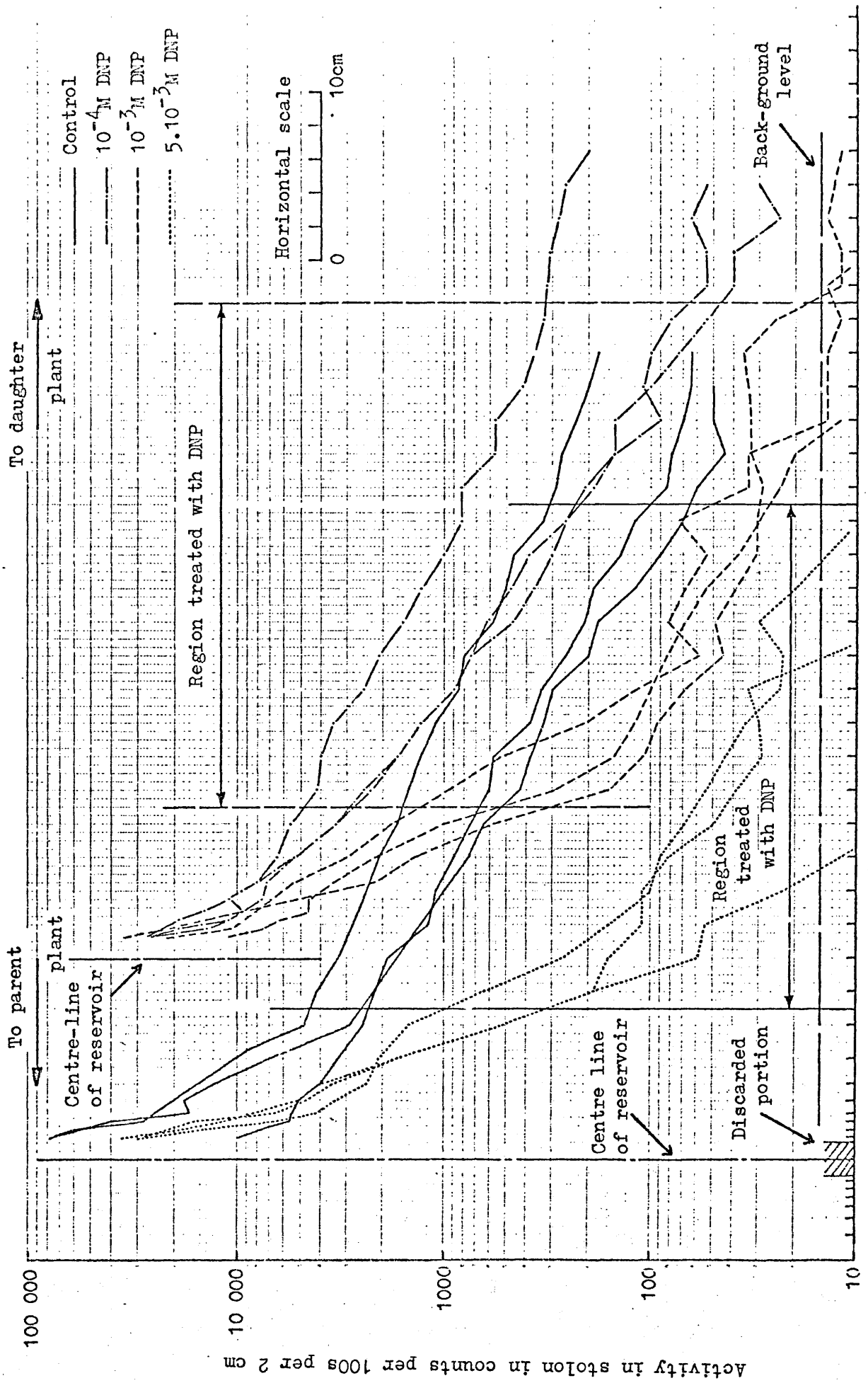
The work reported in this communication is the third in a series dealing with the effect of metabolic inhibitors on phloem transport in the stolon of *Saxifraga*. Both anoxia and cyanide have been found to exert a strong and reversible effect on the sieve tubes (Qureshi & Spanner, 1972 a, c). Careful earlier work with these and other inhibitors (see Willenbrink, 1968) had already reached similar conclusions, although it had been challenged; but in the case of 2,4 dinitrophenol (DNP) the evidence rested on a less solid foundation, Willenbrink reporting that he "could obtain no satisfactory result" with it, and Harel & Reinhold (1966) in an interesting paper concluding that any "inhibitory

effect of DNP .... was probably due to an effect on uptake and/or secretion into the sieve tubes, not to an effect on the conducting cells themselves". Indeed, in their work DNP appeared to actually promote downward transport; and the absence of a definite inhibitory effect on the sieve tubes appeared to them "to exclude the close participation of ATP-dependent processes" at this locus. This would be a most important conclusion if it were inescapable; however, Harel & Reinhold's work leaves open other possibilities, and it was therefore very desirable to re-investigate the action of DNP in circumstances enabling a clearer indication of its behaviour and locus of action to be obtained. The present paper reports some preliminary work to this end.

Materials and Methods

These have been adequately described in previous papers (Qureshi & Spanner, 1971, 1972 a,b,c). In the earlier stages of the work DNP was applied to portions of the stolon only 2 cm long. Results were slight and erratic ; and as in the case of cyanide treatment it was decided to expose longer lengths to the inhibitor. Eventually 30 cm was fixed on, and plants were therefore selected which had particularly long stolons (60 to 70 cm). The inhibitor was made up in 0.05M phosphate buffer of pH7.2, and applied to the stolon by running the latter along a groove milled in a perspex block. The tracers were made up in similar buffer and applied in a small reservoir (Qureshi & Spanner 1971, fig 2), while the $^{14}CO_2$ was given to the subtending leaf in a small chamber of flexible transparent plastic. On termination of the experiment the stolon was cut up as indicated on the horizontal axes of the diagrams; the ^{137}Cs was assayed under an automatic end-window

Fig 1 Unidirectional movement of ^{137}Cs down stolon of Saxifraga under the influence of DNP applied continuously to a 30cm zone. DNP applied 4h prior to ^{137}Cs; total duration of experiment 22h. Curves for lower concentration have been displaced horizontally for clarity.


To daughter plant
To parent plant
Centre-line of reservoir
Region treated with DNP
Control
10^{-4}M DNP
10^{-3}M DNP
5.10^{-3}M DNP
Horizontal scale
0
10cm
Back-ground level
Region treated with DNP
Centre line of reservoir
Discarded portion
100 000
10 000
1000
100
10
Activity in stolon in counts per 10s per 2 cm
Mid-points of segments of stolon

counter and the ^{14}C in an automatic liquid scintillation counter (for details see Qureshi & Spanner 1972 a,b).

Callose was examined by plunging the stolon quickly into acetic alcohol (Eschrich & Currier, 1964) at -20°C and leaving it at this temperature for 24h before processing for electron microscopy. Autoradiographs were prepared in the conventional manner after heat-or freeze drying.

Plants were in continuous light during the experimental period, either daylight or (at night), low pressure mercury illumination of 3750 lux. They were maintained otherwise under ordinary greenhouse conditions, with a temperature ranging from 15° to 25°C depending on the season. The daughter plants were enclosed in small black polythene bags internally dampened to reduce both transpiration and photosynthesis.

Results

Movement of ^{137}Cs

In the earlier work with ^{137}Cs regression lines were fitted by computer to the very straight semi-logarithmic plots. It was the failure to find any relationship between the slope of these lines and the concentration of applied DNP that led to a lengthening of the zone of application to 30 cm. Fig 1 records the experimental results when ^{137}Cs was applied to the stolons between the parent plant and the zone of treatment. Inhibitor treatment was started 4h prior to tracer application, and the duration of the experiment, including this 4h, was 22h. The stolons were then segmented and assayed. It is evident that there is a small effect at $10^{-4}M$ concentration. It increases progressively up to $5 \times 10^{-3}M$. At this level no tracer at all passes through the

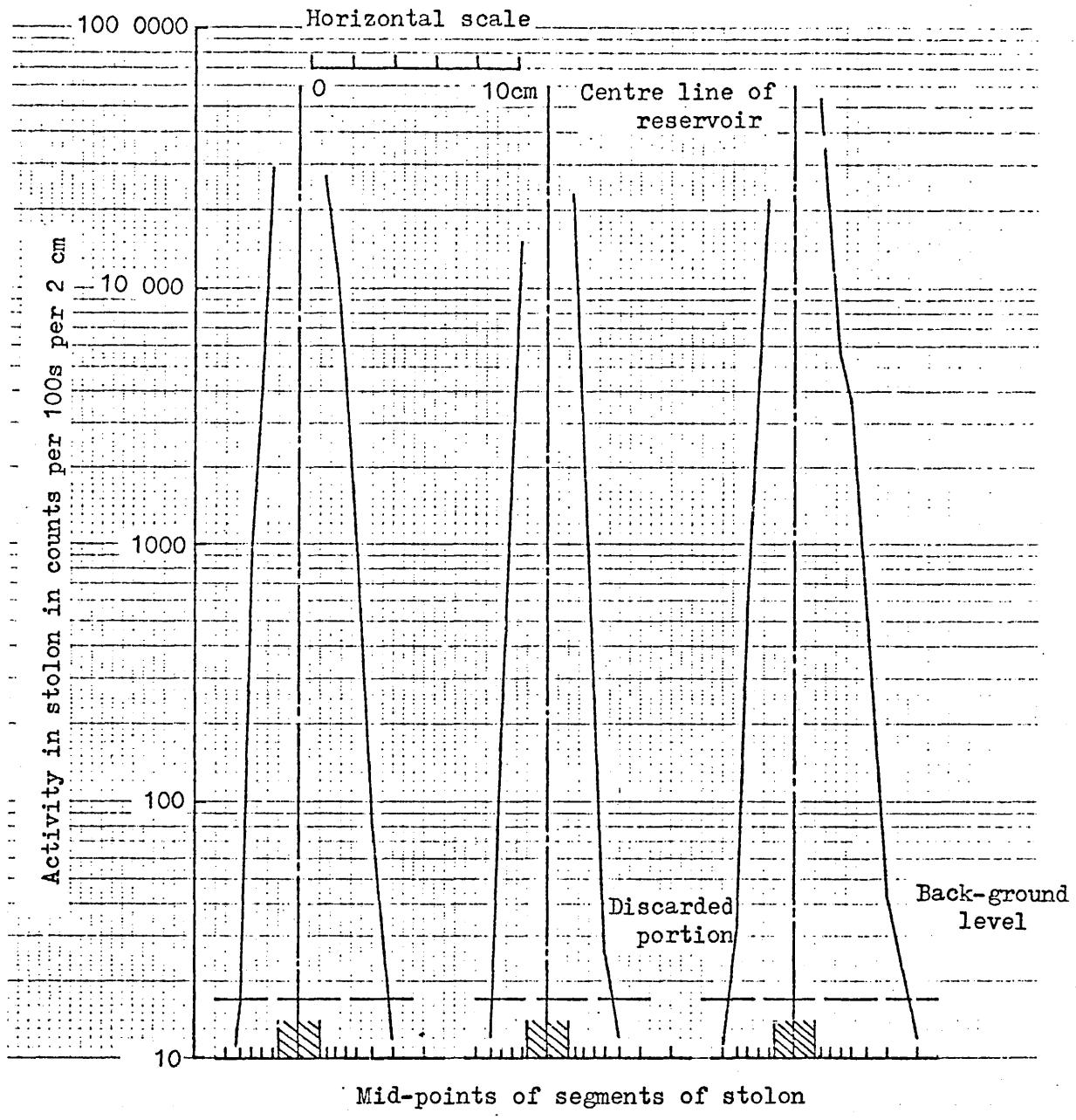


Fig 2 Movement of ^{137}Cs along 40cm lengths of stolon excised at both ends and immersed in 5×10^{-3}M DNP. Treatment started 4h prior to ^{137}Cs application; total duration of experiment 22h. Parent plant to left.

zone of treatment.

In the earlier work (Qureshi & Spanner 1971) it had been found that along with the long distance transport there is always a symmetrical short-distance spread interpreted as taking place probably in the apoplast. It seemed desirable to see whether this movement was affected by DNP. Accordingly, excised pieces of stolon 40 cm long, with vaseline smeared over the cut ends, were soaked in 5×10^{-3}M DNP for 4h. They were then supported so that a short length at the centre was out of the solution; and to this region ^{137}Cs was applied in the usual way. After a further 18h the stolons were assayed. Fig 2 shows the results. It is evident that the inhibitor has not influenced the movement. This seems to confirm that the spread occurs as suggested in the apoplast.

Transport of naturally-assimilated ^{14}C

The slope of the semi-logarithmic lines probably reflects the balance between lateral leakage of tracer and longitudinal transport. It is known that DNP can affect leakage of ions from parenchyma. However, were the change in slope due to increased leakage this would be revealed by a localised raising of the general level of tracer at entry to the zone of treatment. This clearly did not occur; hence we must conclude that it is principally the longitudinal velocity that is influenced by the inhibitor. Where natural assimilates are concerned the leakage in the Saxifraga stolon is believed to be slight (ibid, 1972 b); hence it was of obvious interest to repeat the pattern of the previous experiment using $^{14}CO_2$. Figs 3a and 3b report the results of a series in which $^{14}CO_2$ was administered to the subtending leaf 4h after treatment with DNP began. After a further 4h the stolons were segmented and assayed. It can be seen that there was a small

Figs 3a,b Movement of ^{14}C-assimilate down stolon treated continuously with DNP over central 30cm zone. DNP applied 4h before $^{14}CO_2$ was given to subtending leaf. Total duration of experiment 8h. Curves separated for clarity.

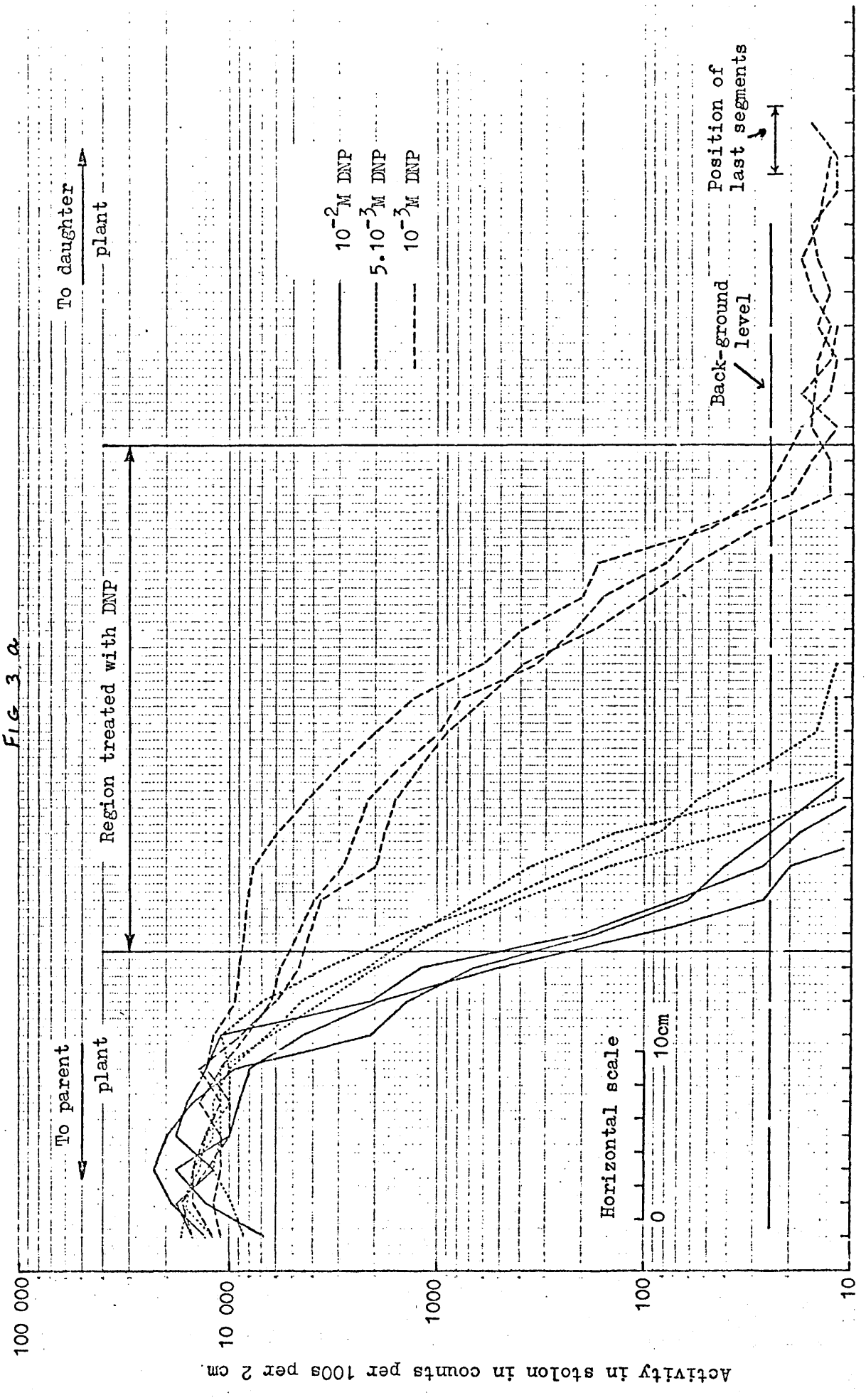


FIG 3 a
To daughter plant
To parent plant
Region treated with DNP
10^{-2}M DNP
5.10^{-3}M DNP
10^{-3}M DNP
Back-ground level
Position of last segments
Horizontal scale
0
10cm
100 000
10 000
1000
100
10
Activity in stolon in counts per 100s per 2 cm
Mid-points of segments of stolon

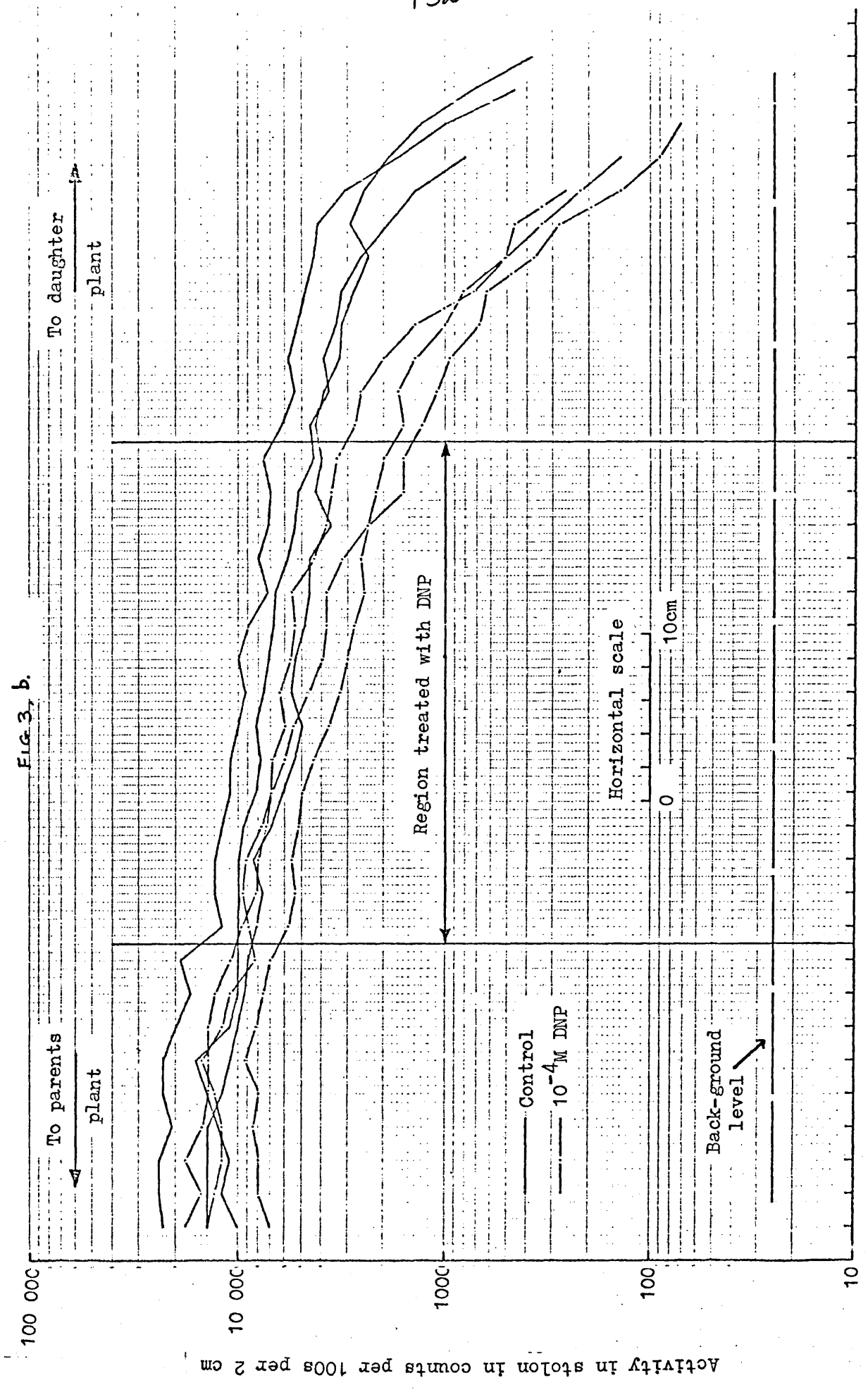
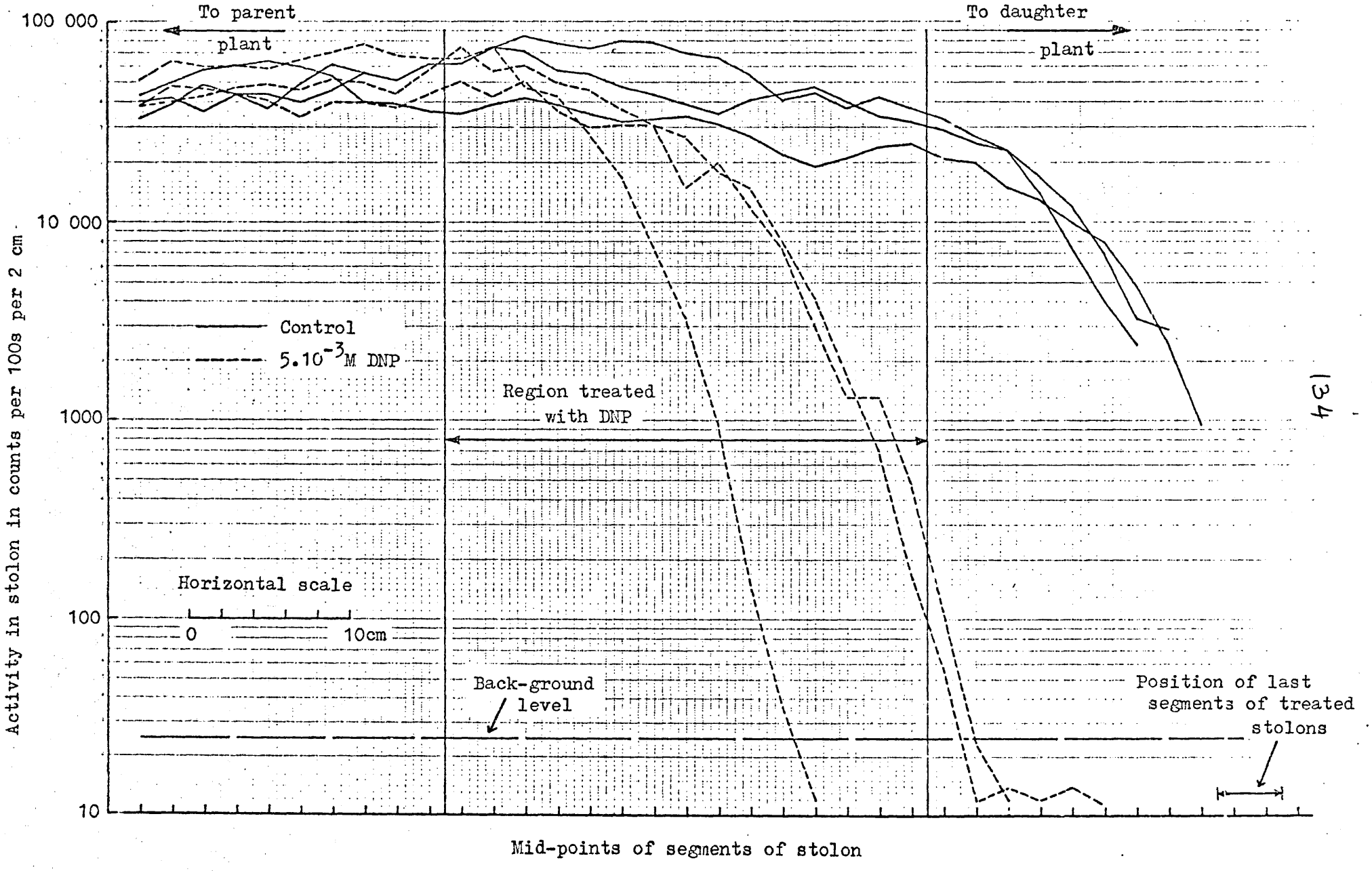

To parents plant
To daughter plant
Region treated with DNP
Horizontal scale
0
10cm
Control
10^{-4}M DNP
Back-ground level
100 000
10 000
1000
100
10
Activity in stolon in counts per 100s per 2 cm
Mid-points of segments of stolon

FIG 3, b.

Fig.4 Details as for fig 3, but $^{14}CO_2$ given at start of treatment with 5×10^{-3}M DNP. Duration of transport as before (i.e.4h).


To parent
plant
To daughter
plant
100 000
10 000
1000
100
10
Activity in stolon in counts per 100s per 2 cm
Control
5.10⁻³M DNP
Region treated
with DNP
Horizontal scale
0
10cm
Back-ground
level
Position of last
segments of treated
stolons
Mid-points of segments of stolon

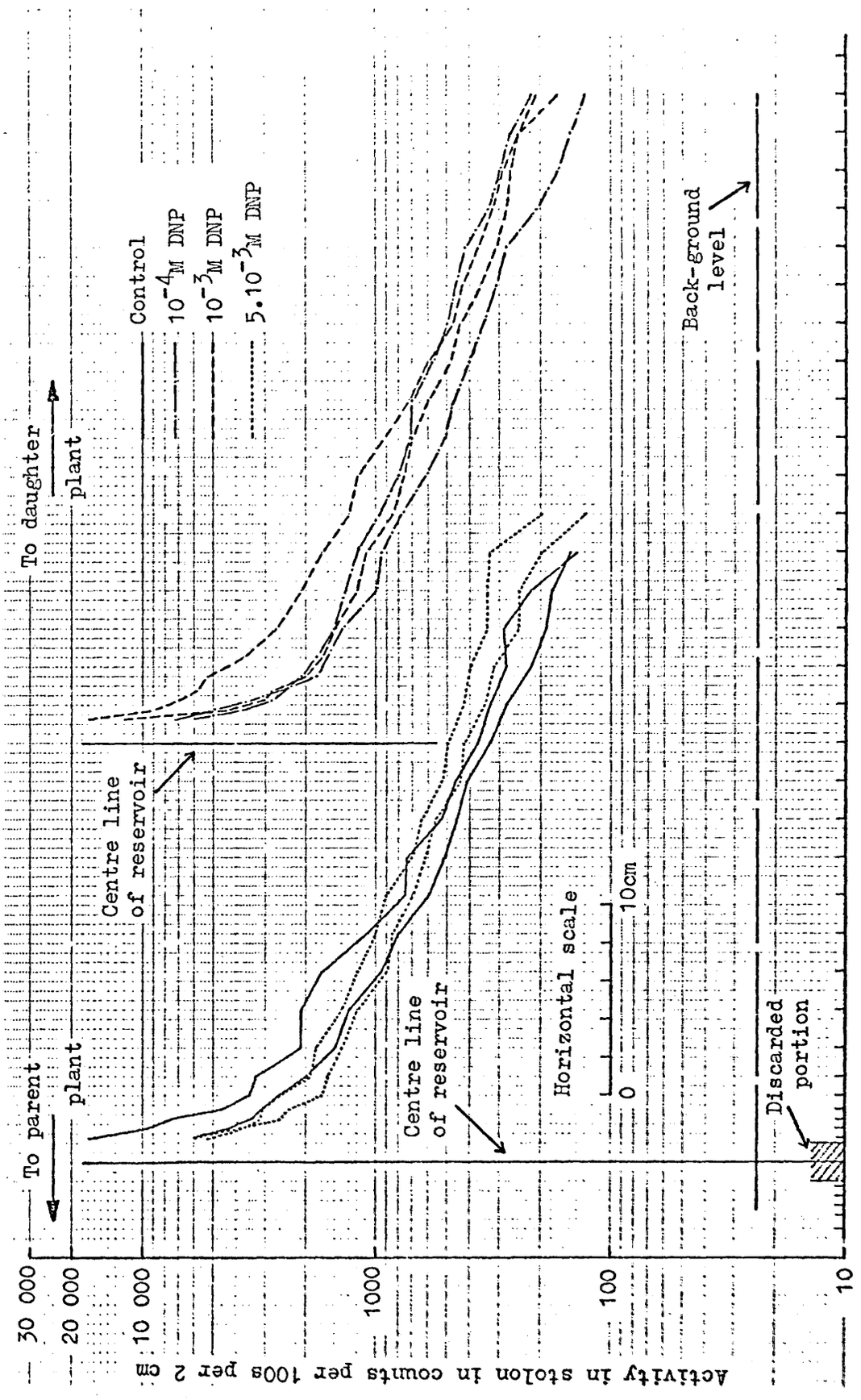
effect with 10^{-4}M DNP. It increased progressively and was extremely pronounced at 10^{-2}M. At this concentration there was occasional evidence of damage to the external tissues. Accordingly treatment was limited subsequently to 5×10^{-3}M.

In order to test how rapidly the poison acts an experiment was run in which the treatment was begun simultaneously with the administration of $^{14}CO_2$. Four hours later the stolons were harvested. It is clear from fig 4 that a somewhat belated inhibition has occurred (cf.fig 3b). However, in view of the general rapidity with which natural assimilate is formed and loaded into the sieve tubes it is still impressive.

Influence of DNP on source leaf

It is very unlikely that any inhibitor reached the parent leaf in these experiments; both the xylem and the phloem movements were in the opposite direction. However, it seemed worth-while to investigate the leaf. In the case of the experiment reported in fig 3b leaves were autoradiographed and nothing abnormal was observed in either the degree of photosynthesis or of vein-loading. In another experiment direct application of DNP was tried. The leaf was closely covered with lens tissue and the latter fed with DNP from a petri dish using a filter paper wick to ensure a slow continuous flow. After 4h the leaf was well washed and ^{137}Cs applied in a small reservoir to the upper surface, midway between the edge and the mid-rib. After a total of 22h the stolons were segmented and assayed. It was found that there was a very low level of activity along the stolon with 10^{-4}M DNP. At 5×10^{-3}M there was virtually none, the little there was being plausibly attributable to xylem movement. Leaves were similarly treated with 5×10^{-3}M solution for 4h, washed and given $^{14}CO_2$. After 4h more

Fig 5 Movement of ^{137}Cs down stolon under influence of DNP applied continuously to subtending leaf. DNP applied 4h before ^{137}Cs; total duration of experiment 22h. Middle curves displaced for clarity.


To parent plant
To daughter plant
Control
10^{-4}M DNP
10^{-3}M DNP
5.10^{-3}M DNP
Centre line of reservoir
Centre line of reservoir
Horizontal scale
0
10cm
Back-ground level
Discarded portion
Activity in stolon in counts per 100s per 2 cm
30 000
20 000
10 000
1000
100
10
Mid-points of segments of stolon

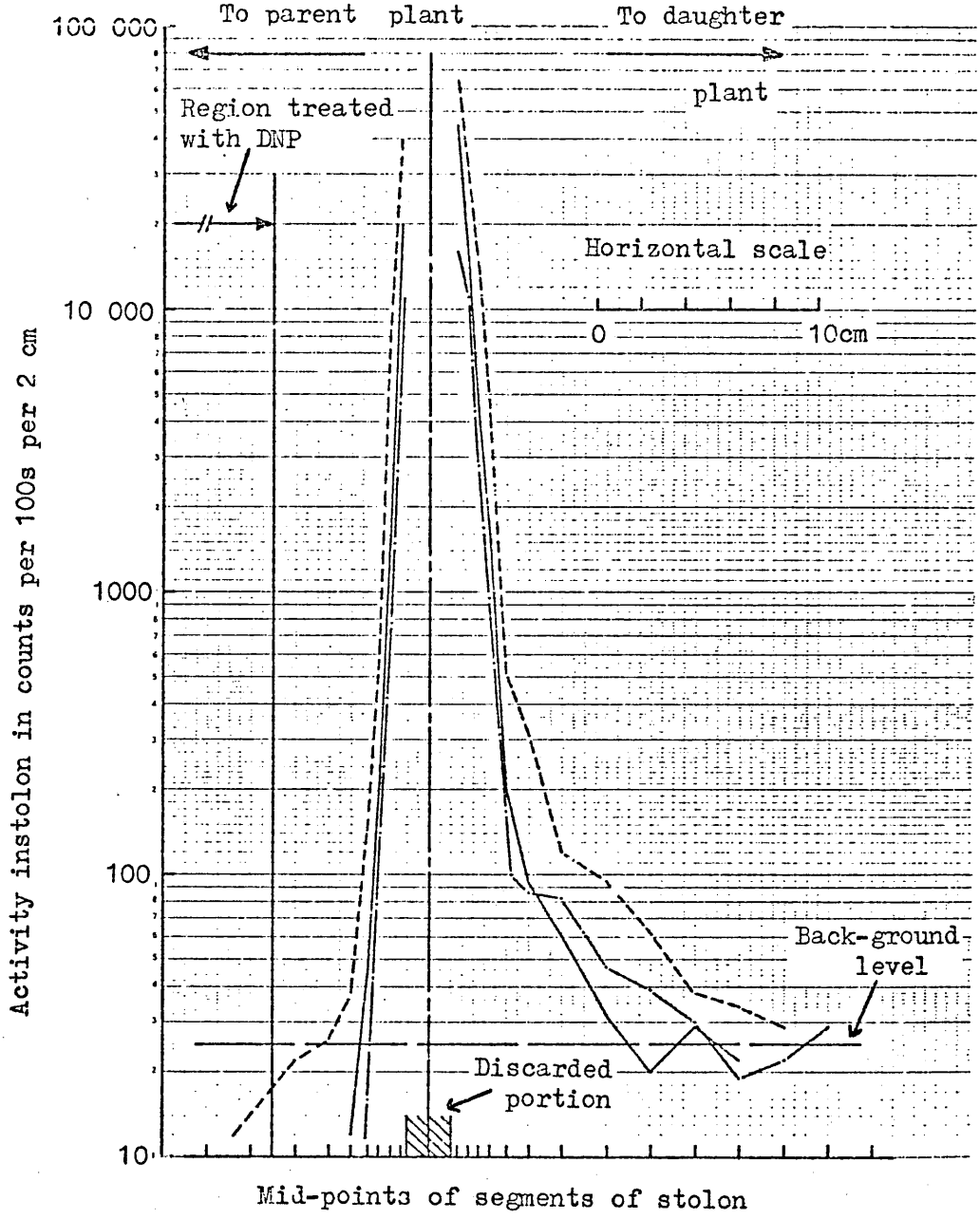


Fig 6 Movement of ^{14}C-sucrose down stolon when applied distally to a 30cm zone treated continuously with 5×10^{-3}M DNP. Treatment applied 4h prior to sucrose; total duration of experiment 8h.

they were freeze-dried and autoradiographed. The autoradiographs indicated reduced photosynthesis and very much reduced vein and petiolar loading.

Under similar conditions of leaf treatment to the above the results of applying ^{137}Cs not to the lamina but to the stolon was surprising. The leaf was treated continuously with DNP throughout the 22h of the experiment. Four hours after the treatment began ^{137}Cs was applied to the stolon about 15 cm from its base. Fig 5 shows the results after a further 18h. Transport surprisingly seems to be quite unaffected. The leaf can hardly have recovered under treatment, and it seems more probable that the task of supplying the daughter plant had been taken over by other leaves. If so, it indicates strikingly the influence of the sink, and the ability of the plant to rearrange its translocation pattern under locally adverse conditions.

Influence of DNP on sink

Of more interest is the possible extent in the foregoing experiments of the influence of DNP on the daughter plant, since to some extent the poison must reach it. Radioactive DNP being unavailable this was investigated in two ways. In the first a 30 cm length of the stolon was subjected to 5×10^{-3}M DNP. After 4h from the start of treatment, ^{14}C-sucrose was applied to the stolon at a point distal to the zone under treatment. Four hours later the experiment was terminated. As in the similar experiment on cyanide it is evident (fig 6) that some long distance transport had occurred. This suggests that the transport mechanism beyond the poisoned zone was still intact, and that all it needed was sugar to activate it. In other words any DNP which had reached

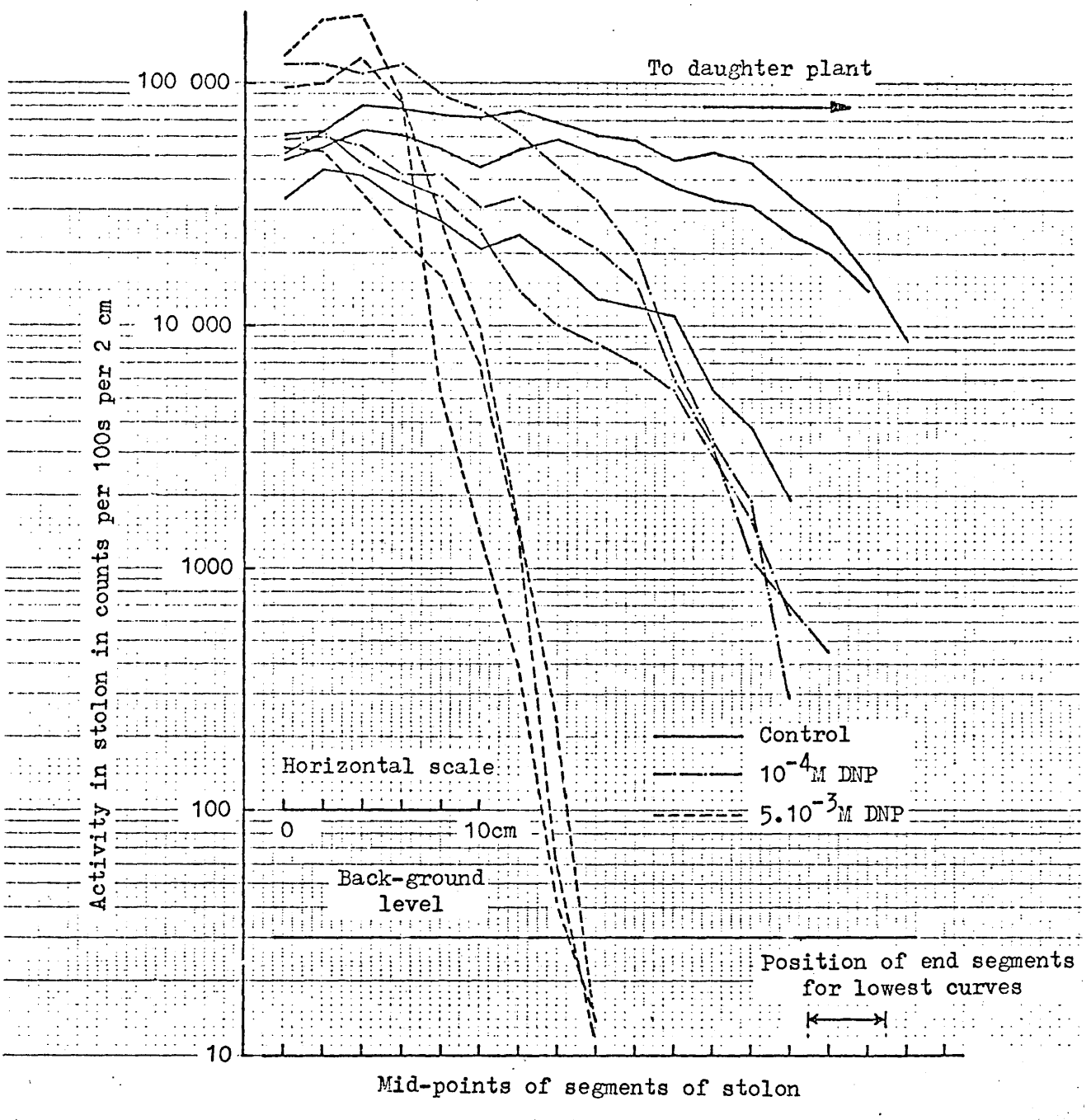
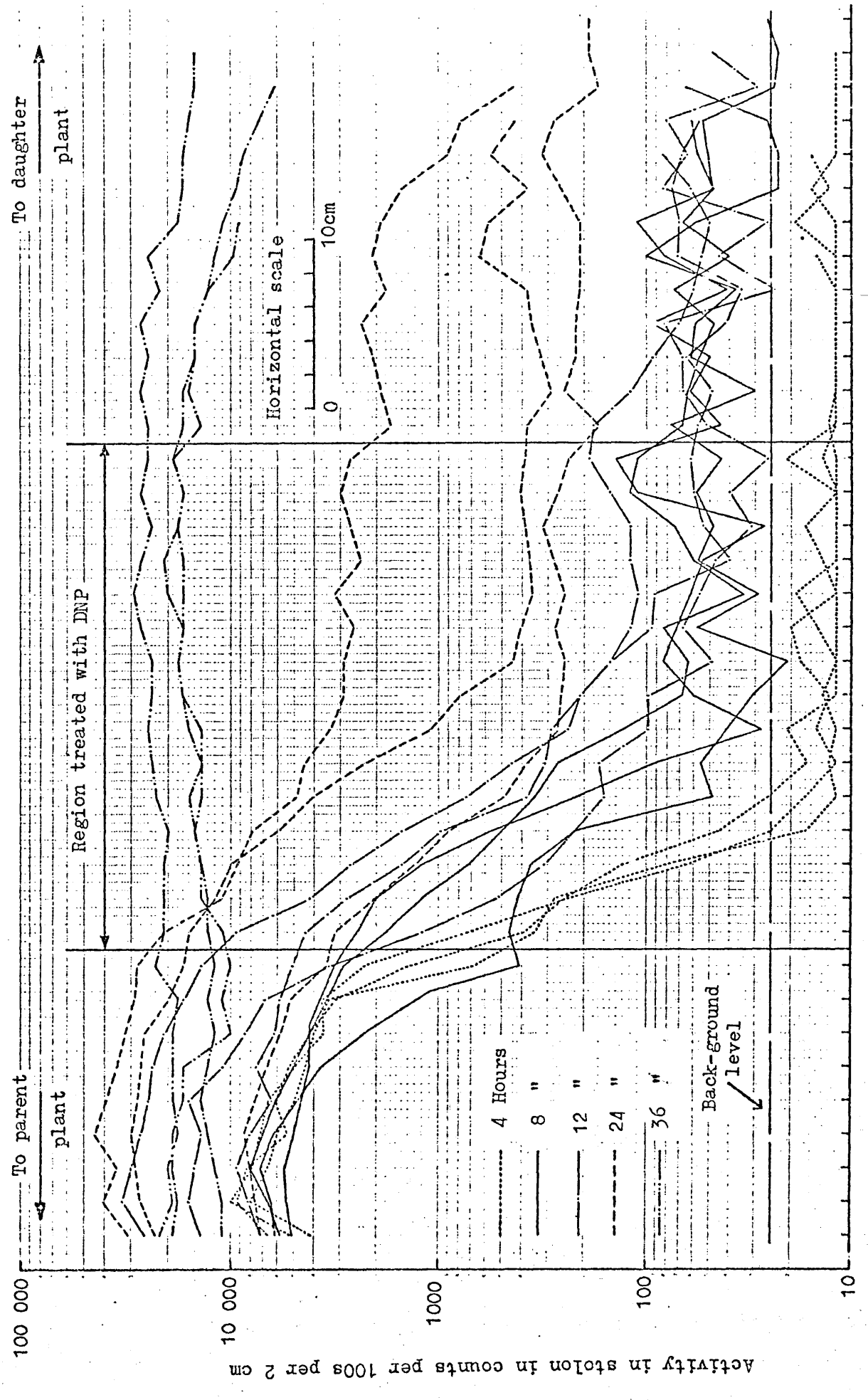


Fig 7 Movement of ^{14}C-assimilate down stolon when daughter plant was treated continuously with DNP.. Treatment applied 4h prior to giving of $^{14}CO_2$ to subtending leaf. Total duration of experiment 8h.

Fig 8 Recovery of transport of ^{14}C-assimilate after 8h treatment of 30cm zone of stolon with 5×10^{-3}M DNP. Times are from cessation of treatment to harvesting. $^{14}CO_2$ given to subtending leaf 4h before harvesting.


To daughter plant
To parent plant
Region treated with DNP
Horizontal scale
0
10cm
4 Hours
8 "
12 "
24 "
36 "
Back-ground level
100 000
10 000
1000
100
10
Activity in stolon in counts per 100s per 2 cm
Mid-points of segments of stolon

this distal region and the sink had not yet attained a significant concentration there.

In the second, DNP was applied directly to the daughter plants. Four hours later $^{14}CO_2$ was given to the subtending leaves, and 4h later again the stolons were harvested . The results (fig 7) show that with 10^{-4}M DNP there was still a considerable degree of transport; even at 5×10^{-3}M it was appreciable considering the inhibitor's 4h start. It would seem therefore that the daughter plant was not extraordinarily sensitive and dominating and that the previous results (e.g.those of fig 3a,b) were not to be attributed to an influence of the translocated poison on the sink. In other words, DNP seems to exert an effect specifically on the sieve tubes themselves.

Is the effect reversible?

To test this, the stolons were treated with DNP for 8h. They were then washed in distilled water and left in light and air. Labelled CO_2 was given to the subtending leaves at such an instant that at the time of harvest each replicate had had 4h to assimilate and transport the tracer. The stolons were harvested at times varying from 4h to 36h after the termination of the DNP treatment. The results are shown in fig 8. They indicate that recovery certainly takes place. It was detectable after 4h, but only complete after 36h. The curves have a peculiar shape; some full-distance transport gets under way very rapidly. The significance of this will be discussed later.

Does DNP promote callose formation?

This pertinent question was investigated by the method

noted earlier. As in the cases of nitrogen-and cyanide treatments (Qureshi & Spanner 1972 a,c) no difference from the controls could be observed. A priori, since DNP interferes with phosphate metabolism,this result was not unexpected. The sieve plate pores, however, as in all the controls, appeared densely filled with P-protein.

Discussion

It is difficult to escape the conclusion that in the stolon of Saxifraga DNP exerts a powerful and reversible inhibitory effect directly on the sieve tubes, and not merely on the terminals; further, that this effect is not due to a mechanical blockage by callose. These conclusions follow from the same arguments as were employed in the cases of cyanide treatment and anoxia (loc.cit.) and there is no need to repeat them. In addition, the shape of the recovery curves in fig 8 supplies an argument not hitherto advanced. It arises from the observation that all the curves reach out horizontally towards the sink end. This appears to indicate that the region of the stolon beyond the zone of treatment either never suffered a loss of transporting power, or else regained it very rapidly; therefore as the 'convalescent' region slowly recovers its ability to deliver assimilate, the latter is carried away by the distal region at unimpaired speed. This is at least a plausible explanation of the shape of the curves; and if accepted it localises the action of the DNP firmly in the zone of treatment.

That this conclusion runs counter to that of Harel & Reinhold (1966) and to a lesser extent to that of Willenbrink (1968), requires some comment. First, however, the interesting question of how the stolon recovers must be raised; for DNP, unlike nitrogen or cyanide, cannot escape by gaseous diffusion. In a personal

communication J.B. Pridham suggested that it might be detoxified by glucosylation. Another suggestion is that it might be involved with the system which metabolises tannin, inclusions of which can often be seen in the phloem cells of the stolon. Whatever the answer, if DNP can be detoxified the concentration it attains will clearly be influenced by the rate at which it is supplied, and if this is inadequate an inhibitory effect may never be exhibited. This may be one explanation of the negative result noticed when the treated zone was only 2cm long; another was suggested in our cyanide paper (1972 c).

The fate of DNP needs further investigation; but clearly here is a consideration which may influence the interpretation of results. Of course, metabolism is not the only way in which the poison may be removed; the transport process itself is another. If the poison slows up both modes of its own removal the level it attains will respond rather sensitively to the rate of supply, by a feed-back mechanism; and inadequate means of applying it may produce much less than the expected result. Perhaps that is why Willenbrink's results with this inhibitor were unsatisfactory.

Harel & Reinhold applied DNP of up to 5×10^{-3}M strength to the cut petioles of the primary leaves of Glycine max. This would introduce it into the xylem, but at an uncontrolled rate, which at least in the experiment of their Table 4 would be low. Harel & Reinhold's conclusions are questionable. The data of their Table 2 show a high import of labelled sucrose from the terminal leaflet into the lateral ones. In a leaf mature enough to export strongly this is unusual, and suggests xylem movement, a possibility their description of method of application is not full enough to rule out. Again, they severed the terminal leaflet in full export, at the instant when DNP was applied to the primary petioles; on

the premise of the Münch hypothesis, which they appear to support, the severance of the central vascular bundles would bring the export process in them to an abrupt halt. Of course there remained the bundles to the lateral leaflets; but if these leaflets had enough sink potential to import as much as they did, why should they turn suddenly to rapid export? Finally, they suggest three explanations for their observation that DNP appears actually to promote downward translocation; and one of these is readily compatible with the view that ATP participates in pumping processes in the sieve tubes. Everything considered, therefore, their evidence is hardly in open conflict with that presented in this paper.

We conclude therefore that our results are antagonistic to the Münch hypothesis and favour a theory of active mass flow, such as that invoking protein contractility or potassium electroosmosis.

References

Eschrich, W., Currier, H.B.: Identification of callose by its diachrome and fluorochrome reactions. Stain Tech. 39, 303-307 (1964)

Harel, S., Reinhold, L. : The effect of 2, 4 dinitrophenol on translocation in the phloem. Physiol. Plant. 19, 634-643 (1966)

Qureshi, F.A., Spanner, D.C. : Unidirectional movement of tracers along the stolon of Saxifraga sarmentosa. Planta (Berl.) 101, 133-146 (1971)

——— ——— : The effect of nitrogen on the movement of tracers down the stolon of Saxifraga sarmentosa, with some observations on the influence of light. Planta (Berl.) in the press (1972a)

——— ——— : Movement of ^{14}C-sucrose along the stolon of Saxifraga sarmentosa. Planta (Berl.) in the press (1972b)

——— ——— : Cyanide inhibition of phloem transport along the stolon of Saxifraga sarmentosa. Planta (Berl.) submitted (1972c)

Willenbrink, J. : Einige Beziehungen zwischen Stoffwechsel und Ferntransport im Phloem. Vort.Gesamt. Bot. 2, 42-49 (1968)

Sub-section: (iv)

Experiments with valinomycin

or

The effects of valinomycin on the transport of ^{14}C-assimilates in plants.

The Effects of Valinomycin on the Transport of ^{14}C-assimilates in Plants

Introduction:

The electro-osmotic theory proposes a circulation of K^+ at each sieve plate associated with a difference in the electro-chemical potential of K^+ across the plate. As the suggested circulation of K^+ is regarded as caused by an active transport taking place across the plasmalemma of the sieve tube. Fig. 1 presents a theoretical scheme of the circulation of K^+ around the sieve plate. If this is the governing mechanism in phloem transport then it would clearly be interesting to find out the effects of upsetting the K^+ circulation on the transport of assimilates.

Some macrocyclic compounds are now known to greatly facilitate passive ion permeation in biological membranes, i.e., to render them extremely "leaky". Tosteson (1968) has reported the comparative effects of various such compounds (Fig. 2) on the electrical resistance of lipid bilayer membranes. Valinomycin, an antibiotic, is one of the three compounds which has the most marked affinity for K^+. This compound forms a complex with K^+ ion, enormously increasing its partition coefficient in lipid phases; thus it causes a marked rise in the selective permeability of the membrane for K^+ ions. On the basis of this property Valinomycin was selected in an attempt to upset the K^+ circulation in the sieve tube. Although this compound did not prove an ideal one because of the large size of the molecule (M.Wt. 1111.36) and its very poor solubility in water, the attempts to test its effects on the transport of assimilates are worth reporting.

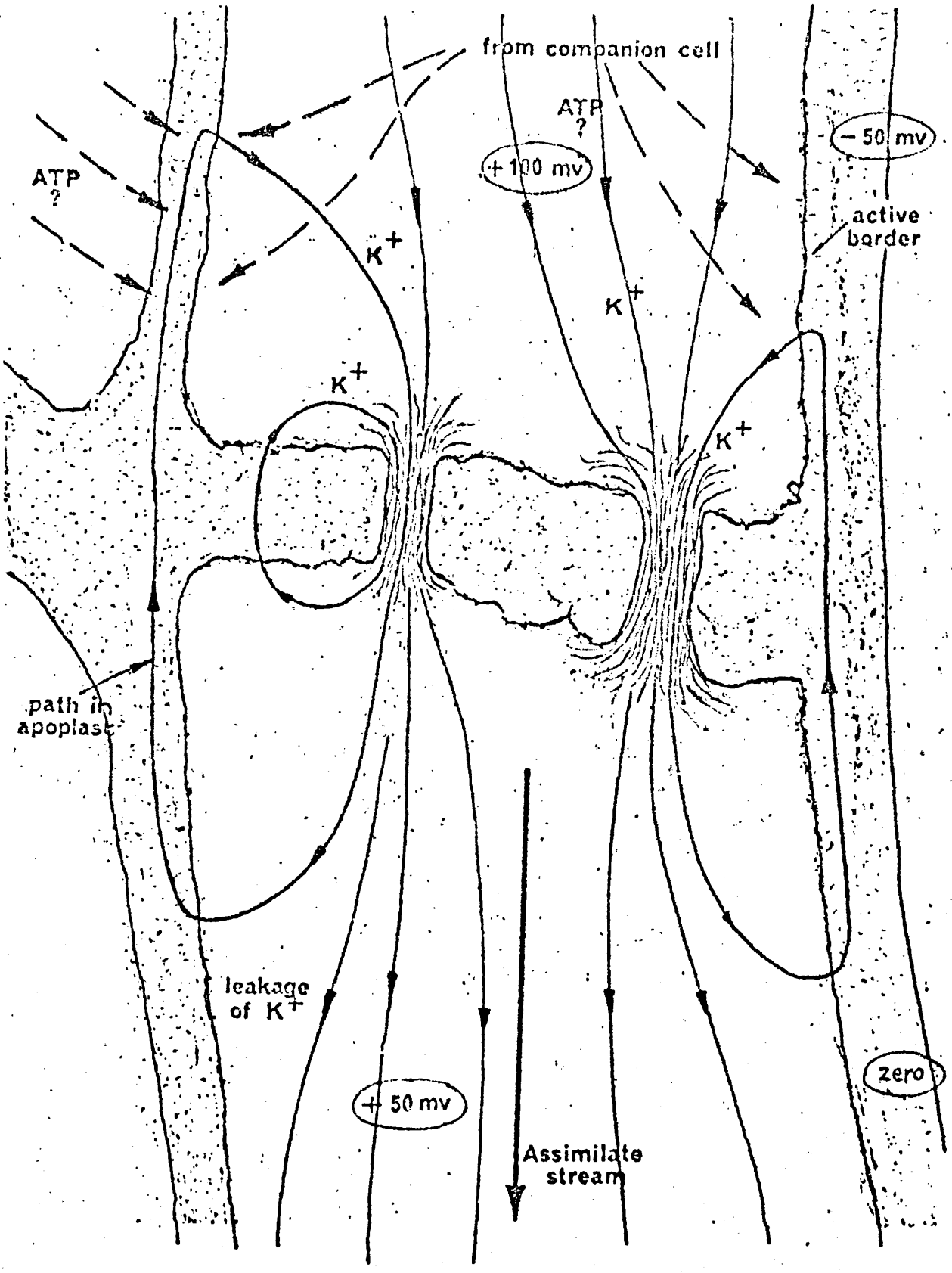


Fig 1 Suggested scheme for K^+ around the sieve plates (From D.C.Spanner and R.L.Jones 1971 after some modifications)

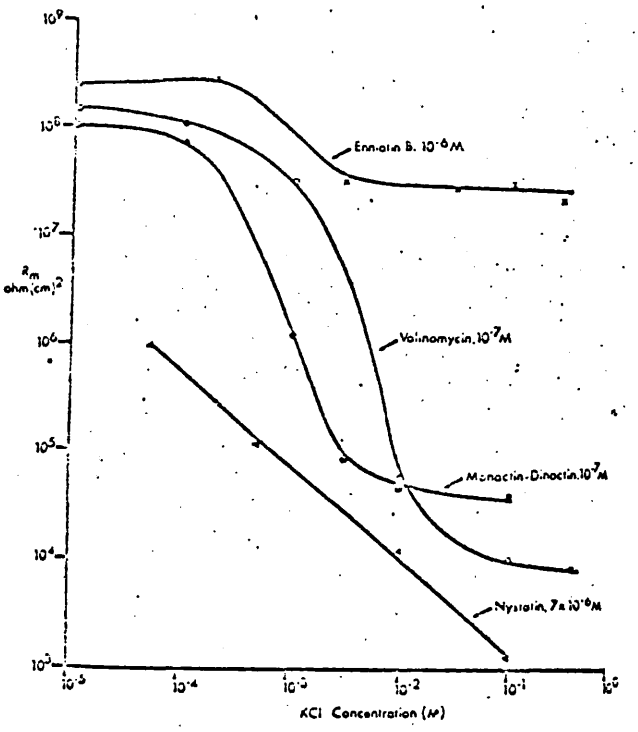


FIG. 2. Effect of macrocyclic compounds on resistance of thin lipid membranes.

Fig 2 Note pronounced effect of Valinomycin in presence of KCl. (From D.C.Tostenson 1968)

Materials and Methods:

Various experimental plants were grown under normal greenhouse conditions and subsequently prepared as follows, the object being to secure the entry of Valinomycin into the plant body. The stolons of Saxifraga were prunned of laterals and the daughter plants were floated on the surface of water in dark chambers. After about 3 weeks, when the daughter plants produced abundant roots, the parent plants were not watered for at least one week. At this stage the plants, having a reversed xylem flow in stolons towards the parent plant and that of phloem towards the daughter plant (Qureshi and Spanner 1971), were used for the experiments. Fully expanded mature leaves of Nymphoides peltatum with long petioles were selected and the cortex was removed from the two sides of the petiole with the help of a double cutting assembly, thus exposing the central vascular tissue. These leaves with attached rootstock were then laid flat in shallow trays of water at least 24 hours before the commencement of the experiment. Plants of maize and barley with at least one fully expanded leaf enclosing another immature leaf was excised just below its node and used as experimental material.

The solution of 5.5 mg of Valinomycin (Messrs. Calbiochem) prepared in 0.5 ml of 100% ethanol, was run with continuously stirring into 500 ml of distilled water in an ultrasonic bath. Sometimes the detergent Brij 35 was added to the distilled water. The final concentration of Valinomycin was 10^{-5}M with 0.1% ethanol and 0.1% Brij 35. About 5 ml of Valinomycin solution were supplied to the shaved petioles of Nymphoides, and 25 ml to the tip-excised roots of the daughter plants of Saxifraga, the decapitated rootstock of Nymphoides or the leaf bases of the

leaves of maize and barley for at least 24 hours before the administration of $^{14}CO_2$ to the appropriate part of the various plants in the manner described elsewhere. Usually 10-40 uCi of radio-active carbon dioxide was provided to each replicate.

At the conclusion of the experiment, the stolons or petioles were harvested, segmented as indicated on the horizontal axes of the figures, and counted by Packards Tri-Carb using the Triton 100-X-toluene scintillator previously described. The leaves of maize and barley were freeze-dried, spread out on a paper and pressed before producing auto-radiographs in the conventional manner.

Results:

In view of the molecular size and insolubility of Valinomycin, it was decided to apply the solution to the daughter plant with detipped roots. It was hoped that the solution would be drawn into the open vessels with the xylem stream and thus reach the phloem tissue. Such a treatment was provided for a lengthen period (about 24 hours) before the administration of $^{14}CO_2$ gas to the subtending leaves of the parent plants of Saxifraga. The stolons were then harvested after a further 4 hours and segmented to be counted for ^{14}C. The results are presented in Fig. 3. Clearly, this reveals no effect of Valinomycin on the pattern of translocation of the ^{14}C-assimilates as compared to the controls.

In an experiment with Nymphoides, Valinomycin was supplied to the shaved petioles (over a 20 cm length) in the manner described earlier for at least 24 hours before the application of $^{14}CO_2$ to the lamina. The $^{14}CO_2$ was supplied for 1 hour. After another 3 hours for translocation, the petioles were harvested and counted with the results shown in Fig. 4. Again, they suggest no difference in the treated plant as compared to the controls.

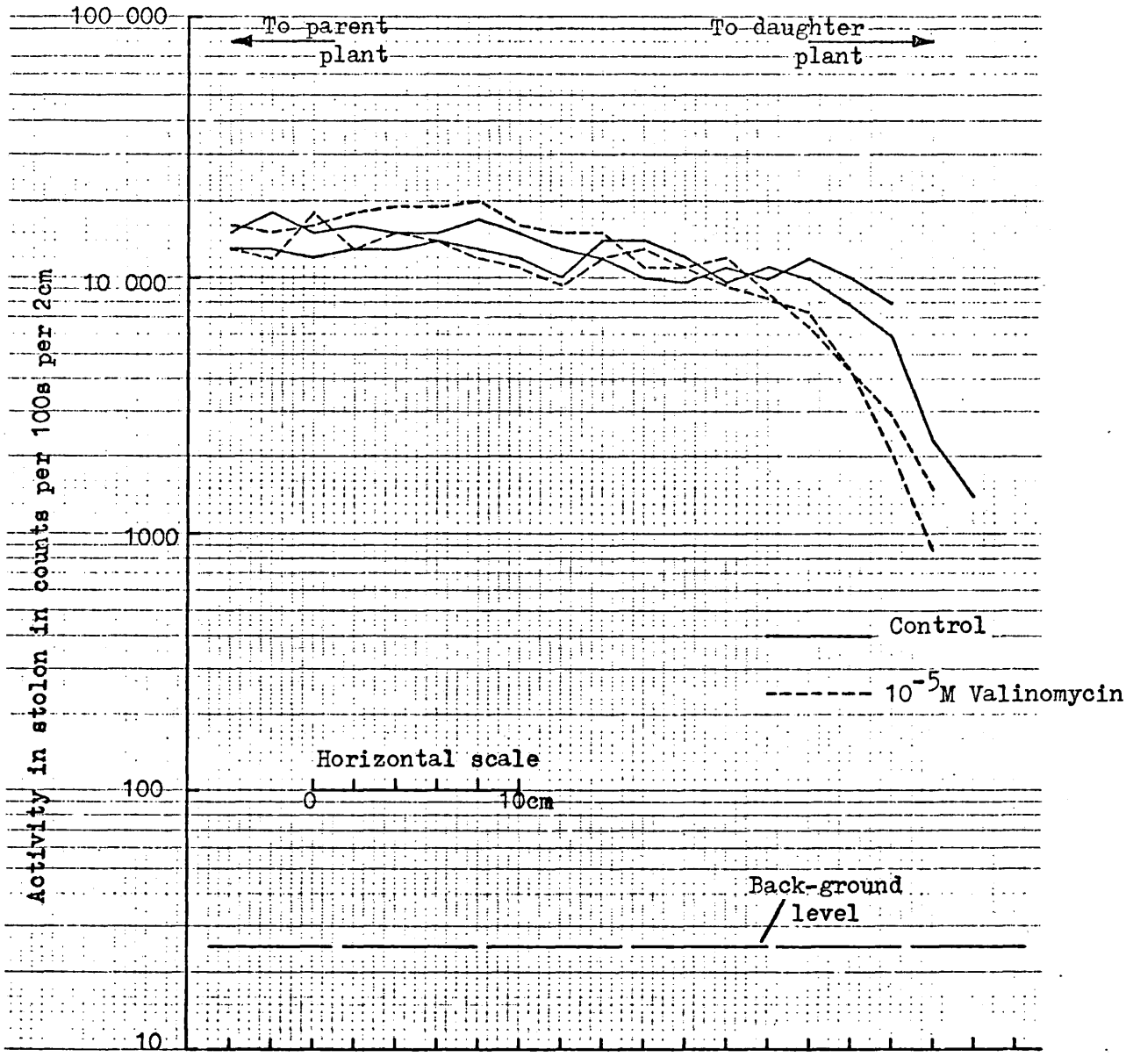


Fig 3 Movement of ^{14}C-assimilates down the stolon when detipped roots of daughter plant were treated continuously with Valinomycin (10^{-5} M). Treatment applied 24h prior to giving of $^{14}CO_2$ to subtending leaf. Total duration of of experiment 28 hours.

Fig 4 Movement of ^{14}C-assimilates down the petiole of Nymphoides with central 30cm immersed in 10^{-5}M Valinomycin. Treatment begun 24h before $^{14}CO_2$ was given to lamina. Total duration of experiment 28 hours.

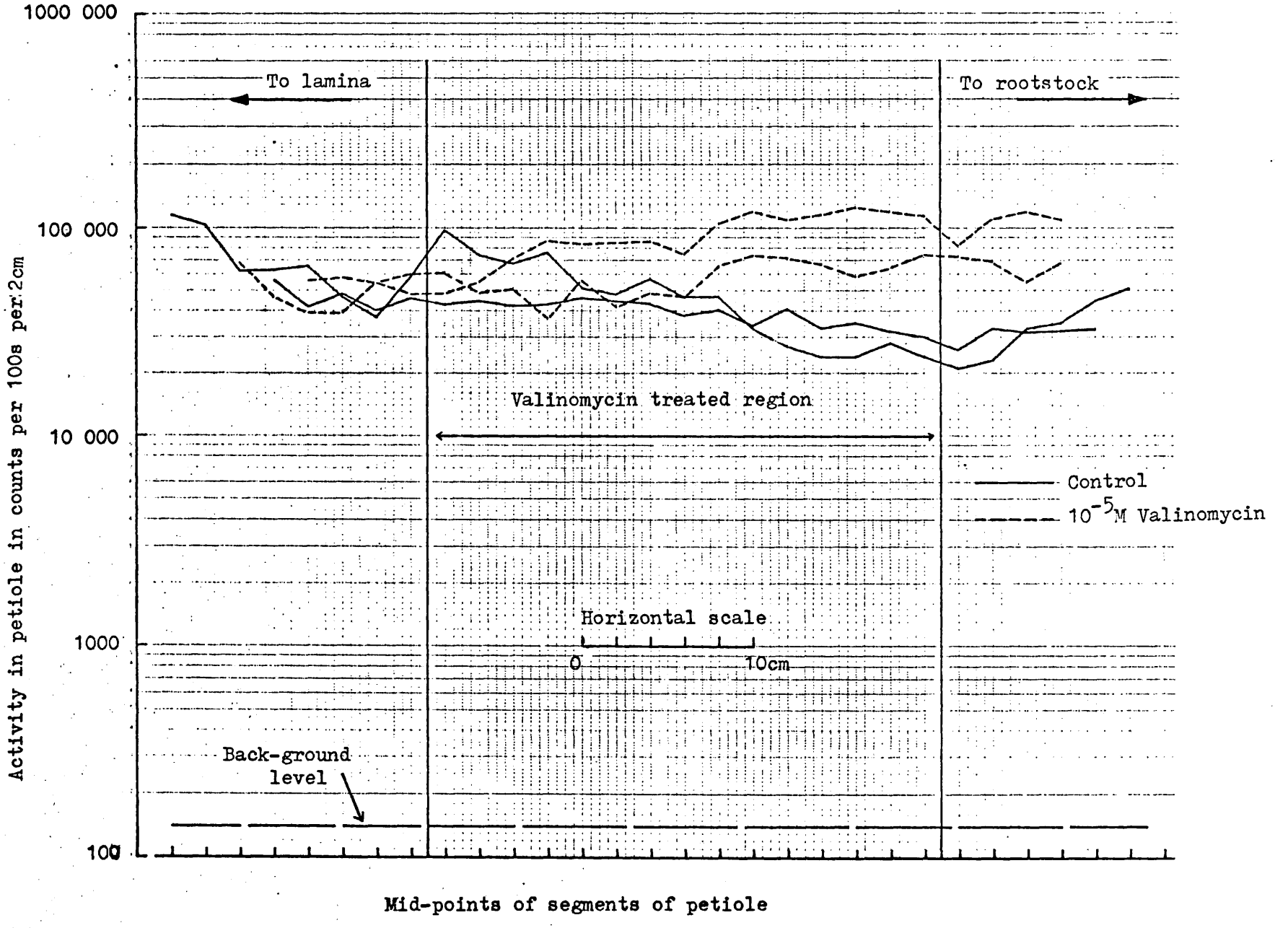
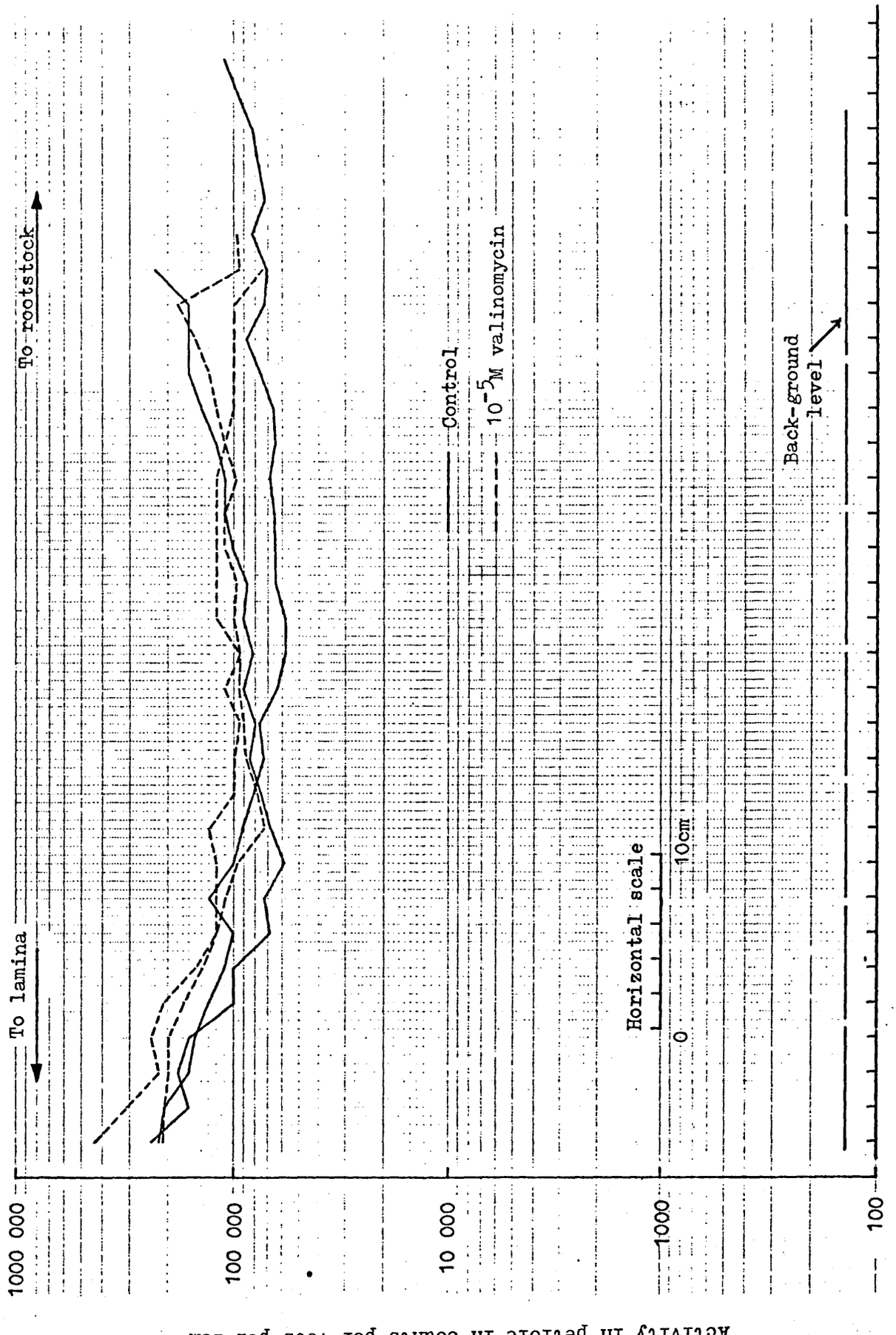

1000 000
100 000
10 000
1000
100
To lamina
To rootstock
Valinomycin treated region
Control
10⁻⁵M Valinomycin
Horizontal scale
0
10cm
Back-ground level
Activity in petiole in counts per 100s per 2cm
Mid-points of segments of petiole

Fig 5 Movement of ^{14}C-assimilates down the petiole when the detipped root stock was treated continuously with Valinomycin ($10^{-5}M$). Treatment applied 24h before the provision of $^{14}CO_2$ to lamina. Duration of the experiment 28 hours.


To lamina
To rootstock
1000 000
100 000
10 000
1000
100
Control
10^{-5}M valinomycin
Horizontal scale
0
10cm
Back-ground level
Activity in petiole in counts per 100s per 2cm
Mid-points of segments of petiole

A further attempt with Nymphoides followed a different pattern making use of the xylem vessels and the xylem flow. Valinomycin solution was supplied to the detipped roots of the Nymphoides plants, the petioles not being shaved. Labelled CO_2 was then supplied to the laminae in the usual way for 4 hours. At the conclusion of the experiment the petioles were segmented and counted and the results are presented in Fig. 5. They clearly are comparable to those presented in Fig. 4.

Finally, in another series of experiments the solution of Valinomycin was supplied to the bases of leaves of maize and barley held in test tubes. After 24 hours labelled CO_2 was provided to the terminal 5-8 cm section of the mature leaves for the usual time (4 h). At the end of the experiment the leaves were freeze-dried and autoradiographs were prepared. The prints of the autoradiographs are presented in Figs. 6 and 7. The treated and the control leaves show more or less the same pattern of distribution of labelled assimilates in the leaf blades and leaf bases, and so again the results must be considered negative.

Discussion:

Valinomycin is lipid-soluble and is reported to "enclose" cations, especially K^+, very efficiently forming charged complexes on which it confers its lipid solubility. These complexes are thus soluble in the hydrophobic interior of the membrane and thus increase enormously the cation permeation. If such a compound does change the selective permeability of the cell membrane, it would, in the case of sieve element, short circuit the mechanism of active influx of K^+ into the sieve tube as is indicated in Fig. 1 on the up stream side. Such a change in ion permeation would prevent the accumulation of K^+ on the up stream side of the sieve tube and destroy the electro-chemical potential gradient across the sieve plate. Under such conditions the

Fig 6 Autoradiograph of frozen dried leaves of barley. Valinomycin (10^{-5}M) was supplied to cut base of leaf 24h before $^{14}CO_2$ (10μCi) was provided to 5cm of apex. Total duration of experiment 28 hours. The region fed with $^{14}CO_2$ was removed before the preparation of autoradiograph. Exposure 4 days.

A= Control
B= 10^{-5}M Valinomycin

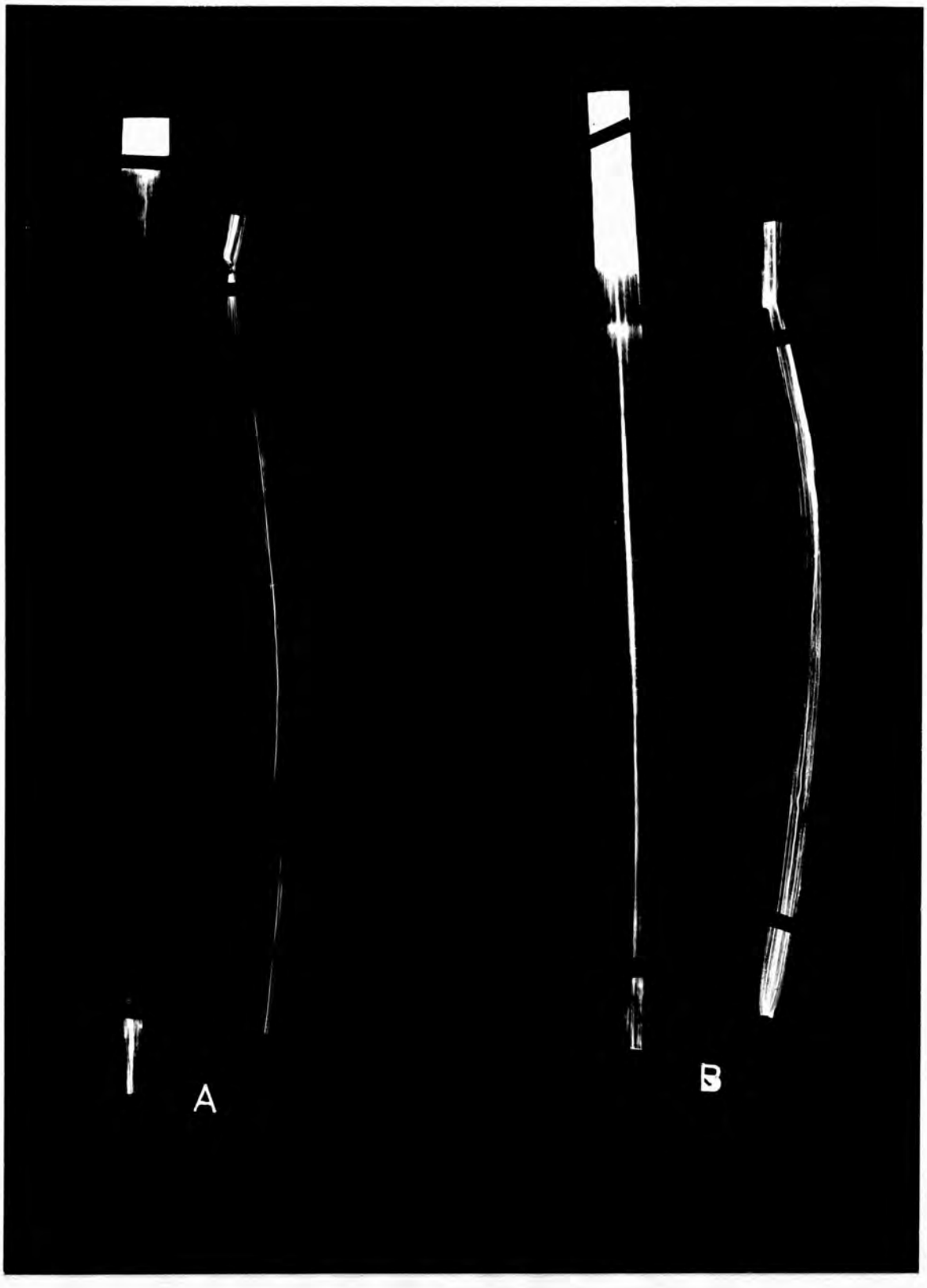

A
B

Fig 7 Autoradigraph of leaves of maize after a similar experiment to that in fig 6. The apex (8cm) was provided with $^{14}CO_2$ (10μCi). Total duration of experiment 28 hours. Exposure 4 days.

A= Control

B= 10^{-5}M Valinomycin


A
B

electro-osmotic mechanism would not be able to operate, causing a possible halt in the transport of assimilates in the sieve elements.

The results presented here do not suggest an effect on the pattern of transport of ^{14}C-assimilates in the four experimental plants. Such results might indicate that K^+ has no role to play in the mechanism of phloem transport; but they are hardly conclusive. The difficulties lie with the large size of the molecule of Valinomycin and especially its property of insolubility in water. This makes it problematical whether or not the antibiotic reached the important site; and if it did, whether its concentration was high enough. Clearly a higher concentration is very desirable, and to achieve this is difficult. Perhaps comprehensive tests with a controlled use of detergents are the best way to approach the problem.

References:

Qureshi, F.A. and Spanner, D.C. - Movement of sucrose-^{14}C along the stolon of Saxifraga sarmentosa. Planta (Berl.) 110, 145-152 (1973).

Spanner, D.C. - The translocation of sugar in sieve tube. J.Expt.Bot., 9(27), 332-342 (1958).

Tosteson, D.C. - Effect of Macrocyclic compounds on the ionic permeability of artificial and natural membranes. Fed. Proc. 27(6), 1269-1277 (1968).

Sub-section: (v)

The effect of inhibitors on sieve plate callose

or

The effects of inhibitors on callose formation in the sieve tubes of Saxifraga sarmentosa.

THE EFFECT OF INHIBITORS ON CALLOSE FORMATION IN THE SIEVE TUBES OF SAXIFRAGA SARMENTOSA L.

ABSTRACT

Electron microscope studies on stolons undergoing transport inhibition under nitrogen, HCN and DNP treatment show no evidence of callose-blockage of the sieve plates. Inhibition must therefore be interpreted in terms of interruption of the sieve tube energy supply; this weights the evidence in favour of theories of active mass flow such as those invoking electroosmosis or protein contractility.

INTRODUCTION

In several recent communications (Qureshi & Spanner 1972 a, b, c) the authors have reported that nitrogen-induced anoxia, cyanide and dinitrophenol exert a strong and reversible inhibitory effect on phloem transport along the stolon of Saxifraga sarmentosa. Further, this effect seems without doubt to be exercised on the sieve tube conduits themselves and not simply on the loading and unloading processes at the terminals; and to be unaccompanied by lateral leakage of the tracers used — assimilated ^{14}C and ^{137}Cs among others — in the region of the inhibited zone. This evidence is very damaging to the pressure-flow hypothesis, for the failure to transport longitudinally cannot simply be explained by damage to

the sieve-tube membranes in the inhibited region. However, the possibility is still open of believing that it might be due to physical blockage of the sieve tubes, a possibility which would not be inconsistent with the pressure-flow theory. The most likely method of blockage involves the callose mechanism. Thus it remained desirable to investigate the state of the sieve plates in the inhibited stolons, more so since it is generally realised (for references see Crafts & Crisp, 1971) that a variety of physical and chemical agencies have been shown to promote callose deposition. It is true that there have been reports (Eschrich et al, 1965; Webster & Currier, 1965) that callose formation has no effect on the transport of assimilates; but this view, a priori unlikely as a complete statement, has been controverted by later work (McNairn & Currier, 1968). In any case, were it true, it would itself deny the possibility just noted, i.e. that the inhibitory effects were to be explained by callose blockage and not by interference with the metabolic energy requirement of sieve tube pumps. If true it would therefore render the present work merely redundant.

MATERIALS AND METHODS

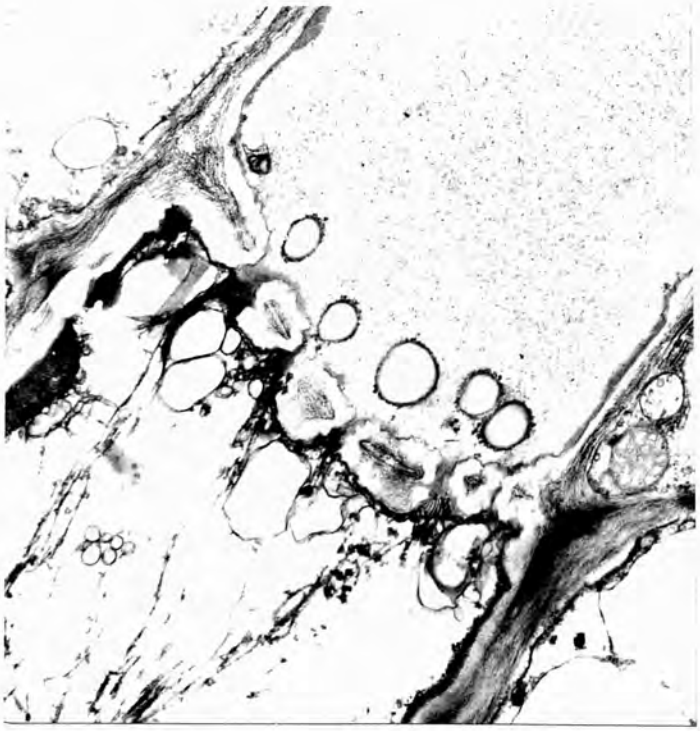
The anatomical work was planned to be parallel with the physiological experiments; reference to the previous papers will therefore provide details of the treatments to which the plants were subjected. In view of the small size of the sieve plates earlier efforts to examine them by light microscopy were abandoned in favour of electron microscopy. For fixation a slight variation of the technique of Eschrich & Currier (1964) was used; the whole stolon was dropped into a mixture of 95% alcohol and glacial acetic acid (3:1 $^{v}/_{v}$) at -20°C and kept in this for at least 24h. At the end of

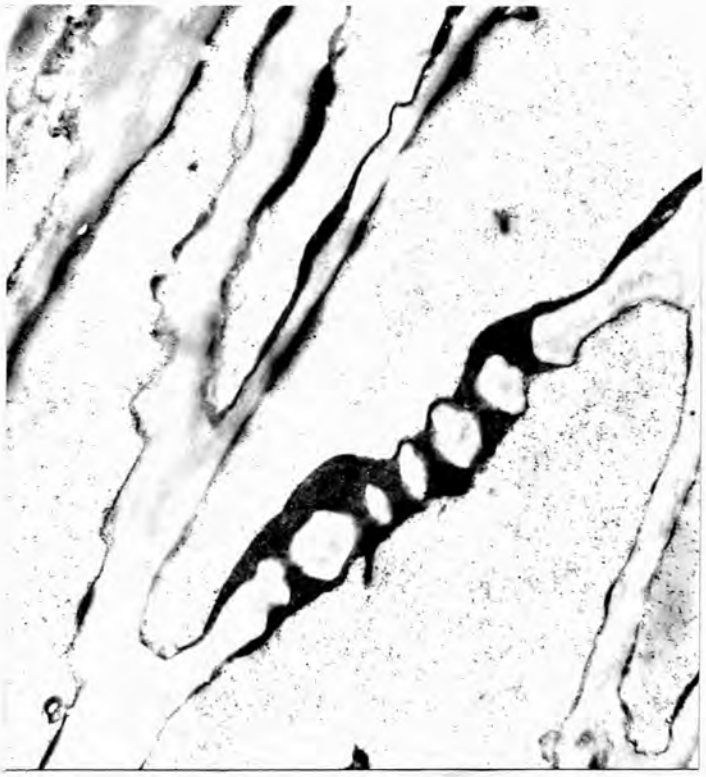
PLATE 1

Longitudinal sections through sieve plates from the stolon of Saxifraga sarmentosa after 20cm lengths had been fixed as described below.

A. Fixed in 6% buffered glutaraldehyde for 4h. Note the thick deposit of callose. The pores contain P-protein.

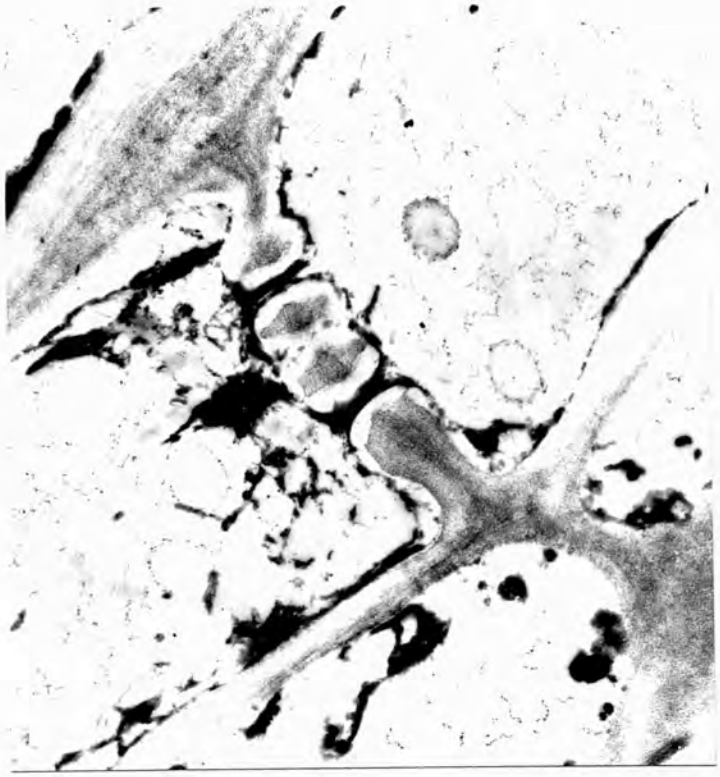
B. Fixed in acetic-alcohol at -20°C for at least 24h. Note absence of callose. Coagulated P-protein fills pores.

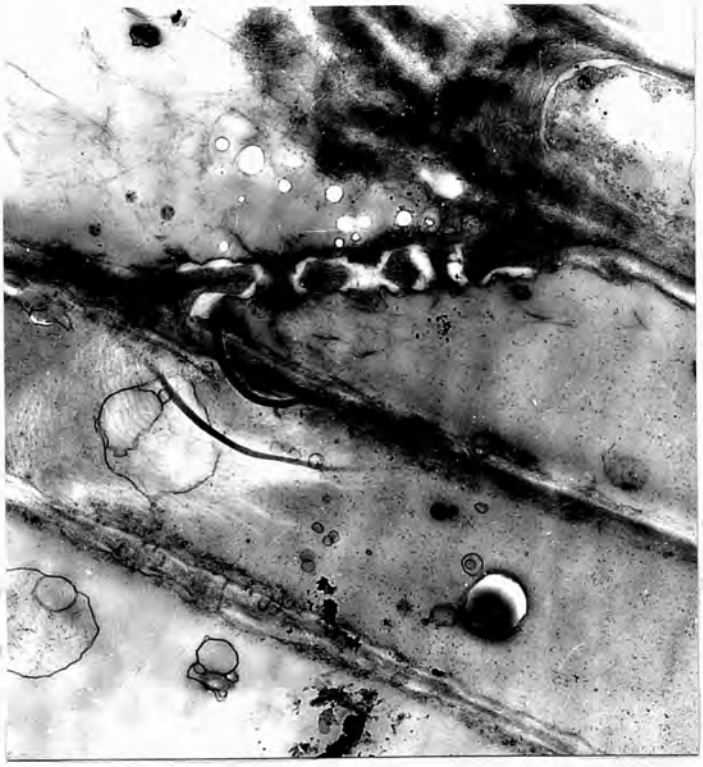




C. Fixed in glutaraldehyde (as A) followed by acetic-alcohol (as B). Note that callose formation is comparable to that in A.

D. Fixed in acetic-alcohol (as B) after treatment with hot water (45°C) for 15 min. Note the fair amount of callose present and the de-natured P-protein in the pores.





this period it was allowed to warm up to room temperature and was then thoroughly washed, dehydrated and prepared for electron microscopy in the usual way. The usual stages of fixation in buffered 6% gluturaldehyde and post-fixing in OsO_4 were included for completeness, sections being examined after staining with lead citrate.

RESULTS

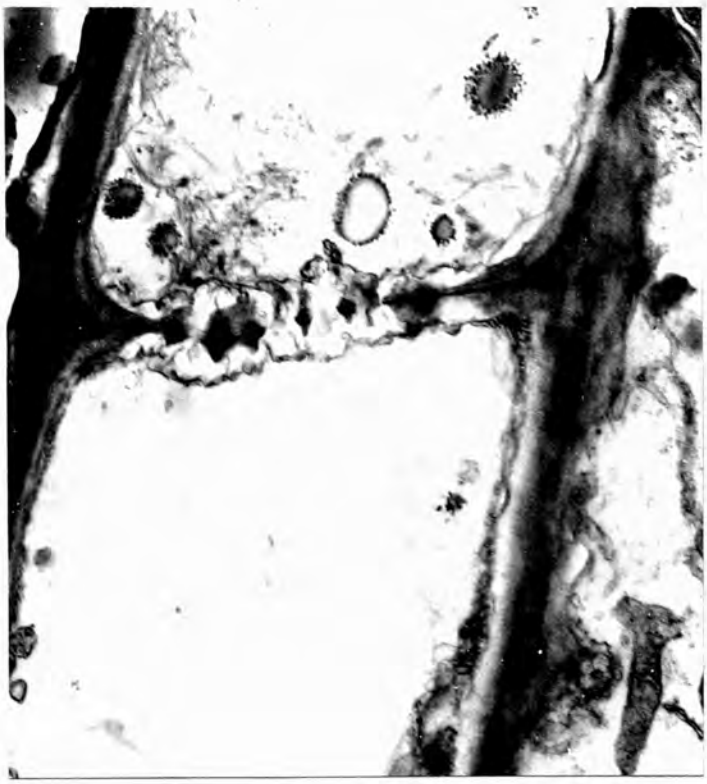
Control treatments

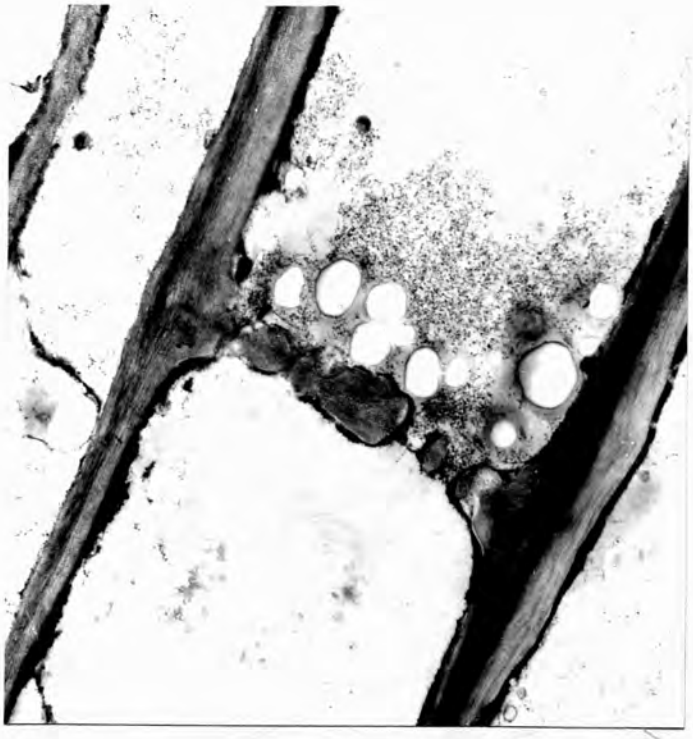
In order to provide a basis for comparison, normally-conducting stolons were fixed in 6% glutaraldehyde at room temperature for 4h, in acetic-alcohol at -20°C for 24h, or in the first followed by the second. The segments immersed were at least 20cm long, i.e. not in such short lengths that the sugar content of the sieve tubes could diffuse rapidly away. Typical results are given in Figs.A, B and C. They show first, that fixation in glutaraldehyde under these circumstances results in the appearance of a fair amount of callose on the plates; second, that fixation in acetic-alcohol at -20°C gives an image showing virtually no callose; and third, that when acetic-alcohol follows glutaraldehyde a thick deposit of callose is present, showing that the second result is not due to dissolution of callose by the acetic-alcohol. The fixation images after acetic-alcohol are, as would be expected, coarse, and the P-protein has been severely denatured.

As a further basis for comparison stolons were subjected to two other treatments; heating to 45°C for 15 mins, and severe wilting, water being withheld for at least two weeks. The results, after fixation in cold acetic-alcohol, are shown in Figs.D and E. Both have

E. Fixed in acetic-alcohol (as B) after severe wilting. Note the heavy deposit of callose and the de-natured P-protein in pores.

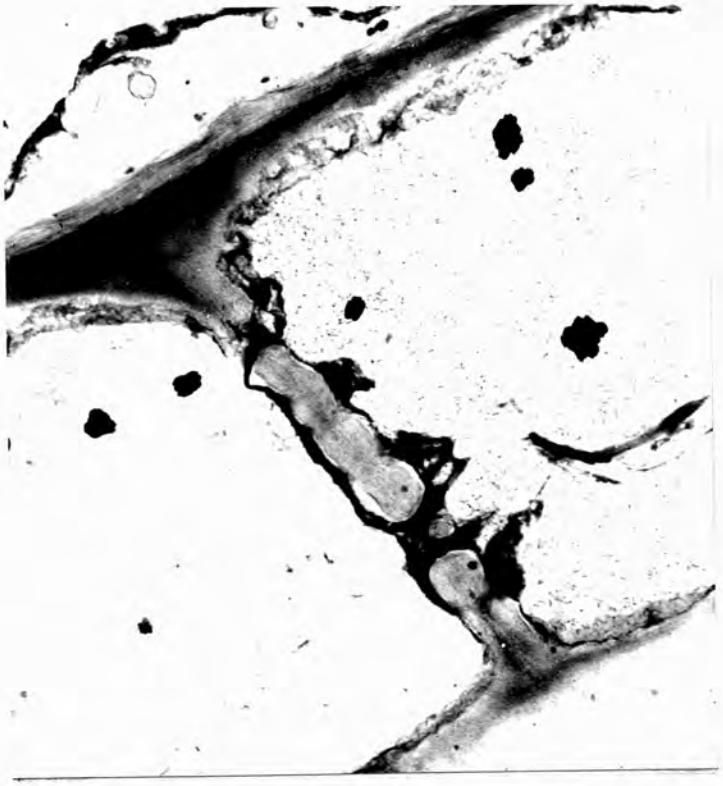
F. Fixed in acetic-alcohol (as B) after subjecting to N_2-anoxia for 5h. Note absence of callose, and usual plugging of pores with P-protein.



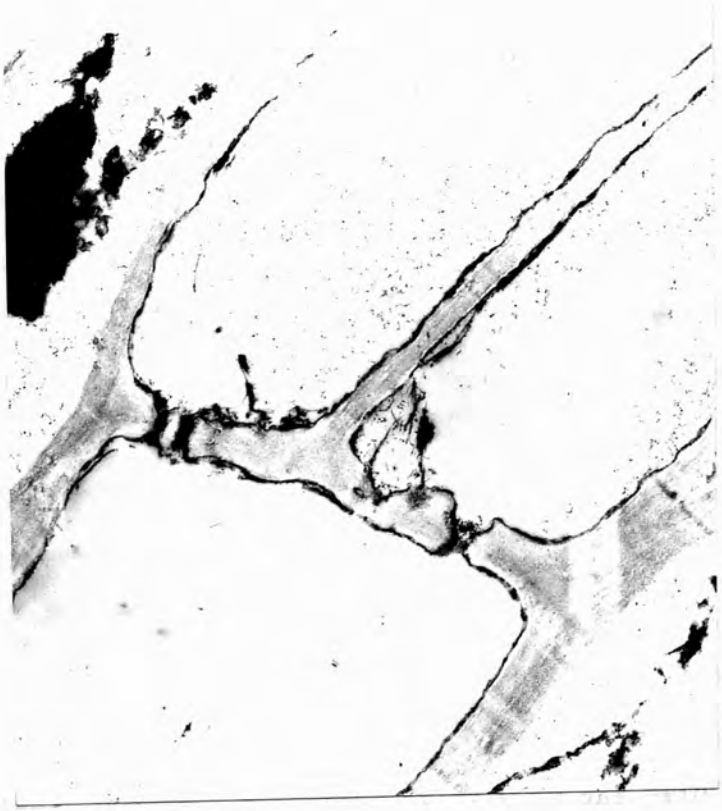


G. Fixed in acetic-alcohol (as B) after subjecting to HCN gas over 10^{-3}M KCN for 7h. Note absence of callose.

H. Fixed in acetic-alcohol (as B) after immersion in $5x10^{-3}$M DNP for 5h. Note absence of callose.



J. Fixed in acetic-alcohol (as B) after treatment in $5x10^{-2}$M $SrCl_2$ for 18h. Note absence of callose.



resulted, in contrast to the treatment of Fig.B, in marked callosing of the plates. This is in accordance with expectations (McNairn & Currier, 1968; Cronshaw & Anderson, 1969).

Inhibitor treatments

When stolons were subjected to nitrogen in the dark either for 5h or 22h there was no difference from the controls in air and light; in all cases callose was practically absent (Fig.F). In the case of gaseous HCN the result was the same; Fig. G shows the results when the stolons was exposed above 10^{-3}M KCN for 7h, but above $5x10^{-3}$M the indications were similar. Nor was the result different with dinitrophenol; Fig.H shows the appearance after 8h in $5x10^{-3}$M DNP. Lower concentrations appeared identical in their effects.

One further experiment was done, relevant to some earlier work in which ^{89}Sr was used as a tracer (Qureshi & Spanner, 1971). The stolon was immersed in $SrCl_2$ for 18h before examination. Calcium at a strength of 1.5×10^{-4}M has been reported to provoke callose formation. However, strontium at $5x10^{-6}$M and $5x10^{-2}$M appeared to be without effect; Fig.J shows the result when the higher concentration was applied.

DISCUSSION

It seems clear from the results reported above that callose formation cannot be the explanation of the transport inhibition exercised by anoxia, cyanide and DNP. The method of fixation employed, while it naturally results in coarse fixation images, would seem to yield reliable information on the abundance of pre-existing callose. Not only would it rapidly denature the enzymes

involved, but the time factor would permit the escape by diffusion of the sugar which forms their obvious substrate. Further, it is clear that the fixation itself does not lead to actual removal of callose (Fig. C). That inhibitors which prevent the metabolic supply of high-energy intermediates required for normal synthesis should fail to stimulate production of callose is not surprising; but the demonstration is important in sharpening the conclusions to which the inhibition work seems to point, namely that the sieve-tubes require energy to perform their transport function.

The negative result with strontium is perhaps to be expected. Unlike the work of Eschrich et al (1965) who injected calcium chloride into the pith cavity the strontium had to penetrate the cuticle. The result therefore merely indicates that in the tracer experiments (Qureshi & Spanner, 1971) the failure of strontium to move in the phloem was not due to callose-blockage. The interpretation there given therefore seems valid.

One final point which seems to emerge is that blockage by P-protein rather than by callose is an unlikely explanation of the inhibitor action; the indications are that the control stolon, presumably translocating normally, possesses the same distribution of this material as the inhibited stolons (compare Fig.B with Figs. F, G and H). If this be accepted, the inhibitor studies constitute evidence against the pressure-flow hypothesis very difficult to refute.

LITERATURE CITED

Crafts, A.S. & Crisp, C.E., 1971. Phloem transport in plants. Freeman, San Francisco.

Cronshaw, J. & Anderson, R., 1969. Sieve plate pores of Nicotiana. J.Ultrastruct. Res. 27, 134-48.

Eschrich, W. & Currier, H.B., 1964. Identification of callose by its diachrome and fluorochrome reactions. Stain Tech. 39, 303-7.

Eschrich, W., Currier, H.B., Yamaguchi, S. & McNairn, R.B., 1965. Der Einfluss verstärkter callosebildung auf den stofftransport in siebröhren. Planta, 65, 49-64.

McNairn, R.B. & Currier, H.B., 1968. Translocation blockage by sieve plate callose. Planta, 82, 369-80.

Qureshi, F.A. & Spanner, D.C., 1971. Unidirectional movement of tracers along the stolon of Saxifraga sarmentosa. Planta, 101, 133-46.

_____ , _____, 1972a. The effect of nitrogen on the movement of tracers down the stolon of Saxifraga sarmentosa, with some observations on the influence of light. Planta, In the Press.

_____ , _____ , 1972b. Cyanide inhibition of phloem transport along the stolon of Saxifraga sarmentosa L. J.exp.Bot. Submitted.

_____ , _____ , 1972c. The influence of dinitrophenol on phloem transport along the stolon of Saxifraga sarmentosa.
Planta, In the Press.

Webster, D.H. & Currier, H.B., 1965. Callose: lateral movement of assimilates from phloem.
Science, 150, 1610-11.

Section: III

Twin tracers

Sub-section: (i)

The comparative movement of two ions

or

The simultaneous movement of two ions in the phloem of Saxifraga sarmentosa.

The Simultaneous Movement of Two Ions in the Phloem of the *Saxifraga* Stolon

Summary Pairs of tracers were applied simultaneously to the long thin stolons of *Saxifraga sarmentosa*. After several hours of translocation the very precise pattern of exponential fall-off was examined and interpreted in the light of a model of mass flow with leakage. ^{42}K appears to leak faster than ^{22}Na; ^{86}Rb is very close to ^{42}K. The anion ^{82}Br shows a lower fall-off than ^{137}Cs; this is tentatively regarded as due to a much-reduced leakage, though it might imply a higher velocity. The implications of these findings for sieve-tube mechanism are uncertain.

Introduction

Comparative studies on the movement of tracers in the phloem have an obvious if limited interest in connection with the problem of mechanism. The hasty view that mass-flow in the sieve tubes necessarily implies that all molecular species travel at the same rate has lost its plausibility since chromatography became such a common tool, and it is now widely realised that the minute scale of the sieve tube conduits makes it a priori very likely that different substances will travel in them at different rates. This makes data on comparative rates less critical but still interesting, for certain hypotheses of phloem mechanism, such as the potassium (electroosmotic) theory, appear to entail important restrictions. In particular, this theory has been held to imply anionic immobility in the sieve tubes (MacRobbie, 1971).

Previous studies on comparative movement have included the pioneering work of Swanson and Whitney (1953) who used ^{32}P with ^{42}K or ^{137}Cs. They concluded that ions showed "independent and different" rates of movement in the phloem. The evidence, while "somewhat damaging" to the pressure-flow hypothesis, was not "crucial". Later Bukovac and Wittwer (1957) reported experiments designed to test the relative mobility of a wide range of ions, but their data hardly permit precise analysis. Biddulph and Cory (1957) conducted a more strictly comparative study with tritiated water, ^{32}P and ^{14}C given simultaneously; amd recently Cataldo, Christy and Coulson (1972) have carried this approach further using tritiated water and ^{14}C-sucrose. Both papers reported a difference in velocity between tracers, but concluded that this was not incompatible with mass flow.

The results available from comparative studies are hardly extensive enough therefore to enable us to invoke them in a critical evaluation of theories of mechanism. Accordingly, it seemed

worthwhile to investigate the question further using the rather favourable opportunities afforded by the long stolons of Saxifraga sarmentosa. This paper is a report of some preliminary work and a discussion of some theoretical implications.

Materials and Methods

The preparation and use of the plants have been adequately described in an earlier paper (Qureshi and Spanner, 1971). The tracers were applied in pairs made up in 50 or 75 µl of neutral 0.05M phosphate buffer. Details of the approximate doses and concentrations applied are given in Table 1. The plants were grown under ordinary greenhouse conditions with supplementary light of 3700 lux from low pressure mercury lamps, this being maintained during the whole course of the experiments. These lasted for 7 or 18h as stated in the legends.

At the conclusions of the experiments the stolons were harvested, segmented and assayed as previously described. The isotopes were separated by means of the differing decay constants. In order to secure comparable statistical accuracy the short-lived isotope should ideally be about twice as active initially as the other; in fact in most cases it was made rather more than this.

Results

Three pairs of tracers were used in these comparisons: ^{42}K and ^{22}Na, ^{86}Rb and ^{22}Na, and ^{137}Cs and ^{82}Br. The results are presented in Figs. 1, 2, 3 and 4, and in Table 2. For reasons of clarity some of the individual curves have been shifted vertically by arbitrary amounts, and in the case of Fig.4, horizontally. Regression lines have been added; the horizontal extent of these indicates the observations they take into account. It will be seen

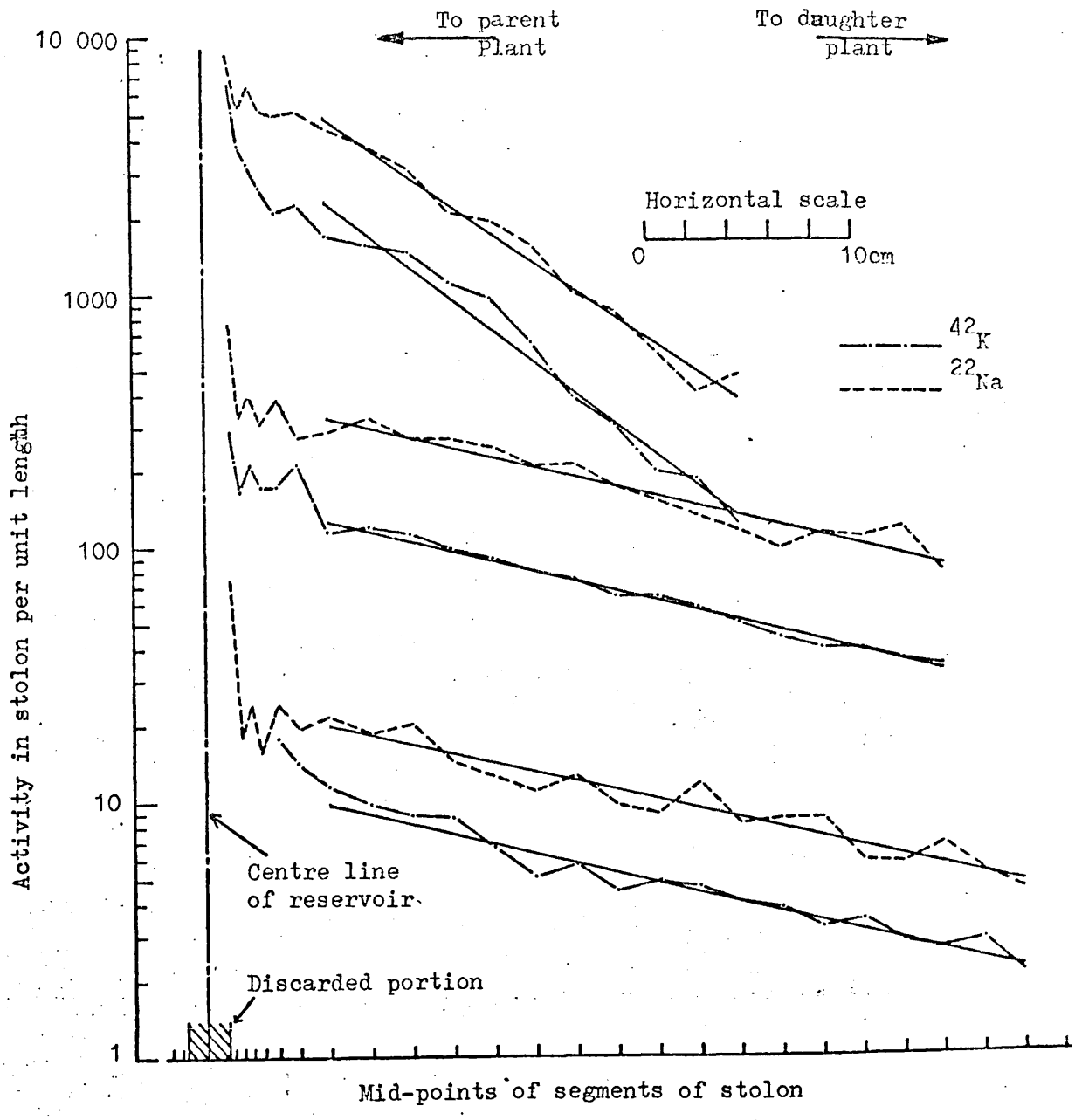


Fig.1 Simultaneous movement of two cations (^{22}Na and ^{42}K) down the stolon of Saxifraga; in triplicate. The vertical position of each pair of curves (relative that is to other pairs) is set arbitrarily for clarity. The extent of the regression lines indicates the points which they cover. For regression constants, see Table 2. Duration 7h.

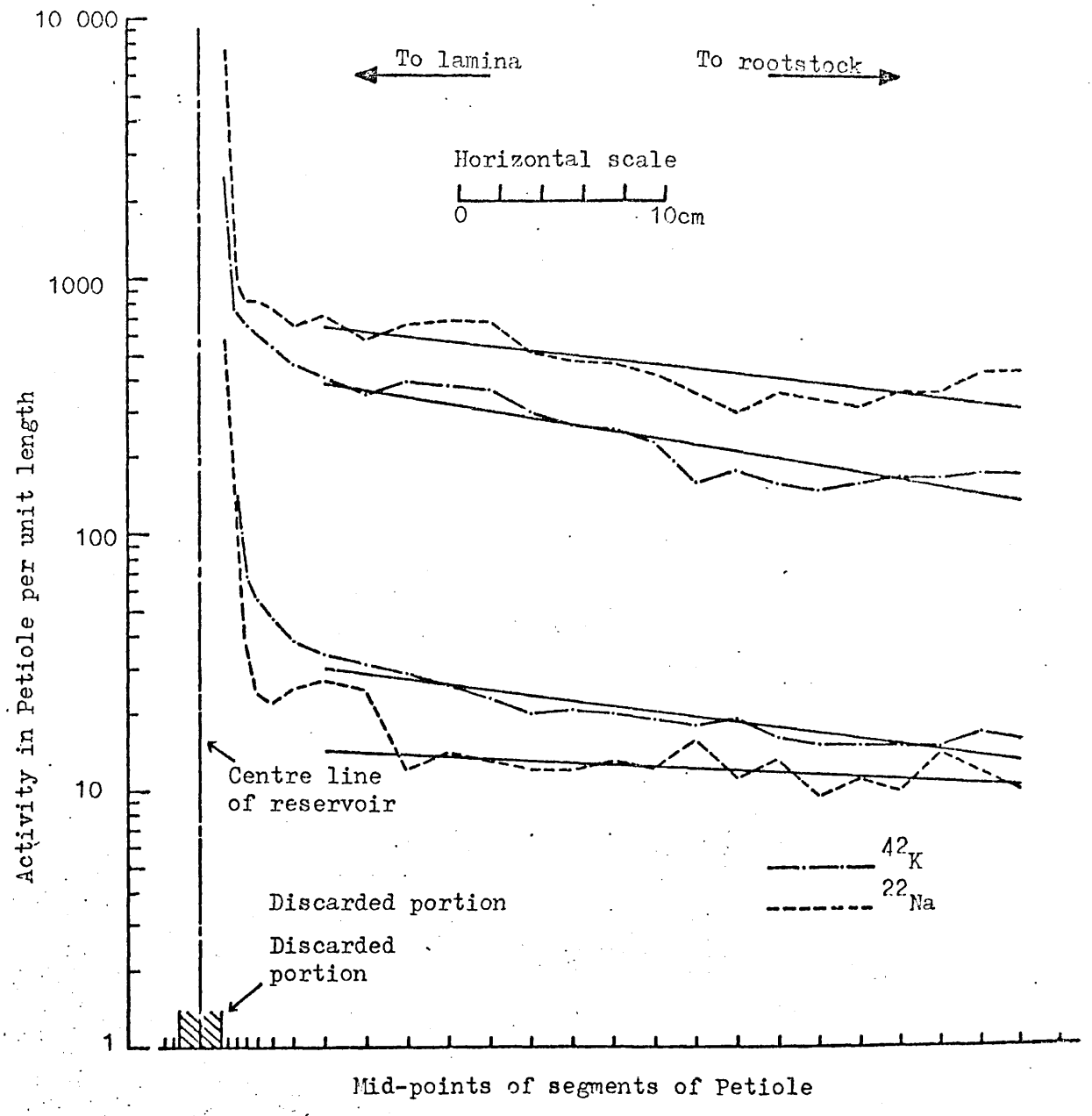


Fig.2 Simultaneous movement of two cations (^{22}Na and ^{42}K) down the petiole of Nymphoides; in duplicate. See further, legend to Fig.1. Duration 7h.

Fig.3 Simultaneous movement of two cations (^{22}Na and ^{86}Rb) down the stolon of Saxifraga; in duplicate. See further, legend to Fig.1. Duration 18h.

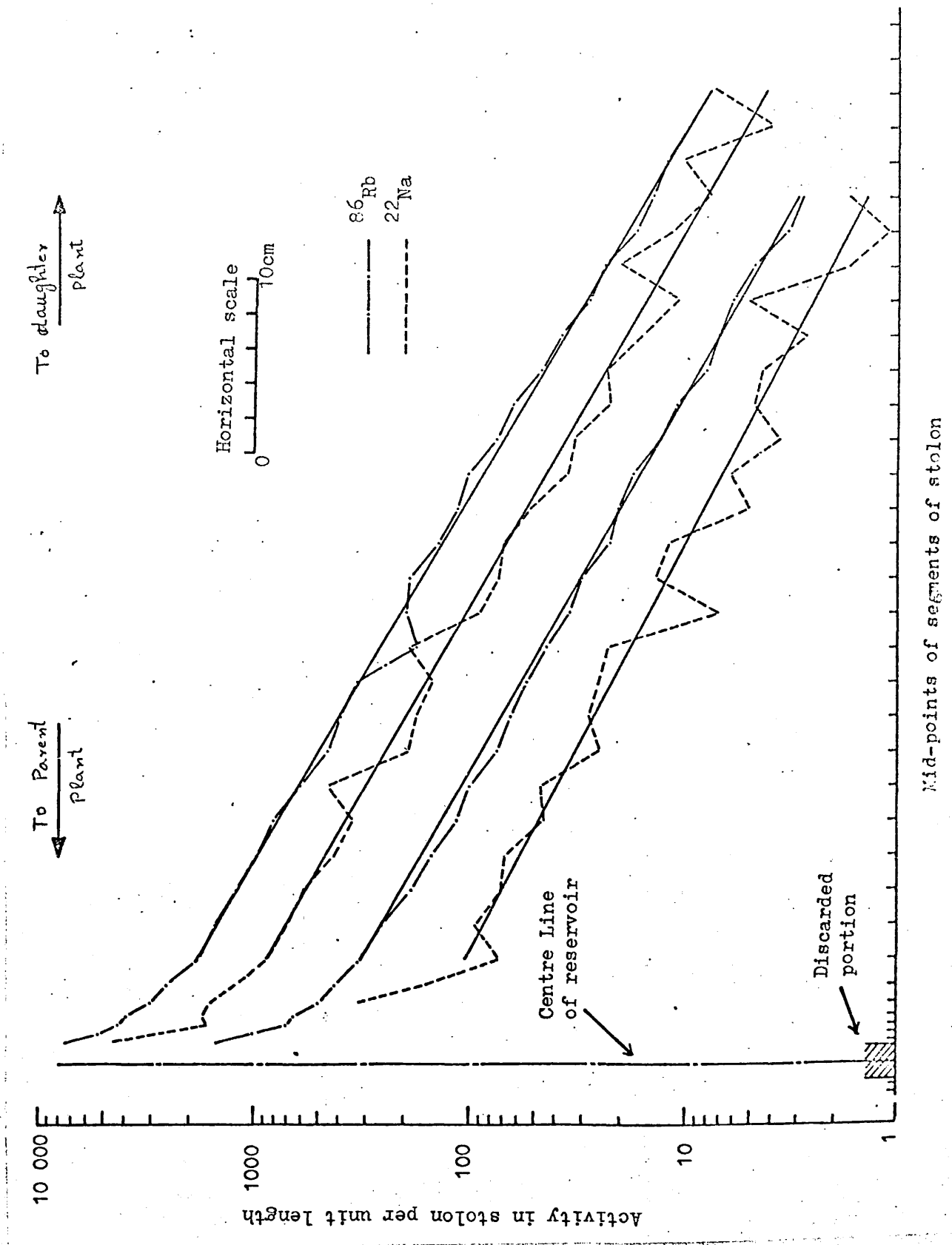
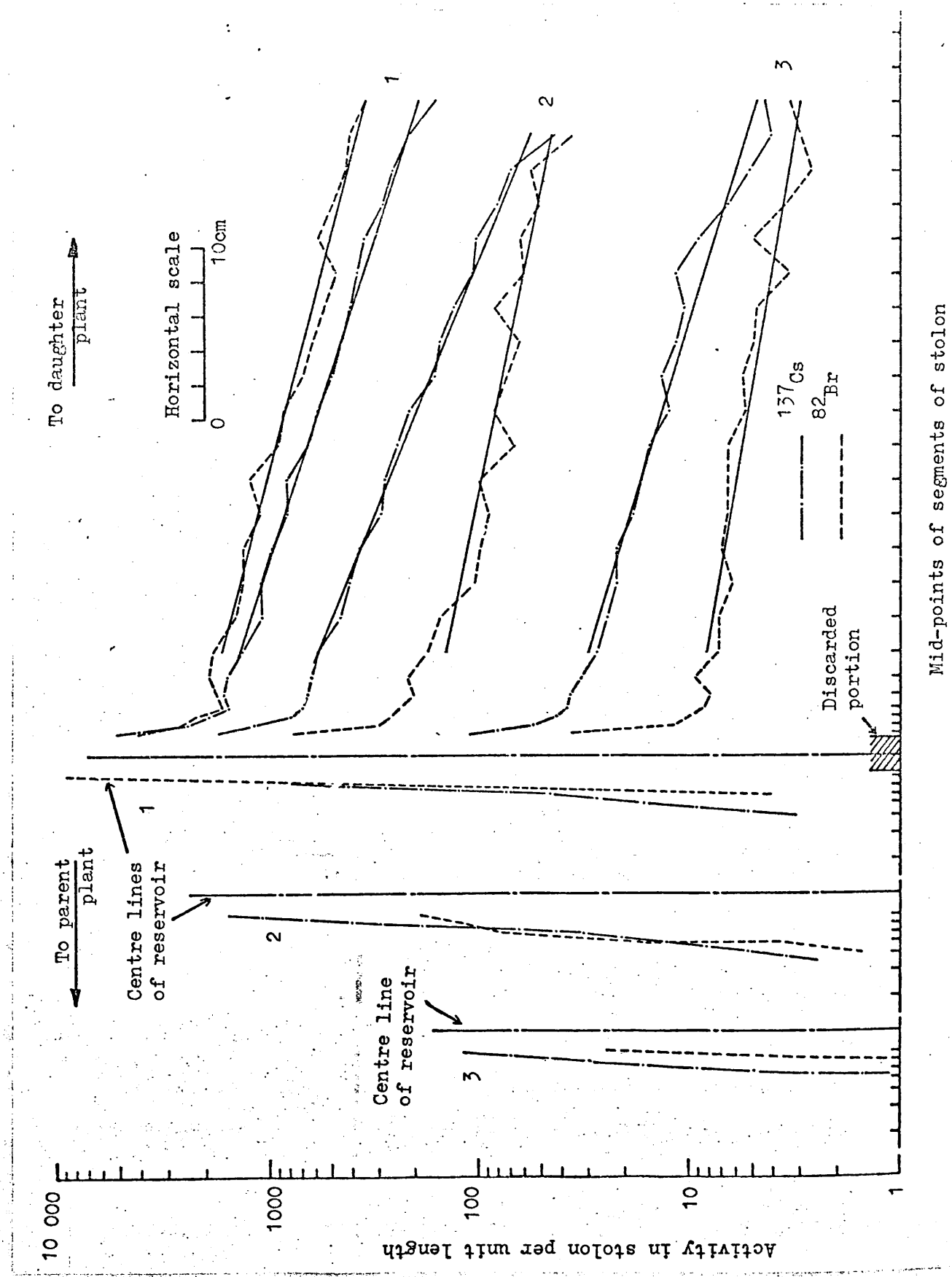

To daughter plant
To Parent plant
Horizontal scale
0
10cm
$^{86}_{Rb}$
$^{22}_{Na}$
Centre Line of reservoir
Discarded portion
10 000
1000
100
10
1
Activity in stolon per unit length
Mid-points of segments of stolon

Fig.4 Simultaneous movement of an anion and a cation (^{82}Br and ^{137}Cs) along the stolon of Saxifraga; in triplicate. See further, legend to Fig.1. In addition, two sets of curves to the left of the reservoir are arbitrarily displaced. Duration 18h.


Activity in stolon per unit length
10 000
1000
100
10
1
Mid-points of segments of stolon
To daughter plant
To parent plant
Horizontal scale
0
10cm
Centre lines of reservoir
Centre line of reservoir
Discarded portion
137Cs
82Br
1
2
3

that this avoids the region of unpolarised movement near the tracer reservoir (Qureshi and Spanner, 1971). The constants for the regression lines in Table 2 enable the actual level of tracer present to be ascertained and compared, where appropriate, with the tracer that has accumulated in the daughter plant.

Discussion

It has already been established that after an extended period of translocation, the fall-off pattern of tracer distribution down the stolon is accurately exponential (Qureshi and Spanner, 1971). That being so, the slope of the semi-logarithmic plot provides a fairly precise parameter to discuss. The earlier studies leave no doubt in our minds that we are dealing with a phenomenon of unidirectional mass-flow, and that the fall-off in activity is probably to be accounted for in terms of lateral leakage. This is not inconsistent with the level distribution curves obtained with ^{14}C (ibid 1973a), since assimilates under certain conditions (probably holding in an ephemeral organ like the stolon) may show little lateral movement. With this understanding, the slope of the semi-logarithmic lines is a measure of $\frac{ks}{Av}$ (Spanner and Pebble, 1962; compare Horwitz, 1958), where v is the linear velocity of the tracer, k the velocity constant of lateral leakage, A the cross-section of the conducting channels, and s the area per cm length open to lateral leakage. In other words, the slope measures the fractional loss of tracer from the stream per unit length. In a given context differences in slope can be related either to v or to k, and in this connection it is important to recognise that v is not a magnitude common to all molecular species; the sieve plates may impose frictional or electrostatic restraints which differ from one solute to another. The electrcosmotic theory, in particular,

has to reckon with this possibility.

The results are perhaps best discussed in terms of the ratios of the slopes for the paired ions (Table 2). Potassium and sodium have a consistent relation here, with the former showing the greater slope, especially in the case of Nymphoides, whose petiole had, significantly perhaps, suffered a certain amount of surgery. The greater slope for potassium may reflect either a lower velocity, or a higher leakage rate. The latter seems more probable, since there is much evidence that of these two ions potassium penetrates the cellular membranes appreciably faster (Dainty, 1962). In the present experiments for radiochemical reasons potassium had to be applied at considerably greater molarity (Table 1); its native abundance in the sieve tubes would also be expected to be considerable. As a result its concentration there would be much greater than that of sodium, and this may have influenced the comparison, though in what way is not immediately clear. The Rb:Na comparison is very close to the K;Na in the case of Saxifraga. Taken together therefore the evidence is not, prima facie, of any striking difference of behaviour for the three ions; and if potassium has some unique relation to the sieve-tube function it is not apparent. Negative as this result is however it does draw attention to an important conclusion: that potassium undergoes major transport. This is not of course novel, but it is one which the theory (cf.Spanner and Jones, 1970) has somewhat overlooked. Its implication is that if any potassium is recirculated at the sieve plate it is probably only a small fraction of what traverses them. Since the entire potassium current would be electroosmotically active, while only the recirculated fraction would be involved in membrane transport, this recognition goes some way to alleviating one of the main

objections to the theory. Of course there is a limit to the extent to which the recirculated potassium current can, in thought, be reduced; if it be imagined too small, the sieve plate potential, short-circuited by the main current, would fall to a value too low to allow for a significant power input. There would seem to be an inverse relationship here between the recirculation current which might be proposed and the density of "plugging" of the sieve-plate pores. It seems premature to try to decide whether this relationship is such as to preclude a potassium recirculation small enough to be consistent with our knowledge of membrane properties and yet large enough to operate effectively in the way the theory requires; the problem needs more experimental and theoretical investigation.

Of rather different interest is the comparison between ^{137}Cs and ^{82}Br, since here a difference of ionic sign is involved with a quite negligible difference in ionic mobility. The result is straightforward: the slope for the anion is about half that for the cation. How do we understand this with reference to $\underline{v}$ and $\underline{k}$? It seems likely that the permeability of the sieve tube membranes to bromide is very appreciably less than that to caesium (cf. MacRobbie and Dainty, 1958; Hope and Walker, 1961; Spanswick, Stolarek and Williams, 1967). This will mean less leakage, with a consequent reduction in slope for bromide. It is argued elsewhere (Spanner, 1973) that the electroosmotic theory does not require the non-transport of anions, but only their transport at a reduced velocity. This reduced velocity will entail a steeping of the bromide line. On balance, it would seem quite possible to maintain that the effect of reduced leakage ($\underline{k}$) would outweigh that of reduced velocity ($\underline{v}$), permitting the conclusion that the observed results while not lending the theory positive support, are not inconsistent

with it. An independent assessment of the relationship between the velocities of caesium and bromide transport is in principle possible by comparing the actual quantities of tracers delivered to the daughter plants (Table 2). This seems to suggest a higher velocity for bromide; however the amounts delivered depend also on the concentrations in the conducting tracts, and owing to the different leakages for the two ions the curves (Fig.4) give little guidance on this point.

We conclude therefore that this preliminary study, while indicating a method of rather precise comparison, throws little further light on mechanism.

Table 1. Details of tracers

Application was in pairs in 50 or 75 µl of neutral 0.05M phosphate buffer.

Tracer	Half-life	Specific Activity	Typical Dose	Concentration as applied (approx.)
^{22}Na	2.6 y	100 mCi/mg	10 µCi	9×10^{-5} M
^{42}K	12.45 h	250 mCi/g	100 µCi	0.2 M
^{86}Rb	18.7 d	2-10 mCi/mg	30 µCi	7×10^{-3} M
^{137}Cs	30 y	25 mCi/mg	10 µCi	5×10^{-5} M
^{82}Br	36 h	200-400 mCi/mg	30 µCi	2.5×10^{-5} M

Table 2. Regression-line constants

The equation fitted is : ln (counts per 2cm per 100s) = a + bx

Where x is distance in cm from centre point of first segment included in regression.

Paired tracers[a] Plant		Regression constants a	b	Ratio[b] of slopes	Content of sink (daughter plant) Counts per 100s.
^{42}K, ^{22}Na Saxifraga (Fig.1)	1	8.319	-0.1424	1.13	
		8.966	-0.1261		
	2	6.478	-0.0448	1.05	
		7.161	-0.0425		
	3	6.753	-0.0448	1.02	
		7.693	-0.0439		
^{42}K, ^{22}Na Nymphoides (Fig.2)	1	8.127	-0.0233	2.68	
		7.321	-0.0087		
	2	8.482	-0.0317	1.55	
		8.899	-0.0205		
^{86}Rb, ^{22}Na Saxifraga (Fig.3)	1	11.686	-0.1094	1.02	
		8.461	-0.1070		
	2	11.576	-0.1037	1.06	
		7.547	-0.0983		
^{137}Cs, ^{82}Br Saxifraga (Fig.4)	1	8.832	-0.0619	1.26	990
		8.793	-0.0493		2010
	2	8.354	-0.0775	2.06	1008
		7.090	-0.0376		2142
	3	9.037	-0.0573	1.83	3530
		8.698	-0.0313		8507
Ditto (not plotted)	4	7.986	-0.0468	1.90	2584
		7.914	-0.0246		10634
"	5	8.943	-0.0454	1.21	
		9.583	-0.0374		
"	6	9.214	-0.0445	1.71	
		9.493	-0.0261		
"	7	10.178	-0.0537	2.08	
		10.585	-0.0258	Mean 1.72 ± 0.135	

[a] The numerals refer to the replicates; against these are listed the isotopes in the order stated in the entry.

[b] With standard deviation of the mean of 7 values.

References

Biddulph, O., Cory, R. : An analysis of translocation in the phloem of the bean plant using THO, P^{32} and C^{14}. Plant Physiol. 32, 608-619 (1957).

Bukovac, M.J., Wittwer, S.H. : Absorption and mobility of foliar-applied nutrients. Plant Physiol. 32, 428-435 (1957).

Cataldo, D.A., Christy, A.L., Coulson, C.L. : Solution-flow in the phloem. II Phloem transport of THO in Beta vulgaris. Plant Physiol. 49, 690-695 (1972).

Dainty, J. : Ion transport and electrical potentials in plant cells. Ann. Rev. Plant Physiol. 13, 379-402 (1962).

Hope, A.B., Walker, N.A. : Ionic relations of Chara Australis. IV Aust.J.biol.Sci. 14, 26-44 (1961).

Horwitz, L. : Some simplified mathematical treatments of translocation in plants. Plant Physiol. 33, 81-93 (1958).

MacRobbie, E.A.C., Dainty, J. : Ion transport in Nitellopsis obtusa. J.Gen.Physiol. 42, 335-353 (1958).

MacRobbie, E.A.C.; Phloem translocation. Facts and mechanisms: a comparative survey. Biol. Rev. 46, 429-481 (1971).

Qureshi, F.A., Spanner, D.C. : Unidirectional movement of tracers along the stolon of Saxifraga sarmentosa. Planta (Berl.) 101, 133-146 (1971).

Qureshi, F.A., Spanner, D.C. : Movement of [^{14}C] sucrose along the stolon of Saxifraga sarmentosa. Planta (Berl.) in the press. (1973).

Spanner, D.C., Prebble, J.N. : The movement of tracers along the petiole of Nymphoides peltatum I. J.expt.Bot. 13, 294-306 (1962).

Spanner, D.C., Jones, R.L. : The sieve tube wall and its relation to translocation. Planta (Berl.) 92, 64-72 (1970).

Spanner, D.C. : The potassium (electro-osmotic) theory of the phloem: a restatement and defence. In preparation (1973).

Spanswick, R.M., Stolarek, J., Williams, E.J. : The membrane potential of Nitella translucens. J.expt.Bot. 18, 1-16, (1967).

Swanson, C.A., Whitney, J.B. : Studies on the translocation of foliar-applied P^{32} and other radioisotopes in bean plants. Amer.J.Bot. 40, 816-823, (1953).

General Conclusions.

The studies on the transport of ^{137}Cs along the stolon of Saxifraga imply that this non-physiological tracer unlike ^{89}Sr undergoes polarised long distance transport in the same direction as the assimilates. Thus its movement must be predominantly in the phloem in contrast to ^{89}Sr which is transported mainly in the xylem. The distribution pattern of ^{137}Cs along the stolon after a suitable length of time shows very precisely the well-known exponential fall-off pattern. This suggests an appreciable leakage out of the sieve tubes and makes it difficult to calculate with any exactitude the linear velocity of movement simply by observations on the 'front' (See Spanner & Prebble 1962). On the contrary, the pattern of distribution of either applied ^{14}C-sucrose or naturally assimilated-^{14}C shows little fall-off and is fairly level. In this case the linear velocity may be estimated as about 20 cm/h. A significant point in connection with the distribution of ^{14}C-assimilates is that the activity at the proximal end of the stolon after a time falls below that lower down, producing a 'hump'. This condition in the translocating axis is difficult to explain in terms of the analogue-diffusion theory. The convincing demonstration of the fact that, apart from a symmetrical short-distance spread of the tracers, ^{137}Cs and ^{14}C-sucrose always show a unidirectional long-distance movement along the stolon again tells against the analogue diffusion theory, and supports the idea of some sort of mass flow mechanism. The evidence of this work thus strongly opposes the validity of any mechanism, based either on protoplasmic streaming or activated diffusion, which permits simultaneous bidirectional movement at a single cellular locus.

That the translocating channels require metabolic energy not exclusively at the source and sink but also all along their length, is a conclusion strongly emphasised by the various experiments performed with nitrogen, cyanide and DNP. When a length (10-30 cm) of the central portion of a translocating stolon is subjected to these metabolic inhibitors, the

transport of both ^{137}Cs and ^{14}C-assimilates is strongly inhibited in a fashion which seems to be clearly both localised and reversible. In the present experiments, that the treatment with inhibitors does not seem to affect the physiological activities of source and sink is indicated in several ways, most directly by the use of radioactive cyanide. Some of this inhibitor travels indeed to the daughter plant either in the xylem stream or with the assimilates; but the concentration of the accumulated cyanide is not of the level which could apparently inhibit the activities of the sink. There is of course no migration of cyanide to the parent plant so the question of source inhibition does not arise. Probably a similar situation applies to the other inhibitors, i.e., DNP, nitrogen. That the inhibiting effect is localised in the treated region of the conduits is further implied by the experiments showing that the portion of stolon beyond the treated region (and the sink itself) is still able to sustain transport when provided locally with sucrose. The presence of the tracer in the proximal region of the stolon is naturally aditional evidence, if more were needed, of exclusion of inhibitor action from this part of the system.

The inhibition of transport caused by the metabolic inhibitors used is reversible conforming the work of others. Recovery is steady and takes different times for different inhibitors; for instance, 4 h suffices for nitrogen, 24 h for cyanide, while DNP requires 36 h.

The present work also supplies evidence against two interpretations of the inhibitory effect which would leave the latter consistent with the Münch hypothesis. In the first place the pattern of distribution of tracers in the treated stolon does not suggest an abnormal leakage and accumulation of the tracers in or before the treated region under the thrust of the assimilate stream. This means that the inhibitors do not merely damage the integrity of the sieve tube membrane and render it permeable. In the second place the transport inhibition is not due to the formation of

callose at the sieve plate in a manner which could block the sieve pores. It is true that the sieve plate pores appear closely occluded with P-protein in treated stolons, but the same is true of controls; thus inhibition of transport cannot be due to this factor either. Further ultrastructural examination of treated sieve tubes is desirable; nevertheless the conclusion seems to be strongly indicated that inhibition must be attributed to interference with an energy-dependet driving mechanism.

These various lines of evidence strongly oppose the Münch hypothesis and favour some sort of activated mass flow mechanism, i.e., a mechanism which required driving energy everywhere along the conduit and most probably at the sieve plates. There are two theories which regard the sieve plates as active pumps, one dependent on protein contractility (Mac Robbie 1971, Fensom 1972) and the other invoking potassium electroosmosis (Spanner 1958, Spanner and Jones 1970). An attempt to test the potassium theory was made using valinomycin. It proved to be a failure, possibly because of the difficulties associated with the big size of the valinomycin molecule and its insolubility in water. It is doubtful if in these experiments valinomycin ever reached the important site in enough concentration to interfere with the proposed recirculation of potassium ions around the sieve plates. This approach calls for further investigation perhaps solubilising the valinomycin by a controlled use of suitable detergents.

The exponential fall-off pattern of distribution of the tracers along the length of the stolon provides a fairly precise parameter for comparative studies of simultaneous tracer movement. This was exploited with pairs of tracers in the hope of gaining evidence of a discriminatory nature, since the electroosmotic theory seems to entail consequences of its own in this respect. The studies with ^{42}K, ^{22}Na, ^{86}Rb, show no striking difference of behaviour between the ions, and if potassium has some unique relation to the sieve tube function, it is not apparent. The results, however, did suggest that there is a major transport of potassium along the sieve tubes and if any potassium is recirculated at the sieve plate it is probably only

a small fraction of what traverses them. The differences between the transport of ^{82}Br and ^{137}Cs were more marked, but more evidence is needed before it can be decided whether or not anions travel at the same velocity in the conduits as cations. However, a start has been made on a promising approach.

The conclusions drawn from these studies may be summarised as follows:

1. Phloem transport in the stolon of Saxifraga is undoubtedly unidirectional.
2. Nitrogen, cyanide and DNP exert a strong effect localized in the sieve tubes themselves (as well as at the terminals). The effect is reversible.
3. The inhibition of transport is not due to callose blockage or membrane damage.
4. Anion transport follows a similar pattern to cation.

These conclusions are very adverse to the Münch hypothesis, and to any diffusion-analogue theory; they favour a theory of active mass flow. It is not possible further to pinpoint the exact mechanism for the propelling forces.

APPENDIX I

Photographs of experimental set-up used

in inhibitor studies

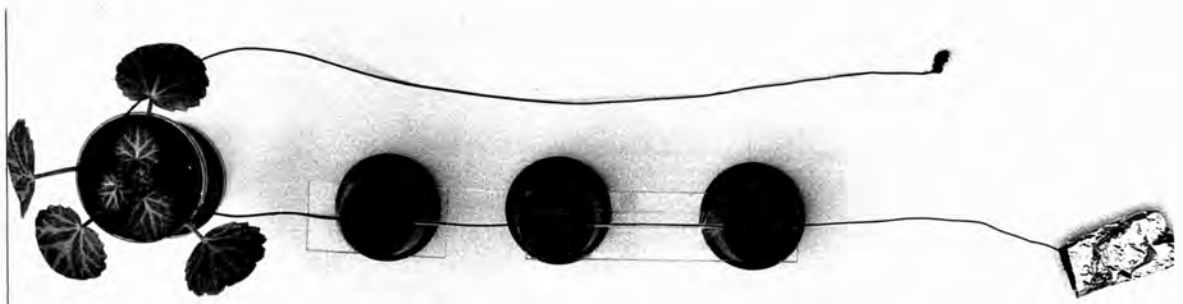


Plate 1 *Saxifraga* stolon under DNP inhibition. Tracer is being applied at the smaller platform on the right. The stolon runs through DNP solution in a groove of the larger (30cm) platform on the left. The daughter plant is held in a dark polythene bag protected with Aluminium foil. The set-up for KCN inhibition was rather similar, but the platform shorter (20cm).

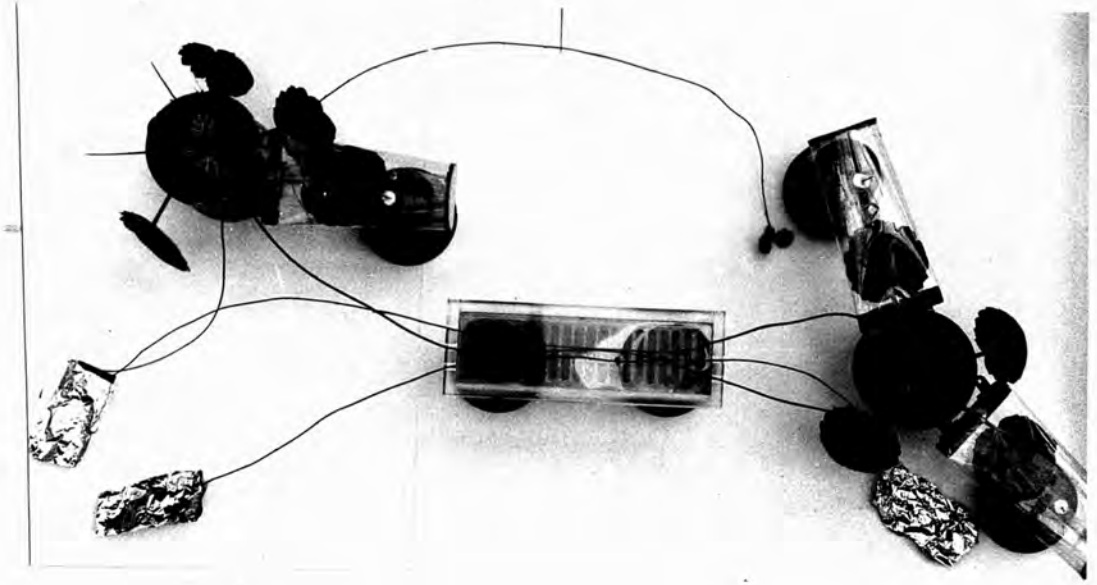


Plate 2 *Saxifraga* stolons under inhibition with gaseous HCN. Three stolons run through the perspex treatment box. The subtending leaves are enclosed in chambers of flexible plastic for feeding with $^{14}CO_2$. The treatment box is 20cm long.

APPENDIX II

Autoradiographs of subtending leaves of *Saxifraga* given $^{14}CO_2$ in inhibitor studies.

Note: light areas represent activity.

Plate 1

(see pp.)

A. Stolon in dark sleeve in air (control)

B. " " " " " N_2

C. Subtending leaf exposed to N_2 except for 15 mins. in in light with $^{14}CO_2$.

Note that there is little difference between A & B; C shows reduced vein loading at base and in petiole.

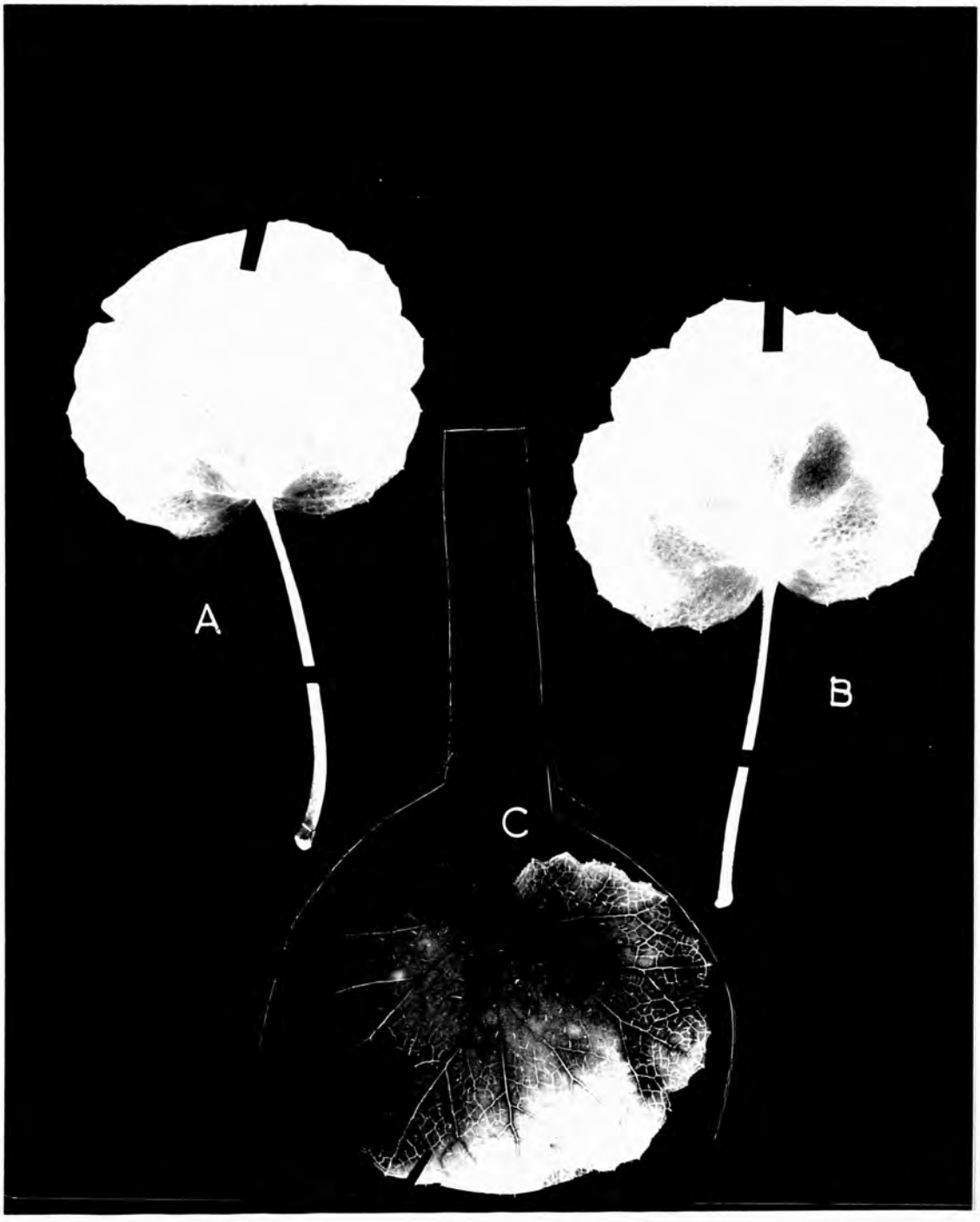

A
B
C

Plate 2

(see pp.)

A. Control

B. Stolon treated with gaseous cyanide above 10^{-4} M solution

C. " " " " " " 10^{-3} M "

D. " " " " " " 5.10^{-3} M "

E. Subtending leaf treated with gaseous cyanide above 5.10^{-3} M solution then given 1h in air with $^{14}CO_2$.

Note that there is little difference between A,B,C & D.

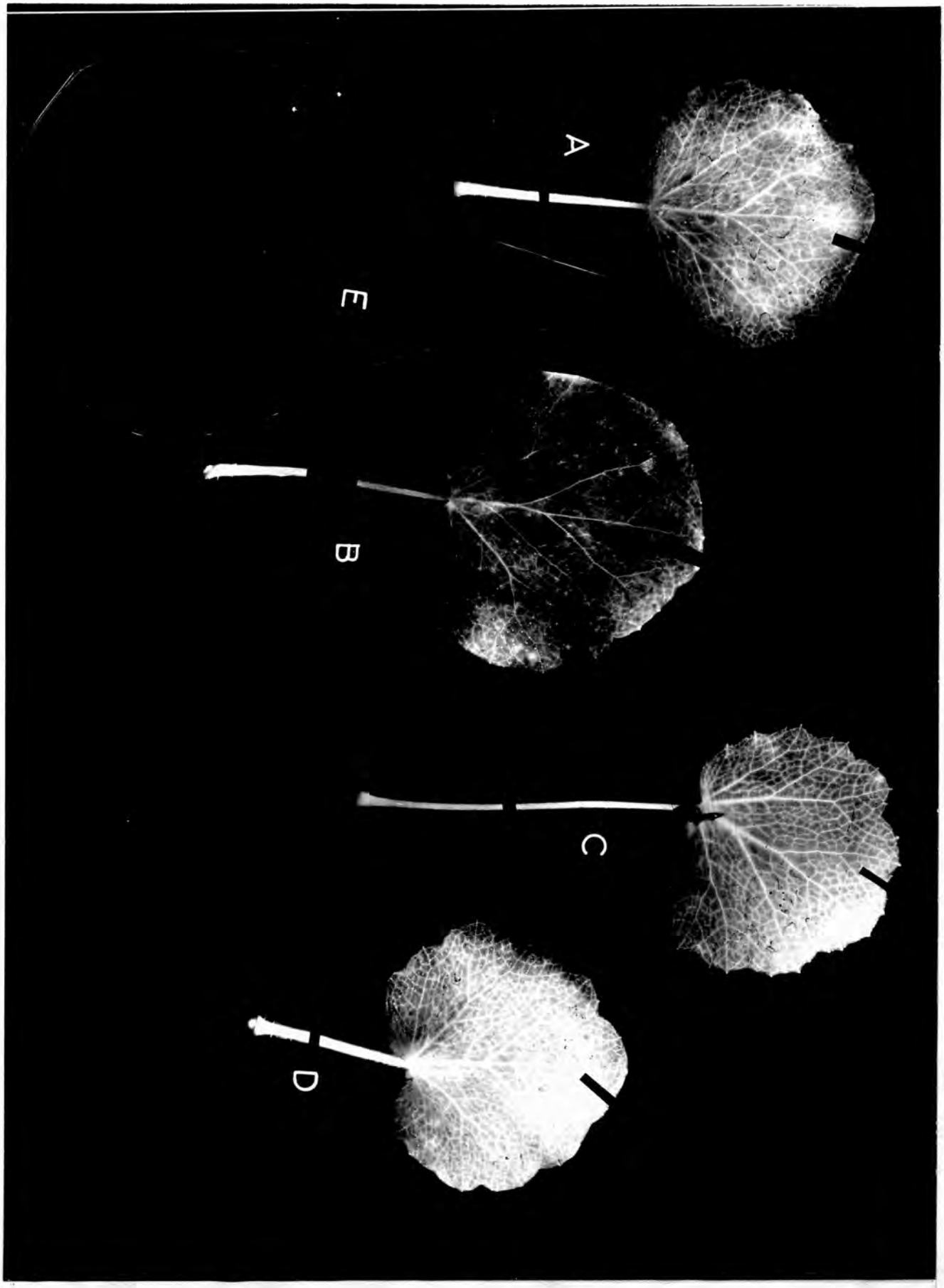

A
E
B
C
D

Plate 3

(see pp.)

A. Control

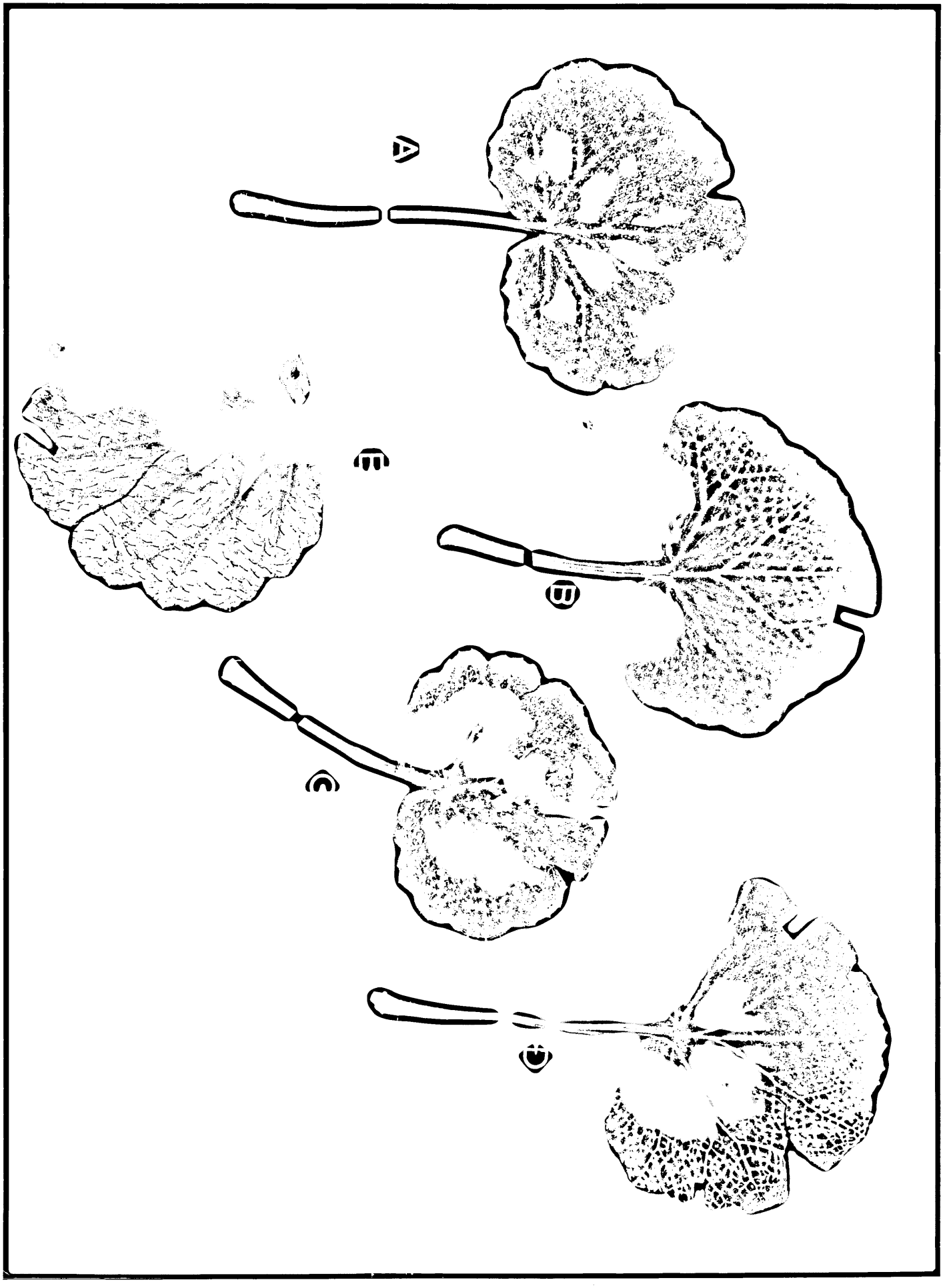
B. Stolon treated with 10^{-4} M DNP

C. " " " 10^{-3} M DNP

D. " " " 5.10^{-3} M DNP

E. Subtending leaf treated with 5.10^{-3} M DNP, then washed and given 1h. $^{14}CO_2$.

Note the E shows no activity in petiole and reduced activity in adjacent veins.



APPENDIX II (cont.)

Autoradiographs showing effects of metabolic inhibitors on photosynthesis and vein loading in leaves of Runner beans (Phaeolus multiflorus).

Plate 4 (see pp.)

A. Leaf treated with N_2 except for 15 min. in air with $^{14}CO_2$.

B. Leaf treated with cyanide (gaseous, above 5×10^{-3}M) KCN.

Note absence of activity in major veins and petioles.

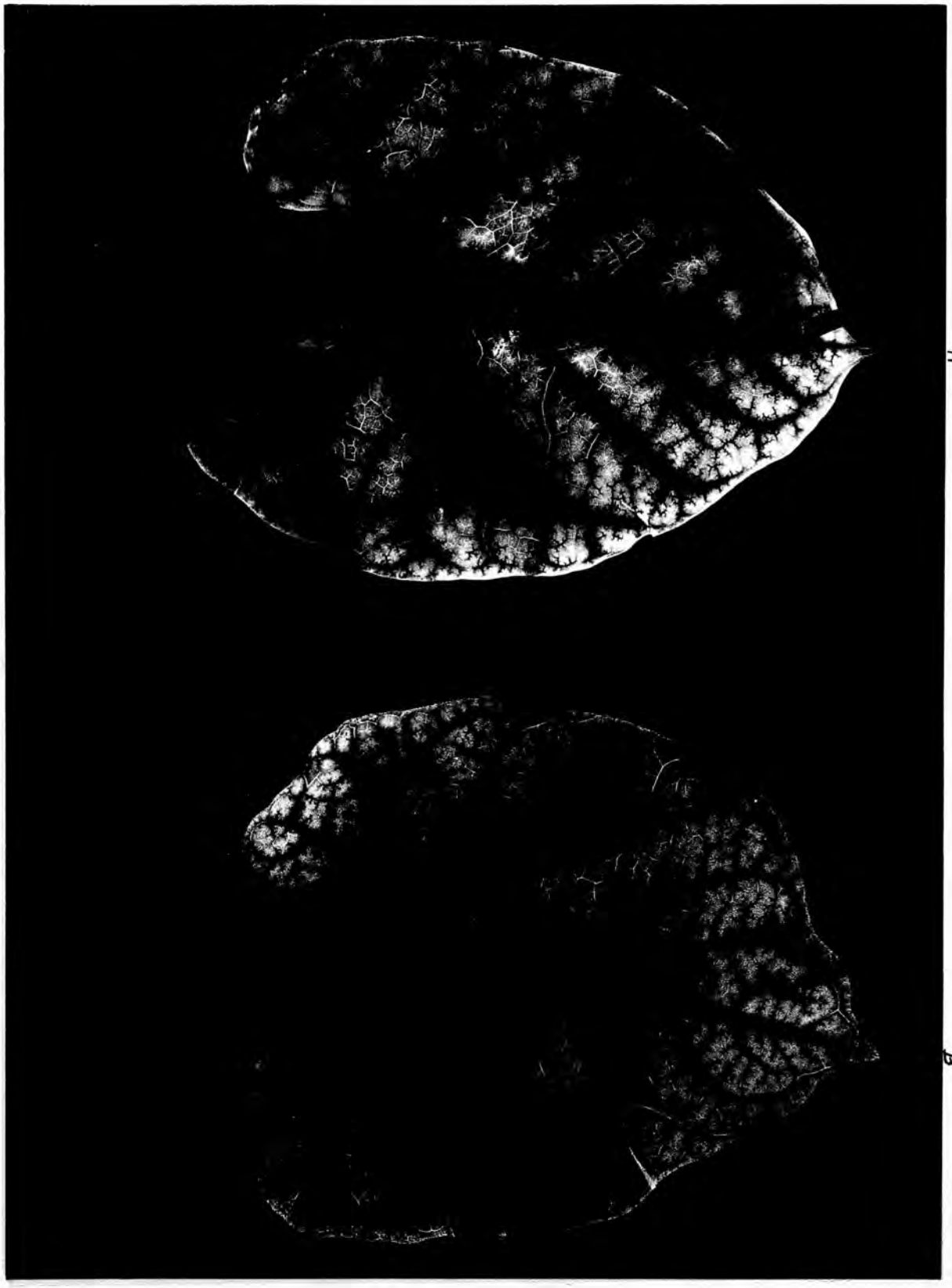
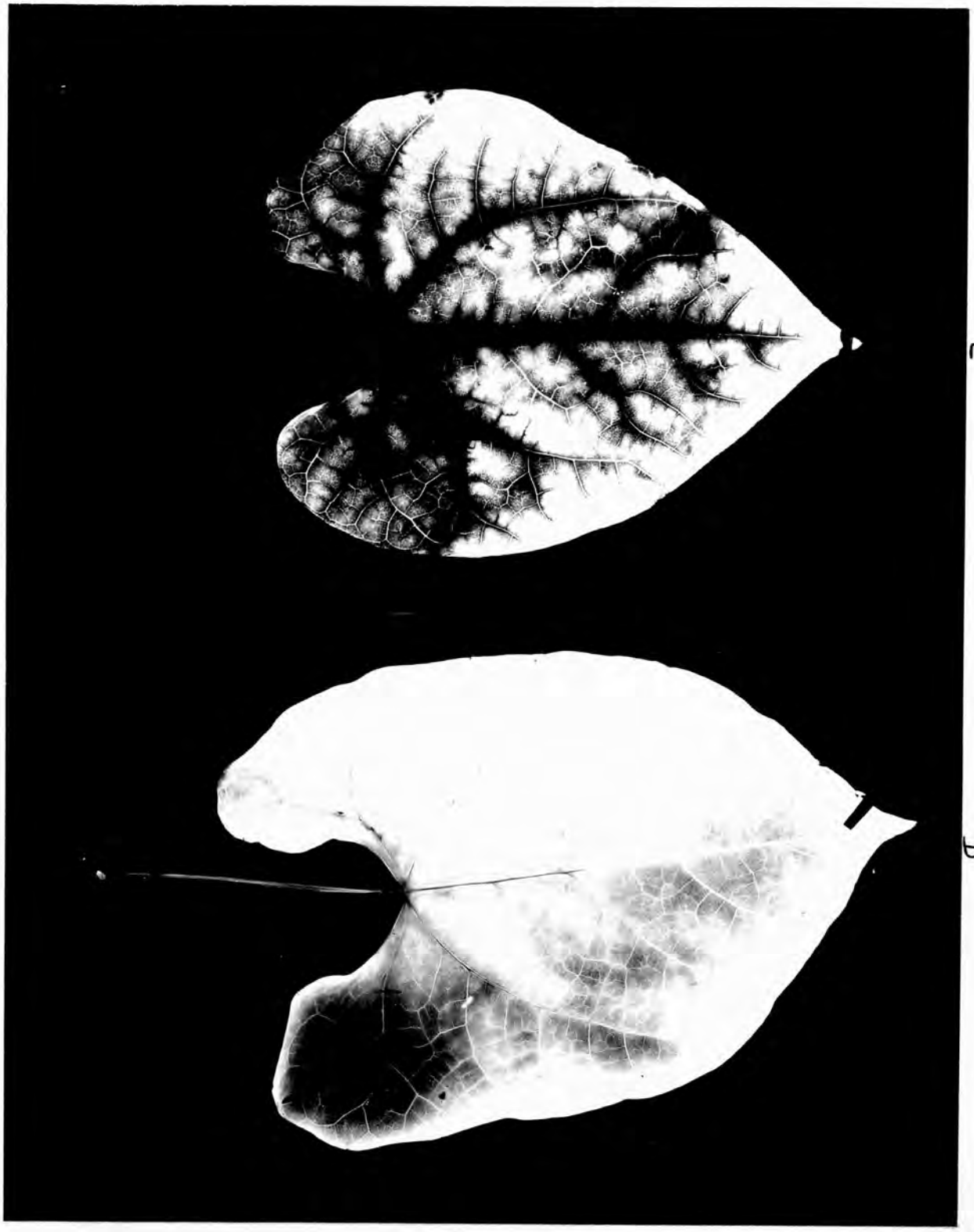

A
B

Plate 5 C. Leaf treated with DNP (5×10^{-3}M).

(see pp.) D. Control.

Note activity in petiole of D.


C
D

APPENDIX III

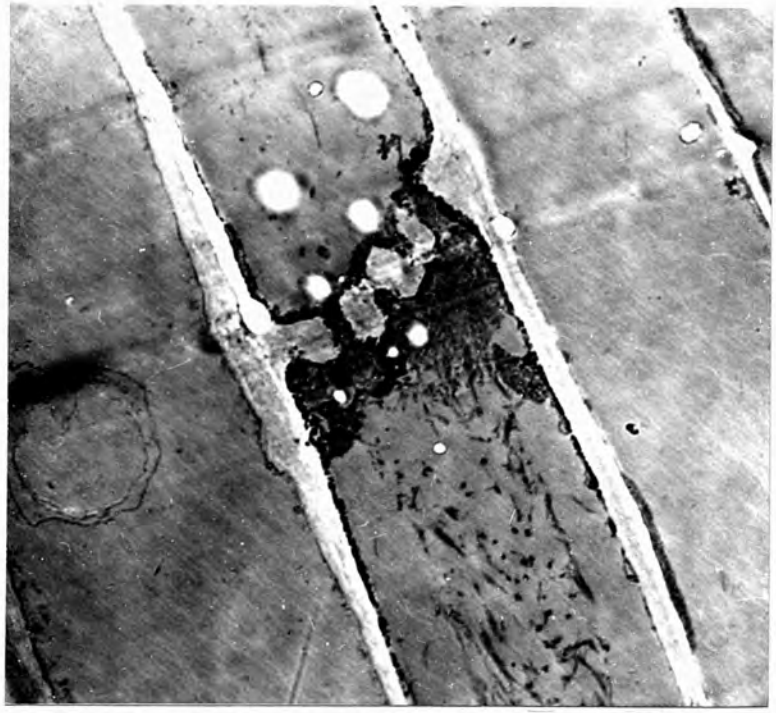
Longitudinal sections through sieve plates from the stolon of Saxifraga after inhibitor treatment and fixation in acetic-alcohol at -20° C.

Plate A. Control: stolon subjected to 5 hours light and air.

Note absence of callose and coagulated P-protein filled pores.

Plate B. Stolon subjected to 5 hours dark and air.

No callose; blocked pores.



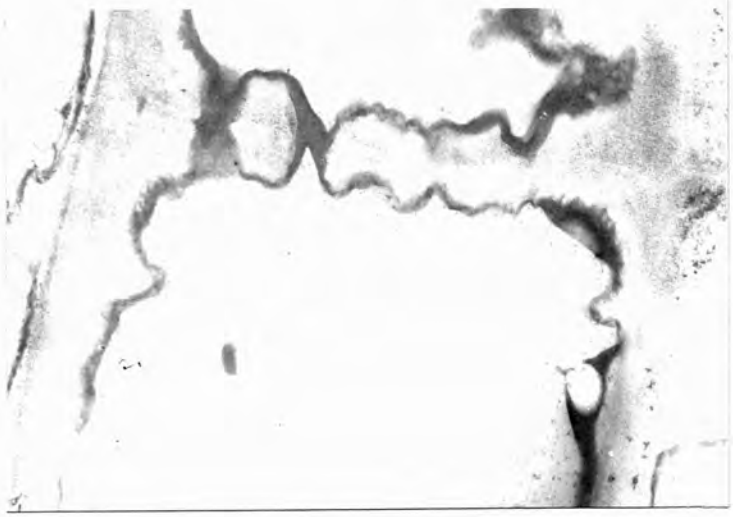


Plate C. Stolon subjected to light and air for 22 hours.
No callose; pores plugged.

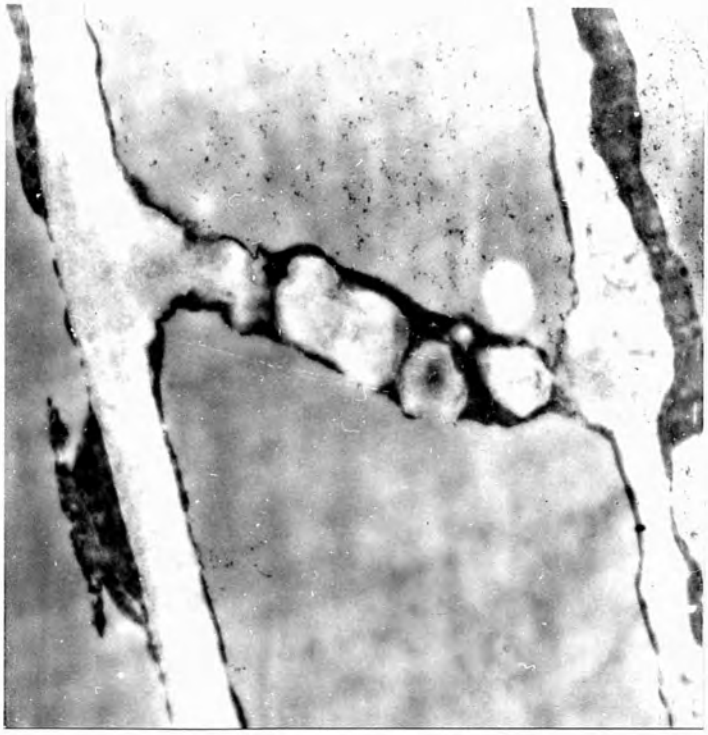


Plate D. Stolon subjected to "zero HCN" (i.e., control) for 7 hours.
No callose but de-natured P-protein present in sieve pores.

Plate E. Stolon subjected to HCN (10^{-4} M) for 7 hours.
Note absence of callose and plugged sieve pores.

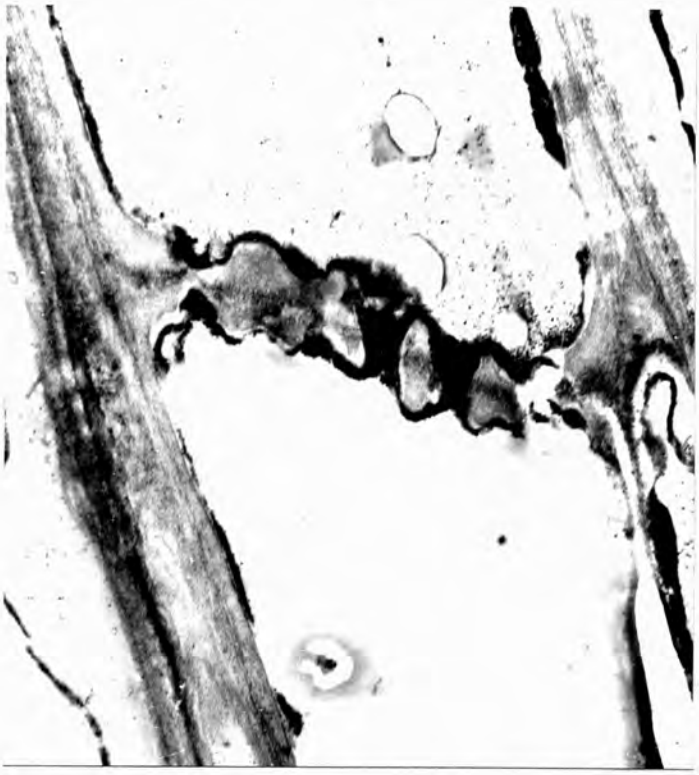


Plate F. Stolon subjected to HCN (5×10^{-3} M) for 7 hours.
Comparable to Plate F. (Control.)

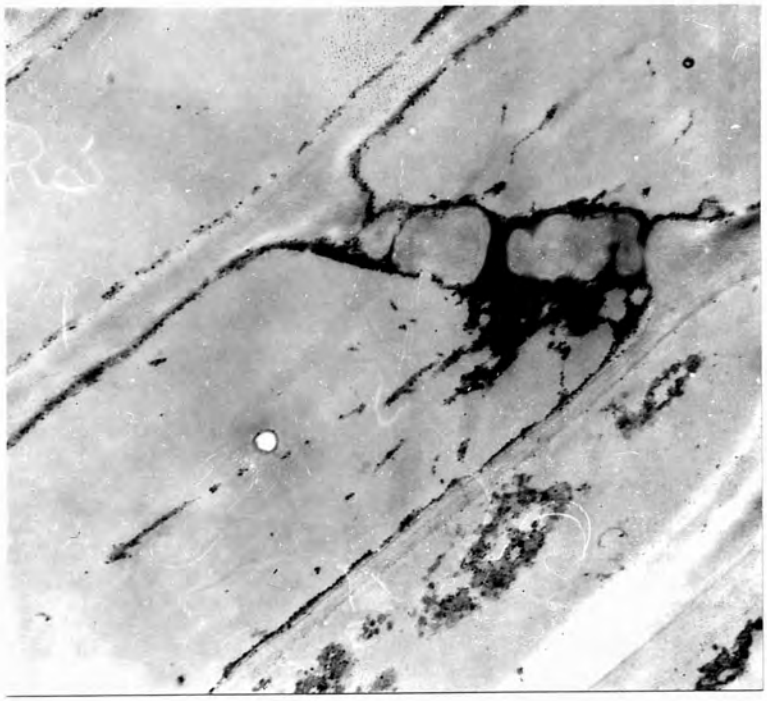
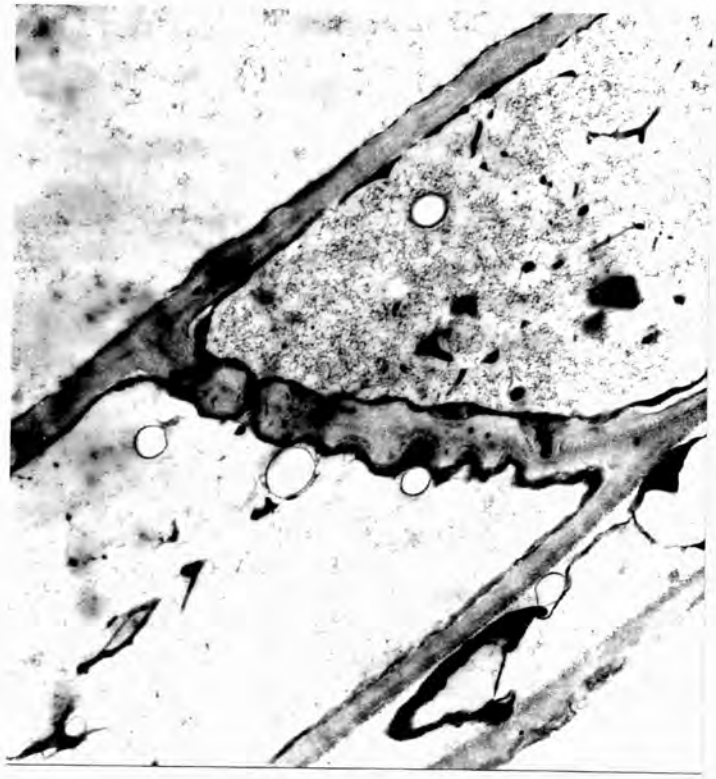


Plate G. Stolon treated with "zero DNP" (i.e., control) for 8 hours. Note the absence of callose and plugged sieve pores.

Plate H. Stolon treated with DNP (10^{-4} M) for 8 hours. Comparable to control (Plate J).



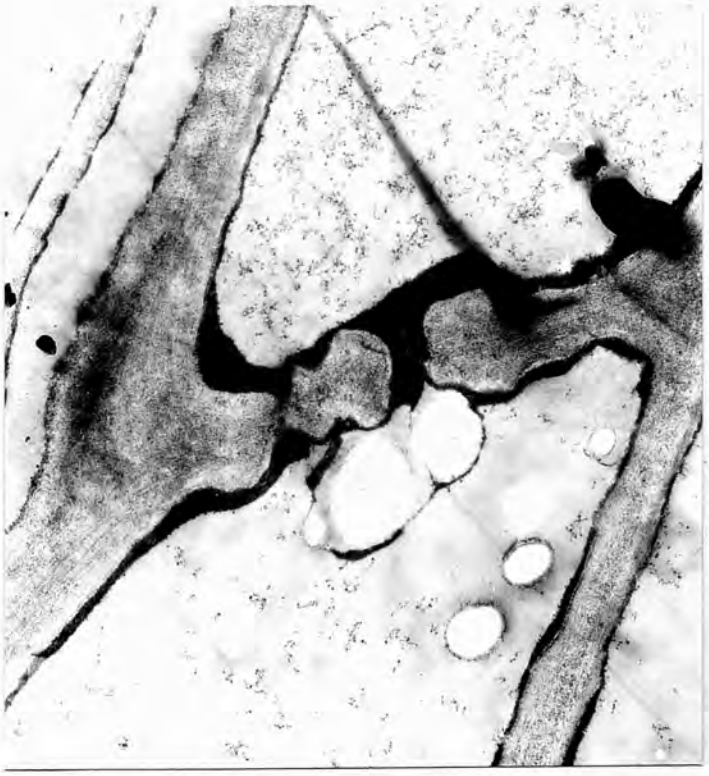
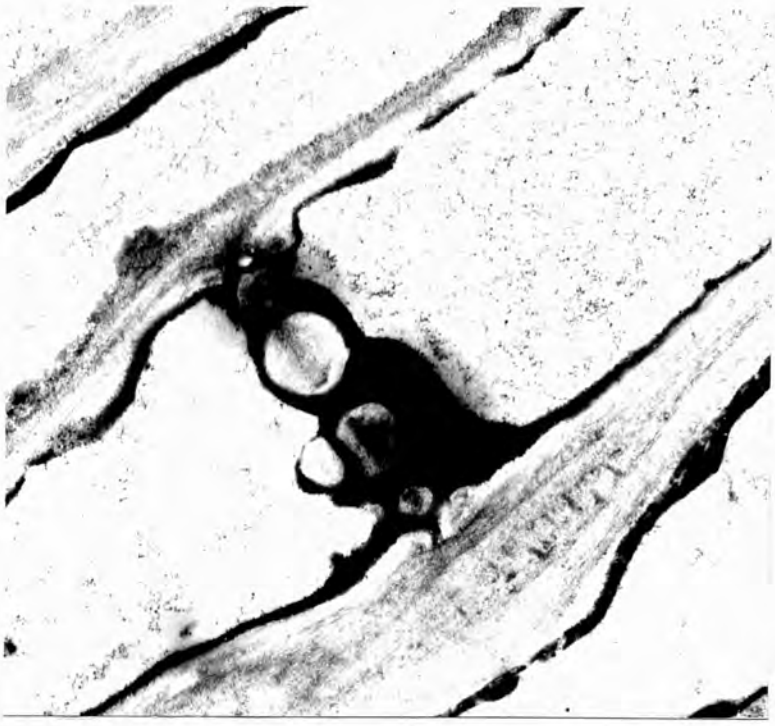


Plate J — Stolon treated with DNP (10^{-3} M) for 8 hours.

Comparable to control (Plate J).

Plate K — Stolon treated with DNP (10^{-2} M) for 8 hours.

Comparable to control (Plate J).



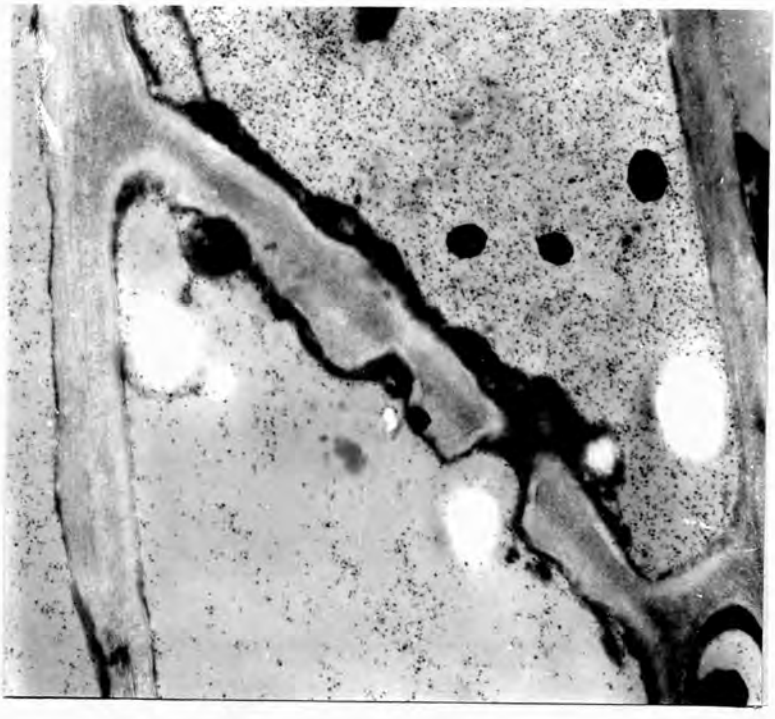


Plate 4. Stolon treated with M $SrCl_2$ for 18 hours.
Note absence of callose and coagulated P-protein filling sieve pores.