STUDIES ON TRANSPORT PHENOMENA IN STOLONS OF
*Saxifraga sarmentosa* WITH PARTICULAR REFERENCE
TO THE ACTION OF AUXIN

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STATEMENT OF THE PROBLEM

The problem of hormone-directed transport has been the subject of a considerable amount of work yet its mechanism still remains unclear.

Two main hypothesis have so far been proposed to explain this phenomenon:

i-The hormone, in particular auxin, acts directly on the whole pathway of transport probably by stimulating protoplasmic streaming in the transcellular strands (Davies and Wareing, 1965). Alternatively, the hormone may directly promote the photosynthetic activity in the leaves (Bidwell, 1973; Bidwell and Quong, 1975) or directly interfere with the mechanism of phloem "unloading" at the "sink" region (Philips, 1975).

ii-The second hypothesis advocates an indirect effect of the hormone on the long-distance transport of metabolites. According to this theory, the hormone stimulates or maintains the rate of synthetic activity at the treated region, therefore creating a "metabolic sink" towards which the nutrients and/or plant growth regulators are attracted.

This hypothesis stems from the "nutrient diversion theory" put forward by Went (1936; 1939) who, in connection with the problem of apical dominance, suggested that the nutrients moved within the plant towards areas of highest auxin concentration.

Objections have been raised against both theories, and up to now, there is no direct and definitive proof which might enable us
to accept one or reject the other.

In the present study, an attempt was made to verify the validity of either of these two theories in stolons of *Saxifraga sarmentosa* by using the following approaches:

1. Blockage of the downward stream of auxin by using several inhibitors of auxin polar transport, and observing their effect on the auxin-directed translocation of metabolites and their metabolism.

2. Reduction of the demand for assimilates at the treated region by either inhibiting the synthesis of RNA and/or proteins, or supplying directly to that area metabolites or high concentrations of mannitol.

Other points were also investigated, namely the effect of several substances on the movement and metabolism of IAA-5-\(^3\)H, as well as an attempt to localize the pathway of the basipetal transport of applied auxin.
SUMMARY

Plant hormones, especially auxins, have been known to influence the movement of solutes within the plant.

Despite the considerable amount of work done on the subject the mechanism of hormone-directed transport still remains unclear.

In the present study, an attempt was made to investigate further this problem in stolons of Saxifraga sarmentosa, using isotopically-labelled metabolites and particularly auxin-transport inhibitors or inhibitors of RNA and protein synthesis.

An attempt was also made to investigate the effect of these inhibitors on the movement of tritiated indol-3yl-acetic acid, IAA-5-3H.

It has been shown for young decapitated stolons of Saxifraga sarmentosa, that application of IAA to the cut surface greatly increased the accumulation of 14C-labelled assimilates in the treated tissues. Aqueous solutions of gibberellic acid (GA3) also increased the import of 14C-photosynthates into the treated region, whereas a solution of kinetin had little or no effect. Combinations of these plant growth substances and IAA did not show any significantly greater effect than IAA alone.

When "rings" of aqueous solutions of auxin transport inhibitors such as 2,3,5-triiodobenzoic acid (TIBA), N-1-naphthylphthalamic acid (NPA) or morphactin were interposed between the "source" of labelled metabolites and the IAA-treated region, no significant effect on the IAA-induced movement of labelled assimilates was recorded. However,
when applied in combination with IAA at the decapitated region, TIBA (10^{-4} M) inhibited almost completely the effect of IAA, whereas NPA (10^{-4} M) or morphactin (10^{-4} M) slightly enhanced the effect of IAA. All three substances blocked almost completely the basipetal movement of IAA-5^{-3}H in intact stolons of the same plant.

The inhibitors of RNA and protein synthesis (actinomycin-D, puromycin and cycloheximide) when applied in solution as a "ring" between the source of radioisotope and the IAA-treated region had little or no effect on the movement of labelled assimilates; when applied in combination with IAA at the decapitated end, these substances inhibited to a considerable extent the IAA-induced accumulation of ^{14}C-photosynthates in the treated tissues. The polar movement of IAA-5^{-3}H was little affected by both actinomycin-D (20 mg/l) and puromycin (2x10^{-4} M) whereas cycloheximide (10 mg/l) had a strong inhibitory effect on the same movement.

Other substances, such as endothal and 2,4-dinitrophenol, at concentrations which caused callose formation, had only a small effect on the movement of ^{14}C-labelled assimilates in intact stolons of Saxifraga, but both compounds, at the same and lower concentrations, inhibited the basipetal transport of IAA-5^{-3}H.

Application of sucrose or high concentrations of mannitol directly to the IAA-treated region, considerably reduced the amount of ^{14}C-photosynthates which accumulated in this area. Similarly, the polar transport of IAA-5^{-3}H was also interfered with, but not completely stopped, by high concentrations of mannitol.

The results of the present study strongly suggest that the
action of the hormone on the long-distance translocation of metabo-
lites is a localized one, confined to the treated region and nearby,
probably mediated through a local stimulation of the metabolism.
They do not support the hypothesis that IAA acts by activating the
whole pathway of transport.
PART I

Plants show some very complicated and highly organized
structures with different parts serving different functions. All
plants contain a transport system in order to keep the plant
in a steady condition. For instance, leaves have the job of synthesizing
food, while roots are the locations where water and nutrients
are obtained in order to live. These components then be
translated to other parts of the plant and the whole maintains the ideal trans-
port system through which their nutrients flow.

It is a well-established fact that in desert plants, such as
desert and other growth factors (Clayton and Ohlson 1977) have lower
levels of pollen growth. Moreover, changing leaves into other
mechanisms increases their ability to photosynthesis. Their ability, then, increases, their need, and
succession. These plants can also have a very high
transpiration (Clayton, 1981; Thomson and Pierce, 1989; Van Bokhoven
in May 1980; Duncan, 1981; Smith and Orgy, 1986).

The Ch. 2: reaction mechanism is still very simple in bacteria in
specific cases (Van Bokhoven).

Hard-like, 1981, in association with the mechanism of ammonia
production, had become a major source of nitrogen in the absence
of ammonia and adequate levels within the plant. However,
these levels are in higher when needed. Thus, hard conditions such as
over the years, are unlikely to affect the flow of ammonia through
these pathways.
I-INTRODUCTION

Higher plants are very complicated and highly organized entities in which different organs perform different functions, all working together in an integrated system in order to keep the plant as a living organism. For instance, leaves have the task of synthesizing carbohydrates and other nutrients, which are utilized in other parts of the plant. These compounds have to be rapidly translocated to other areas of the plant and the phloem provides the ideal transport system through which these nutrients move.

It is a well established fact that, in intact plants, nutrients and other growth factors (Morris and Winfield 1972) move towards regions of active growth, such as young developing leaves and apical meristems (Kursanov, 1961; Hartt, 1962; Humphries, 1963; Hartt and Kortschak, 1964). These regions are also known to have a high auxin concentration (Thimann, 1934; Thimann and Skoog, 1934; Van Overbeek et al., 1947; Jacobs, 1952; Scott and Briggs, 1960).

Now the question arises - is there any causal relationship between these two phenomena?

Went (1936, 1939), in connection with the phenomenon of apical dominance, put forward a theory known as "Nutrient Diversification Theory" in which he suggested that nutrients moved within the plant towards areas with highest auxin level. Thus, Went considered that auxin had, in some way, the ability to direct the flow of solutes towards those regions.
Mitchell and Martin (1937), and Stuart (1938), working with bean plants, provided direct evidence that nitrogenous compounds and carbohydrates accumulated in the region of the stem treated with IAA. The experimental period, in these cases, extended for several days, but the IAA-induced effect was already noticeable after 12 hours.

The advent of radioactive tracers, and their application to the study of several phenomena in plants, provided a considerable impetus to the effort of trying to clarify the role played by plant hormones in the control of the translocation of metabolites in the phloem. For example, Booth et al., (1962) showed that decapitation of Pisum sativum and Solanum andigena plants, followed by treatment with IAA in lanolin, produced an increased accumulation of $^{14}C$-labelled assimilates and $^{14}C$-sucrose in the stumps. In this case, a period of only a few hours (6-12 hours) elapsed between the application of IAA and the end of the experiment.

The ability of certain plant hormones, and in particular IAA, to induce the accumulation of nutrients in the regions of its application has been given the name of "Hormone-directed Transport" (H. D. T.). Although a considerable amount of study has been made of this phenomenon, its mechanism is still far from clear.

In the next pages, I shall try to present some of the theories that have been put forward to try and explain the mechanism of H. D. T., and some of the evidence, so far produced, relating to this subject.
The stimulus due to IAA treatment may act at three different sites of the pathway of transport.

1. At the treated region

In this case, IAA may produce its effects on the long distance transport of metabolites in three different ways:

a. Indirectly, by stimulating or maintaining the local rate of metabolic activity, so that, the treated region becomes a "metabolic sink", that is, an area of high demand for nutrients, in which those compounds are used in synthetic activities.

b. Auxin may act in a more direct way, for instance, stimulating the rate of phloem "unloading" in the treated region. This hypothesis assumes that in the movement of metabolites out of the sieve tubes, at the "sink" region, some active process is involved. This means that the rate of phloem unloading should not be entirely dependent upon the demand for metabolites of the surrounding tissues, and should be able to proceed against a concentration gradient.

c. Still in this context, it is possible that IAA may prevent the formation of callose or any other "plugging mechanism" in reaction to the decapitation, which might render the phloem unfunctional (or restrict the movement of solutes) in that region.

2. IAA affects the whole length of the transport pathway.

In this case, the hormone would have a direct effect on the translocation of metabolites, by stimulating the activity of phloem sieve tube elements, perhaps on the protoplasmic streaming of the
transcellular strands (Thaine 1961, 1962), or maintaining the phloem integrity.

This interpretation implies that induced movement of metabolites is dependent upon the basipetal transport of auxin. Any factor that blocks such a transport should also necessarily stop the auxin-directed long distance transport of nutrients.

3. IAA acts at the "source" region

Two different ways may be considered:

a. IAA treatment might increase the photosynthetic rate in leaves, therefore enhancing the amount of carbohydrates available for translocation. Here again, the stimulation of photosynthesis might be brought about, indirectly, as a response to increased demand for metabolites at the "sink" site. Alternatively, auxin might act directly on some photosynthetic process in the leaves and, in such a case, it would have to be transported from the point of application to the point where the action would take place.

b. Another possibility is that IAA might promote the rate of phloem loading at the source.

Some evidence indicates that the transfer of metabolites from the mesophyll of the leaf into the vascular tissue is an energy driven process (Phyllis and Mason, 1933; Brovchenko, 1965; Bieleski, 1966) and IAA could, in some way, influence this process.

As we have mentioned above, Mitchell and Martin (1937) and Stuart (1938) were the first to produce evidence showing that
nutrients (nitrogenous compounds and carbohydrates) accumulated in the portion of the stem treated with IAA, and below. These authors used intact bean plants and the experiment was carried out over a period of several days. In such an extended experimental period, it is reasonable to assume that the treatment with auxin caused the appearance of a new growth centre, which has the ability to draw metabolites from other parts of the plant where they are available.

Booth et al. (1962) tried to avoid the possibility of any IAA-induced growth by reducing the experimental period. They showed that $^{14}$C-sucrose and $^{14}$C-labelled assimilates accumulated in decapitated internodes of Solanum andigena and Pisum sativum plants treated with IAA in lanolin, within only a few hours after the application of the hormone.

Davies and Wareing (1965), in the same plants, observed a similar response to IAA on the transport of $^{32}$P, but neither 2,4-dichlorophenoxy acetic acid (2,4-D), a synthetic auxin, nor gibberellic acid or kinetin had any significant effect. These results were extended to disbudded poplar cuttings. The same workers were also able to show that auxin-induced transport took place in the phloem and that the auxin effect was greatly reduced when 2,3,5-triiodobenzoic acid (TIBA), an inhibitor of IAA basipetal transport, was applied together with IAA at the decapitated internode, or as a ring between the sources of IAA and $^{32}$P. On the basis of these observations, they suggested that hormone-directed transport was independent of growth, and was probably due to some direct effect of the hormone.
acting along the phloem.

A similar effect of TIBA on the auxin-induced transport of $^{32}$P was obtained by Sebassak (1966), in etiolated pea seedlings.

On the other hand, Panigrahi and Audus (1966), in *Vicia faba* plants, and Mullins (1970), in *Phaseolus vulgaris* L., failed to observe any significant reduction of the accumulation of $^{14}$C-2-uracil and $^{14}$C-photoassimilates respectively, in the IAA-treated stumps, due to TIBA application.

Similar results were obtained by Morris, Kadir and Barry (1973) who noticed that a TIBA ring applied to the stem of intact pea seedlings, although inhibiting the transport of $^{14}$C-IAA, did not prevent the movement of labelled sugars into the apical region of the shoot.

The effect of auxins in directing the flow of metabolites within the plant has since been repeatedly confirmed by several workers in different plants (Seth and Wareing, 1964, 1967; Morris and Thomas, 1968; Phillips, 1968, 1969; Mullins, 1970; Patrick and Wareing, 1970, 1973; Bowen and Wareing, 1971; Morris and Winfield, 1972; Breen and Muraoka, 1973; Tupy, 1973; Bidwell and Quong, 1975; Wardlaw and Moncur, 1976). However, it appears that the effectiveness of a particular hormone in directing the flow of metabolites depends greatly on the plant studied, as well as on the nutrient translocated (Bowen and Wareing, 1971). Furthermore, different synthetic auxins may either be effective in different systems, or may produce different degrees of response in the same plant.
The same workers, when investigating the basipetal movement of four different applied auxins in the stem of Phaseolus vulgaris L. and Pisum sativum L., could not find any clear correlation between the extent of the basipetal transport of the hormone, and the hormone efficacy in inducing the transport of nutrients.

Effect of other plant growth regulators.

In detached leaves, cytokinins have been found to cause the movement of labelled amino acids, $^{32}$P, and $^{14}$C-photosynthates (Mothes, 1960; Mothes and Engelbrecht, 1961; Gunning and Barkley, 1963; Muller and Leopold, 1966), from untreated to treated areas. However, other radioisotopes, such as $^{22}$Na, $^{36}$Cl, $^{88}$Rb and $^{131}$I, were not equally attracted to kinetin-treated regions (Muller and Leopold, 1966).

The data available regarding the effect of cytokinins in other organs of the plant are somewhat conflicting. For instance, cytokinins, when applied alone to decapitated stems and pedicels of several plants, have generally failed to produce any significant response (Seth and Wareing, 1964; Davies and Wareing, 1965; Sebanek, 1965; Morris and Thomas, 1968). A few exceptions have been reported; Seth and Wareing (1964), and Mullins (1970) found increased transport of $^{32}$P, in Pisum, and $^{14}$C-sucrose, in Phaseolus, in response to 6-benzylaminopurine (BA) and 6-(benzylamino)-9-(2-tetrahydropyranyl)-9-H purine (SD 8339) treatment, respectively. Benzyladenine and kinetin were also found to increase the movement of $^{14}$C-labelled assimilates into shoots and roots of Vitis vinifera plants (Shindy
and Weaver, 1967, 1970; Shindy, Kliewer and Weaver, 1973), and into young *Citrus* fruits (Kriedemann, 1968).

When applied together with indol-3yl-acetic acid (IAA), cytokinins considerably promoted the IAA-induced directional movement of nutrients (Seth and Wareing, 1964, 1967; Sebanak 1965; Morris and Thomas, 1968; Mullins, 1970) which points to a synergistic interaction between the two hormones.

Similarly, gibberellins by themselves have been found to have little or no effect on "Hormone-directed Transport" in several cases (Seth and Wareing, 1964, 1967; Davies and Wareing, 1965). Some evidence, however, has shown increased transport of nutrients in decapitated flowering carnation shoots (Jeffcoat and Harris, 1972), decapitated internodes of *Phaseolus vulgaris* (Mullins, 1970), and in flowers and young fruits of *Vitis vinifera* L. (Weaver et al., 1969), after gibberellic acid (GA₃) treatment.

If gibberellic acid is applied together with IAA, the effect of the latter is considerably increased (Seth and Wareing, 1964, 1967). The greatest effect, however, was obtained when a mixture of the three substances (auxin, gibberellic acid and cytokinin) was used (Seth and Wareing, 1967; Mullins, 1970).

These results suggest that the three groups of hormones are involved in the control of "Hormone-directed Transport"; in decapitated stems IAA is probably the natural limiting factor, whereas in the leaves cytokinins are probably the factor in deficit.
Effect of auxins in nucleic acid and protein metabolism.

It is now clear that plant growth substances, especially auxins, can influence nucleic acid and protein metabolism in a variety of plant tissues. For instance, Silberger and Skoog (1954) demonstrated that indol-3-yl-acetic acid enhanced DNA and RNA synthesis in tobacco pith tissue, and that the nucleic acid synthesis increase took place before any growth response could be detected. Since then, several authors have been able to confirm these results in other plant materials, such as: Rhoeo leaves and bean endocarp (Sacher, 1967), yeast cells (Shimoda et al., 1967), green pea stem sections (De Hertogh et al., 1965), oat coleoptile sections (Masuda, 1966, 1968; Masuda et al., 1967), sterile tobacco tissue (Klambt, 1974), elongating and maturing soybean hypocotyl (Key and Shannon, 1964; Key and Ingle, 1968; Key, 1969).

By measuring the rate of incorporation of labelled precursors into RNA and proteins, it has been possible to determine the extent to which auxins affect nucleic acid and protein metabolism. Using this process, several workers have observed increased incorporation of such precursors following auxin treatment of different plant tissues (Basler and Hansen, 1964; Key and Shannon, 1964; Hamilton et al., 1965; Masuda et al., 1967; Patterson and Trewavas, 1967; Masuda, 1968; Key, 1969; Mullins, 1970).

Some variability has been observed in response to auxin treatment in some tissues (Carpenter and Cherry, 1966). In such
cases, the inefficacy of auxin in increasing the overall level of protein in these tissues does not mean that auxins are completely without effect on protein synthesis. It is possible that qualitative, rather than quantitative, changes in the pattern of protein synthesis may have taken place, leading to the formation of certain specific enzymes to the detriment of others. Evidence supporting this point of view has been provided by the data of Trewavas (1968a and b) and Patterson and Trewavas (1967). Also, it has been observed that auxin may promote the synthesis of certain types of RNA polymerase while having little or no effect in promoting the synthesis of others (Hardin and Cherry, 1972; Teissere et al., 1973; Guilfoyle et al., 1975; Rizzo and Cherry, 1976).

In auxin-responsive tissues, enhanced synthesis has been reported to take place within minutes up to 2-3 hours of incubation (Key and Shannon, 1964; Truelsen and Galston, 1966; Masuda et al., 1967; Key, 1969; Masuda and Kamisaka, 1969).

Auxin-induced promotion of RNA and protein synthesis has usually been associated with auxin-induced cell elongation (Masuda et al., 1967; Key et al., 1967; Cherry, 1967; Masuda, 1968). There is little doubt now that sustained growth requires continuous RNA and protein synthesis, since the inhibition of the latter, by specific inhibitors, also brings about a parallel inhibition of growth (Nooden and Thimann, 1963; Masuda and Wada, 1966; Coartney et al., 1967; Key et al., 1967; Key and Ingle, 1968). However, the auxin effect on nucleic acid and protein synthesis does not seem to be a consequence
of auxin-induced growth, for it can still occur in conditions of osmotic stress, in which no elongation takes place (Masuda et al., 1967; Masuda, 1968).

Some authors believe that the effect of plant hormones in H.D.T. is brought about through their stimulation of metabolic activities. For instance, Mullins (1970) has found that, in decapitated stems of Phaseolus vulgaris L., an increased incorporation of $^{14}$C-leucine into protein, and $^{14}$C-orotic acid into RNA, was visible 2.5 hours after treatment with a mixture of IAA, gibberellic acid, and cytokinin, whereas promotion of accumulation of $^{14}$C-photosynthates was detectable only 3 hours after treatment with the same mixture. Further support comes from the findings of Tupy (1973), who demonstrated that the sucrose-mobilizing action of three auxins in Hevea brasiliensis depended on previous activation of the overall metabolism.

Breen and Muraoka (1973) have similarly noticed that in softwood cuttings of plum treated with indolebutyric acid (IBA) at the base, the pattern of $^{14}$C-photosynthates remained unchanged, relative to the untreated ones, until a callus developed.

On the other hand, Patrick and Wareing (1970), when investigating the metabolic activity of decapitated internodes of bean plants 9 hours after treatment with IAA, could not find any significant change, either in the respiration rate, or in sucrose uptake and metabolism. However, 12 hours after decapitation, there was a pronounced fall in the rate of incorporation of $^{14}$C-leucine into protein, and of total protein content, in untreated internodes,
whereas IAA treatment prevented both.

Mullins (1970) also has observed, 24 hours after decapitation, a 20 per cent reduction in the incorporation of $^{14}$C-leucine into protein relative to the original rate, and the fall in the protein content is accompanied by a decline in the accumulation of $^{14}$C-assimilates. He, therefore, suggested that the senescence-delaying effect of auxins might provide some understanding of the mechanism of "Hormone-directed Transport".

The involvement of plant hormones in the control of senescence of excised plant parts had been known for some time (Wollgishn, 1961; Osborne, 1962; Sacher, 1965, 1967, 1968; Beavers, 1966; Von Abrams and Pratt, 1968). Thus, application of Kinetin, or other cytokinins to detached leaves of herbaceous plants prevents yellowing, whereas auxins are less effective in preventing senescence in this system. The reverse seems to be true in excised stem segments, in which the delay of senescence rests almost entirely with auxins (Sacher, 1967).

The hormonal control of senescence appears to be, at least in part, mediated through its effect on nucleic acid and protein metabolism (Osborne, 1962).

The retarding effect of hormonal treatment on the loss of RNA and protein may be due, either to sustained or increased synthesis of RNA and protein (Carpenter and Cherry, 1966; Sacher, 1967; Burdett and Wareing, 1968), or to suppressed nuclease and protease activities.
in the tissues (Srivastava and Ware, 1965; Balz, 1966; Pilet and Braun, 1970; Trüelsen, 1967), or perhaps both.

In the face of this evidence, it is reasonable to admit that the stumps of decapitated internodes, deprived of their main source of auxin, are senescent systems, and that the auxin-induced accumulation of metabolites is a reflection of a higher metabolic activity, relative to untreated ones. On the other hand, it is also possible that in such a system the ability of the phloem to translocate may decline, and that auxin may be required to maintain the phloem integrity.

Evidence against the hypothesis that the retention of RNA and protein in auxin-treated tissues of decapitated internodes may be the cause of the hormone-induced transport of nutrients was produced by Patrick and Wareing (1973). These authors demonstrated that decapitated internodes "aged" for 3 days with plain lanolin were still able to respond to IAA treatment, in terms of increased transport of nutrients. Yet, after similar treatment, no difference in the protein level between IAA-treated and lanolin controls could be found.

These results seem to indicate that hormone-directed transport cannot be entirely explained in terms of protein loss.

Possible effects of auxins at the metabolites source level.

One way by which plant hormones, auxins in particular, could increase the rate of translocation is by, directly or indirectly,
stimulating the source.

Indeed, some evidence has been produced suggesting that IAA treatment of parts of the plant may stimulate the photosynthetic rate in the leaves (Bidwell and Turner, 1966; Bidwell et al., 1968; Bidwell, 1973; Bidwell and Quong, 1975).

The effect of auxins on photosynthesis might be a secondary response, due to an increased demand for carbohydrates, which had been created by the hormone at the "sink" level. Some data has been produced suggesting that the demand for metabolites at the "sink" region can control the speed of nutrients through the phloem (Wardlaw, 1965, 1974; Wardlaw and Moncur, 1976) and, in some cases, the rate of photosynthesis (Wardlaw, 1968; Habeshaw, 1973). These results would also explain the directional characteristic of hormone-directed transport. However, there are objections to this interpretation; as we have already mentioned, Patrick and Wareing (1970) could not find any effect of IAA on "sink" activity.

Alternatively, auxin might directly increase the efficiency of the photosynthetic process (Tamas et al., 1972), consequently increasing the amount of carbohydrates available for export. This, by itself, would hardly account for the directional feature of the auxin-induced mobilization of metabolites.

To obviate this deficiency, Bidwell (1973), and later on Bidwell and Quong (1975), tried to explain the auxin-induced movement of nutrients by suggesting that IAA might move from its site of application, or production, to the leaf through a specific channel,
probably a vascular connection. He further suggested that IAA might affect only certain areas of the leaf associated with that particular vascular system and, in response, those parts would react by increasing their photosynthetic rate. Then, the export of carbohydrates would occur only through the same vascular connection and move towards the IAA source.

This explanation of H.D.T. implies that the auxin would have to be transported from its site of application, or production, and reach the source of metabolites.

Wardlaw and Moncur (1976) showed that in wheat plants whose ears had been removed and replaced with plain agar or agar plus auxin, the insertion of a 10 cm cold jacket between the site of IAA application and the source of metabolites considerably reduced the basipetal movement of IAA, but did not prevent the auxin-induced accumulation of $^{14}$C-assimilates in the top of the peduncle. For the same reason, the results of experiments in which triiodobenzoic acid (TIBA) was used as an inhibitor of IAA basipetal transport, and in which no restriction on the movement of metabolites was observed (Mullins, 1970, Morris, Kadir and Barry, 1973), must be regarded as evidence against such an explanation.

Hew et al., (1967) showed that application of IAA or gibberellic acid (GA) to decapitated soybean plants increased the export of photosynthate from the primary leaves, and affected the pattern of distribution of assimilates within the plant, but had no effect on the rate of photosynthesis. Because the applied IAA could be recovered
only from the stems, the authors suggested that the IAA effect was on the longitudinal translocation in the stem, and not on the leaves.

Carbohydrates synthesized in the chloroplasts in the leaves have to be transferred to the sieve tubes, before they are ready to be translocated. The movement of sugars (sucrose) into the phloem is an energy-driven process, and, as such, requires metabolic energy and takes place against a concentration gradient (Kursanov, 1963; Hartt, 1965; Bieleski, 1966; Ford and Peel, 1967; Milthorpe and Moorby, 1969). It is possible, then, that IAA may act at this level by stimulating "loading" of the sieve tubes.

Lepp and Peel (1970, 1971) produced some evidence which indicates that in willow bark strips both IAA and kinetin can increase the flux of sucrose into the phloem, the effect being particularly evident in the case of IAA.

Contrary to the findings of several workers (Booth et al., 1962; Seth and Wareing, 1964, 1967; Davies and Wareing, 1965; Morris and Thomas, 1968; Bowen and Wareing, 1970; Mullins, 1970; Patrick and Wareing, 1970; Jeffcoat and Harris, 1972), Lepp and Peel (1970) found out that applied 14C-sucrose moves away from the place of IAA and kinetin application.

**Effect of plant growth regulators on callose formation.**

Decapitation of a plant organ is a rather drastic operation and, as such, may well produce a considerable disturbance in the
biochemistry and physiology of the organ and of the plant as a whole. In such circumstances, it is only logical to assume that the plant may react to injury by trying to counterbalance the disturbance produced. For example, damage to the phloem, as well as some other treatments, have been known to result in callose deposition in the sieve plates of functioning sieve tubes (Currier, 1957; Evert and Derr, 1964; Engleman, 1965; Eshrich, 1965). This reaction of the plant to wounding is a rather rapid one, and must be regarded as a defence against excessive loss of sieve tube content. It is therefore perfectly possible that such "plugging" mechanism may have some restricting effect on translocation. It is also likely that auxins, and perhaps other plant growth regulators, may affect, in some way, the formation of callose.

Some evidence has now been produced (Thomas and Hall, 1975), showing that IAA or gibberellic acid, at the appropriate conditions, inhibit the formation of wound callose. The IAA effect could be detected 30 minutes after the treatment. On the other hand, kinetin and abscisic acid (ABA) had no effect, and ethrel promoted the deposition of callose.

Treatment of plant organs with other foreign chemicals (Crafts and Currier, 1963) as well as other stimuli (Currier and Webster, 1964; Webster and Currier, 1965) have also been known to induce callose deposition. For instance, Ullrich (1963) noticed that cyanide treatment (KCN) of central bundles of *Pelargonium zonale* petioles, clearly stimulated callose formation on many sieve plates.
Similarly, endothal (disodium-3,6-endoxohexahydrophthalate), an herbicide, has also proved to promote callose deposition (Maestri, 1967).

Although it appears that a considerable amount of callose can be deposited around the sieve plate pores before interfering too much with the longitudinal translocation of assimilates (Ullrich, 1963; Currier and Webster, 1964; Webster and Currier, 1965), blockage of lateral transport of metabolites into and out of the sieve tubes, as well as longitudinal translocation, have been observed in some cases, apparently due to callose deposition (Webster and Currier, 1965; Currier, McNairn and Webster, 1966; Maestri, 1967; McNairn and Currier 1968; McNairn, 1972).

In the light of these observations, it seems probable that, under certain conditions, callose deposits in the sieve plates can to some extent control translocation; thus, IAA and some other plant growth regulators, by inhibiting callose formation, may also affect the translocation of metabolites.

As I said earlier, the problem of the mechanism of "Hormone-directed Transport" is still not completely understood. I have briefly outlined some of the possible ways by which IAA might stimulate the transport and accumulation of metabolites.

Any available evidence, which may help to support or discredit any of the theories put forward, will help to confine and simplify the problem and, therefore, will be a valuable step in the direction of finding a solution to the problem.

One of the main theories advanced assumes that the IAA-induced
movement of nutrients is dependent on the basipetal transport of auxin (Davies and Wareing, 1965). If this theory were to be correct, it would follow that any substance which, in some way, has the ability to block the movement of auxin will also inhibit the auxin-induced transport of metabolites. In this respect are of particular interest those substances which can block the basipetal movement of auxin without interfering with the metabolic reactions of the cell.

Except for Caldeira's work (1971), who noticed that a ring of NPA (0.25% or 1%) in lanolin applied around the stem of Pisum plants did not block the upward movement of $^{32}$P, the only inhibitor of IAA transport used so far in this connection has been TIBA (Davies and Wareing, 1965; Panigrahi and Audus, 1966; Sebanek 1966; Mullins, 1970) with somewhat conflicting results.

In these circumstances, I decided to investigate further the effect of TIBA, and other inhibitors of IAA polar movement, on the IAA-induced transport of labelled assimilates and its metabolism.

Another approach to the same problem, also used in this study, was the application of substances which are known to inhibit, specifically, the synthesis of RNA and proteins. It was hoped that these compounds would have little effect on the basipetal transport of IAA.

In Part I of this study, I shall present the results of experiments dealing with the effect of inhibitors of auxin transport, as well as of inhibitors of RNA and protein synthesis, on the IAA-induced mobilization of $^{14}$C-labelled assimilates in the stolons of Saxifraga sarmentosa.
I shall also present some data concerning callose formation in the sieve tubes of the phloem, due to treatment of the stolons with 2,4-dinitrophenol (DNP) and endothal.

Part II will deal mainly with the effects of all these substances on the basipetal movement of tritiated IAA (IAA-5-\(^3\)H).
II. MATERIAL AND GENERAL EXPERIMENTAL METHODS

1. Plant material.

The experiments concerning the movement of labelled assimilates were performed in stolons of *Saxifraga sarmentosa*. These plants, when grown under appropriate conditions, produce rather long and uniform stolons, which are suitable for the investigation of the movement of metabolites in the phloem, since there is little interference from lateral appendages as is often the case in normal stems.

Young plants, supplied by the University of London Botanical Supply Unit, were grown in 9 cm diameter plastic pots in a heated greenhouse (temperatures varying between 15-17°C in winter to 25-27°C in summer) under normal conditions, except for some extra light provided during the winter by a bank of fluorescent lamps.

When the plant had reached the adequate stage of development, single stolons started to be produced at the axil of the mature leaves. Early in their development, these stolons were inserted through small holes bored in the platform on which the plant stood, and were allowed to hang vertically into a darkened enclosure below the platform. Under these conditions, they could be grown quite long (up to 60 cm) and uniform, whereas those exposed to the light remained comparatively short.

In some early experiments, I used stolons (henceforward referred as "old stolons") which had finished elongating and which supported a small daughter plant at the apical end. These "old stolons", besides
presenting considerable variability, also showed little response to plant growth-regulator treatment in general, and to indol-3yl-acetic acid (IAA) in particular. Later on, young stolons (about 25-30 cm long), which I found to be fairly responsive to IAA-treatment, were employed.

2. Preparation of solutions.

Plant growth substances, and other compounds used in this study, were usually applied in aqueous solution. Certain inhibitors of RNA and protein synthesis, such as actinomycin-D, puromycin and cycloheximide (purchased from Sigma Chemicals Co. Ltd., St. Louis U.S.A.), and the respiratory inhibitors, cyanide and 2-4-dinitrophenol, are readily soluble in water and, therefore, present no special problem when bringing them into solution. Endothal was supplied to me in a 21.4% aqueous solution and it was diluted to the required concentration.

However, with other substances which are not directly soluble in water or are only slightly soluble, different ways had to be employed in order to bring them into aqueous solution. For instance, indol-3yl-acetic acid (IAA) (B D H Chemicals Ltd., Poole, England) was first dissolved in a few drops of ethanol and then distilled water was added up to make the desired concentration; kinetin (6-furfuryl amino purine) (Sigma Chemicals Co. Ltd., St. Louis, U.S.A.) was first dissolved in a few drops of 0.1N HCl and the required concentration was obtained by adding up the right volume of distilled water; 2,3,5-triiodobenzoic acid (TIBA) (Koch Light Labs., Colnbrook, U.K.),
N-1-naphthylphthalamic acid (NPA), 2-chloro-9-hydroxyfluorene-9-carboxylic acid butyl ester (morphactin) (E. Merck, AG, Darmstadt, Germany), gibberellic acid (GA$_3$), were dissolved in a concentrated ammonia solution to which some distilled water was added and, finally, the excess of ammonia was boiled off in a water bath. The desired concentration was obtained by adding up the appropriate volume of distilled water.

In each case, the pH of the solution was checked and brought to about 6.5-7, by applying a few drops of a 1N solution of HCl or ammonia as the case required.

3. Treatment of the stolons.

Saxifraga plants bearing one or more young stolons, about 25-30 cm long, were selected, well watered and placed under continuous light for 24 hours before any treatment was started. At the same time all lateral buds or shoots and scales of the stolons were removed.

After 24 hours under such conditions, each stolon was placed horizontally in a 6.5 mm wide and 3 mm deep groove running along a perspex block. The apical 1 cm of the stolon was removed with a sharp razor blade and the epidermis of the terminal 2 cm of the decapitated stolon slightly abraded with fine emery paper. This region was immediately treated with an aqueous solution of the compound or mixture of compounds under investigation contained in a small polythene vial with an open end and a small hole on the opposite side. The decapitated end of the stolon was inserted through this small hole into the
container (fig. 1 and plate I). The stolons kept as controls were similarly treated with distilled water.

Some experiments were designed for investigating the effect of the plant growth regulators applied at some distance from the decapitated end on the movement of labelled assimilates. In these cases, in addition to the procedure already described, the epidermis of the 3–4 cm long chosen region was also slightly abraded with fine emery paper, and then carefully immersed in the aqueous solution of the compound in test. This solution was contained in a small reservoir limited by the lateral sides of the groove and two small amounts of soft white petroleum jelly. The reservoir was finally covered with transparent wrap film to avoid variation in the solution concentration due to evaporation (fig. 1 and plate I).

4. Application of radioactivity to the plant.

In early experiments, the movement of nutrients in the phloem of Saxifraga stolons was measured by using $^{32}$P as a "tracer". After a pre-treatment period of 18–20 hours, the radioisotope was applied directly to the stolon as an aqueous solution of $^{32}$P-labelled sodium orthophosphate using a procedure similar to that described by Qureshi and Spanner, (1971). Thus, the epidermis of a small area of the stolon (25 cm apart from the decapitated end) was slightly scratched with fine emery paper and embedded over a 2 cm length in soft white petroleum jelly, great care being exercised to avoid smearing the upper surface. The scratched region was then covered with a
Plate I

Set-up used in experiments designed to study the effect of several plant growth regulators on the transport of $^{14}$C-assimilates in stolons of *Saxifraga sarmentosa*.

1-Stolon
2-"Reservoir"
3-Small container with an open end and a small hole on the opposite side through which the apical region of the stolon was inserted.
4-Assimilation chamber with the subtending leaf enclosed.

Fig. 1

Diagramatic representation of part of the experimental set-up shown in the photograph above.

1-Stolon
2-"Reservoir"
3-Small container with open end
small reservoir of transparent tubing and luted around with petroleum jelly. 15-20 µl of radioactive solution containing 1.5-2.0 µCi of ³²P were then injected into the reservoir with a small syringe, and the reservoir covered to avoid evaporation.

This technique did not prove to be entirely satisfactory due to the fact that different amounts of radioisotope were taken up by different stolons giving rise to a considerable variability in the results. This variability might have been due in part to the fact that, at this stage, I was using "old stolons" which did not respond very well to the treatment.

Later on, ¹⁴CO₂, instead of ³²P, was used as an indicator of the translocation in the phloem and, for that reason, an entirely different technique had to be employed. Also at this stage, it was decided to change to young growing stolons which proved to be more responsive to treatment.

Thus, 18-20 hours after the treatment had started, ¹⁴CO₂ was supplied to each leaf subtending a stolon by enclosing it inside a transparent "poly glass" chamber. Two small drops of ¹⁴C-labelled sodium carbonate (Na₂¹⁴CO₃; specific activity 60 mCi/mM; Radiochemical Centre, Amersham, U.K.), containing 15-20 µCi of radioisotope were spotted on a small piece of filter-paper, dried under an infrared lamp, and then placed inside each chamber enclosing a leaf. The chamber was immediately sealed and the ¹⁴CO₂ released by flooding the piece of paper with a 1N HCl solution, by means of a syringe.

The chambers were removed after 1 hour of assimilation under
continuous light provided by an overhead lamp, and a further period, usually 4-5 hours, was allowed for translocation before the stolons were harvested.

5. Assessment of the distribution of radioactivity in the stolons of Saxifraga.

At the end of the translocation period, the stolons were harvested and immediately divided in 2 cm long segments. The sections were taken in sequential order starting from the apical end, so that 20 cm of the stolon were assayed and the remainder was discarded.

Each 2 cm was further cut in small pieces and dropped in 8 ml of a 2:1 v/v mixture of toluene-based scintillant fluid and triton -X-100 (Turner, 1968). The samples were kept in the dark for 48 hours and then vigorously shaken before being counted in an automatic scintillation spectrometer (N.E. 8312, Nuclear Enterprises Ltd., Sighthill, Edinburgh, Scotland.).

This procedure was adopted because it was noticed that the count rate rose with time until it levelled off after a period of about 48 hours, probably due to extraction of the cell content by the scintillant mixture. It was also observed that if dried instead of fresh sections were used, a considerable reduction in the count rate occurred, which seems to confirm that the rise of the number of c.p.m. in the first 48 hours, in fresh sections, was due to extraction of the cell content by the scintillant fluid.

There were a few cases in which the distribution of radioacti-
ivity had to be determined in the stele and in the cortex separately. Thus, the stele of each section had to be surgically removed and its radioactivity content, and that of the cortex, assayed individually.

The separation of cortex and stele was performed by making a very superficial incision along the section with a scalpel. Then the edges of the incision were gently pulled apart with the help of the scalpel and a pair of forceps and finally the stele was carefully removed. In stolons of Saxifraga this operation is not a difficult one due to the very favourable anatomy of this organ. Thus, the pith and the conducting tissues form a central cylinder surrounded by several layers of pericycle which are separated from the cortex by a well defined endodermis. The separation occurs at this level as I have been able to confirm by observing stained free-hand sections under a light microscope (plate II).

The c.p.m. obtained using the procedure described above were corrected for background radiation, but no attempt was made to correct quenching or self-absorption, having in mind the fact that the stolons were fairly uniform and, therefore, the samples should be equally quenched.

6. Effect of plant growth substances on the metabolism of $^{14}$C-labelled assimilates.

The fate of the $^{14}$C-labelled photosynthates after treatment with plant growth regulators was one of the points investigated. Several stolons were set up and treated in the way already
Plate II

Microphotographs of cross sections taken from stolons of *Saxifraga sarmentosa* showing the zone through which the cortex and the stele were separated.

A—Cortex and stele before separation

1—Zone of separation

B—Tissues of the stele after separation

C—Cortical tissues after separation
Plate II

A

B

C
described and, after a 5 hour transport period, the apical 4 cm of each stolon were harvested, the stele and the cortex separated and then cut in small pieces. The soluble fraction of $^{14}$C-labelled carbohydrates, in both cortex and stele, was then extracted by boiling the small pieces in 80% ethanol for a period of 10 hours, followed by two more changes of 3 hours each.

The three extracts relative to each treatment were combined, and the volume reduced to 2 ml under an infra-red lamp. A 1 ml sample was taken, added to a counting vial which already contained 10 ml of a 2:1 mixture of toluene-based scintillant fluid and triton-X-100, and counted in an automatic scintillation spectrometer.

After being dried in an oven at 65°C for 48 hours, the extracted tissues were incinerated inside the counting vials using a MicroMat Bf 5010 apparatus. The $^{14}$CO$_2$ released by this process was fixed by 1 ml of β-phenyl ethylamine contained in the vial and, later on, the radioactivity counted after adding 10 ml of the scintillant fluid mixture.

The radioactivity present in the samples of both ethanol soluble and insoluble $^{14}$C-labelled carbohydrates was corrected for background, and the external standard ratio of each sample determined.

The external standard ratio was considered to be a reasonable indicator of the level of quenching of each sample, so that, for purposes of comparison between the different samples, I decided to bring them to the same level of quenching by dividing the given counts of each sample by its external standard ratio.
Effect of DNP and endothal on callose formation in intact stolons of Saxifraga and its identification.

Intact stolons of Saxifraga were treated with a range of concentrations of DNP and endothal applied in solution at some distance from the apical end.

The effect of treatment with these compounds on callose formation was assessed 18-20 hours later. Immediately after harvested all the stolons were rapidly fixed in alcohol-acetic acid (3:1 v/v) cooled to $-20^\circ$C by dry ice (solid CO$_2$).

Callose was identified by its reaction with resorcinol blue, following the procedure of Eschrich and Currier (1964). The relative amount of callose present in freehand made sections, taken from the treated region, was determined by counting the number of blue sieve plates in the microscope field, after staining with resorcinol blue.
It is now abundantly clear that plant hormones and especially auxin can somehow regulate the long-distance movement and distribution of nutrients and other growth factors, within the plant. However, despite the considerable amount of work already done on this subject, the way by which this phenomenon takes place is still not well understood. Our deficiencies in understanding the mechanism of phloem transport are undoubtedly one of the main obstacles to solving the problem of the mechanism of "hormone-directed transport".

So far two main theories have been advanced for such a mechanism. One proposes that the hormone-induced movement of metabolites is brought about indirectly, through a localized effect of the hormone by stimulating or maintaining the sink activity at the treated region. The other favours a direct effect of the hormone on the phloem, probably by activating the whole pathway of transport; alternatively, the hormone might, as suggested by Phillips (1975), directly stimulate some active process of phloem "unloading" at the treated region.

As far as I can tell, no definitive experimental evidence, which might enable us to accept one or reject the other, has yet been produced.

In the present study I tried to gather some evidence which might help to understand the mechanism of hormone-directed transport.
The problem was investigated in stolons of *Saxifraga sarmentosa* using the following approaches:

1. Effect of plant growth substances, and in particular of inhibitors of auxin transport, on the distribution of labelled metabolites;
2. Inhibition of RNA and protein synthesis and its effect on the auxin-induced transport of $^{14}$C-labelled photosynthates;
3. Effect of solutions of sucrose or mannitol on the IAA-induced transport of labelled assimilates;
4. Effect of the application of endothal and 2,4-dinitrophenol on the movement of labelled assimilates and callose formation in intact stolons of *Saxifraga*.

It has been suggested by Davies and Wasing (1965) that the auxin-induced accumulation of nutrients in the treated tissues is due to a direct stimulation by the hormone of the whole system of transport, and is therefore dependent on the basipetal movement of the auxin. If such an explanation is correct, it should be possible to inhibit the hormone-induced mobilization of nutrients by applying inhibitors of auxin transport, such as TIBA, NPA or morphactin,
either in combination with the hormone or interposed, as a solution "ring", between the source of metabolites and the auxin-treated region.

On the other hand, if the auxin effect is restricted to the treated region, regardless of being a direct or indirect one, the interposition of a "ring" of auxin transport inhibitors should not interfere to a great extent with the distribution of nutrients. The same principle should have applied if these inhibitors were applied in combination with auxin, provided that they did not interfere with the metabolism of the region.

An attempt was made to clarify these problems by observing the distribution of labelled metabolites, namely $^{32}P$-orthophosphate and $^{14}C$-photosynthates, in both "old" and young stolons of Saxifraga, in response to treatment with plant growth regulators.

a. Interaction between IAA, GA and kinetin on the translocation of $^{32}P$ in "old" stolons of Saxifraga.

The effect of IAA or mixtures of IAA, kinetin and/or GA$_3$ in lanolin on the long-distance transport of $^{32}P$ was investigated in "old" stolons of Saxifraga.

The plants were used when the stolons had finished elongating and already bore a small daughter plant at the apical end. The daughter plant with a 1 cm long portion of the stolon was removed and the experiment set up in a way similar to that described under MATERIAL AND GENERAL EXPERIMENTAL METHODS, except that the decapitated region was treated with lanolin or lanolin containing either IAA.
(0.1 % w/w), or mixtures of IAA, kinetin (0.1 % w/w) and/or GA₃ (0.1 % w/w), instead of the aqueous solutions of these substances.

The lanolin pastes were prepared by adding a known quantity (weight) of the plant growth substance(s) dissolved in a small volume of either ethanol or 1N HCl (in the case of kinetin), to the required weight of molten lanolin and stirring the mixture for 5 minutes at 35°C.

After a 20 hour pre-treatment period under continuous light, 15-20 μl of ³²P-orthophosphate solution (containing 1.5-2.0 μCi of ³²P) was applied to a point on the stolon situated 20 cm from the decapitated end. 28 hours later, under the same conditions, the stolons were harvested and, after discarding a 2 cm long section of the stolon near the point of application of ³²P, the remaining portion was cut into 2 cm long sections and their radioactivity assayed in an automatic scintillation spectrometer.

The results of this experiment are shown in fig. 2 (see also Appendix), in which each profile is the average of at least 3 stolons and the activity is expressed in c.p.m./2 cm of the stolon. The vertical lines represent the standard error of the mean. A study of fig. 2 shows an almost semi-logarithmic pattern of distribution of the activity with the distance.

It is also quite clear that treatment of the apical region with pastes of lanolin containing either IAA (0.1 % w/w) or mixtures of IAA, kinetin and/or GA₃ produces only a slight increase in the amount of ³²P which accumulates in the treated tissues, compared
Fig. 2. Profiles of the distribution of $^{32}$P-sodium orthophosphate in "old" decapitated stolons of *Saxifraga sarmentosa* treated at the apical end with lanolin (full squares) ($n=3$), or lanolin containing:

- IAA (0.1% w/w) open squares ($n=4$)
- IAA + GA$_3$ (0.1% w/w) open circles ($n=4$)
- IAA + GA$_3$ + kinetin (0.1% w/w) open triangles ($n=4$)

The treatments were made 20 hours before the application to each stolon of $1.5-2.0$ μCi of $^{32}$P-sodium orthophosphate, and the translocation period was 24 hours. The experiment was carried out under continuous light at 20°C.

The radioactivity, expressed as c.p.m./2 cm of stolon, was plotted against the distance in cm from a point on the stolon 18 cm away from the apical end. The activity present in each 2 cm long segment is assumed to be concentrated in the mid-point of the segment. At each point of the profiles, the radioactivity is a mean for at least 3 replicates ($n$), and the vertical lines represent ± the standard error of the mean.
with similar region of stolons treated with plain lanolin.

It seems then that the application of pastes of lanolin containing plant growth substances to "old" stolons of Saxifraga had little or no effect on the distribution of $^{32}P$, compared with similar stolons treated with plain lanolin.

b. Effect of a 0.25 % "ring" of TIBA in lanolin on the translocation of $^{32}P$ in both intact and decapitated stolons.

In a first experiment, a group of 3 intact stolons of Saxifraga, each bearing a small daughter plant at the apical end, was treated with a 0.25 % "ring" of TIBA in lanolin placed 10-14 cm from the apex. Another group of intact stolons in similar conditions was left untreated as control. The stolons of a third group were decapitated and the cut surface was smeared with lanolin. Twenty hours after the beginning of the treatment, 15-20 $\mu l$ of $^{32}P$-labelled-sodium orthophosphate (containing 1.5-2.0 $\mu Ci$) were applied to each stolon at a point located 22 cm from the decapitated end. Twenty four hours later, the stolons were harvested, the region contiguous to the point of application of $^{32}P$ as well as the small daughter plants, when present, being discarded and the remainder of the stolons divided in 2 cm long segments. Finally, the radioactivity of each section was determined in the usual way.

During the whole experiment, which was carried out under continuous light (intensity of illumination about 350 lux), the small
daughter plants at the end of the stolons were kept enclosed in small black polythene bags containing moist filter paper to increase their sink capacity and avoid transpiration.

A study of the profiles of distribution of $^{32}$P shows quite clearly that, under the stated conditions, a "ring" of TIBA (0.25 %) in lanolin does not significantly interfere with the amount of $^{32}$P reaching the apical region (fig. 3). On the other hand, decapitation of the stolons, followed by treatment of the cut surface with plain lanolin, caused by the end of the experimental period a pronounced reduction in the amount of radioisotope which accumulated in the foremost 6 cm. This effect was to be expected after the removal of the "sink" region.

In a second experiment, somewhat younger stolons were employed. These stolons had just finished elongating and a small daughter plant was beginning to emerge. In this experiment the stolons were decapitated and divided into three groups. The four stolons of one group were treated at the distal end with a lanolin paste containing IAA (0.1 %); a second group of three stolons was similarly treated with IAA, but a "ring" of TIBA (0.25 %) in lanolin was also interposed between the source of tracer and the IAA-treated region; finally the third group, also comprising three stolons, was treated with lanolin only applied at the decapitated end of each stolon.

After a 20 hour pre-treatment period under continuous light, 15-20 µl of $^{32}$P-sodium orthophosphate solution was applied to a point on each stolon 22 cm from the cut end, and a further period of 24-hours was allowed for translocation. At the end of this period of
translocation the stolons were harvested and the distribution of radioactivity determined as in the previous experiment.

The results from each group of stolons, after being averaged, are plotted in fig. 4. They show that the amount of radioactivity reaching the foremost 2 cm treated with IAA is similar in both groups irrespective of the presence or absence of the TIBA "ring". However, it can also be seen that the TIBA "ring" caused a considerable reduction in the level of activity present at, and beyond, the region where the TIBA "ring" was located. This effect is probably due in part to a direct toxic effect of the relatively high concentration used, but it might also be partly ascribed to the presence in the lanolin paste of a fair amount of ethanol used in dissolving the TIBA and which has, most probably, caused some damage to the tissues.

Fig. 4 also indicates that in stolons of this age more radioactivity accumulates in the apical region when treated with IAA, than in the corresponding area of stolons treated with plain lanolin.

In the light of these results, one may perhaps conclude that in stolons at this stage of development IAA, at the concentration used, was able partially to replace the "sink" effect produced by the developing daughter plant. Perhaps more important, however, is the suggestion that the degree of response to IAA treatment may very much depend on the age of the tissues employed.
Fig. 3. Effect of a 0.25% (w/w) lanolin "ring" of TIBA on the
distribution of $^{32}$P-sodium orthophosphate in "old" intact stolons of
Saxifraga sarmentosa.

Profiles of distribution of radioactivity in both intact and
decapitated stolons. Intact stolons were either treated with a
0.25% lanolin ring of TIBA (circles; n=3), applied some 9-13 cm below
the apex, or kept untreated (triangles; n=3); decapitated stolons
(squares; n=3) were treated with lanolin at the cut surface. $^{32}$P-
sodium orthophosphate (1.5-2.0 μCi/stolon) was applied directly to
the stolon at a point 22 cm below the extremity. 20 hours after the
commencement of the treatment. Translocation period 24 hours. The
experiment was carried out under continuous light at 18-20°C.

The radioactivity, expressed as c.p.m./2 cm of stolon, was
plotted against the distance in cm from a point on the stolon 18 cm
away from the apical end.

At each point of the profiles, the activity is the mean of
3 replicates (n), and the vertical lines represent the standard error
on either side of the mean.

The TIBA treated region is indicated in the profiles by the
vertical dashed lines.
To the apical region

Distance in cm

Activity in c.p.m./2 cm of stolon x 10^-2 (log-scale)

Intact.

Decap.

TIBA
Fig. 4. Effect of a 0.25% (w/w) TIBA "ring" in lanolin on the distribution of $^{32}$P-sodium orthophosphate in decapitated stolons of *Saxifraga* treated at the apical end with 0.1% IAA in lanolin.

The cut surface of decapitated stolons was treated with either lanolin (triangles; n=3) or lanolin containing 0.1% (w/w) IAA. The IAA-treated stolons were then treated with a "ring" of 0.25% TIBA (circles; n=3) in lanolin, or plain (squares; n=3).

$^{32}$P (1.5-2.0 $\mu$Ci/stolon) was applied 20 hours after the beginning of the treatment, and the translocation period was 24 hours. The experiment was carried out under continuous light at 18-20°C.

Radioactivity, expressed as c.p.m./2 cm of stolon, was plotted against the distance, in cm from a point on the stolon 20 cm away from the apical end. Each point of the profiles is the mean of 3 replicates (n), and the vertical lines represent $\pm$ standard error of the mean.

The vertical dashed lines indicate the area on the stolon treated with TIBA.
To the apical region

Distance in cm

Activity in c.p.m./2 cm of stolon x 10^-2 (log. scale)

Distance in cm
c. IAA-induced movement of $^{14}\text{C}$-labelled photosynthates in "old" stolons of *Saxifraga*.

The profiles presented in fig. 5 represent the means of the results of an experiment in which "old" stolons of *Saxifraga*, each one supporting a small daughter plant at the apical end, were decapitated and the distal region treated either with an aqueous solution of IAA (5x10$^{-5}$ M) or distilled water.

Twenty hours after the beginning of the treatment 15$\mu$Ci of $^{14}\text{CO}_2$ were fed to each leaf subtending a stolon in the manner described under MATERIAL AND GENERAL EXPERIMENTAL METHODS. After an 8-hour translocation period under continuous light, each stolon was harvested and then divided into 2 cm sections over a distance of 20 cm starting at the decapitated end. The stele of each segment was at this stage separated from the cortex, and the radioactivity of both tissues assayed as described earlier.

The distribution of $^{14}\text{C}$-assimilates present in the stele and in the cortex, as well as the sum of their activities in both IAA-treated and untreated stolons, are shown in fig. 5. The number of c.p.m./mg of fresh weight present in the stele and in the cortex was reduced before being plotted for reasons of clarity.

The results of this experiment show that in "old" stolons of *Saxifraga*, IAA, at the concentration used, had only a very small stimulating effect on the translocation of $^{14}\text{C}$-labelled assimilates, this effect probably not being significant. They also indicate that the distribution pattern of $^{14}\text{C}$-photosynthates is similar in the
Fig. 5. Distribution profiles of $^{14}$C-labelled photosynthates in "old" decapitated stolons of Saxifraga.

Decapitated stolons were treated at the apical region with an aqueous solution of IAA ($5 \times 10^{-5}$ M) (open symbols) or distilled water (full symbols). Twenty hours later 15 µCi of $^{14}$CO$_2$ were supplied to each leaf subtending a stolon and left to translocate for 8 hours.

The experiment was carried out under continuous light at 22-24°C. Cortex and stele were assayed separately. The radioactivity expressed in c.p.m./mg. fr. weight, of both stele (circles; n=3) and cortex (triangles; n=3) is shown divided by 4; the sum of the activities of cortex and stele (squares; n=3) is presented in full.

The results are, as usual, plotted against the distance in cm from a point on the stolon 20 cm from the apical end, and each point of the profiles is the mean of 3 replicates (n). The vertical lines represent ± the standard error of the mean.
To the apical region

Activity in c.p.m./2 cm of stolon/mg fr. wt. \(\times 10^{-1}\) (log scale)

Distance in cm

IAA
Water

Cortex

Stele
stele and in the cortex, despite the much higher level of activity/mg of fresh weight present in the tissues of the stele of both IAA-treated and untreated stolons.

The results of the experiments reported so far seem to indicate that in *Saxifraga* the extent of response to IAA treatment, in terms of translocation of metabolites, depends upon the age of the treated tissues. For this reason, and also because of the considerable variability among the replicates in old stolons, it was decided at this stage to change to young growing stolons, in the hope that they would respond better to the treatment with plant growth regulators. Therefore, the experiments reported henceforward were carried out in young growing stolons about 25–30 cm long.

d. IAA-directed transport of $^{14}$C-labelled assimilates in young stolons of *Saxifraga*.

The previous experiments dealing with the effect of IAA, and some other plant growth regulators, on the transport of metabolites were carried out in stolons which had finished elongating. In the next experiment the effect of three different concentrations of IAA on the transport of $^{14}$C-photosynthates, was examined using young growing stolons.

After decapitation, the stolons were treated with one of the aqueous solutions of IAA ($10^{-6}$ M; $10^{-5}$ M; $5 \times 10^{-5}$ M) or distilled water. Eighteen hours later, $^{14}$CO$_2$ was supplied to each leaf subtending a stolon for 1 hour, after which the assimilation chambers were
removed and the stolons allowed to translocate for the next 5 hours.

During the whole experimental period, the plants were kept under a continuous illumination of 350 lux provided by a low pressure mercury lamp. Finally the stolons were harvested and radioassayed as mentioned under MATERIAL AND GENERAL EXPERIMENTAL METHODS.

The results of this experiment are shown in fig. 6 in which each point in the profiles represents the average of at least three stolons. They show quite distinctly that IAA in all concentrations used increased by several-fold the import of ¹⁴C-labelled assimilates by the treated tissues, compared with the amount of label that reached similar regions of untreated stolons. It is also evident that, of the three concentrations employed, 10⁻⁵M and 5x10⁻⁵M were equally effective. On the other hand, treating decapitated stolons with water resulted, at the end of the experimental period, in a much smaller amount of radioactivity reaching the distal region.

The results of yet another experiment designed to investigate the effect of IAA on the mobilization of metabolites, when applied to tissues of different ages, are shown in fig. 7. In this case, IAA in aqueous solution was applied to either the apical region of decapitated stolons or to an area 12-15 cm from the decapitated end.

Twenty hours after the beginning of the experiment, ¹⁴CO₂ was fed to the plants in the usual way and, 6 hours later, the stolons were harvested and cut into 2 cm pieces. The stele and cortex of each section were separated, weighed and their radioactivity content determined.
Fig. 6. Effect of different concentrations of IAA on the transport of $^{14}$C-photosynthates in young decapitated stolons of *Saxifraga*.

The apical regions of decapitated stolons were treated with distilled water (full squares; n=6) or one of the following aqueous solutions of IAA: $10^{-6}$M (open triangles; n=3); $10^{-5}$M (open circles; n=3); $5 \times 10^{-5}$M (open squares; n=3).

$^{14}$CO$_2$ (15 μCi) was supplied to each leaf subtending a stolon 18 hours after the beginning of the treatment and the translocation period was 6 hours.

The experiment was performed under conditions of continuous light and 20-22°C of temperature.

The results, expressed in c.p.m./2 cm of stolon, were plotted against the distance, in cm, from a point on the stolon 20 cm from the apical end. The points in the profiles are the mean of at least 3 replicates (n) and the vertical lines represent ± the standard error of the mean.
To the apical region

Distance in cm

Activity in c.p.m./2 cm of stolon x 10^-3 (log-scale)

IAA
Water

64
Fig. 7. Effect of IAA on the translocation of \( ^{14} \text{C}-\text{photosynthates} \) in decapitated stolons of *Saxifraga*.

Young decapitated stolons were treated with an aqueous solution of IAA (5x10^{-5} M) either at the apical end (squares) or at a region 12-15 cm below the cut surface (circles), indicated in the profiles by the dashed vertical lines. Other stolons were treated with distilled water at both places (triangles).

\( ^{14} \text{CO}_2 \) (15 μCi) was applied to each leaf subtending a stolon, 18 hours after the beginning of the experiment, and 6 hours later the radioactivity present in both the stele (open symbols; n=3) and the cortex (full symbols) was determined.

The experiment was carried out under continuous light, at 21-24°C. The results expressed as c.p.m./mg. fr. weight/2 cm of stolon, were plotted against the distance in cm, from a point on the stolon 20 cm from the apical end. Each point in the profiles is the mean of 3 replicates, and the vertical lines indicate the standard error on either side of the mean.
To the apical region

IAA Water

IAA Water

Activity in c.p.m./mg fr. wt./2 cm of stolon x 10^-2 (log scale)

Distances in cm

2 4 6 8 10 12 14 16 18 20

Stebe

cortex

WaterWater

( D O U 6 8 10 12
2468101214161820

Distance in cm
The profiles shown in fig. 7, each point representing the mean of three stolons, demonstrate that IAA is much more effective in promoting the accumulation of labelled assimilates when applied to the apical region of the stolon, that is, to younger tissues than to older ones. Furthermore, the IAA effect is particularly evident in the tissues of the cortex of the stolons treated with auxin at the apical region. In cases where IAA was applied some 12-15 cm down the stolon, and the apical end treated with water, less radioactivity reached this region than in stolons treated with water in both places. This suggests that IAA might have promoted the accumulation of labelled assimilates in the treated area at the expense of the apical region, although no indication of increased lateral movement of $^{14}$C-assimilates out of the stele could be detected.

It must however be stressed that, despite a larger relative effect being elicited by IAA in the tissues of the cortex at the apical region, there was, at every point, a higher level of activity per mg. of fresh weight in the stele than in the cortex of stolons belonging to the same treatment.

The different degrees of response elicited by the treatment with IAA of tissues of different ages is a clear indication that the IAA-induced effect is, at least in part, somehow associated with the metabolic activity of the tissues. However, one of the main problems which arises when one uses young growing parts of plants, such as young stolons, is the difficulty in determining which part of the IAA-increased import of assimilates by the treated tissues can be
ascribed to IAA-induced growth (or synthetic activities) and what part, if any, is independent of growth.

e. Timing of auxin application and its effect on the IAA-induced mobilization of $^{14}$C-photosynthates.

It has been observed that in seedlings of Phaseolus vulgaris, removal of the apex is followed, in a matter of 12-24 hours, by a marked decline in both the overall protein level, and the rate of incorporation of $^{14}$C-leucin into protein, in the stumps of the decapitated plants (Mullins, 1970; Patrick and Wareing, 1970). Treatment of the cut surface with IAA prevents such a decline.

In the face of these observations, Mullins (loc. cit.) suggested that decapitated internodes, deprived of their main source of auxin, are senescent systems and, consequently, the IAA-induced transport of metabolites might be better understood on the basis of an IAA senescence-delaying effect. Since then, Patrick and Wareing (1973) have questioned the validity of this hypothesis by showing that decapitated stems of bean plants treated with plain lanolin for three days were still able to respond to IAA treatment in terms of increased transport of nutrients.

Here, I also report the results of a similar experiment designed to investigate to what extent the IAA-induced mobilization of $^{14}$C-labelled assimilates is affected by delaying the application of IAA, after decapitation of the stolons.

Several young stolons of Saxifraga were decapitated, divided
into four groups, and then the apical region treated as follows: One group of stolons was treated with an aqueous solution of IAA (5x10^{-5} M) from the beginning; another group was treated with distilled water also for the entire period; a third group was treated with distilled water for 48 hours which was then exchanged for an IAA solution (5x10^{-5} M) where they remained for the rest of the experimental period (25 hours); the last group of stolons was also treated with distilled water but for 66 hours and then with an IAA solution until the end of the experiment (i.e. for 8 hours).

Six hours before the end of the experiment, $^{14}$CO$_2$ (15 μCi) was supplied to each subtending leaf for 1 hour, and then a further 5-hour period was allowed for translocation, after which the stolons were harvested and radioassayed in the usual way.

The results of this experiment (fig. 8) show that decapitated stolons kept in water for a period of 48 hours or even 66 hours were still able to respond to IAA treatment. These results are certainly in agreement with the findings of Patrick and Wareing (1973) in stems of bean plants.

When observed in more detail, the profiles corresponding to the four types of treatment show different patterns of distribution of $^{14}$C-photosynthates. For instance, the profile for those stolons treated with IAA for the whole experimental period shows an almost even distribution of radioactivity over the entire length assayed; the stolons treated with water for 48 hours and then transferred to an IAA solution for the rest of the experiment show a similar level
Fig. 8. Effect of delaying the application of IAA, after decapitation, on the translocation of $^{14}$C-photosynthates in young stolons of *Saxifraga*.

The apical region of young stolons was treated with a $5 \times 10^{-5}$ M aqueous solution of IAA at different times after decapitation:

- Open squares immediately after decapitation ($n=5$)
- Open circles 48 hours after decapitation ($n=6$)
- Open triangles 66 hours after decapitation ($n=4$)
- Full squares the entire experimental period in distilled water ($n=5$)

The duration of the whole experiment was 73 hours and the translocation period was 6 hours. The experiment was carried out under continuous light and the temperature varied between 19 and 23°C.

The results, expressed in c.p.m./2 cm of stolon, were plotted as in fig. 7. The points in the profiles are the means of at least 4 replicates ($n$), and the vertical lines represent the standard error on either side of the mean.
To the apical region

Distance in cm

Activity in c.p.m./2 cm of stolon x 10^-2 (log scale)

Water

IAA
of radioactivity at the treated region compared with the previous lot, but there is a steady decline in the number of c.p.m. as we move away from the apical region; the third group of stolons, which was treated with water for 66 hours before being transferred to IAA (8 hours) shows that considerably less radioactivity accumulated in the treated tissues when compared with the other two groups; finally, in the stolons treated with water during the whole experimental period, very little activity was recorded in the apical region. The results of this experiment, besides showing that the IAA-induced response is a relatively rapid one (it was easily visible within 8 hours, including the translocation period), they also suggest that the IAA-effect starts at the treated region and then spreads down the stolon as the time progresses. In the light of this evidence, it appears then that the IAA-directed transport of nutrients cannot be entirely explained on the basis of a senescence-delaying effect.

f. Effect of kinetin and gibberelllic acid, and their interaction with IAA on the movement of $^{14}$C-labelled assimilates.

The role of other plant hormones on the long-distance movement of nutrients is a matter of some confusion. Cytokinins, for example, have been found to promote the movement of labelled amino acids, $^{32}$P and $^{14}$C-photosynthates from untreated to treated areas of detached leaves (Mothes and Engelbrecht, 1961; Gunning and Barkley, 1963; Muller and Leopold, 1966). In other organs, however, cytokinins
have in several cases failed to produce any significant response in terms of increased transport of metabolites to the treated areas (Seth and Wareing, 1964; Davies and Wareing, 1965; Sebanek, 1965; Morris and Thomas, 1968), although some exceptions have also been reported (Seth and Wareing, 1964; Mullins, 1970; Shindy and Weaver 1967, 1970; Kriedemann, 1968) especially in response to 6-benzylaminopurine (BA).

With gibberellins a similar picture emerges. Thus, Seth and Wareing (1964; 1967) and Davies and Wareing (loc. cit.) could not find any significant increase in the accumulation of 32P and 14C-assimilates in the decapitated internodes of Phaseolus and Pisum plants in response to gibberellic acid treatment. On the other hand, Mullins, (loc. cit.) and Jeffcoat and Harris (1972) observed increased translocation of labelled metabolites in bean and carnation plants due to GA3 treatment.

Combinations of IAA and cytokinins and/or gibberellic acid have been found to have greater effect than IAA alone (Seth and Wareing, 1964; 1967; Sebanek, 1965; Mullins, 1970).

In view of the contradictory results reported, it was decided to investigate these problems further, using to that end young growing stolons of Saxifraga.

In a first experiment, the apical region of young decapitated stolons was treated with water or aqueous solutions of either kinetin (K) (2x10^{-4}M) or gibberellic acid (GA3) (10^{-4}M). Eighteen hours later, 14CO2 was supplied to the plants and, after a 5-hour trans-
location period under continuous light, the stolons were harvested, cut into 2 cm segments and radioassayed as usual.

The results of this experiment, shown in fig. 9, indicate very clearly that in young Saxifraga stolons treatment with kinetin had no significant effect on the translocation of $^{14}$C-labelled assimilates. On the other hand, gibberellic acid increased considerably the amount of $^{14}$C-photosynthates imported by the treated tissues.

In a similar experiment, young decapitated stolons were treated at the apical end with water or aqueous solutions of either IAA ($5 \times 10^{-5} \text{M}$) or mixtures of IAA and gibberellic acid ($10^{-4} \text{M}$) or kinetin ($10^{-4} \text{M}$).

Eighteen hours after the beginning of the treatment, the plants were fed with $^{14}$CO$_2$ and then allowed to translocate for another 5 hours under continuous light. At the end of this period, the stolons were collected, divided into 2 cm segments and their radioactivity counted.

The results of this experiment are shown in fig. 10. They show a several-fold increase in the accumulation of $^{14}$C-photosynthates at the treated region, compared with the amount of radioactivity present in the corresponding zone of stolons treated with water. Application of GA$_3$ in combination with IAA produced a slightly higher effect than IAA alone, whereas a mixture of IAA and kinetin had the same effect as IAA alone.
Fig. 9. Effect of kinetin and gibberellic acid on the distribution of $^{14}$C-photosynthates in young decapitated stolons of Saxifraga.

The apical region of young decapitated stolons was treated with distilled water (full squares; n=3) or aqueous solutions of either kinetin ($2 \times 10^{-4}$M; open triangles; n=3) or GA$_3$ ($10^{-4}$M; open circles; n=3). $15 \mu$Ci of $^{14}$CO$_2$ were applied to each subtending leaf, 18 hours after the commencement of the treatment, and a further 5 hours were allowed for translocation. Plants were kept under continuous light throughout the experiment at 20-22°C.

The radioactivity, expressed as c.p.m./2 cm of stolon, was plotted as in fig. 6. Each point in the profiles is the mean of 3 replicates (n) and the vertical lines have the same meaning as in fig 8.

Fig. 10. Interaction of IAA, kinetin and gibberellic acid on the distribution of $^{14}$C-labelled assimilates.

The apical region of young decapitated stolons was treated with distilled water (full squares; n=3) or aqueous solutions of either IAA ($5 \times 10^{-5}$M; open circles; n=3) or mixtures of IAA and kinetin ($10^{-4}$M; open triangles; n=3) or IAA and GA$_3$ ($10^{-4}$M; open squares; n=3). $^{14}$CO$_2$ was fed to the plants 18 hours after the beginning of the treatment and the translocation period was 5 hours.

The experiment was carried out under continuous light at 20-22°C. Radioactivity was plotted as in fig. 6. Each point is the mean of 3 replicates (n), and the vertical lines represent ± the standard error of the mean.
These results indicate that in young stolons of *Saxifraga* the hormone-directed movement of nutrients in the phloem is mainly controlled by IAA, although gibberellic acid, particularly in the presence of low levels of IAA, may partially replace the effect of this substance.

The failure of GA₃ to produce any significant increase in the IAA effect when these two substances are applied together, is perhaps an indication that GA₃ and IAA act through the same channels, that is, stimulate similar physiological reactions.

The absence of any significant effect due to kinetin, either alone or in association with IAA, may be interpreted as suggesting that cytokinins are not the limiting factor of hormone-directed transport in these tissues.

g. Effect of inhibitors of auxin transport in the translocation of ¹⁴C-labelled photosynthates.

If the auxin-induced effect on the translocation of nutrients depends on the basipetal movement of auxin down the stem, as suggested by Davies and Wareing (*loc. cit.*), it should be possible to eliminate such an effect by selectively blocking the basipetal transport of auxin. On the other hand, if the auxin effect is a localized one, that is, restricted to the treated region, then the auxin-induced movement of nutrients should not be hampered by the application of specific inhibitors of auxin transport, provided that they do not interfere with the metabolism of the cell. Thus, the use of inhibit-
ors of auxin transport should enable us to discriminate between these two possibilities.

Since TIBA, NPA and morphactin are the most commonly used inhibitors of auxin polar movement, I started by investigating the effect of aqueous solutions of these compounds on the translocation of $^{14}$C-assimilates. The decapitated stolons were treated at the apical end with water or solutions of either TIBA ($10^{-4}$M) or morphactin ($10^{-4}$M). $^{14}$CO$_2$ was supplied to the plants 18 hours after the commencement of the treatment and the radioactivity counted as mentioned before.

The results of this experiment, presented in figs. 11 and 12, show quite clearly that TIBA by itself, at the concentration used ($10^{-4}$M), has no significant effect on the distribution of labelled assimilates. These observations do not agree with the findings of Mullins (loc. cit.) who noticed that TIBA, at this and higher concentrations, enhanced the accumulation of $^{14}$C-photosynthates in decapitated internodes of Phaseolus vulgaris.

On the other hand, both NPA ($10^{-4}$M) and morphactin ($10^{-4}$M) increased to some extent the amount of $^{14}$C-labelled assimilates which reached the treated region (figs. 11 and 12 respectively).

This effect of NPA and morphactin on the movement of metabolites may well be a genuine promoting effect of these substances; an alternative explanation is that it may be a consequence of the blockage of residual endogenous auxin basipetal transport, thus preventing a rapid depletion of auxin in the apical tissues by basipetal dispersal.
Fig. 11. Effect of TIBA and NPA solutions on the movement of \(^{14}\text{C}\)-photosynthates in stolons of *Saxifraga*.

Young decapitated stolons were treated at the apical region with distilled water (squares; n=5) or aqueous solutions of TIBA (10^{-4}\text{M}; triangles; n=5) or NPA (10^{-4}\text{M}; circles; n=5). Eighteen hours later, 15 \(\mu\text{Ci}\) of \(^{14}\text{CO}_2\) were applied to each subtending leaf, and the plants were left to translocate for the next 5 hours. Throughout the experiment the plants were kept under continuous light at 21-22°C. The radioactivity, in c.p.m./2 cm of stolon, was plotted against the distance in cm from a point on the stolon 20 cm from the apical end. The profiles are the mean of 5 replicates (n), and the vertical lines represent the standard error on either side of the mean.

Fig. 12. Effect of morphactin on the translocation of \(^{14}\text{C}\)-labelled assimilates in young stolons of *Saxifraga*.

The apical region of young decapitated stolons was treated with water (squares; n=3) or an aqueous solution of morphactin (10^{-4}\text{M}; triangles; n=3). \(^{14}\text{CO}_2\) (15 \(\mu\text{Ci}\)) was applied 18 hours after the beginning of the treatment; translocation period 5 hours. The experiment was carried out under continuous light at 22-24°C. Radioactivity plotted as in fig. 11. Each profile is the mean of 3 replicates (n), and the vertical lines have the same meaning as in the previous figure.
If this is so, absence of a similar effect of TIBA is difficult to explain only on the basis of an inhibition of auxin transport and would suggest that TIBA may have some other secondary effect, perhaps on the metabolism.

In a further group of experiments the effect of TIBA, NPA and morphactin on the IAA-induced transport of metabolites was also investigated. These compounds were applied in aqueous solution either in combination with IAA at the decapitated region or as a "ring" located some 10-14 cm behind the area treated with IAA, as described under MATERIAL AND GENERAL EXPERIMENTAL METHODS.

The results of these experiments, which followed a schedule similar to the previous ones, are shown in figs. 13, 14 and 15 respectively.

Observing the profiles of distribution of activity, one can see that TIBA \(10^{-4}\) M applied together with IAA \(5\times10^{-5}\) M at the apical end of the stolon inhibits almost completely the IAA-induced transport of labelled-photosynthates. When interposed as a "ring" between the source of radioactivity and the IAA-treated area some 10-14 cm behind the decapitated end, TIBA did not significantly interfere with the IAA-induced import of labelled assimilates into the apical region. Nevertheless, TIBA caused a reduction in the level of activity at and near the area where the TIBA "ring" was located (fig. 13).

NPA (fig. 14) or morphactin (fig. 15) solutions applied either in combination with IAA at the decapitated end, or as a "ring" at
Fig. 13. Effect of a TIBA solution on the IAA-induced transport of $^{14}$C-assimilates in stolons of Saxifraga.

A $10^{-4}$M solution of TIBA was applied at the apical region of young decapitated stolons, either in combination with IAA (full circles; n=5) or alone as a "ring", indicated by the vertical dashed lines, located some 10-14 cm below the decapitated region which was treated with a $5 \times 10^{-5}$M IAA solution (Open circles; n=10). Other stolons were treated only with a $5 \times 10^{-5}$M aqueous solution of IAA applied at the apical region (open squares; n=9).

$^{14}$CO$_2$ was supplied to the plants after an 18 hour treatment period, and the translocation period was 5 hours. The experiment was carried out, as usual, under continuous light at 22-24°C.

The results expressed as c.p.m./2 cm of stolon were plotted against the distance in cm from a point on the stolon, 20 cm from the apical end.

The points in the profiles are the mean of at least 5 replicates (n) and the vertical lines represent ± the standard error of the mean.
To the apical region

Activity in c.p.m. / 2 cm of stolon x 10^(-2) (log scale)

Distance in cm
Fig. 14. Effect of a $10^{-4}$ M NPA solution on the IAA-induced translocation of $^{14}$C-photosynthates.

NPA ($10^{-4}$ M) was applied to young decapitated stolons, either in combination with IAA (full circles; n=3), at the apical region, or alone, as a "ring" (open circles; n=3), indicated by the vertical dashed lines, located some 10-14 cm below the terminal region treated with IAA; other stolons were simply treated at the decapitated region with a $5\times10^{-5}$ M solution of IAA (open squares; n=3).

$^{14}$CO$_2$ (15 µCi) was fed to each subtending leaf 18 hours after the beginning of treatment; translocation period, 5 hours; experiment run under continuous light at 20-23°C. Radioactivity, in c.p.m./2 cm of stolon, plotted as in fig. 13. The profiles are the means of 3 replicates (n) and the vertical lines represent $\pm$ the standard error of the mean.

Fig. 15. Effect of a solution of morphactin ($10^{-4}$ M) on the IAA-induced movement of $^{14}$C-labelled assimilates in stolons of Saxifraga.

A $10^{-4}$ M morphactin solution was applied to young decapitated stolons, either in combination with IAA (full circles; n=5) at the apical region, or alone, as a "ring" (open circles; n=5), indicated by the dashed vertical lines, some 10-14 cm below the terminal area treated with IAA; other stolons were simply treated with a $5\times10^{-5}$ M solution of IAA (open squares; n=5) applied at the decapitated end. Label was applied as in the previous figure, and the experiment was run under similar conditions of illumination at 18-20°C. Radioactivity was plotted as in fig. 13, and each point in the profiles is the mean of 5 replicates; the vertical lines represent the standard error on either side of the mean.
To the apical region

Distance in cm

C.P.M./2 cm of stolon x 10^{-3}

0 2 4 6 8 10 12 14 16 18 20

Distance in cm

To the apical region

C.P.M./2 cm of stolon x 10^{-3}

0 2 4 6 8 10 12 14 16 18 20

Morph.
some distance behind the IAA-treated region, had no inhibitory effect on the IAA-induced accumulation of $^{14}$C-labelled assimilates; on the contrary, the application of NPA or morphactin, either together with IAA or as a "ring", caused a slight increase in the amount of label reaching the apical region, compared with the same area of stolons treated with IAA alone.

However, when the apical region was treated with a combination of IAA and NPA or morphactin, there was a small reduction in the level of activity in the zone adjacent, as was to be expected if the IAA basipetal transport was blocked. A similar effect, although perhaps less evident, can also be observed in the case where the stolons were treated with a "ring" of these two plant regulators.

The results reported here argue strongly against the hypothesis of an effect of the auxin on the whole pathway of transport and show very clearly that the auxin-induced movement of metabolites is dependent on a localized effect of auxin at the treated region. The results of these experiments also demonstrate that TIBA effectively inhibits the auxin-induced accumulation of labelled assimilates when applied in combination with IAA, but not when these two substances were applied to zones spatially separated. This seems to indicate that the inhibitory effect of TIBA in the first case cannot be ascribed to inhibition of basipetal auxin transport, but has to be imputed to some other effect of TIBA, probably on the metabolic actions of auxin.
As we have seen, the application of a TIBA "ring" around the stolons caused a localized reduction of the level of activity at the region where the TIBA "ring" was located. In order to reveal whether this effect of TIBA was equally evident in the cortex and in the stele, a further experiment was run, differing from those described above only in that, at the end of the translocation period, the distribution of labelled assimilates was determined separately in the cortex and in the stele.

The results of this experiment show, quite clearly, that the effect of TIBA is particularly evident in the tissues of the cortex, whereas the stele appears to have been little affected (fig. 16).

They, therefore, suggest that TIBA may have affected mainly the lateral movement of metabolites at the region of its application, either indirectly by lowering the metabolic rate of the region and therefore the demand for metabolites, or directly by interfering with some process involved in the "unloading" of the sieve tubes.

h. Effect of plant growth substances on the metabolism of $^{14}C$-labelled photosynthates in stolons of Saxifraga.

One possible way by which auxin may influence the long-distance transport of nutrients is by stimulating the overall metabolism of the treated region, therefore creating a "sink" for metabolites, that is, a region of high demand for nutrients. The high rate of consumption of metabolites at the "sink" region would most probably create a gradient of concentration between the source and
Fig. 16. Effect of a TIBA "ring" on the distribution of $^{14}$C-labelled assimilates in both the cortex and the stele of young decapitated stolons of *Saxifraga*.

Young decapitated stolons were treated at the apical region with a $5 \times 10^{-5}$M solution of IAA (squares; $n=3$); other stolons were similarly treated, but an additional TIBA "ring" ($10^{-4}$M), indicated in the profiles by the vertical dashed lines, was applied some 10-14 cm below the decapitated end (circles; $n=3$).

$^{14}$CO$_2$ was applied to the plants after 18 hours of treatment and the translocation period was 5 hours.

On conclusion of the experiment, run under continuous light at 19-22°C, the radioactivity present in the cortex (full symbols) and in the stele (open symbols) was determined individually.

The results, in c.p.m./2 cm of cortex or stele, were plotted against the distance in cm from a point on the stolon, 20 cm from the apical end.

Each profile is the mean of 3 replicates; the vertical lines represent ± the standard error of the mean.
To the apical region

Distance in cm

Activity in c.p.m./2 cm of stolon x 10^-3 (log. scale)

IAA

TIBA
the sink which would encourage the flow of metabolites towards the zone of its utilization. The effect of the hormone would then be, essentially, to create or reinforce the "sink" activity as a consequence of increased metabolic rate.

With the objective of investigating to what extent the rate of utilization of $^{14}$C-photosynthates was altered by the application of IAA and other plant growth substances, an experiment was set up in which young stolons of Saxifraga were used either intact or with the apex removed. In the latter case the decapitated region was treated with water or an aqueous solution of either IAA ($5 \times 10^{-5}$ M) or mixtures of IAA and TIBA ($10^{-4}$ M), IAA and NPA ($10^{-4}$ M) or IAA and morphactin ($10^{-4}$ M).

Eighteen hours after the beginning of the treatment, $^{14}$CO$_2$ was supplied to the plants in the usual way. Five hours later, the apical 4 cm of each stolon were harvested, the cortex and stele separated and their radioactivity extracted with 80% boiling ethanol. After extraction of the tissues, both ethanol soluble and insoluble fractions of $^{14}$C-photosynthates were radioassayed following the procedure described under MATERIAL AND GENERAL EXPERIMENTAL METHODS.

The results of such experiments are shown in table I.

It is evident that the incorporation of $^{14}$C-labelled photosynthates in the insoluble fraction, expressed as percentage of the total c.p.m./mg of fresh weight, very much depends on the kind of treatment applied to the stolons. For instance, young decapitated stolons treated with a solution of IAA were found to have a higher
TABLE I

Interaction between IAA and inhibitors of auxin transport on the metabolism of $^{14}$C-photosynthates in the terminal 4 cm of young stolons of *Saxifraga*.

The apical region of young decapitated stolons was treated with water (WAT) or solutions of IAA ($5 \times 10^{-5}$M) alone, or in combination with $10^{-4}$M TIBA (IAA + TIBA), or $10^{-4}$M morphactin (IAA + M). Intact stolons (INT) were kept as controls.

$^{14}$CO$_2$ was applied 18 hours after the beginning of the treatment, and the plants were left to translocate for 6 hours. The results are expressed in % of total c.p.m./mg. of fr. weight.

<table>
<thead>
<tr>
<th>80% alcohol insol. fraction</th>
<th>Treatment</th>
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<tbody>
<tr>
<td></td>
<td>INT</td>
</tr>
<tr>
<td>EXP. 1</td>
<td>9.2</td>
</tr>
<tr>
<td>EXP. 2</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9.2</td>
</tr>
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</table>
rate of incorporation of $^{14}$C-photosynthates in the ethanol insoluble fraction (14.9 %) as compared with intact untreated ones (9.2 %). In decapitated stolons treated with distilled water, there was a considerable drop in the rate of incorporation (7.2 %). This means that IAA-treated stolons have a rate of incorporation twice as large as the water-treated ones.

TIBA applied in combination with IAA not only completely inhibited IAA-induced increase in the rate of incorporation of $^{14}$C-assimilates in the insoluble fraction, but also caused it to drop to a lower level (4.4 %) than that of water-treated stolons.

On the other hand, decapitated stolons treated with mixtures of IAA and NPA (16.5 %), or IAA and morphactin (16.2 %), had slightly higher rates of incorporation than stolons treated with IAA alone.

These data, therefore, entirely agree with the results of experiments reported in the previous section dealing with the effect of inhibitors of auxin transport on the IAA-induced movement of $^{14}$C-labelled photosynthates. For instance, the suggestion that the inhibition by TIBA of the IAA-induced accumulation of metabolites might be due to interference with metabolic reactions of the cell seems to be confirmed here.

The effect of plant growth regulators on the metabolism of $^{14}$C-photosynthates in young stolons of Saxifraga strongly suggests that the hormone-directed transport of nutrients is dependent, at least under the conditions of our experiments, on the stimulation of the metabolism at the treated region.
2. Inhibition of RNA and protein synthesis and its effect on the auxin-induced transport of $^{14}\text{C}$-labelled photosynthates.

There is a considerable amount of evidence showing increased synthesis of RNA and protein in response to treatment with plant growth substances, in particular with auxins, in a number of different tissues. This increase in RNA and protein synthesis has been shown in a few cases to occur within a relatively short period of time (Datta and Sen, 1965; Esnault, 1965; Masuda and Kamisaka, 1969).

It has also been demonstrated that the control of some physiological phenomena is mediated through hormone-induced changes in RNA and protein metabolism.

Assuming that the hormone-directed transport of nutrients is dependent on stimulation of maintenance of the metabolism at the treated region, and there are several indications suggesting that that might be the case, it is therefore likely that the auxin-induced long distance transport of metabolites might be associated with an increased RNA and protein metabolism.

There are a number of substances known to block, selectively, some steps in the process leading to the synthesis of protein. Among the most commonly used are the antibiotics actinomycin-D, puromycin and cycloheximide (actidione). These compounds act at different levels, but all of them have as a final consequence the inhibition of protein synthesis. Thus, actinomycin-D by binding itself to the guanine base of DNA prevents the formation of DNA-governed RNA (mRNA); puromycin is an analogue of the amino acyl-
tRNA complex, and therefore may become linked, through the amino group, to the growing polypeptide chain being formed on the ribosome. Because puromycin cannot detach itself from the polypeptide chain, and also because it cannot pair with the mRNA for lack of anti-codon, the growth of the polypeptide chain is stopped and subsequently freed from the ribosome; cycloheximide is also an inhibitor of translation, and seems to inhibit the transfer reaction in peptide bond formation, and also to affect polypeptide chain initiation.

The possible relationship between the IAA effect on RNA and protein metabolism, and the IAA-induced transport of labelled assimilates was examined by applying the mentioned protein synthesis inhibitors, either together with IAA at the distal end of young decapitated stolons, or alone as a "ring" some 10-14 cm behind the IAA-treated region, and then monitoring their effect on the distribution of $^{14}$C-labelled photosynthates.

In a first set of experiments, young stolons of *Saxifraga* were decapitated and the apical region treated with water or solutions of either IAA ($5 \times 10^{-5}$M) or mixtures of IAA and actinomycin-D (15mg/l), IAA and puromycin ($2 \times 10^{-3}$M) or IAA and cycloheximide (10mg/l). Eighteen hours after the beginning of the treatment with actinomycin or puromycin (treatment with cycloheximide was done only 3 hours before the supply of radioactivity), the plants were supplied with $^{14}$CO$_2$ and then allowed to translocate for the next 5 hours under continuous light. At the end of the experimental period, the stolons were harvested and radioassayed as mentioned before.
The results of these experiments are shown in figs. 17, 18 and 19. From the analysis of the profiles, it is evident that all three substances, when applied in combination with IAA, inhibited almost completely the IAA-induced import of $^{14}$C-photosynthates by the treated region. In this respect, actinomycin appears to be somewhat less effective than either cycloheximide or puromycin.

In a similar set of experiments, solutions of the mentioned inhibitors of protein synthesis were applied at some distance below the IAA-treated tissues. The results presented in figs. 20 and 21 show that under these conditions the inhibitors of RNA and protein synthesis had little or no effect on the IAA-directed transport of $^{14}$C-photosynthates. The slightly lower activity present in stolons treated with a "ring" of these compounds may be due to the fact that small amounts of inhibitor may have been translocated in the phloem and reached the "sink" region, where they have an inhibitory effect.

The results of experiments employing inhibitors of RNA and protein synthesis show, quite clearly, that, at least under the conditions of our work, the IAA-induced accumulation of metabolites is dependent on the metabolic activity of the treated tissues. When this "sink" capacity is reduced to a greater or lesser extent by the use of inhibitors of protein synthesis, the IAA-induced flow of metabolites is also affected. This effect cannot be ascribed to inhibition of the basipetal transport of auxin, for in the case of both actinomycin and puromycin such a movement is not greatly disturbed by these substances, as I shall prove later on.
Fig. 17. Inhibition of IAA-induced transport of $^{14}$C-photo-
synthates by actinomycin 15 mg/l in stolons of *Saxifraga*.

The apical region of young decapitated stolons was treated
with distilled water (full squares; n=3), IAA (5x10^-5 M; open squares; n=3) or a mixture of IAA and actinomycin (15 mg/l; open triangles; n=3).

$^{14}$CO$_2$ (15 μCi) was applied to each leaf subtending a stolon 18 hours after the beginning of the experiment and the plants were allowed to translocate for another 5 hours.

The experiment was carried out under continuous light at
18-20°C. The results were plotted as usual, each profile being the mean of 3 replicates (n) and the vertical lines representing the standard error on either side of the mean.
To the apical region

Activity in c.p.m./2 cm of stolon x 10^-2 (log scale)

Distance in cm
Fig. 18. Inhibition of IAA-induced transport of $^{14}$C-photosynthates by puromycin ($2 \times 10^{-4}$M) in stolons of Saxifraga.

The apical region of young decapitated stolons was treated with distilled water (full squares; n=3) or aqueous solutions of either IAA ($5 \times 10^{-5}$M; open squares; n=3) or a combination of IAA and puromycin ($2 \times 10^{-4}$M; open circles; n=3).

18 hours later, 15 $\mu$Ci of $^{14}$CO$_2$ were supplied to each subtending leaf and the plants allowed to translocate for the next 5 hours.

The entire experiment was run under continuous artificial light and the temperature varied between 20–22°C.

The results were plotted as in previous cases, each profile representing the mean of 3 replicates (n). The vertical lines are ± the standard error of the mean.
To the apical region

Activity in c.p.m./2 cm of stolon x 10^-2 (log scale)

Distance in cm
Fig. 19. Inhibition of IAA-induced transport of $^{14}$C-photosynthates by cycloheximide (10 mg/l) in stolons of Saxifraga sarmentosa.

The apical region of young decapitated stolons was treated with distilled water (full squares; n=3) or aqueous solutions of either IAA ($5 \times 10^{-5}$M); open squares; n=3); or with IAA and 15 hours later changed to a mixture of IAA and cycloheximide (10 mg/l; open circles; n=3).

The "tracer" was applied 18 hours after the beginning of the treatment, and the translocation period was 5 hours.

The experiment was run under continuous light at 23-25°C. The activity was plotted against the distance in cm from a point on the stolon 20 cm from the apical end; the profiles are the mean of 3 replicates (n) and the vertical lines represent the standard error on either side of the mean.
To the apical region

Activity in c.p.m. / 2 cm of stolon x 10^-3 (log scale)

Distance in cm

IAA

IAA + CH,
Water
The failure to observe any significant reduction in the response to IAA treatment when inhibitors of protein synthesis are applied between the source of metabolites and the IAA-treated tissues, strongly suggests that the inhibition of IAA-induced accumulation of labelled assimilates by these substances, when applied together with IAA, is essentially due to inhibition at the "sink" region, and not to an effect of these compounds on the pathway of transport. It also precludes the possibility that the inhibition of auxin-induced transport may be due to disruption by the inhibitor of de novo synthesis of some special protein needed for the phloem transport. If this were the case, then the protein synthesis inhibitors should have a similar effect when applied either in combination with IAA or in separate places, which, obviously, is not the case.

In the whole, the results of experiments with protein synthesis inhibitors do not support the hypothesis of an effect of auxin on the entire transport system. They also show quite clearly, that hormone-directed transport is a localized phenomenon essentially confined to the treated region and nearby tissues, and is dependent on the stimulation of some local process, most probably a metabolic one.
Fig. 20. Effect of a "ring" of cycloheximide on the IAA-directed transport of $^{14}C$-photosynthates.

A "ring" of cycloheximide (15 mg/l), indicated in the profiles by the vertical dashed lines, was applied 10-14 cm below the apical region of decapitated stolons treated with IAA (circles; n=3); other stolons were simply treated with IAA ($5 \times 10^{-5}$ M) at the distal region (squares; n=3).

$^{14}CO_2$ (15 µCi) was supplied to each subtending leaf 18 hours after the beginning of treatment; the translocation period was 5 hours. The experiment was carried out under continuous light at 20-23°C. Activity plotted as in fig. 19. The profiles are the mean of 3 replicates; the vertical lines represent the standard error on either side of the mean.

Fig. 21. Effect of a "ring" of either actinomycin-D or puromycin on the IAA-directed transport of $^{14}C$-photosynthates.

"Rings" of actinomycin-D (12 mg/l; triangles; n=6) or puromycin ($10^{-4}$ M; circles; n=6), indicated by the vertical dashed lines, were applied around young decapitated stolons, treated with IAA ($5 \times 10^{-5}$ M) at the apical end. Other stolons were simply treated with $5 \times 10^{-5}$ M IAA solution (squares; n=6).

$^{14}CO_2$ (15 µCi) was applied 18 hours after the treatment began and the plants were allowed to translocate for 5 hours. The experiment was carried out under continuous light at 18-20°C. Radioactivity plotted as in fig. 19. The profiles are the means of 6 replicates (n); the vertical lines have the same meaning as in fig. 20.
As we have seen, the effect of plant hormones on the long-distance transport of metabolites is restricted to the treated region and nearby. One way in which IAA might increase the accumulation of nutrients in this area is by, directly or indirectly, stimulating the "unloading" of metabolites out of the sieve tubes. Unfortunately very little is known about the mechanism of the unloading process itself, let alone the effect of plant growth substances on it.

We do not know whether it is an energy-driven process or a diffusional one entirely controlled by the demand of the surrounding tissues. There is some evidence suggesting that sieve tube "unloading" may not be too energy-dependent. For instance, Ford and Peel (1967) showed that temperatures as low as 2°C did not inhibit the loss of 14C-labelled carbohydrates from the phloem, although this same temperature did stop the movement of 32P-labelled phosphates into the sieve tubes of willow, which is known to be an energy-driven process (King, 1971). Weatherley et al. (1959) proposed a scheme to explain the "unloading" of metabolites out of the sieve tubes. They suggested that the movement of sucrose out of the sieve tubes is regulated by differences in sucrose "potential" between the phloem and the surrounding tissues. These differences in sucrose "potential" are not necessarily the same as differences of concentration, since they considered the possibility that the "unloading" of the sieve tubes might occur against a concentration gradient. In this scheme
the regions of sucrose utilization (sinks) would have a low sucrose "potential" and therefore sucrose would be "pumped" into them. The same mechanism working in reverse would also explain the movement of metabolites into the phloem at the source region.

The role of auxin in such a scheme can only be speculated. For instance, an auxin-enhanced metabolic rate would fit in this mechanism; on the other hand, a direct effect of auxin on the "unloading" process is more difficult to envisage.

Working in sugar cane, Sacher, Hatch and Glasziou (1963) found that sucrose was broken down during its transport out of the phloem by an acidic invertase located in the free space. This invertase controlled the rate at which the sucrose was removed from the sieve tubes and supplied to the growing tissues. In the same tissues, auxin has been shown to promote the synthesis of this invertase within 2-3 hours from the beginning of the treatment (Hatch and Glasziou, 1963; Glasziou et al., 1966). Here again, we have some evidence suggesting an indirect effect of auxin on the "unloading" of the sieve tubes.

On the other hand, Patrick and Wareing (1970), in Phaseolus vulgaris seedlings, could not find any stimulatory effect of IAA on the uptake of $^{14}$C-sucrose either by excised segments or by attached internodes; as a matter of fact, IAA caused inhibition of uptake. Inhibition of sucrose uptake in other tissues had also been reported by Sacher (1967) who suggested that this inhibition was perhaps due to non-competitive inhibition of enzymes.
As I said earlier, the problem concerning the effect of auxins on the unloading process of sieve tubes still remains far from clear.

In the next experiments, I tried to discover whether reducing the demand for nutrients by applying a sucrose solution directly to the IAA-treated tissues, or altering their osmotic properties with an hypertonic solution of mannitol, would in any way affect the IAA-induced long distance transport of labelled assimilates.

Thus, sucrose (2x10⁻³ M) was applied in combination with IAA (5x10⁻⁵ M) to the apical region of young decapitated stolons. The controls were treated with IAA alone. The experiment was then carried out in the usual way.

The results show that application of sucrose directly to the treated region caused a pronounced reduction in the IAA-induced accumulation of ¹⁴C-photosynthates in the treated area (fig. 22).

In a similar experiment, the decapitated region of young stolons was treated with solutions of either IAA (5x10⁻⁵ M) or mannitol (0.3 M) applied alone or in combination with IAA.

The results of this experiment show that a 0.3 M solution of mannitol applied together with IAA reduces to some extent the effect of the latter substance on the import of ¹⁴C-photosynthates by the treated tissues (fig. 23). Stolons treated with mannitol alone show a further decline in the amount of radioactivity reaching the treated area. However, in both cases, plasmolysis of the tissues of the distal region had as a consequence an appreciable accumulation of radioactivity in great part of the stolon behind the treated region.
Fig. 22. Inhibition of IAA-induced accumulation of $^{14}$C-photosynthates by direct application of a $2 \times 10^{-3}$M sucrose solution.

The apical region of young decapitated stolons was treated with a solution of IAA ($5 \times 10^{-5}$M; squares; $n=3$) or a combination of IAA and sucrose ($2 \times 10^{-3}$M; circles; $n=3$).

$^{14}$CO$_2$ (15 μCi) was applied to each leaf subtending a stolon 18 hours after the beginning of treatment; translocation 5 hours. Experiment run under continuous light at 23-25°C.

Radioactivity plotted as in fig. 19. Each profile is the mean of 3 replicates ($n$); vertical lines represent ± the standard error of the mean.

Fig. 23. Effect of hypertonic solutions of mannitol on the translocation of $^{14}$C-assimilates in stolons of Saxifraga.

Young decapitated stolons were treated at the apical region with IAA ($5 \times 10^{-5}$M; open squares; $n=3$) or a 0.3 M solution of mannitol either alone (full triangles; $n=3$) or in combination with IAA (open triangles; $n=3$).

18 hours later $^{14}$CO$_2$ (15 μCi) was fed to each subtending leaf, and the plants were allowed to translocate for another 5 hours.

The experiment was carried out under continuous light at 22-25°C. The results were plotted as in fig. 19. The profiles represent the mean of 3 replicates, and the vertical lines are the standard error on either side of the mean.
as compared with similar area of stolons treated with IAA alone.

The effect of sucrose in curbing the action of IAA on the long distance transport of assimilates is to be expected if one considers that any IAA-increased demand for metabolites is met by the readily available sucrose applied locally. In this circumstances the demand for assimilates, labelled or not, from other parts of the plant is therefore considerably diminished. In the end, we have here a simple case of competition between two sources of metabolites, one of which happens to be advantageously located in relation to the common sink.

The effect of hypertonic solutions of mannitol on the IAA-directed transport of labelled assimilates can probably be explained on the basis of an alteration of the osmotic relationship between the "source" and the "sink".

4. Effect of endothal and 2,4-dinitrophenol on the movement of 14C-labelled photosynthates and callose formation in young stolons of Saxifraga.

It is still a matter for controversy whether the longitudinal transport of solutes in the phloem is an energy-requiring process or a passive one. A considerable amount of work has been done, in which low temperatures, anoxia, and respiratory inhibitors have been used as tools in trying to clarify this problem. Despite all this, the problem still remains unsolved. Of course, it must be recognized
that the phloem, being a living tissue, needs some energy, albeit small, in order to maintain its integrity. But the issue is whether the longitudinal movement is itself energy dependent. Experiments using metabolic inhibitors, in particular dinitrophenol (DNP), seem to indicate that the longitudinal transport in the phloem can be reduced or stopped by them (see e.g. Qureshi and Spanner, 1973), although this is by no means absolute proof in favour of an active mechanism. For instance, such an effect could also be explained in terms of physical obstruction of the conducting channels, due for instance to the promotion of callose formation on the sieve plates, or the triggering of some other plugging mechanism. There is some evidence suggesting that treatment of plants with foreign chemicals may cause callose formation on the sieve plates (Eschrich et. al., 1965; Ullrich, 1963) which may block the movement of solutes in the phloem.

The effect of DNP and endothal (an herbicide) on both the movement of 14C-labelled assimilates and callose formation was investigated in intact growing stolons.

A region, some 10-14 cm behind the apex of a few stolons, was treated with a range of concentrations of either DNP or endothal in aqueous solution. The controls were kept untreated. Four hours later, 14CO2 was supplied to the plants. and, after a 6 hour translocation period under continuous light, the distribution of radioactivity in both treated and untreated stolons was determined.

Fig. 24 shows that treatment of stolons with endothal
Fig. 24. Effect of a "ring" of endothal on the translocation of $^{14}\text{C}$-photosynthates in intact stolons of *Saxifraga*.

Young stolons were treated 10-14 cm below the apex with a "ring" of one of the following concentrations of endothal: 0.02% (open squares; n=6) 0.05% (open triangles; n=5); 0.1% (open circles; n=4). The controls were kept untreated (full squares; n=9).

$^{14}\text{CO}_2$ (15 μCi) was applied to each leaf subtending a stolon 4 hours after the beginning of the experiment, and then allowed to translocate for the next 6 hours. Throughout the experimental period the plants were kept under continuous light at 18-20°C.

The radioactivity was finally determined and the results, in c.p.m./2 cm of stolon, plotted against the distance in cm from a point on the stolon 20 cm from the apex. Each profile represents the mean of at least 4 replicates (n); the vertical lines are the standard error on either side of the mean, and the position of the "ring" is indicated by the vertical dashed lines.
solutions ranging to 200 mg/l up to 1000 mg/l results in slightly lower amounts of $^{14}$C-activity reaching the apical region, although this effect is probably not significant. However, at least with the highest concentration used, endothal produced a considerable reduction in the level of radioactivity present at and near the region treated with this substance. Behind the treated area, there was a build up of activity suggesting a blockage of the pathway of transport.

Treatment of the stolons with a $10^{-5}$M DNP solution appears to have little effect on the movement of $^{14}$C-assimilates (fig. 25). On the other hand, a $5\times10^{-5}$M DNP solution inhibited completely the movement of $^{14}$C-photosynthates, but this concentration also proved to be lethal to the tissues.

In a similar experiment, some stolons were treated for an 18 hour period with aqueous solutions of either endothal or DNP applied to areas on the stolons located some 12–18 cm below the apex. Some untreated stolons were used as controls. At the end of this period, the stolons were harvested and immediately plunged into a $-20^\circ$C alcohol-acetic acid (3: 1 v/v) mixture, which was then allowed to warm up at room temperature. Later on, freehand sections were made from pieces taken either from the treated region of treated stolons, or from a corresponding region of the controls. The sections were stained with resorcinol blue according to the procedure described by Eschrich and Currier (1964). The presence of callose was assessed by counting the number of blue coloured sieve plates in the field of the microscope.
Fig. 25. Effect of a "ring" of 2,4-dinitrophenol (DNP) on the translocation of \(^{14}\)C-assimilates in intact stolons of *Saxifraga*.

Young stolons were treated 10-14 cm below the apex with a "ring" of one of the two concentrations of DNP: \(10^{-5}\)M (open circles; \(n=3\)) or \(5\times10^{-5}\)M (open triangles; \(n=4\)). The controls were kept untreated (full squares; \(n=4\)).

\(^{14}\)CO\(_2\) was applied to the plants at the same time as in fig. 23, and the translocation period was also 6 hours.

The experiment was run under continuous light at 18-20\(^\circ\)C, and the results, in c.p.m./2 cm of stolon, were plotted as previously. The profiles are the means of at least 3 replicates (\(n\)) and the vertical lines represent \(\pm\) the standard error of the mean; The vertical dashed lines indicate the position of the ring.
The results of such an experiment are presented in table II. These results indicate that both endothal and DNP promote callose formation, especially at high concentrations. In stolons treated with endothal, callose formation may account for the build up of radioactivity shown in the profiles immediately behind the treated area; it might also explain the lower levels of activity observed at the place where the endothal solutions were applied, perhaps by impeding lateral transport of nutrients.

As far as DNP is concerned, there is little doubt that it promoted callose formation in many sieve plates, particularly at the higher concentrations, although the functional meaning of these deposits of callose cannot be ascertained due to the lethal effects of these high DNP concentrations. What these results, both of endothal and DNP, suggest is that considerable amounts of callose can be formed without interfering too much with the transport of solutes. For this reason it is not possible to establish any firm correlation between the number of sieve plates presenting callose deposits and the degree of blockage of the transport system.
Effect of a range of concentrations of endothal and 2,4-dinitrophenol (DNP) on callose formation in stolons of *Saxifraga*.

A large area of intact stolons was treated with one of a range of concentrations of either endothal or DNP for 18 hours. Untreated stolons were used as controls.

The callose present in the sieve plates of freehand sections made from pieces of stolon taken from the treated region or from the corresponding area of untreated stolons was identified by staining the sections with resorcinol blue. The results are presented as the means ± S.E. (standard error of the mean), for 20 replications, of the number of coloured sieve plates counted in the microscope field.

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<th>Treatment</th>
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<td>5×10⁻⁶ M</td>
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<tr>
<td>10⁻⁵ M</td>
<td>3.17 ± 0.29</td>
</tr>
<tr>
<td>5×10⁻⁵ M</td>
<td>10.23 ± 0.54</td>
</tr>
<tr>
<td>10⁻⁴ M</td>
<td>9.43 ± 0.42</td>
</tr>
<tr>
<td>Control</td>
<td>1.40 ± 0.18</td>
</tr>
</tbody>
</table>
PART II

INTRODUCTION

One of the main requisites in the definition of plant hormone is that the substance be translocated from the source of its origin to the site of its action.

Auxins, and in particular indol-3-y1-acetic acid (IAA), which is perhaps universally represented among the species of higher plants, adequately fit into this definition. Indeed, indol-3-y1-acetic acid is generally agreed to be synthesized mainly in the very young leaves and terminal axilinums of stems and shoots (Went, 1928; Skoog, 1938; Davenport, 1947; Jacobs, 1952; Scott and Briggs, 1950), and is then transported to other parts of the plant where it controls growth and other physiological phenomena.

PART II

Went (1928) and van der Vel (1957) were the first to show that the movement of auxin is polar in nature, that is, auxin moves preferentially in the basipetal direction. Since then, this property of auxin transport has been repeatedly confirmed in a variety of tissues, not only with indol-3-y1-acetic acid but also with synthetic auxins, such as 2,4-D (2,4-dichlorophenoylactic acid) (McCrady, 1953; 1958; McCrady and Jacobs, 1963; 1967), NAA (naphthaleneacetic acid) and IBA (indolebutyric acid) (Leopold and Cox, 1961; Morris and Thomas, 1974).

Most of the work concerning the movement of auxine and supplied IAA has been done with the technique devised by Went (1928), which employs excised segments of plant organs as the experimental
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Auxins, and in particular indol-3yl-acetic acid (IAA), which is perhaps universally represented among the species of higher plants, adequately fit into this definition. Indeed, indol-3yl-acetic acid is generally agreed to be synthesized mainly in the very young leaves and terminal meristems of stems and shoots (Went, 1928; Skoog, 1938; Oserkowsky, 1942; Jacobs, 1952; Scott and Briggs, 1960), and is then transported to other parts of the plant where it controls growth and other physiological phenomena.

Went (1928), and van der Weij (1932) were the first to show that the movement of auxin is polar in nature, that is, auxin moves preferentially in the basipetal direction. Since then, this property of auxin transport has been repeatedly confirmed in a variety of tissues, not only with indol-3yl-acetic acid but also with synthetic auxins, such as 2,4-D (2,4-dichlorophenoxyacetic acid) (McCready, 1963; 1968; McCready and Jacobs, 1963a; 1967), NAA (naphthaleneacetic acid) and IBA (indolebutyric acid) (Leopold and Lam, 1961; Morris and Thomas, 1974).

Most of the work concerning the movement of endogenous and applied IAA has been done with the technique devised by Went (1928), which employs excised sections of plant organs as the experimental
system; auxin is applied to one or the other end of the isolated segment, usually by means of an agar block (Donor block), and then received, after a transport period, in another agar block (Receiver block) applied to the opposite end. This method has been very useful in unravelling the properties of auxin transport.

However, such an experimental system is a rather artificial one and, as such, the results that it yields may not reflect the characteristics of auxin transport under natural conditions. Lately, particularly since labelled auxins became available, a considerable amount of work has also been done with intact plants, which has made it possible to confirm the results produced by the method of Went regarding polarity and other characteristics of the auxin transport (Morris et al., 1969; Bonnemain, 1971; Hollis and Tapper, 1971; Morris and Kadir, 1972; Morris et al., 1973; Morris and Thomas, 1974).

As I have said earlier, one of the principal features of auxin transport is its polarity (Went, 1928; van der Weij, 1932; Went and Thimann, 1937; Leopold and Lam, 1961; McCready, 1963; 1966; 1968; Pilet, 1965; Gorter and Veen, 1966). Polar transport of auxins has some important characteristics of its own. For instance, it has a velocity independent of concentration and distance (van der Weij, 1932; McCready, 1963; 1966; Pilet, 1965), and requires metabolic energy (duBuy and Olson, 1940; Gregory and Hancock, 1955; Niedergang-Kamen and Leopold, 1957; Reiff and von Guttenberg, 1961; Goldsmith, 1966; Wilkins and Martin, 1967).
In so far as the velocity of the polar transport of auxin is independent of donor concentration and length of the section used, it rules out the possibility of diffusion being the main process involved. Also, the observed velocities of polar auxin movement are much too high to be accounted for only by diffusion. The fact that it requires metabolic energy and can proceed against a concentration gradient (van der Weij, 1934; McCready, 1963) is also additional evidence against diffusion being the principal method of transport, and suggests that the transport of auxin is to some extent an "active transport".

Several factors can affect the efficiency and polarity of auxin transport. They can be divided in two groups:

a. **Internal factors** e. g. age of the tissue used. The younger the tissue the more efficient it is in transporting the auxin and the more marked is the polarity of transport (Jacobs, 1961; Leopold and Lam, 1962; McCready and Jacobs, 1963b).

b. **External factors**. Among them deserve to be mentioned temperature, light and other compounds.

**Temperature.** Gregory and Hancock (1955), Kaldewey, (1965) and McCready (1968) and Hollis and Tepper (1971) showed that the basipetal transport of auxin can be considerably reduced by low temperatures, and that it follows an optimum curve. On the other hand, it seems that acropetal movement is not affected to a great extent by a drop in the temperature (McCready, 1968).

**Light.** The effect of light on the movement of auxin is still
not completely clear. Conflicting reports have been produced. For instance, Naqvi and Gordon (1967), Thornton and Thimann (1967), in etiolated coleoptiles, and Leopold and Lam (1964) in green pea plants, showed that light inhibited basipetal transport. On the other hand, Guttenberg and Zetsche (1956) and Scott and Briggs (1963) noticed that light promoted transport. To complicate further the picture, Thimann and Wardlaw (1963), in light grown pea plants (var. Alaska), found out that light had no effect on transport, but promoted uptake.

Other compounds. There is a certain number of synthetic substances which can interfere, to a greater or lesser extent, with the basipetal transport of indol-3yl-acetic acid and, for that matter, of some other auxins. In this study I will refer to some of these substances, some of which have the ability to inhibit the transport of auxin. These include respiratory inhibitors, other auxins, and substances structurally related to them.

Respiratory inhibitors. Having in mind that the polar movement of auxin is dependent on metabolic energy, it is not unreasonable to expect that any substance which in some way interferes with the normal supply of energy of the cell will also interfere with the polar transport of auxin.

It is well known that the oxidative reactions of respiration are the main source of energy of the cell; therefore, those substances which inhibit or restrict respiration or the transfer of energy from the oxidative reactions of respiration to other compounds, will, necessarily, inhibit auxin transport. For instance, 2,4-dinitro-
phenol (DNP), an uncoupler of phosphorylative reactions, and cyanide (KCN), a poison of the cytochrome system, both inhibit the basipetal transport of auxin (duBuy and Olson, 1940; Niedergang-Kamien and Leopold, 1957). Sodium azide (Na$_3$N) is yet another respiratory inhibitor which has the capability of inhibiting the polar movement of auxin (McCready, 1966).

**Auxins and structurally related substances.**

The most extensively used inhibitor of auxin polar transport has been 2,3,5-triiodobenzoic acid (TIBA). Its inhibitory action has been observed in a number of plant organs (Kuse, 1953; Hay, 1956; Niedergang-Kamien and Skoog, 1956; Niedergang-Kamien and Leopold, 1957; Pilet, 1965; Keitt and Baker, 1966; McCready, 1966; Winter, 1967; Goldsmith, 1968, 1969; Morris et al., 1972; Thomson et al., 1974; Katekar and Geissler, 1975). Several physiological phenomena which are thought to involve the transport of auxin, such as phototropism and geotropism, apical dominance and abscission, have also been shown to be affected by TIBA application.

The mechanism by which TIBA brings about the inhibition of basipetal transport of auxin is still a matter for some speculation. For instance, it has been suggested that TIBA might block the movement of auxin by competing for auxin binding sites in the plasmalemma (Niedergang-Kamien, 1954; Niedergang-Kamien and Leopold, 1959). Recently some direct evidence has been produced showing that, in fact, TIBA competes with auxin for binding sites in a particulate fraction.
taken from homogenates of corn coleoptiles (Hertel et al., 1972; Thomson et al., 1973; Batt et al., 1976; Batt and Venis, 1976) and pea seedling buds (Jablanovic and Nooden, 1974), and in a suspension
of crown gall cells (Rubery and Sheldrake, 1974).

Little is known about the distribution of these attachment sites on the plasmalemma. For instance, one does not know whether they are uniformly distributed all over the plasma membrane, or whether they are preferentially located (Leopold and Hall, 1966; Hertel and Flory, 1968), or restricted to one side of the cell (Goldsmith and Ray, 1973).

These considerations are quite important in establishing which is the metabolic step in the transport of auxin, that is, the step responsible for the polarity of such a movement. In this connection the use of inhibitors, like TIBA, may be of great help in trying to solve these problems. For instance, observations of Christie and Leopold (1965a and b) in corn coleoptiles sections showed that TIBA is more effective in blocking the movement of auxin when applied to the basal rather than to the apical end of the section. It was then suggested that the metabolic step is secretion out of the cell, whereas uptake is a passive process (Christie and Leopold, 1965a and b). A similar theory had also been presented by Hertel and Leopold, (1963). This assumption also implies that TIBA acts essentially through inhibition of the secretion process, whereas uptake is essentially TIBA-insensitive.

An alternative explanation for the inhibition of the polar
movement of auxin by TIBA is that this compound may promote reactions which lead to the lowering of the levels of auxin in the tissues (Audus and Thresh, 1956), or to its immobilization (Winter, 1967, 1968). Another possibility is that TIBA may inhibit uptake of auxin, at least at high concentrations (Sabnis and Audus, 1967; Rubery and Sheldrake, 1974).

Several other synthetic plant growth regulators have been shown to have similar or even stronger inhibitory action than TIBA. For instance, N-1-naphthylphthalamic acid (NPA) (Morgan and Soding, 1958; Morgan, 1964; Pilet, 1965; McCready, 1966, 1968; Keitt and Baker, 1966; Thomson et al., 1973; Katekar and Geissler, 1975), derivatives of fluorenolcarboxylic acid, generally known as "Morphactins" (Krelle and Libbert, 1968; McCready, 1966, 1968; Parups, 1970; Pilet, 1970; Naqvi, 1971; Beyer, 1972; Bridges and Wilkins, 1973), DRX 1840 (3,5a-dihydro-2-(p-methoxyphenyl)-8H-pyrrolo[5,1-α] isoindol-8-one) (Beyer, 1972; Quebedeaux and Beyer, 1972) and fluororescines, particularly eosin (Katekar and Geissler, 1975), all have proved to be strong inhibitors of auxin polar transport in a variety of tissues. At the same time, some physiological phenomena, such as phototropism, and geotropism, abscission and apical dominance, which are thought to be controlled to some extent by auxin, are also affected by these substances (see e. g. Schrank, 1961; Mentzer and Netien, 1950; Ching et al., 1966, for NPA; Harada, 1967; Khan, 1967; Parups, 1970; Schneider, 1970; and Bopp, 1972, for morphactins; Beyer, 1972; Morgan and Durham, 1972; Gaither and Abeles, 1975, for
The mode of action of these plant growth regulators is still far from clear. It has been suggested that NPA and morphactin may bring about the inhibition of auxin polar transport by binding to some site in the plasmalemma. Indeed, Lembi et al., (1971), Thomson et al., (1973) and Thomson and Leopold (1974) have produced data showing that NPA and morphactin do, in fact, bind to some specific loci which are thought to be associated with the plasma membrane, in particulate fractions of corn coleoptiles.

It has been suggested that the common attachment site for NPA and morphactin is distinct from the attachment site for auxin and TIBA, for no competitive inhibition of auxin binding to its specific receptor by NPA or morphactin could be observed (Hertel et al., 1971; Thomson and Leopold, 1974). However, evidence has just come out (Batt et al., 1976; Batt and Venis, 1976) showing that IAA and NPA have at least two sets of high affinity binding sites in membrane preparations from corn coleoptiles. One of these sites has been shown to be quite specific to active auxins and auxin transport inhibitors and appears to be associated with the plasmalemma. The other site is less specific and seems to be associated with Golgi membranes and/or endoplasmic reticulum.

Both TIBA and NPA have been found to compete for both sites. In the case of NPA, however, it seems possible that a third binding site with higher affinity may be present, for the binding kinetics of the sites reported by Batt et al., (1976) and Batt and Venis
(1976), are quite different from the kinetics of the NPA binding site described by Lembi et al., (1971), Thomson (1972) and Thomson et al., (1973).

One may then assume that the inhibitory effect of NPA and morphactin on the transport of auxin may not result from competition for the auxin receptor, but could be due to some other effect on the mechanism of auxin movement (Thomson and Leopold, 1974).

Inhibition of auxin uptake as the possible cause for the reduction of basipetal transport has to be ruled out, especially in the case of NPA, for it has been shown that this substance promotes IAA uptake, particularly at low IAA concentrations, in both mesocotyl segments (Sabnis and Audus, 1967) and coleoptiles of Zea mays L. (Cande and Ray, 1976).

With respect to DPX 1840, Gaither and Abeles (1975) are of opinion that it may act in a way similar to that of TIBA and NPA and they even suggested a common attachment site for these three substances. Little is known about the mode of action of fluoresceins.

Effect of inhibitors of RNA and protein synthesis on the transport of auxin.

There is a certain number of substances that, although extensively used in other connections, have not yet been employed, as far as I can tell, in the investigation of the movement of auxin. Among them some compounds used as inhibitors of RNA and protein
synthesis, deserve special reference. In this study I will make a brief reference to three of these inhibitors of RNA and protein metabolism, the roles of which as possible inhibitors of auxin transport were investigated in intact stolons of *Saxifraga sarmentosa*.

Cycloheximide (actidione) has been widely used as a specific inhibitor of protein synthesis (Kerridge, 1958; Young *et al.*, 1963; Siegel and Sisler, 1963, 1964; Wettstein *et al.*, 1964; Ross, 1968). However, some evidence has been produced lately which suggests that cycloheximide may have secondary effects, acting perhaps as an uncoupler of oxidative phosphorylations (MacDonald and Ellis, 1969; Ellis and MacDonald, 1970; Webster, 1973; McMahon, 1975). Thus cycloheximide, apart from inhibiting protein synthesis, may also interfere with the normal supply of energy to the cell, falling in this case in the same category as DNP. In view of this evidence, one should expect an inhibitory effect of cycloheximide on the movement of auxin. In the next section I shall present some data showing that in fact cycloheximide is able to prevent the transport of auxin.

Actinomycin-D (Hurwitz *et al.*, 1962) and puromycin (Villa-Trevino *et al.*, 1964) are two antibiotics which are known to inhibit the synthesis of mRNA and protein, respectively. They act at different levels in the process of protein synthesis and are being used more and more to check the dependence of a particular process upon transcription and translation.

These two compounds are regarded as being rather specific, and there is no evidence suggesting that they might interfere with
the energy supply of the cell. Such qualities made their utilization as tools in the study of auxin transport even more interesting, since any apparent inhibition of auxin basipetal movement by actinomycin-D or puromycin would suggest the involvement of de novo protein synthesis in that process.

The effect of the herbicide endothal on the movement of indol-3yl-acetic acid was also investigated.

Endothal (disodium 3,6-endoxohexahydrophthalate) has been known for a long time to cause defoliation in plants. When applied at high concentrations, this plant growth regulator destroys the permeability of the cell and causes the death of the tissues; when applied at lower concentrations, it promotes abscission of the leaves (Crafts, 1953; Osborne, 1968). As I said earlier, abscission is one of those physiological phenomena which appears to be controlled to some extent by auxin; it seems, therefore, possible that the effect of endothal on abscission may be due to interference with the mechanism of auxin transport.

The polar movement of auxins is generally accepted as being associated with young growing tissues (Leopold and Lam, 1962; Jacobs, 1961). However, the basipetal movement of auxin still takes place under conditions where no elongation occurs, for instance, under osmotic stress caused by application of hypertonic solutions of mannitol to segments of plant organs (McCready and Jacobs, 1967; McCready, 1968; Cande and Ray, 1976). The effect of a "ring" of solutions of
mannitol, at concentrations which prevent elongation, on the basi­
tal movement of auxin was further investigated in intact stolons of
Saxifraga.

Inactivation and metabolism of auxin in the tissues during
transport.

In experiments involving the transport of plant hormones, one
important consideration to have in mind is the possible metabolism of
the hormone in the tissues during the transport period. Indeed, there
is now enough evidence showing that part of the auxin taken up by the
tissues is removed from the transport stream and immobilized. One
process of immobilization involves conjugation of the auxin molecule
with a molecule of some other compound, particularly aspartic acid
or glucose (Andreae and Good, 1955; Good et al., 1956; Zenk, 1961;
Morris et al., 1969; Patrick and Woolley, 1973). Another type of im­
mobilization seems to involve formation of a complex with protein
(Winter and Thimann, 1966). Auxin may also be degraded by enzymatic
reactions, giving rise to the formation of other compounds which
might be immobile; alternatively, these compounds may be transported
masking the true movement of auxin. In experiments on auxin transport,
it is therefore important to ensure that the hormone is transported
in its original form, and that one is not measuring the transport of
the products of its metabolism. Further, it is important to discover
whether the application of other plant growth regulators alters in
any way the metabolism or immobilization of the auxin.
Path of auxin transport.

As we have said, polarity is one of the most outstanding features of auxin transport. One point that is still not completely clear is which tissue or tissues are involved in this process. For instance, in *Avena* coleoptiles, where vascular strands are small and rudimentary, it is quite clear that auxin moves essentially through parenchyma cells. The same can be said of the movement of auxin through sections of pith tissue taken from *Coleus* plants (Jacobs and McCready, 1967). On the other hand, Avery (1935) observed polar transport in the veins of tobacco leaves. At first glance, these results seem to indicate that both parenchymatous and vascular tissues may be involved in polar transport. However, one must bear in mind, as was pointed out by Goldsmith (1969), that in short segments of tissue it is most unlikely that either phloem or xylem can operate normally under such conditions. It is then perhaps reasonable to rule out the phloem and the xylem as the likely pathway of polar transport. Support for this assumption comes from data of Morris and Kadir (1972), who showed that no activity was found in the honeydew of aphids after IAA-1-\(^{14}\)C was applied to the apical bud of intact pea plants, even though there was a considerable amount of radioactivity present in the internode upon which the aphids were feeding.

Some recent results, some of them obtained by autoradiographic techniques, suggest that the polar movement of applied IAA takes place in the cells of the vascular cambium and in the cells which result from its activity, that is, the very young elements of the phloem
and xylem still not completely differentiated and perhaps some parenchymatous cells associated with the xylem (Sabnis et al., 1969; Bonnemain, 1971; Sheldrake, 1972; Bourbouloux and Bonnemain, 1973; Wangermann, 1973).

At this point, we should, perhaps, call attention to the fact that indol-3-yl-acetic acid affects vascular differentiation in many plant systems (Snow, 1935; Camus, 1949; Jacobs, 1952; Wangermann, 1967; Sachs, 1969), and it is not unreasonable to suggest that transport and vascular differentiation might be, to some extent, associated (Sachs, 1975).

In intact plants, under certain conditions, auxin can move in the phloem together with other substances. For instance, exogenous auxins applied to fully developed leaves are exported and translocated in the phloem with assimilates (Little and Blackman, 1963; Eschrich, 1968; Brossard and Tepper, 1969; Bonnemain, 1971; Hoad et al., 1971; Morris and Kadir, 1972; Goldsmith et al., 1974). Labelled auxin applied to the bark of segments of willow stem was also detected in the sieve elements by means of aphids feeding upon these strips of bark (Lepp and Peel, 1971 a and b; Hoad et al., 1971). However, the characteristics of the movement of auxins in the phloem of intact plants are entirely distinct from those of the polar movement. Thus, movement in the phloem is non-polar (its direction is determined by the relative position and strength of "source" and "sink"), is unaffected by treatment with TIBA (Morris et al., 1973; Goldsmith et al.,...
1974), and is quite rapid (velocities up to 24.0 cm per hour, not much different from those reported for the movement of assimilates) (Little and Blackman, 1963; Eschrich, 1968; Bonnemain, 1971; Goldsmith et al., 1974). On the other hand, polar transport of auxin is independent of the position of metabolic "sinks" in the plant, is readily inhibited by TIBA and other specific inhibitors (Morris et al., 1973; Goldsmith et al., 1974), and is much slower (velocities in the range of 0.3 to 1.5 cm per hour) (Morris et al., 1969; Hollis and Tepper, 1971). These velocities agree well with published values of the polar transport of IAA in isolated sections of plant organs (Goldsmith, 1969).

It appears then, that in intact plants there are two separate transport pathways: one, taking place in the phloem, is rapid, non-polar and insensitive to treatment with TIBA; the other, much slower and essentially basipetal, moves probably in the vascular cambium and young undifferentiated vascular elements, and is inhibited by TIBA.

In chapter I, I have essentially investigated the effect of several substances on the IAA-induced transport and distribution of labelled assimilates in decapitated stolons of Saxifraga.

In the present chapter, I shall produce data dealing with some aspects of auxin transport, particularly the effect of the mentioned substances on the polar movement of tritiated indol-3yl-acetic acid (IAA-5-^3H) in intact stolons of the same plant.

These two subjects are not separated; in fact they are com-
plementary. For instance, one of the theories put forward to try and explain the mechanism of "Hormone-directed Transport" suggests that the IAA-induced transport of nutrients is due to a direct stimulation of the whole pathway of transport. Such a theory considers the phenomenon of "Hormone-directed Transport" as being dependent on the basipetal movement of auxin, hence the need to investigate the effect of these compounds on the transport of IAA. Further, the results of experiments on IAA-induced transport of metabolites using inhibitors of auxin transport and other plant growth regulators will be meaningless, unless one can complement those results with the knowledge of their effects on the transport of auxin.

2. Preparation of solutions:

a. Nonspecific solutions of plant growth regulators were prepared following the procedure described in Part I of this thesis.

b. Radioactive solution of IAA-$^{15}$N was prepared from a 1.5 ml sample of IAA-$^{15}$N of specific activity 37 Ci/mmol (C.E.A., Saclay, France) obtained in ethanolic solution with a stated purity of not less than 99 per cent.

The solution was evaporated under a gentle nitrogen stream and the IAA-$^{15}$N redissolved in 1 ml of distilled water giving a 0.1% stock solution. All these operations were carried out under a dim green light and the prepared solution was then stored in the dark.
II-MATERIAL AND GENERAL EXPERIMENTAL METHODS

A. Movement of tritiated indol-3yl-acetic acid (IAA-5-\(^3\)H) in intact stolons of *Saxifraga sarmentosa*.

1. Material.

Young intact stolons of *Saxifraga sarmentosa* were used in the investigation of the transport of applied IAA-5-\(^3\)H.

The stolons, about 30 cm long, were grown according to the process described under MATERIAL AND GENERAL EXPERIMENTAL METHODS, Part I, of this study.

Any lateral bud or shoot which had developed, as well as all the scale leaves present, were removed prior to the stolon being used in auxin transport experiments.

2. Preparation of solutions.

a. Aqueous solutions of plant growth regulators were prepared following the procedure described in Part I of this thesis.

b. Radioactive solution of IAA-5-\(^3\)H was prepared from a 1 mCi sample of IAA-5-\(^3\)H of specific activity 21 Ci/mM (C.E.A., Gif-sur-Yvette, France) obtained in ethanolic solution with a stated purity of not less than 99 per cent.

The alcohol was evaporated under a gentle nitrogen stream and the IAA-5-\(^3\)H redissolved in 1 ml of distilled water giving a 5x10^{-5}M stock solution. All these operations were carried out under a dim green light, and the prepared solution was then stored in the dark, in
a deep freezer (−20°C), till the moment of its utilization.

3. Experimental procedure.

Intact stolons of Saxifraga were placed horizontally in a groove running along a perspex block, and a solution of the plant growth regulator under investigation, or distilled water, was applied as described before to a 2 cm wide region, 4–6 cm behind the apical end of the stolon. The epidermis of the treated area had been previously abraded with fine emery paper to facilitate penetration of the solution.

A minute amount of a 5x10⁻⁵ M IAA-5⁻³H solution was injected near the apical end of the stolon by means of a thin capillary "pipette" attached to the arm of a three-way moving stage which allows small vertical and horizontal movements. The capillary "pipette" was allowed to penetrate the outer tissues of the stolon only for 45 seconds, after which it was pulled back and the pierced surface smeared with petroleum jelly. All these manipulations were carried out under a dissecting microscope. Finally, the plants with the stolons were thoroughly watered and placed in the dark, where they remained for the transport period (usually 16–18 hours).

In a particular case, IAA-5⁻³H was injected about the midway down the stolon and "rings" of 2,3,5-triiodobenzoic acid (TIBA) or morphactin were applied on both sides of the point of the injection by the technique described above.
4. Analysis of the distribution of radioactivity in the stolons of Saxifraga.

At the end of the transport period, the stolons were harvested, the region near the point of injection discarded and the remaining part or parts divided in 1 cm sections, taken in sequence. Each section was then cut in small pieces and dropped in a counting vial to which 8 ml of scintillant fluid were added. The samples were then stored in the dark for a 24 hour period, after which they were well shaken and counted in an automatic scintillation spectrometer (N.E. 8312, Nuclear Enterprises Ltd., Sighthill, Edinburgh, Scotland), for a period of 60 seconds each.

Later on, when a Micro-Mat BF 5010 incinerator apparatus became available, it was decided to verify if there was any improvement in the counting rate by incinerating the previously dried sections, and fixing the released \(^3\)H\(_2\)O in 1 ml of \(\beta\)-phenyl ethylamine, before adding the scintillant fluid. From the comparison of the results obtained, it became clear that the original technique, besides being much simpler and less time consuming, compared favourably with the latter.

The counts expressed as c.p.m./cm of stolon were corrected for background radiation, but, although some quenching and self-absorption is bound to have taken place, no attempt was made to correct them, assuming that all samples were similarly affected.

In a few cases, it was necessary to investigate the distribution of the radioactivity in the cortex and in the stele separately.
In such cases, after the normal period of transport, the stele and the cortex of each section had to be surgically separated and their radioactivity content assayed separately as mentioned in Part I.

5. Extraction of the tissues and chromatography.

In some experiments it was thought necessary to check to what extent applied IAA-5-\(^3\)H was metabolized during its transport through the stolons and what effect, if any, TIBA, NPA, morphactin and endothal had on IAA-5-\(^3\)H metabolism. Accordingly, after the usual transport period, each stolon was harvested, the region close to the point of application of tritiated-IAA discarded, and the remaining portion of the stolon cut in small pieces, dropped in 4 ml of chilled ether and extracted for 24 hours in the dark, at 0°C. The extraction was repeated twice more for periods of 8 hours, after which the three extracts were bulked. The ether extracts were then reduced to dryness by evaporation under a stream of air, at room temperature, and redissolved in 0.5 ml of methanol.

Aliquots taken from these samples were spotted on to strips of Whatman n° 1 chromatography paper (5.0 cm wide and 25.0 cm long) using 10 \(\mu\)l capillaries (Drumond Scientific Co.).

Ascending chromatograms were developed over a distance of 20 cm at 25°C in darkness.

Two solvent systems were used, namely isopropanol : 30 % ammonia : water (8:1:1 v/v) and n-butanol : glacial acetic acid : water (5:1:2.2 v/v).
Developed chromatograms were divided into 20 equal sections, 1.5 cm wide, each of which was eluted in 1 ml of methanol for 2 hours in the counting vials. 10 ml of scintillant fluid were added to each vial and its radioactivity content determined by scintillation counting. Counts expressed as c.p.m. were corrected for background radiation, but no attempt was made to correct quenching, having in mind that all samples must have been equally quenched.

B. Movement of IAA-5-^3H in hypocotyl sections of *Helianthus annuus* seedlings.

Seeds of *Helianthus annuus* were soaked in running tap water for 24 hours and sowed in moistened potting compost in large plastic trays.

The seedlings were raised for 10 days in a growth cabinet under controlled environmental conditions of temperature (25°C.) and photoperiod (16 hours). At this stage, the only developed leaves present were the cotyledonary leaves.

10 day-old seedlings were then selected for uniformity and 13 mm long segments were excised from the region immediately below the cotyledonary leaves. Batches of 20 segments were pre-treated for 2 hours by immersion either in different concentrations of endothal or in distilled water. After this period, the segments were blotted dry and transferred to small Petri dishes, where they were assembled in a vertical position, supported by means of small holes made in the Petri dishes lid. Each Petri dish contained 10 µCi of 5x10^-7 M IAA-5-^3H incorporated in 1 ml of 1.5 per cent agar.
Ten out of each batch of 20 segments relative to a particular pre-treatment were orientated so that the morphologically apical end was left in contact with the agar containing the tritiated IAA. The remaining 10 sections were orientated in the opposite direction, that is, the basal instead of the apical cut surface in contact with the agar.

The movement of IAA-5-^3^H through the hypocotyl segments was assessed by collecting tritiated IAA at the opposite end in 1.5 per cent agar blocks.

The Petri dishes, with the segments assembled as described, were next placed inside a plastic box lined with wet filter paper in order to maintain humidity high. The plastic box with the Petri dishes was then transferred to darkness and left at 25°C for the transport period. All operations which involved handling of IAA-5-^3^H were carried out under dim green light.

At the end of the 5 hour transport period, the agar blocks were removed. The blocks for each treatment were pooled together, and their radioactivity extracted with 1 ml of pre-chilled 85 % methanol for 18 hours in the dark at 0°C.

Similarly, each segment was cut transversely into two equal parts, one of which was called "donor section" and the other "receiver section" depending on the end of the segment from which they came. Sections which had undergone the same treatment were pooled together and their radioactivity extracted as described before.
III-RESULTS

Transport of Tritiated IAA

Basipetal movement of auxin has been considered an essential requirement in the explanation of the mechanism of hormone-directed transport of nutrients in plants (Davies and Wareing, 1965).

Indeed, their suggestion that hormone-directed transport is due to activation of the whole pathway of transport implies that the hormone has to move from the place of its application in order to be effective. It is a well known fact that indol-3yl-acetic acid (IAA), and other auxins, when applied to young growing tissues of either intact plants or to excised segments of plant organs, move mainly in a basipetal direction, that is, polarly (Went, 1928; van der Weij, 1932; McCready, 1968; Morris, et. al., 1969; Bonnemain, 1971; Hollis and Tepper, 1971). This polar movement, which seems to be common to most active compounds, has been shown to be associated with several physiological phenomena in which IAA plays some important role, such as cambial activation (Snow, 1935; Wareing et al., 1964), vascular regeneration (Jacobs, 1952, 1967) and xylem differentiation (Sachs, 1969). It is, therefore, little wonder the claim that it might be also associated with the auxin-directed transport of metabolites.

In intact plants, under certain circumstances, applied auxin can also move in a non-polar way (Little and Blackman, 1963; Eschrich, 1968; Morris and Kadir, 1972; Goldsmith et al., 1974). This movement has, however, completely different characteristics when compared to the polar transport. Whether it occurs under natural conditions, and
its possible physiological role, still remain obscure.

There are certain compounds such as 2,3,5-tri-iodobenzoic acid (TIBA), N-1-naphthylphthalamic acid (NPA) and derivatives of fluorenolcarboxylic acid (morphactins) which have been shown in several occasions to inhibit, allegedly in a specific way, the polar movement of auxin (Niedergang-Kamien and Leopold, 1957; Morgan and Soding, 1958; Pilet, 1965; 1970; Keitt and Baker, 1966; McCready, 1966 1968; Krelle and Libbert, 1968).

In Part I of this study I have attempted to investigate the effect of these and some other substances on the transport of nutrients in the phloem; here I shall try to complement those results with the investigation of the effect of such compounds on the movement of labelled IAA, particularly in intact stolons of *Saxifraga sarmentosa*.

1. Effect of TIBA, NPA and morphactin on the transport of tritiated indol-3yl-acetic acid (IAA-5-$^3$H) in intact stolons of *Saxifraga*.

   a. Inhibition of IAA-5-$^3$H basipetal transport by TIBA, NPA and morphactin in intact stolons of *Saxifraga*.

In a set of experiments, intact growing stolons were placed along wide central grooves in perspex blocks; the surface of an area on each stolon some 4-7 cm from the apex was slightly abraded with fine emery paper and then submerged in a 10^{-4} M aqueous solution of one of the three compounds under investigation, and left in these conditions for the whole experimental period. Stolons used as controls were similarly treated with distilled water. At the same
time, a minute amount of a $5 \times 10^{-5}$ M IAA-5-^3H solution was injected for 45 seconds immediately below the apex as described under MATERIAL AND GENERAL EXPERIMENTAL METHODS.

After an 18-hour translocation period carried out in the dark at 19-21°C, the stolons were harvested; a 0.5 cm piece of stolon immediately below the point of IAA-5-^3H application was discarded, and a 15 cm long portion of the remainder divided into 1 cm pieces, starting from the distal end, and taken in sequence. Each section was then radiassayed individually as mentioned before.

The results of these experiments are shown in figs. 26, 27 and 28. They clearly show that a $10^{-4}$ M solution of TIBA, NPA or morphactin respectively, blocks almost completely the basipetal movement of IAA-5-^3H. Most of the radioactivity applied to the treated stolons accumulated near the point of injection, so that little activity could be detected beyond the treated region.

On the other hand, the controls show what appears to be a "pulse" of activity near the front. A rough estimate of the velocity of this "pulse" of activity indicates that it is about 6.7 mm/hour, a value which is well inside the limits of the published values for the polar transport of IAA in tissues of aereal organs.

b. Effect of a $10^{-4}$ M solution of TIBA or morphactin on the basipetal and acropetal movement of IAA-5-^3H in intact stolons of Saxifraga.

In another experiment designed to investigate the effect of solutions of TIBA or morphactin on both the basipetal and acropetal movements of IAA-5-^3H, the radioisotope was injected about midway
Fig. 26. Inhibition of the basipetal movement of IAA-5-\(^3\)H by a 10\(^{-4}\)M solution of TIBA in intact stolons of _Saxifraga_.

A 5\times10\(^{-5}\)M solution of radioisotope was injected for 45 seconds near the apex of each stolon. The transport period was 18 hours, carried out in the dark at 19-21\(^{\circ}\)C. The activity, expressed in c.p.m. per cm of stolon, was plotted against the distance in cm from a point near the site of IAA-5-\(^3\)H injection. Each profile is the mean of 3 replicates, and the vertical lines represent ± the standard error of the mean. The vertical dashed lines indicate the position of the area treated with TIBA (open circles) or water (full circles). The background level is indicated by the dash-point line.
Fig. 27. Inhibition of the basipetal movement of IAA-5-3H by a 10^{-4}M solution of NPA in intact stolons of Saxifraga.

A 5x10^{-5}M solution of radioisotope was injected for 45 seconds near the apex of each stolon. The transport period was 18 hours, carried out in the dark at 19-21°C. Activity, expressed in c.p.m./cm of stolon, was plotted against the distance in cm from a point near the site of IAA-5-3H application. The points in the profiles are the means of 3 replicates each, and the vertical lines represent ± the standard error of the mean.

The vertical dashed lines indicate the position of the area of the stolons treated with NPA (open circles) or water (full circles). The background level is indicated by the dash-point line.
Basipetal Dir.

Distance in cm

Activity in c.p.m./cm of stolon x 10^-1 (log. scale)

NPA
Fig. 28. Inhibition of the polar movement of IAA-5-$^3$H by a $10^{-4}$M solution of morphactin in intact stolons of *Saxifraga*.

A 5x$10^{-5}$M solution of tritiated IAA was injected for 45 seconds near the apex of each stolon. The transport period was 18 hours in the dark at 19-21°C. The activity expressed in c.p.m./cm of stolon is plotted against the distance in cm from a point near the site of IAA-5-$^3$H injection. Each point in the profiles is the mean of 3 replicates, and the vertical lines represent the standard error on either side of the mean. The vertical dashed lines indicate the position of the portion of the stolon treated with either morphactin (open circles) or water (full circles). The background level is indicated by the dash-point line.
down the stolon. Areas on both sides of the point of injection of IAA-5-$^3$H were treated with aqueous solutions of TIBA (10^{-4}M) or morphactin (10^{-4}M). The equivalent regions of the controls were treated with water.

After a 6-hour transport period in the dark, the stolons were harvested; the area comprising 0.5 cm on either side of the point of IAA-5-$^3$H application was discarded and the remainder divided sequentially in both directions into 1 cm sections. The radioactivity content of each of these sections was determined in an automatic scintillation spectrometer, as described before.

The results presented in fig. 29 show that both TIBA and morphactin at the concentrations used inhibited almost completely the movement of tritiated IAA in the basipetal direction. The controls show the usual "pulse" of activity near the front of the profile. The velocity of this "pulse" was tentatively estimated as being roughly 6.6 mm/hour, a value which is very similar to that reported in the previous experiment.

On the other hand, the acropetal movement of labelled IAA was not greatly affected by either TIBA or morphactin. However, there are some differences in the patterns of distribution of radioactivity that must be reported. Thus, in stolons treated with TIBA there was a greater accumulation of activity in the area comprising the 2 cm immediately below the point of IAA-5-$^3$H application than in the corresponding region of the controls; morphactin-treated stolons had the lowest level of activity of them all in that same area. On the other
Fig. 29. Effect of solutions of TIBA ($10^{-4}$ M) and morphactin ($10^{-4}$ M) in both basipetal and acropetal movement of IAA-$5^{-3}$H in intact stolons of *Saxifraga*.

A $5 \times 10^{-5}$M solution of tritiated IAA was injected for 45 seconds midway down the stolon. The transport period was 6 hours in the dark at 18-20°C. The activity, in c.p.m./cm of stolon, was plotted against the distance in cm, in both directions, from near the site of IAA-$5^{-3}$H application. The profiles are the means of 3 replicates each, and the vertical lines represent $\pm$ the standard error of the mean. The treated areas on the stolons are indicated by the vertical dashed lines.

**Full Symbols** - Basipetal movement

**Open Symbols** - Acropetal movement

**Circles** - TIBA-treated stolons

**Triangles** - Morphactin-treated stolons

**Squares** - Controls

The background level is indicated by the dash-point line.
hand, TIBA-treated stolons showed a comparatively low level of activity in the portion of stolon above the point of injection, whereas morphactin-treated ones showed a higher relative radioactivity content particularly in the region where the morphactin "ring" was located. It appears then that in stolons treated with morphactin "rings" the inhibition of the basipetal movement of IAA-5-\(^3\)H had as consequence an increase in the amount of label transported acropetally, most probably in the phloem. However, I have no explanation for the accumulation of activity in the region treated with morphactin.

The effect of TIBA seems to be somewhat different. In this case, inhibition of the basipetal transport of IAA-5-\(^3\)H does not promote the movement of radioactivity in the acropetal direction; instead, most of the activity remains just below the point of injection. One possible explanation for this may well be an inhibition by TIBA of the entry of label into the sieve tubes, as suggested by Goldsmith et al. (1974).

The results of fig. 29 show, very clearly, that, in the same period of time, labelled IAA was transported much further in the acropetal than in the basipetal direction, and that a considerable accumulation of radioactivity took place at the apical region of the stolons.

We may therefore conclude that in intact stolons of *Saxifraga* applied IAA moves both basipetally and acropetally, having these two types of transport entirely different features; the acropetal movement takes place most probably in the phloem together with sugars, and, as
such, is dependent on the "source-sink" relationships of the organ, and is relatively insensitive to treatment with morphactin or TIBA. The basipetal transport is inhibited by these substances and is independent of both the position and the strength of the "sink".

2. Effect of inhibition of RNA and protein synthesis on the transport of IAA-5-\(^3\)H in intact stolons of Saxifraga.

Inhibitors of RNA and protein synthesis, such as actinomycin-D, puromycin and cycloheximide, have been extensively used in the study of several phenomena where protein synthesis is suspected to be involved in their control. However, the compounds mentioned have not yet been used, as far as I know, in the investigation of the problem of the polar transport of auxin, despite the fact that a mechanism involving specific protein carriers has been put forward as a possible explanation for this phenomenon (Osborne and Mullins, 1969).

In the present study I have decided to investigate the effect of the above inhibitors of protein synthesis on the basipetal transport of tritiated IAA in intact stolons of Saxifraga.

a. Inhibition of polar transport of IAA-5-\(^3\)H by cycloheximide in stolons of Saxifraga sarmentosa.

A small amount of IAA-5-\(^3\)H was injected over a period of 45-seconds near the apex of growing intact stolons in the manner described before. An area on the stolons situated 4-6 cm below the point of the label application was treated with an aqueous solution of cycloheximide...
(10 mg/l) after its epidermis had been slightly abraded with fine emery paper. The controls were similarly treated with distilled water.

After a 16-hour transport period in the dark at 20–21°C, the stolons were harvested, a 0.5 cm portion immediately below the point of IAA-5-HT application discarded, and part of the remainder of each stolon divided into 1 cm sections. The cortex and the stele of each section were next separated and their radioactivity determined.

The results of these experiments are shown in figs. 30 and 31. The activity profiles in fig. 30 are the sum of the activities present in the cortex and in the stele, whereas in fig. 31 the distribution of radioactivity in the cortex and in the stele are shown separately. It is evident from both figures that a "ring" of cycloheximide effectively inhibits the basipetal transport of auxin. However, the effect of cycloheximide on the movement of auxin appears to be in some respects different from that of TIBA, NPA or morphactin in as much as in the case of cycloheximide a big peak of activity developed immediately above the treated region, whereas in stolons treated with any of the above mentioned inhibitors of auxin transport most of the activity remained near the point of application of the radioisotope.

Another very important point which emerges from fig. 31 is that the applied IAA-5-HT moves mainly in the tissues of the stele. This is particularly evident in stolons kept as controls in which the "pulse" of activity is clearly visible in the stele and practically non-existent in the cortex. However, there is still a considerable
Fig. 30. Inhibition of IAA-5-\(^3\)H polar movement by cycloheximide (10 mg/l) in intact stolons of *Saxifraga*.

The radioisotope, in a original solution of 5x10\(^{-5}\)M, was injected near the apex, for 45 seconds. The plants were left in the dark for 16 hours at 19-21°C, after which the stolons were harvested and the radioactivity determined.

The activity, in c.p.m./cm of stolon, was plotted against the distance in cm from a point near the site of IAA-5-\(^3\)H application. Each point in the profiles is the mean of 3 replicates, and the vertical lines stand for ± the standard error of the mean. The vertical dashed lines indicate the position of the areas of the stolons treated with cycloheximide (open circles) or water (full circles).
Fig. 31. The same as in fig. 30, except for the fact that the activities in the cortex and in the stele are shown individually.

Open Symbols - Stele

Full Symbols - Cortex

Circles       - Cycloheximide-treated stolons

Triangles    - Controls
amount of radioactivity present in the tissues of the cortex, especially near the point of IAA-$5^3$H application, but this activity appears to be somehow immobilized and therefore not available for transport.

b. **Effect of actinomycin-D and puromycin on the polar movement of IAA-$5^3$H in intact stolons of Saxifraga.**

In another experiment, a region of the stolons located some 5-7 cm below the apex was treated with distilled water or an aqueous solution of either actinomycin-D (20 mg/l) or puromycin ($2 \times 10^{-4}$M). Four hours later, IAA-$5^3$H ($5 \times 10^{-5}$M) was injected about 0.5 cm away from the apex. After a 16-hour transport period in the dark, the stolons were harvested and, after discarding a small portion of the stolon near the point of IAA-$5^3$H injection, the remainder was cut into 1 cm sections over a distance of 15 cm and their radioactivity counted.

The results presented in fig. 32 show that both actinomycin and puromycin have little effect on the basipetal movement of labelled IAA. However, when more closely inspected, the profiles show that puromycin caused some accumulation of label near the treated region and there was a small reduction in the amount of radioactivity transported beyond this region; actinomycin did not produce any such effects.

In any case, the "pulse" of activity is clearly visible near the front of the profiles and appears to have moved down the stolon.
Fig. 32. Effect of actinomycin-D (20 mg/l) or puromycin (2x10^{-4} M) on the basipetal transport of IAA-5-^{3}H in intact stolons of Saxifraga.

A 5x10^{-5} M solution of tritiated IAA was injected for 45 seconds near the apex of each stolon, 4 hours after the beginning of the treatment with actinomycin-D (squares), puromycin (circles) or water (triangles).

The translocation period was 16 hours in the dark at 18-21°C. The activity, in c.p.m./cm of stolon, was plotted against the distance in cm from a point near the site of IAA-5-^{3}H application. Each profile is the mean of 3 replicates, and the vertical lines represent the standard error on either side of the mean. The position of the treated areas of the stolons is indicated by the vertical dashed lines.
Basipetal Dir.

Activity in c.p.m./cm of stolon \times 10^{-1} (log. scale)

Distance in cm

Actino

Puro
at an estimated velocity of about 7 mm/hour, a value which is similar to those reported in previous experiments.

3. Effect of the respiratory inhibitor KCN and the uncoupling agent DNP on the basipetal transport of IAA-$^3$H in intact stolons.

The polar movement of auxin has been known to be a metabolically-driven process, therefore dependent on metabolic energy (Goldsmith, 1968). Substances which can either inhibit respiration, like cyanide (KCN), or interfere with the transfer of energy from the oxidative reactions of the respiration to other compounds, such as DNP, have also the ability of blocking the polar movement of auxin (duBuy and Olson, 1940; Niedergang-Kamien and Leopold, 1957).

The effect of both KCN ($5 \times 10^{-4}$M) and several concentrations of DNP on the basipetal transport of IAA were once more investigated using this time intact stolons of Saxifraga. In a first experiment, intact stolons to which a minute amount of IAA-$^3$H ($5 \times 10^{-5}$M) had just been applied near the apex were treated with distilled water or aqueous solutions of either KCN ($5 \times 10^{-4}$M) or DNP ($10^{-4}$M) some 5-7 cm below the point of IAA-$^3$H injection.

After an 18-hour transport period in the dark, the stolons were harvested, divided into 1 cm segments, and the radioactivity determined in the usual manner.

The results presented in fig. 33 show that both DNP and KCN were very effective in blocking the polar movement of labelled-IAA.

Since it has been found out, in other instances, that high concentrations of DNP may have toxic effects on the tissues, it was
Fig. 33. Inhibition of IAA-\(5-^3\text{H}\) polar movement by cyanide (KCN; \(5\times10^{-4}\text{M}\)) or 2,4-dinitrophenol (DNP; \(10^{-4}\text{M}\)) in intact stolons of Saxifraga sarmentosa.

A \(5\times10^{-5}\text{M}\) solution of tritiated IAA was injected for 45 seconds near the apex of each stolon. After an 18-hour transport period in the dark at 20-23°C, the stolons were harvested, divided in 1 cm sections and radioassayed. The activity, in c.p.m./cm of stolon, was plotted as in previous cases. The profiles are the means of 3 replicates each, and the vertical lines have the same meaning as in fig. 32.

The vertical dashed lines indicate the position of the treated portions of the stolons.

Squares - KCN-treated stolons

Circles - DNP-treated stolons

Triangles - Controls

The dash-point line indicates the level of background activity.
decided to investigate the effect of lower concentrations of DNP on the polar transport of IAA-5-\(^3\)H also in intact stolons of *Saxifraga*. Thus, the stolons were treated in the same way as in the previous experiment with one of the following concentrations of DNP: DNP \(5 \times 10^{-5}\)M or DNP \(10^{-5}\)M; the controls were treated with distilled water.

After a 17-hour transport period carried out in the dark, the radioactivity was assayed as mentioned before. The results of this experiment are shown in fig. 34. They indicate, quite clearly, that DNP at either concentration is still able to inhibit, to a great extent, the basipetal movement of IAA-5-\(^3\)H. It is, however, also clear that a \(10^{-5}\)M concentration of DNP is considerably less effective in terms of inhibition of labelled IAA transport than the higher concentration of \(5 \times 10^{-5}\)M.

It is perhaps interesting to call the attention to the fact that the inhibition of IAA-5-\(^3\)H transport by KCN or by different concentrations of DNP is similar to that caused by cycloheximide in so far as all of them caused a peak of activity to accumulate in the upper section of the treated area and immediately above. This is probably an indication that the effect of cycloheximide on auxin transport is not mediated through inhibition of protein synthesis but must be due to interference with the normal supply of energy of the cell.

4. Effect of endothal (disodium 3,6-endoxohexahydrophthalate) on the transport of IAA-5-\(^3\)H.

*Endothal* is an herbicide which was initially developed as a defoliant (see e.g. Crafts, 1953). Since auxins are known to be
Fig. 34. Effect of two different concentrations of DNP ($10^{-5}$M and $5 \times 10^{-5}$M) on the polar movement of IAA-$3^\text{H}$.

A small amount of a $5 \times 10^{-5}$M solution of tritiated IAA was injected near the apical end of intact stolons of *Saxifraga*. After a 17-hour transport period in the dark at 20-23°C, the stolons were harvested and the radioactivity determined. The activity, in c.p.m/cm of stolon, was plotted as in previous cases. The profiles are the mean of 3 replicates (n) each, and the vertical lines represent ± the standard error of the mean. The position of the treated regions of the stolons is signalled by the vertical dashed lines.

- Squares - $5 \times 10^{-5}$M DNP treated stolons
- Circles - $10^{-5}$M DNP treated stolons
- Triangles - Controls

The dash-point line indicates the background level of activity.
Basipetal Dir,

Distance in cm

16 14 12 10 8 6 4 2

Activity in c.p.m./cm of stolon x 10^-1 (log. scale)

169
involved in the control of abscission (Addicott and Lynch, 1955; Addicott et al., 1955; Jacobs, 1968; Halliday and Wangermann, 1972), it was thought to be of interest to investigate what effect endothal might have on the transport of IAA-5-3H.

a. Inhibition of IAA-5-3H basipetal transport by endothal in intact stolons of Saxifraga.

In a first experiment, a minute amount of IAA-5-3H (5x10^-5M) was injected over a period of 45 seconds near the apex of intact growing stolons. An area 4-7 cm below the point of IAA-5-3H injection was treated with water or one of the three concentrations of endothal: 100 mg/l, 200 mg/l or 500 mg/l. 18 hours later, the stolons were harvested and a 0.5 cm long portion, immediately below the point of injection, discarded.

Fig. 35 shows the results of this experiment. It can be seen that the three concentrations of endothal were all extremely effective in inhibiting the basipetal transport of tritiated IAA. Here again, there was an accumulation of activity immediately above the endothal-treated region. In the controls there was the normal "pulse" of activity which must have travelled down the stolon at an average velocity estimated at about 6.5 mm/hour.

In a similar experiment, the stolons were treated with a 200 mg/l solution of endothal or water. At the end of the 15-hour transport period carried out in the dark, and after discarding the 0.5 cm below the point of IAA-5-3H application, the stolons were divi-
Fig. 35. Effect of a range of concentrations of endothal on basipetal transport of IAA-5-$^3$H in intact stolons of Saxifraga.

IAA-5-$^3$H ($5 \times 10^{-5}$M) was injected near the apical end of intact stolons. The transport period was 18 hours in the dark at 21-23°C. The radioactivity in c.p.m./cm of stolon was plotted against the distance in cm from near the point of application of labelled IAA. The profiles are the mean of at least 3 replicates (n); the vertical lines have the same meaning as in previous cases. The vertical dashed lines indicate the location of the treated areas of the stolons. The dash-point line indicates the level of background radiation.

Full Circles - Stolons treated with 500 mg/l endothal; n=3
Open Squares - Stolons treated with 200 mg/l endothal; n=4
Open Circles - Stolons treated with 100 mg/l endothal; n=3
Open triangles - Controls; n=3
ded into 1 cm sections. The stele and the cortex of each section were then separated and their radioactivity content assayed individually.

The results in fig. 36 confirm earlier findings. Thus, they show that the basipetal transport of IAA-5-\(^3\)H in stolons treated with endothal (200 mg/l) is almost completely blocked. On the other hand, the controls show that the "pulse" of activity is almost exclusively confined to the stele, therefore confirming the findings of fig. 31. The estimated velocity of the "pulse" of activity in this case seems to be just a bit lower (6 mm/hour) than in other cases.

b. Effect of endothal solutions on the movement of IAA-5-\(^3\)H in segments excised from the hypocotyl of Helianthus annuus seedlings.

Thirteen millimeters long sections were excised from the region immediately below the cotyledonary leaves of 10-day-old Helianthus annuus seedlings. Batches of 20 sections were treated for 2 hours with distilled water or with one of the four different concentrations of endothal: 1 mg/l; 5 mg/l; 50 mg/l; 500 mg/l. After this treatment, the sections were assembled in a vertical position in small petri dishes containing 10 µCi of IAA-5-\(^3\)H (5x10^-7 M) incorporated in 1.5% agar, so that the apical cut surface of 10 out of the batch of 20 sections were left in contact with the agar. The other 10 sections were orientated in the opposite direction, that is, with the basal cut surface in contact with the agar. The tritiated IAA passing through the sections was collected at the opposite side in 1.5% agar blocks.
Fig. 36. Effect of a 200 mg/l endothal solution on the distribution of IAA-5-^3^H in both the cortex and the stele of intact stolons of *Saxifraga*.

The radioisotope was applied as in previous experiments. After a 15-hour translocation period in the dark, the stolons were collected and the radioactivity present in the stele and in the cortex assayed individually. The activity was plotted as usual, the results being the means of 3 replicates each. The vertical lines represent ± the standard error of the mean, and the vertical dashed lines have the same meaning as in fig. 35.

Open Symbols - Stele

Full Symbols - Cortex

Circles - Stolons treated with endothal (200 mg/l)

Triangles - Controls
Distance in cm

Activity in c.p.m./cm of stolon x 10^{-1} (log. scale)

Basipetal Dir.

Endot.
At the end of the 5-hour transport period, the activity in the agar blocks, as well as in the segments, was determined and the rest of the experiment carried out according to the procedure described under MATERIAL AND GENERAL EXPERIMENTAL METHODS.

The results of this experiment are presented in Table III. They show that, as in intact stolons of Saxifraga, endothal has a marked inhibitory effect on the basipetal transport of IAA-5-3H in excised sections of Helianthus annuus seedlings. This is revealed not only by the dramatic reduction in the amount of radioactivity reaching the receiver blocks, especially at higher concentrations, but also by the distribution of activity within the segments themselves. On the other hand, the acropetal movement seems to be little affected or perhaps even increased, although, due to the low activity reaching the receivers and its variability, no definitive conclusion could be drawn.

One of the reasons for the observed reduction in the basipetal transport of tritiated IAA might be ascribed to inhibition of uptake by endothal. Indeed, it can be seen that endothal does reduce uptake considerably in comparison with the controls, even at the lowest concentration used. However, the reduction of IAA-5-3H basipetal transport by endothal cannot be entirely accounted for by such an inhibition of uptake, since the movement of label inside the segments is also enormously reduced. This is clearly shown by the ratios between the amount of activity present in the "donor" and the "receiver" halves of the segments.
TABLE III

Effect of a range of concentrations of endothal on the polar movement of IAA-5-\(^3\)H in isolated segments of Helianthus annuus.

Radioactivity present in both the basipetal and acropetal receivers, as well as in both halves of the segments, after 5 hours transport in the dark at 25°C. 13 mm long segments were excised immediately below the cotyledonary leaves of 10-day old Helianthus seedlings, and pretreated for 2 hours with water or one of the concentrations of endothal. IAA-5-\(^3\)H was applied to one of the cut ends in the original concentration of 5x10^{-7} M, incorporated in agar (10 µCi in 1 ml of agar/20 segments).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Direction of transp.</th>
<th>C.P.M. in Basipetal receivers</th>
<th>C.P.M. in the tissues</th>
<th>Ratio</th>
<th>Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Basipetal</td>
<td>13420</td>
<td>49808</td>
<td>16834</td>
<td>2.96</td>
</tr>
<tr>
<td></td>
<td>Acropetal</td>
<td>24</td>
<td>25932</td>
<td>134</td>
<td>193.52</td>
</tr>
<tr>
<td>Endothal:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mg/l</td>
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</tr>
<tr>
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<td>13894</td>
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<tr>
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<td>Acropetal</td>
<td>35</td>
<td>11138</td>
<td>192</td>
<td>58.01</td>
</tr>
</tbody>
</table>
An interesting point which also emerges from this experiment is the difference in uptake observed between the cut surface at both ends of the sections, particularly in the controls. Thus, we can see that much more IAA-$^{3}$H was taken up by the apical cut surface than by the basal one. This seems to suggest that, if it is true that the basipetal movement of tritiated IAA depends on the uptake, it is also true that uptake appears to be dependent on transport.

In these experiments dealing with the transport of IAA-$^{3}$H, it is important to ensure that the substance reaching the receivers is still in the original chemical form, that is, we have to make sure whether the labelled IAA was metabolized during its transport. Thus, aliquots were taken from the extracts of basipetal receivers of both the controls and sections treated with a 1 mg/l solution of endothal, and spotted in Whatman no 1 chromatography paper. Ascending chromatograms were run using as chromatographic solvent iso-propanol: ammonia: water (10: 1: 1, v/v), and the distribution of activity along the chromatograms was determined by liquid scintillation counting, as described before.

Fig. 37 shows that the activity reaching the agar blocks appears essentially in a single peak at a Rf similar to that of IAA-$^{3}$H.

5. Effect of plant growth regulators on the metabolism of IAA-$^{3}$H in stolons of Saxifraga sarmentosa.

During its transport through the tissues, the auxin molecule may be metabolized or conjugated with the molecules of other compounds.
Fig. 37. Distribution of radioactivity along chromatograms of ethanolic extracts from the basipetal receiver blocks of *Helianthus annuus* segments pre-treated with (A) 1 mg/l solutions of endothal, (B) distilled water (controls). Transport period 5 hours. IAA-5-3H (5x10^-7 M) was applied to the apical cut surface of the segments incorporated in 1.5 % agar. Chromatographic solvent iso-propanol: 30 % ammonia: water (10: 1: 1 v/v).

The horizontal bar indicates the position of IAA-5-3H, chromatographed in parallel.
giving rise to immobile or inactive products. Auxin transport inhibitors, and other plant growth regulators, may promote such reactions. It was then thought important to investigate the effect of solutions of TIBA ($10^{-4}$ M), NPA ($10^{-4}$ M), morphactin ($10^{-4}$ M) and endothal (200 mg/l) on the metabolism of tritiated IAA in intact stolons of *Saxifraga*.

An area on each stolon located some 5-8 cm below the apex was treated with either water or a solution of the above compounds, immediately before a minute amount of IAA-$5^{-3}$H was applied near the apex in the usual way. After a transport period of 18 hours in the dark at 23-25°C, a 10 cm apical section was taken, and the apex and a small portion of the stolon, including the point of IAA-$5^{-3}$H injection, were removed. The remainder was cut into small pieces and its radioactivity extracted in pre-chilled ether in the dark, as described under MATERIAL AND GENERAL EXPERIMENTAL METHODS.

Aliquots taken from the extracts were spotted in Whatman n° 1 chromatography paper and ascending chromatograms run over 20 cm using two different chromatographic solvents, namely isopropanol: 30% ammonia: water (8: 1: 1 v/v) and n-butanol: glacial acetic acid: water (5: 1: 2.2). The distribution of radioactivity along the chromatograms was determined by liquid scintillation counting, as described before.

Fig. 38 shows the results obtained from chromatograms developed with isopropanol: ammonia: water. It can be seen that in all cases there are two main peaks of activity, one at the Rf 0.45-0.55 which more or less coincides with the Rf of IAA-$5^{-3}$H chromatographed in parallel. The other peak of activity located near the starting line
Fig. 38. Distribution of the radioactivity along chromatograms of ether extracts of *Saxifraga sarmentosa* tissues treated with (A) TIBA (10⁻⁴ M), (B) NPA (10⁻⁴ M), (C) morphactin (10⁻⁴ M), (D) endothal (200 mg/l) and (E) water (controls).

IAA-5⁻³H, at the original concentration of 5x10⁻⁵ M, was injected near the apex of intact stolons. Transport period 18 hours. Chromatographic solvent—isopropanol: 30 % ammonia: water (8: 1: 1 v/v). The position of IAA-5⁻³H chromatographed in parallel is indicated by the horizontal bar.
is probably associated with indol-3-acetylaspartic acid, a product of the conjugation of IAA with aspartic acid. Of interest is the fact that all treatments appear to have increased quite considerably the amount of radioisotope associated with this peak in comparison with the controls. Such an effect may well result from an increased concentration of IAA-5-\(^3\)H in the apical tissues due to inhibition of the basipetal transport by the plant growth regulators employed.

The results of similar chromatograms run in n-butanol: glacial acetic acid: water are presented in fig. 39. In this case the radioactivity is mainly concentrated in a single peak of activity comprising the Rfs 0.75-0.95, most of this radioactivity being probably free IAA-5-\(^3\)H. A small peak of activity is also present at lower Rf (0.55-0.65) representing some unidentified compound (perhaps IAA-glucose, Zenk, 1961), particularly in chromatograms of extracts from stolons treated with NPA, morphactin and endothal.

In conclusion, the results of this experiment indicate that treatment with the mentioned plant growth regulators does not greatly alter the pattern of IAA-5-\(^3\)H metabolism although it may promote the formation of IAA conjugates such as IA-aspartic acid or IAA-glucose.

6. Effect of high concentrations of mannitol on the polar movement of IAA-5-\(^3\)H.

The polar movement of auxin has been associated with young growing tissues. As the age of the tissues progresses, its capacity for polar transport declines (McCready and Jacobs, 1963; 1967). It is
Fig. 39. As in fig. 38 except for the chromatographic solvent that was n-butanol: glacial acetic acid: water (5: 1: 2.2 v/v).
therefore interesting to see if under conditions where expansion growth is prevented transport can still take place.

There are a few reports in the literature in which solutions of mannitol were used to investigate the transport of auxins. Thus, McCready and Jacobs (1967) showed that 0.2 M solutions of mannitol, which reduced elongation of segments cut from petioles of Phaseolus vulgaris L. plants, increased the basipetal transport of 2,4-dichlorophenoxyacetic acid (2,4-D). On the other hand, Cande and Ray (1976) found that plasmolysis of maize coleoptile segments markedly reduced the basipetal movement of auxin, but did not stop it completely.

Further, in isolated Helianthus annuus sections solutions of mannitol inhibited the polar movement of auxin (Prof. Audus, personal communication). However, as far as I can tell, no similar experiment has yet been performed using intact plants, therefore I decided to investigate the effect of solutions of mannitol (0.3 M and 0.4 M) on the polar movement of tritiated IAA in intact stolons of Saxifraga sarmentosa.

A 3 cm wide area of each stolon located some 4-7 cm below the apex was treated with high concentrations of mannitol (0.3 M or 0.4 M) or distilled water. IAA-5-\(^{3}\)H was next applied near the apex as described before. The stolons were then allowed to transport for 16 hours in the dark at 22-23°C, after which a small portion of each stolon including the point of IAA-5-\(^{3}\)H application was removed and the remainder divided over a certain distance in 1 cm sections. The radioactivity was determined by liquid scintillation counting.
The results of this experiment are presented in fig. 40. They show that in intact stolons the concentrations of mannitol used (0.3 M and 0.4 M) did not block completely the polar movement of IAA-5-\(^3\)H, but its velocity appears to have been to some extent reduced, especially at the higher concentration, where the estimated velocity was only about 4.4 mm/hour compared with approximately 6.8 mm/hour in the controls.

7. Pathway of IAA-5-\(^3\)H basipetal movement.

There are several reports in the literature, which our own observations seem to confirm, suggesting that the polar movement of auxin takes place in the tissues of the stele.

However, as I have shown (figs. 31 and 36), some activity is still present in the cortex. What we do not know is whether this activity is the result of some form of longitudinal transport still taking place in the cortex, or due to lateral "leakage" of the radioisotope out of the stele and into the cortical tissues.

To distinguish between these two possibilities an experiment was designed in which the cortices and the steles of intact growing stolons were carefully separated over a 3 cm long area located some 4 cm below the apex, and a small piece of transparent non-permeable wrap film interposed between them immediately before the application of IAA-5-\(^3\)H. The region in which the operation was performed was thoroughly smeared with white soft petroleum jelly and wrapped up in another piece of wrap film to avoid desiccation. After an 18-hour
Fig. 40. Effect of hypertonic solutions of mannitol (0.3 M and 0.4 M) on the polar movement of IAA-5-3H in stolons of *Saxifraga*.

A small amount of a $5 \times 10^{-5}$ M solution of tritiated IAA was injected for 45 seconds near the apical end of intact stolons. After 16 hours in the dark at 22-23°C the stolons were harvested and the radioactivity counted. The activity in c.p.m./cm of stolon was plotted against the distance in cm from a point near the site of IAA application. The results are the mean of 3 replicates and the vertical lines stand for $\pm$ the standard error of the mean.

The vertical dashed lines indicate the position of the areas of the stolons treated with 0.4 M mannitol (triangles), 0.3 M mannitol (circles) or water (squares).
transport period in the dark at about 20°C, the apex and a small portion of each stolon, the point of IAA-5-^3^H injection included, were removed and the remainder divided into 1 cm sections over a 10 cm distance. The cortex and the stele of each section were then separated and their activity determined individually.

The results (fig. 41) show that separation of the cortex and the stele stopped the basipetal movement of IAA-5-^3^H in both tissues. However, they also show that radioactivity moved at least 2 cm further in the stele than in the cortex. The failure of the stele to transport IAA-5-^3^H under the mentioned conditions is difficult to understand, unless, despite all the care, some damage was done to the tissues which might have made the stele unfunctional. This point needs further investigation.
Fig. 41. Separation of the cortex and the stele over a small distance and its effects on the polar transport of IAA-5-3H in intact stolons of *Saxifraga*.

IAA-5-3H (5x10^-5 M) was injected for 45 seconds near the apex of stolons which had the stele separated, over a small distance, from the cortex.

After an 18-hour transport period in the dark, the stolons were harvested and the radioactivity in the cortex (full circles) and in the stele (open circles) assayed individually. The results, were plotted as in fig. 40. The profiles are the means of 3 replicates each, and the vertical lines have the same meaning as previously.

The region where the stele was separated from the cortex is signalled by the vertical dashed lines.
GENERAL DISCUSSION OF THE RESULTS

The results obtained from experiments designed to study the influence of indol-3-yl-acetic acid and other plant growth substances on the long-distance transport of metabolites in stolons of Saxifraga sarmentosa have shown that the hormone-directed transport of solutes is the result of a localized effect of the hormone at the treated region. They do not support the hypothesis that the hormone acts by directly activating the entire length of the transport system, as suggested by Davies and Wareing (1965).

There seems to be little doubt that the effect of plant growth substances, particularly auxins, is somehow directly or indirectly connected with the metabolic status of the tissues, for it is quite obvious that the degree of response to auxin treatment depends very much on the age of the tissues employed (figs. 2, 5, 6 and 7). This, in itself, is neither in favour of nor against a direct or indirect involvement of the hormone in the translocation of metabolites. Indeed, if it is true that in young tissues we should expect a larger effect of the hormone on the metabolic rate and, therefore, a greater demand for nutrients than in older tissues, it is equally true that the basipetal movement of IAA, being dependent on metabolic energy, also declines to some extent in older tissues (Naqvi and Gordon, 1965; Jacobs, 1967). However, there is a considerable amount of evidence showing that the basipetal movement of IAA is not involved in the control of the mechanism of IAA-induced transport of metabolites.

In the present study, this conclusion was reached mainly on the
basis of results from experiments using auxin-transport inhibitors, or inhibitors of RNA and protein synthesis.

Other points, although not directly related to the problem of hormone-directed transport, were also investigated, namely the effect of endothal and DNP on both the movement of labelled assimilates and callose formation in intact stolons of Saxifraga, and how the movement and metabolism of tritiated IAA were affected by a number of substances.

There are in the literature contradictory reports about the effect of TIBA (2,3,5-triiodobenzoic acid) on the IAA-directed movement of metabolites within the plant. For instance, Davies and Waring (loc. cit.) found that in Pisum sativum TIBA applied together with IAA or as a "ring" at some distance below the area treated with IAA, reduced considerably the effect of auxin on the translocation of $^{32}$P. Similar results were recorded by Sebanek (1965) in etiolated pea seedlings. On the other hand, Panigrahi and Audus (1966) and Mullins (1970) failed to register any significant reduction on the IAA-induced transport of $^{14}$C-2-uracil and $^{14}$C-photoassimilates respectively, after TIBA treatment.

It has also been observed that in intact pea and Coleus plants, a "ring" of TIBA did not greatly affect the movement in the phloem of either labelled sugars or labelled IAA (Morris, et. al., 1973; Goldsmith et. al., 1974).

In the stolons of Saxifraga I found that the effect of a $10^{-4}$M solution of TIBA on the IAA-directed movement of $^{14}$C-assimilates.
depends on whether IAA and TIBA are applied together, or in places spatially separated. Thus, when TIBA was applied in combination with IAA, the auxin-induced transport of $^{14}C$-assimilates was largely reduced (fig. 13), therefore partially confirming the findings of Davies and Waring (loc. cit.) and Sebanek (1965). On the other hand, application of a TIBA solution between the source of radioisotope and the IAA-treated region did not significantly affect the IAA-induced transport of $^{14}C$-photoassimilates (fig. 13), which explains the lack of effect of TIBA on the IAA-directed movement of $^{14}C$-2-uracil recorded by Panigrahi and Audus. However, they do not agree with some of the data of Davies and Waring (1965) who found that a "ring" of TIBA (1%) in lanolin, interposed between the source of radioisotope and the IAA-treated region, caused a considerable reduction in the import of $^{32}P$ by the IAA-treated tissues. One possible explanation of their results may be perhaps sought in the far too high concentration of TIBA used which appears to have phytotoxic effects on the plant (Whiting and Murray, 1948; Mullins, 1970). Alternatively, TIBA might have, somehow, reached the IAA-treated region where it inhibited the IAA-induced movement of metabolites.

Contrary to its lack of effect on the translocation of nutrients in the phloem, a ring of TIBA ($10^{-4}M$) was extremely effective in blocking the basipetal movement of applied IAA-$^{3}H$ (figs. 26 and 29), but had little effect on its acropetal movement (fig. 29).

In the face of these results, the inhibition of IAA-induced transport of labelled assimilates by TIBA, when applied in combination
with IAA, cannot be ascribed to blockage of the polar movement of IAA, for, if that was the case, similar results should be expected from experiments in which TIBA was applied at some distance from the IAA-treated region. Thus, TIBA must have some other effect besides inhibiting the basipetal transport of auxin, for example by interfering in some way with the auxin action on the cell metabolism. One simple explanation might be based on competition with IAA for action sites in the cell, which might or might not be identical to the sites involved in the IAA polar movement. Indeed, evidence has been produced showing that there are in the cell IAA-attachment sites, and that TIBA can compete with auxin for these binding sites (Hertel et al., 1972; Thomson et al., 1973; Batt et al., 1976). One set of these IAA-binding sites appears to be associated with a particulate fraction rich in Golgi membranes and/or endoplasmic reticulum (Batt and Venis, 1976), and these organelles are thought to be centers of synthetic activities.

In cases where IAA and TIBA were applied at different places, there was less radioactivity in the portion of the stolon more or less coinciding with the area treated with TIBA, compared with the same region of stolons treated with IAA alone; however, similar amounts of label reached the distal area treated with IAA in both cases (figs. 13 and 16). When the distribution of radioactivity in the cortex and in the stele was determined separately, it was found that the effect of TIBA was particularly evident in the cortex, while the stele was less affected (fig. 16).
Further evidence supporting the view of an inhibitory role of TIBA on the metabolism comes from an experiment in which the effect of IAA alone, or IAA in combination with inhibitors of auxin polar transport, on the metabolism of $^{14}$C-labelled assimilates was investigated. The results (Table I) show very clearly that while IAA greatly increased the incorporation of $^{14}$C-photosynthates into the ethanol insoluble fraction, TIBA ($10^{-4}$M) not only inhibited completely the IAA promoting effect but even brought the rate of incorporation, to a level below that of the controls (decapitated stolons treated with distilled water).

The effect of other auxin transport inhibitors on the IAA-directed translocation and metabolism of $^{14}$C-labelled photosynthates is quite different from that of TIBA. Thus, solutions of NPA ($10^{-4}$M) or morphactin ($10^{-4}$M) applied either in combination with IAA at the decapitated end of the stolons, or alone at some distance of the IAA-treated region, had no inhibitory effect on both the IAA-induced transport and the metabolism of $^{14}$C-labelled assimilates. On the contrary, the effect of IAA on both processes appears to be slightly enhanced by the application of either NPA or morphactin in combination with IAA (figs. 14, 15 and table I).

When applied alone, TIBA showed no effect of its own on the translocation of labelled assimilates in stolons of *Saxifraga* (fig. 11). On the other hand, both NPA ($10^{-4}$M) and morphactin ($10^{-4}$M) applied to the apical end of decapitated stolons, increased to some degree the import of $^{14}$C-photosynthates into the treated
tissues (figs. 11 and 12). This effect may well be the result of a direct action of NPA and morphactin on growth, since morphactins in general seem to exhibit auxin-like activities (Katsumi, 1973) and it is possible that NPA may act in a similar way. Alternatively, the effect mentioned may be due to a delay in the depletion, by basipetal dispersal, of endogenous auxin from the apical tissues.

Contrary to their promoting effect of the long-distance transport of assimilates, both NPA and morphactin, at the concentration stated above, are very strong inhibitors of the basipetal movement of IAA-5-³H in intact stolons of Saxifraga (figs. 27, 28, and 29).

On the other hand, morphactin (10⁻⁴M) did not greatly affect the acropetal movement of tritiated IAA in the same plant (fig. 29), as one would expect bearing in mind that this type of auxin transport takes place in the phloem together with carbohydrates and other metabolites and should therefore be equally unaffected.

The results reported above dealing with the effect of inhibitors of auxin polar movement on both IAA-directed transport of labelled assimilates and the movement of tritiated IAA in young stolons of Saxifraga show, very clearly, that auxin-directed transport is not dependent on the basipetal movement of the hormone, as would be required if the directional flow of metabolites were to be mediated by a direct activation of the whole pathway of transport. Neither can, for the same reason, the process be ascribed to a direct hormonal stimulation of the photosynthesis in the leaves as described by Bidwell (1973) and Bidwell and Quong (1975). It is therefore
evident that the IAA-induced long-distance transport of nutrients is the result of a localized stimulus confined to the treated tissues and nearby. We are left then with two possible modes of action of auxin: either the hormone acts directly on some process of phloem transport at the "sink" region, perhaps on the "unloading" of metabolites out of the sieve tubes as suggested by Phillips (1975), or indirectly by controlling the metabolism of the area.

As far as the first possibility is concerned, so very little is known about the mechanism of phloem "unloading", apart from suggestions that it might be an active process, as to make it practically impossible to discuss its validity. It remains however a possibility which, at least for the moment, cannot be entirely ruled out.

The second hypothesis proposes that the stimulus is the result of increased or maintained synthetic activities at the treated region which brings about an increased demand for metabolites. Such an effect of auxins on the metabolism of the cell is not an unknown phenomenon. Indeed, there is a considerable amount of evidence showing that auxins and other plant growth substances can stimulate the synthesis of RNA and proteins in several plant tissues (Key and Shannon, 1964; De Hertogh et al., 1965; Masuda, 1966, 1968; Masuda et al., 1967; Sacher, 1967; Key and Ingle, 1968; Key, 1969).

Bearing in mind that hormone-directed transport is a relatively rapid phenomenon (it can be measured within a few hours of the beginning of treatment) the question then is whether the hormonal activation
of the metabolic process can be rapid enough to account for the auxin-induced flow of nutrients. Several reports in the literature showing that in auxin-responsive tissues enhanced synthesis of protein can be detected within minutes up to 2-3 hours of incubation (Key and Shannon, 1964; Masuda et al., 1967; Key, 1969; Masuda and Kamisaka, 1969; Mullins, 1970), do not seem to rule out such a possibility.

In young decapitated stolons of Saxifraga, application at the apical region of inhibitors of RNA and protein synthesis, such as actinomycin-D, puromycin and cycloheximide in combination with IAA, caused a pronounced reduction of the IAA-induced accumulation of \(^{14}\)C-assimilates at the treated region (figs. 17, 18, and 19). At least in the case of cycloheximide the inhibition was complete within 3 hours of application.

On the other hand, when applied around a portion of the stolon located some distance below the area treated with IAA, the above substances had little or no effect on the directional flow of \(^{14}\)C-photosynthates (figs. 20 and 21).

The effect of these RNA and protein synthesis inhibitors on the basipetal movement of tritiated IAA in intact stolons of Saxifraga is not the same for the three compounds. For instance, while cycloheximide (10 mg/l) proved to be very effective in blocking the polar transport of IAA-5-\(^{3}\)H (figs. 30 and 31), actinomycin-D (20 mg/l) and puromycin (2x10^{-4}M) had little effect on such a movement (fig. 32).

The results of experiments using inhibitors of RNA and protein
synthesis provide additional evidence against the hypothesis of a direct action of the hormone on the whole pathway of transport. On the contrary, they strongly suggest that, at least under the conditions of these experiments, the directional transport of assimilates is controlled by the demand at the "sink" region, that is by the rate of synthetic activity taking place, the role of auxin being, as I said earlier, to activate or maintain that metabolic rate. In cases where the tissues still have capacity for growth, as was the case in our experiments, the application of IAA will eventually result in expansion growth. In older tissues which have already finished elongating, it is still possible that IAA may enhance the metabolic rate despite the absence of any immediate visible growth.

The fact that no significant inhibition of the IAA-directed transport of assimilates could be registered when the above inhibitors were applied at some distance from the IAA-treated area shows that their effect is not exerted at the phloem level, as would be the case should the synthesis of any special protein be required so that translocation could take place.

Mullins (1970) found that decapitation of bean plants was followed within 12-24 hours by a marked decline in both the rate of incorporation of $^{14}$C-labelled leucine into protein and the overall protein level at the decapitated internodes. Application of IAA to the cut surface prevented both.

Patrick and Wareing (1970) obtained similar results. Mullins then suggested that decapitated plants, deprived of their main source
of auxin are probably senescent systems and, consequently, auxin-directed transport of metabolites might be better understood on the basis of an IAA senescence-delaying effect. However, Patrick and Wareing (1973) showed that bean plants, decapitated for 3 days before application of IAA, were still able to respond to the treatment with enhanced accumulation of \( ^{14}\text{C}\)-sucrose in the treated tissues.

In young stolons of *Saxifraga*, treated with IAA solutions at different times after decapitation, I was able to confirm the findings of Patrick and Wareing. Thus, stolons decapitated for 66 hours before application of IAA did respond to IAA treatment within 8 hours of IAA application with a several-fold increase in the amount of \( ^{14}\text{C}\)-photosynthate reaching the treated area, compared with stolons decapitated and treated with distilled water for the whole period.

Stolons treated with IAA for a period of 25 hours, 45 hours after decapitation, showed an even greater increase, similar to that of stolons kept in the IAA solution for the entire experimental period (fig. 8).

The results indicate that although the depletion of auxin from the tissues causes a pronounced decline in the metabolic rate, the metabolic apparatus is maintained in a functional condition, ready to be activated, at least for the periods of time reported above. This can hardly be considered a senescent condition in so far as it is fully reversible. However, it is possible that in older tissues, deprived of auxin for prolonged periods of time, the integrity of both the synthetic apparatus and the conducting channels may suffer, and
enter in a steady irreversible decline.

Under the conditions of these experiments, it is reasonable to say that the effect of IAA on the directional movement of metabolites cannot be satisfactorily explained on the basis of a senescence-delaying effect. It seems rather to be the result of a reactivation of the metabolism at the treated region, and it can be seen that this effect spreads gradually down the stolon with time (fig. 8).

In agreement with this view are the findings of Tupy (1973) who found that in *Havea brasiliensis* Mull. Arg. the sucrose-mobilizing action of auxins was dependent on the previous activation of the overall metabolism.

There are however some objections to this interpretation. For instance, Patrick and Wareing (1973) failed to register any increase in the protein level of stolons treated with IAA in lanolin for 12 hours, after an "ageing" period of 48 hours, relative to stolons treated with plain lanolin for the whole period, in spite of auxin still being able to exert its normal mobilizing effect. These results are difficult to reconcile with the previous hypothesis. One possibility is that IAA might have promoted the synthesis of certain types of enzymes to the detriment of other proteins, without affecting too much the overall level of protein in the tissues. Some evidence has been gathered from several systems supporting the view that auxin may produce qualitative changes in the protein metabolism (Patterson and Trewavas, 1967; Trewavas, 1968; Hardin and Cherry, 1972; Teissere *et al.*, 1973; Guilfoyle *et al.*, 1975; Davies, 1976;
Rizzo and Cherry, 1976).

An interesting point which has been observed is that cells of different ages in the same organ may respond in a different way to treatment with auxin or other plant growth substances (Holm et. al., 1970; Goto and Esashi, 1974; Rizzo and Cherry, 1976).

The effect of other plant hormones in the directional movement of nutrients is somewhat confusing. For instance, cytokinins have been shown to be able in certain circumstances to promote the accumulation of labelled metabolites in the treated areas of detached leaves (Gunning and Barkley, 1963; Muller and Leopold, 1966; Mullins, 1970), whereas in other cases they have proved to be ineffective in terms of enhanced accumulation of nutrients (Seth and Wareing, 1964; Davies and Wareing, 1965; Sebans, 1965; Morris and Thomas, 1968).

A similar picture has emerged in relation to gibberellins. Thus, Seth and Wareing (1965) could not find any effect of GA₃ on the translocation of nutrients in Pisum sativum, whereas Mullins (1970) in bean plants, and Jeffcoat and Harris (1972) in carnation shoots, recorded increased transport of labelled metabolites in response to GA₃ treatment.

In Saxifraga plants, treatment of the distal region of young decapitated stolons with a solution of gibberellic acid (10⁻⁴M) resulted in a considerable increase in the amount of ¹⁴C-photosynthates accumulating in the treated region, whereas a solution of kinetin (10⁻⁴M) had little or no effect (fig. 9).

When applied in combination with IAA, GA₃ produced only a
slightly greater effect than IAA alone, while kinetin together with IAA had more or less the same effect as IAA alone (fig. 10). Our results relative to the effect of mixtures of IAA and gibberellic acid or kinetin on the movement of labelled assimilates do not confirm earlier reports of a substantial synergistic effect of IAA and GA$_3$ and/or cytokinin on the same process (Seth and Wareing, 1964, 1967; Sebanek, 1965; Mullins, 1970).

In the face of the present results and other data from the literature, it seems possible that the phenomenon of hormone-directed transport may be controlled by the three groups of hormones, auxin being in this system the main limiting factor.

The different results obtained in this study and in some other cases, relative to the effect of GA$_3$ alone or in combination with IAA, may have been due to the different ages of the tissues used. For instance, it has been found that young tissues can respond to GA$_3$ treatment with increased growth rates, especially in the presence of auxin (Ockerse and Galston, 1967; Katsumi et al., 1965; Katsumi, 1973), and it seems very likely that the same may occur in the case of the translocation of metabolites which is, at least partially, associated with growth. Thus, the small effect of GA$_3$ in the presence of IAA can be explained only if one assumes that the treated region of the stolon can obtain at least part of the endogenous gibberellins, and for that matter of cytokinines, that it needs from other parts of the plant, in response to IAA treatment. Alternatively, gibberellins may act by stimulating the synthesis of auxin (Kuraishi and Muir,
1962; 1964) or by preventing its inactivation (Pilet, 1957; Brian and Hemming, 1958), although both hypothesis are a bit difficult to envisage since we are dealing with decapitated stolons, therefore deprived of their main source of endogenous auxin.

In young elongating tissues, kinetin does not seem to play any part in promoting growth; furthermore, it even tends to inhibit auxin-stimulated longitudinal growth and to promote transverse growth, whereas in older tissues, in the presence of auxin, it can initiate cell division leading eventually to the formation of a callus (Galston and Davies, 1970). The lack of any real growth-stimulating effect of kinetin in young growing tissues would probably explain the lack of effect of kinetin in promoting the translocation of labelled assimilates, provided that growth (demand) and transport of nutrients are directly related. Another possibility is that there is usually an adequate supply of cytokinins, probably synthesized in the roots (Kende, 1965; Weiss and Vaadia, 1965) and transported in the xylem, so that any application of exogenous cytokinins would be superfluous.

The results of experiments described so far suggest that the movement of metabolites in the phloem is controlled by the demand for nutrients at the sink region, and that the effect of IAA, and probably other plant hormones, is to increase, or at least maintain, the sink strength. If that is so, it should be possible to quench the demand for natural assimilates by supplying a sucrose solution directly to the treated area. Indeed, the results of an experiment (fig. 21) show, very clearly, that the application of a solution of
sucrose \((2 \times 10^{-3} \text{M})\) together with IAA at the apical end of decapitated stolons reduced by several-fold the import of \(^{14}\text{C}\)-photosynthates into the treated region, compared with the amount reaching similar area treated with IAA alone.

Similarly, when the osmotic relationships between the sink and other parts of the stolon are altered by the application of high concentrations of mannitol, either alone or in combination with IAA, a reduction in the amount of labelled assimilates reaching the treated tissues occurs, compared with the amount which accumulates in similar region of stolons treated with IAA alone. On the other hand, more activity is present in the remaining part of the stolons under osmotic stress (treated with mannitol alone or in combination with IAA) than in those treated simply with IAA (fig. 22).

The inhibition of the IAA-induced accumulation of \(^{14}\text{C}\)-photosynthates at the apical region of decapitated stolons treated with a mixture of IAA and mannitol is only partial; considerably more radioactivity is still able to reach the treated area of such stolons than that of stolons treated with mannitol alone. This indicates that the IAA-induced transport of metabolites cannot be entirely ascribed to an expansion growth response elicited by IAA treatment, since no expansion growth could take place under the stated conditions. It must then be regarded as a genuine effect of the hormone on the metabolic activity of the treated area. Of course, one cannot dismiss as unimportant the effect that IAA-stimulated elongation might have in drawing up nutrients from other parts of the stolon. It is quite
clear that in cases where elongation was allowed to take place, considerably more activity was imported by the treated area than in stolons treated with IAA and mannitol; the difference in the responses elicited by IAA under these two conditions might even reflect the role played by expansion growth in such phenomenon, although this is perhaps an oversimplified view of the problem.

One point which was also investigated, in spite of not being directly connected with the main problem of this study, was the effect of the herbicide endothal and the uncoupling agent of phosphorylation, 2,4-dinitrophenol (DNP), on both the movement of labelled assimilates and callose formation in the phloem of intact stolons of Saxifraga. This task was taken up with the aim of trying to get an insight in the mechanism of the transport of metabolites in the phloem. Indeed, one of the main difficulties in understanding the mechanism of hormone-directed transport is our lack of knowledge on how the translocation of solutes in the phloem takes place. The main issue here rests as to whether the mechanism of longitudinal movement of metabolites in the sieve tubes is a passive or an active process.

Temperature (Swanson and Geiger, 1967) and metabolic inhibitors such as DNP (Qureshi and Spanner, 1973) seem to interfere with such a movement, therefore suggesting that it may be an energy-requiring process, although this has not yet been proved beyond doubt (see e.g. Harel and Reinold, 1966).

An alternative possibility is that the application of DNP, or
some other compounds, may cause deposition of callose or of some other "plugging" material on the sieve plates which may obstruct the transport of solutes.

Although Qureshi and Spanner (loc. cit.) could not find any increased callose formation in stolons of Saxifraga in response to DNP treatment, our results show that in the same plant both DNP and endothal, especially at the higher concentrations used, enhanced considerably callosing in the sieve plates (table II). On the other hand, a "ring" of endothal in all concentrations employed (200 mg/l; 500 mg/l; 1000 mg/l) did not inhibit to a great extent the translocation of $^{14}$C-photosynthates, even though less radioactivity reached the apical region of treated stolons than similar areas of the controls (fig. 24).

DNP at the concentration of $10^{-5}$ M had only a small inhibitory effect on the movement of $^{14}$C-labelled assimilates, whereas a $5 \times 10^{-5}$ M DNP concentration stopped almost completely such a movement, most certainly through the lethal effect it had on the tissues (fig. 25).

Although the callossing effect of both endothal and DNP may be responsible for some accumulation of radioactivity below the area treated with either of these substances, it is however evident that considerable amounts of callose can be formed without greatly affecting the phloem transport; in other words, there is no direct correlation between the number of sieve plates with callose deposits and the extent of transport inhibition, as shown by comparing figs. 24, 25 and table II.
When the effect on the translocation of metabolites in stolons of *Saxifraga* of both endothal and DNP is compared with the effect of both compounds on the polar movement of tritiated IAA in the same plant, it is clear that these two types of transport are not equally sensitive to treatment with the substances mentioned. Thus, the basipetal movement of IAA-5-\(^3\)H, which is known to be dependent on metabolic energy, is completely inhibited by all of the concentrations of both endothal (figs. 35 and 36) and DNP (figs. 33 and 34) used in the study of the movement of \(^{14}\)C-photosynthates, whereas the translocation of metabolites is relatively unaffected by the same concentrations, except for DNP 5x10\(^{-5}\)M which causes the death of the tissues (fig. 25).

Although we do not know the mode of action of endothal, however, the results obtained from treatment of stolons with DNP may be an indication that, after all, phloem transport is not entirely dependent on metabolic energy, or at least, that it has different energy requirements from those of auxin polar transport.

The differences between the results presented in this study and those of Qureshi and Spanner (1973) may well have resulted from treating different lengths of stolons of different ages with different DNP concentrations.

Part II of this thesis was essentially concerned with the study of the effect of several compounds on the movement of IAA-5-\(^3\)H. Reference to the action of some of them on the movement of tritiated IAA has already been made when discussing their effects on the trans-
location of $^{14}$C-labelled assimilates. However, there are still a few further points that, in my opinion, deserve to be discussed. For instance, it has been confirmed in intact stolons of *Saxifraga* the existence of two different pathways of transport for applied IAA-5$^-{3}$H. Thus, when tritiated IAA was injected near the apex of young growing stolons, there was in the controls a peak of activity near the front of the transport stream. Evidence gathered from comparing the position of this peak after different transport periods (compare e.g. figs. 28, 29, 31 and 36) suggests that a pulse of activity moved down the stolons at an average velocity tentatively estimated as ranging from about 6.0 mm/hour to about 7.0 mm/hour. When the radioisotope was applied halfway down the stolon, besides this basipetal movement there was also a certain amount of radioactivity travelling in the acropetal direction. The fact that this acropetal movement seems to take place at higher velocities, together with some other characteristics of this movement such as its relative insensitivity to treatment with inhibitors of auxin polar transport and the accumulation of radioactivity in regions of active growth, indicate that such a movement takes place probably in the phloem together with carbohydrates and other metabolites, as has already been shown by Morris and Kadir (1972), Morris et al. (1973), Goldsmith et al. (1974) in other plants.

Although the apical region is supposed to be the main source of endogenous auxin of the stolon and, therefore, any movement should necessarily be in a basipetal direction, it is however possible that
some of the basipetally transported auxin may get into the sieve tubes and be rapidly redistributed in the phloem.

On the other hand, the tissue or tissues involved in the polar transport of auxin are still not exactly known, despite the considerable amount of work done on this subject. There is some evidence suggesting that the basipetal movement of auxin may take place in the cells of the vascular cambium and in very young undifferentiated phloem and xylem elements (Sabnis et al., 1969; Bonnemain, 1971; Sheldrake, 1972; Bourbouloux and Bonnemain, 1973; Wangermann, 1973).

In Saxifraga, I also found that the polar movement of applied IAA-5-³H took place essentially in the tissues of the stele (figs. 6 and 11) although some activity was also present in the cortex, probably due to lateral leakage.

Bearing in mind the very different characteristics of the polar movement of auxin and those of the phloem and xylem transport, and also their opposite directions, it is most unlikely that the basipetal transport of auxin should take place in functional phloem elements and even less in the dead vessels of the xylem. We are left then with the pericycle, the vascular cambium, phloem and xylem parenchyma cells, and the pith tissue as the possible pathways of auxin polar transport.

The origin of the activity present in the tissues of the cortex has not yet been elucidated. The results of an experiment in which the cortex and the stele were carefully separated over a 3 cm distance immediately before the application of IAA-5-³H, and a piece of trans-
parent non-permeable wrap film inserted between them, failed to produce a clear and definitive answer; separation of cortex and stele stopped the basipetal movement of tritiated IAA in both, although the radioisotope travelled about 2 cm further in the stele than in the cortex (fig. 41). This might be indication that the cortex receives its activity laterally from the stele, but, as I said, these results are not conclusive proof due to the failure of the stele to keep on translocating IAA-5\(^{-3}\)H after separation from the cortex. I have no idea why it should not translocate, unless some damage was done during the operation which might have rendered the stele non-functional.

There are several substances which can block the basipetal movement of auxin. Some of them I have already mentioned such as TIBA (figs. 26 and 29), NPA (fig. 27), morphactin (figs. 28 and 29) and the protein synthesis inhibitor cycloheximide (figs. 30 and 31). The first three substances have been widely used for that purpose and are supposed to act in a rather specific manner; the last compound has been known to inhibit protein synthesis, but had never before, as far as I know, been used in the study of auxin transport. Its inhibitory effect on auxin polar movement is probably due to disruption of the energy supply of the cell, for there is now substantial evidence pointing in that direction (MacDonald and Ellis, 1969; Ellis and MacDonald, 1970; Webster, 1973; McMahon, 1975). That it is not simply due to inhibition of protein synthesis is shown by the lack of any real blocking effect on the part of the other protein synthesis inhibitors, namely actinomycin-D and puromycin (fig. 32).
The effect of cycloheximide on auxin polar transport appears then to be somewhat similar to that of the uncoupling agent of phosphorylation, DNP, which by interfering with the energy supply of the cell brings about the inhibition of the basipetal auxin transport (figs. 33 and 34). Similar effect was also observed in response to treatment with cyanide (KCN; fig. 33) which is an inhibitor of the cytochrome system, leading therefore to the inhibition of the respiration.

These results illustrate well the dependence of the polar transport of auxin on metabolic energy.

Yet another compound which I found to be very effective in blocking the polar movement of IAA-5-\(^3\)H, both in intact stolons of Saxifraga (figs. 35 and 36) and in hypocotyl segments of Helianthus annuus L. (table III), is the herbicide endothal. This substance has been known to cause abscission of the leaves in plants (see e.g. Crafts, 1953; Osborne 1968).

Although we do not know the way by which endothal causes the inhibition of auxin transport, it is possible that the abscission-promoting effect of this compound may result from a blockage of the transport of auxin, since it is a well known fact that auxin retards abscission.

The distribution profiles of IAA-5-\(^3\)H in stolons treated over a short distance with a solution of endothal are similar to those of stolons treated with a solution of either DNP, KCN or cycloheximide, therefore suggesting that endothal may act in a similar way, that is,
by interfering with the energy supply. The common feature of these profiles is the presence of a peak of activity immediately above the treated area which shows a local inhibition of basipetal transport by the compounds mentioned. It would appear then that the mechanism of auxin transport above the treated region keeps on working normally, "pumping" IAA-5-\(^3\)H into the upper section of the treated area where it fails to be transported further, so that it keeps on accumulating.

The inhibitory effect of solutions of endothal on the polar movement of auxin was also observed in hypocotyl segments of Heli- anthemus annuus plants, at concentrations as low as 1 mg/l (table III). Such an effect is revealed not only by the reduction of the amount of label reaching the agar receiver blocks, but also by the distribution of activity within the segments themselves.

As table III shows, uptake was also greatly affected. However, such an effect on uptake cannot be held as the sole reason for the observed inhibition of IAA-5-\(^3\)H basipetal transport, since the basipetal movement of label within the segments is also reduced, as indicated by the ratio between the activities present in the "donor" and "receiver" halves of the segments. On the other hand, the effect of endothal on the acropetal movement seems to be due in great part to its action on uptake, even though it also appears that it might have been somewhat increased by the endothal concentrations used.

During its transport through the tissues, auxin may become partially immobilized (Goldsmith and Thimann, 1962; Winter and Thimann, 1966; Goldsmith, 1969), or may be to some extent metabolized
giving rise to immobile products, so that part of the original auxin is removed from the transport stream.

Winter (1967; 1968) claims that the inhibition of auxin polar movement by TIBA may be accounted for by increased immobilization and decarboxylation of IAA. Assuming that such a suggestion may be correct it is not unreasonable to think that some other inhibitors of auxin transport may also have some effect on auxin metabolism.

In intact stolons of Saxifraga sarmentosa, the pattern of the metabolism of applied IAA-5-\(^3\)H did not appear to be greatly altered by either of the following compounds: TIBA (10\(^{-4}\)M), NPA (10\(^{-4}\)M), morphactin (10\(^{-4}\)M) and endothal (200 mg/l). The same substances, mainly free IAA-5-\(^3\)H, were present in the chromatograms of extracts from treated and untreated stolons. However, larger amounts of a compound, probably labelled IA-aspartic acid (Rf 0-0.1 in isopropanol: NH\(_4\)Cl: water, 8:1:1), were present in stolons treated with the above substances than in the controls (fig. 38).

This effect can either be the result of a direct action of these compounds on the metabolism of IAA-5-\(^3\)H or, more likely, a consequence of higher levels of hormone in the tissues above the treated region due to inhibition of the basipetal transport.

In any case, it is in my opinion very unlikely that the recorded increase in the immobilization of IAA-5-\(^3\)H in treated stolons may account entirely for the inhibitory effect of the mentioned substances, since a great proportion of the applied IAA-5-\(^3\)H still remained in its original chemical form (figs. 38 and 39), whereas
the inhibition of the basipetal transport of tritiated IAA was practically complete (figs. 26, 27, 28 and 35).

Although polar transport of auxin is usually associated with young growing tissues, it can also take place under conditions of osmotic stress where elongation has been prevented (McCready and Jacobs, 1967). However, under more severe conditions causing extensive plasmolysis of the tissues, the polar movement of auxin was considerably reduced, but not completely stopped (Cande and Ray, 1976).

In intact stolons of Saxifraga treated with either a 0.3 M or a 0.4 M solution of mannitol, the results obtained generally confirm the above findings. Thus, a 0.3 M mannitol solution, which caused moderate osmotic stress, had only a very limited effect on the basipetal transport of IAA-5-\(^3\)H. A higher concentration (0.4 M), however, greatly affected the polar movement of tritiated IAA (fig. 40) even though it was not completely stopped.

The profiles of distribution of IAA-5-\(^3\)H suggest that the velocity of transport in mannitol-treated stolons may have been reduced particularly at the higher concentration. A tentative estimate of the velocity in stolons treated with a 0.4 M solution of mannitol suggests that the "pulse" of activity travelled down the stolons at an average velocity of 4.4 mm/hour, compared with a value of about 6.8 mm/hour in the controls. In this case, the effect of mannitol on the movement of IAA-5-\(^3\)H can hardly be ascribed to a reduction of the rate of transport at the treated region, as suggested
by Cande and Ray (1976), for in such a case one would expect a build-up of activity immediately above the treated region and not below it, as can be seen in fig. 40. The reduction of the basipetal transport velocity can perhaps be explained in terms of a much larger free-space where the movement of auxin would be achieved essentially by diffusion which is a much slower process than active transport.
CONCLUSIONS

On the basis of the results of the experiments reported in this thesis, the following conclusions are drawn:

1. Solutions of NPA ($10^{-4}$M) or morphactin ($10^{-4}$M), which proved to be extremely effective in blocking the basipetal movement of tritiated IAA, had no significant effect on the IAA-induced translocation of $^{14}$C-labelled assimilates, therefore suggesting that the basipetal movement of auxin is not required for the IAA-directed transport of metabolites to take place.

2. TIBA ($10^{-4}$M) stops the IAA-induced accumulation of labelled assimilates and inhibits its IAA-stimulated incorporation into the ethanol insoluble fraction when applied together with IAA, but has little or no effect when both substances are applied at different places. This strongly suggests that the inhibitory effect of TIBA on the IAA-induced transport of labelled photosynthates does not depend on the blockage of the polar transport of auxin, but is probably due to inhibition of the auxin action on the metabolism.

3. The results of experiments showing an increase of the rate of incorporation of $^{14}$C-labelled assimilates into the ethanol insoluble fraction in response to IAA treatment (alone or in combination with NPA or morphactin), in addition to the inhibition of IAA-induced translocation of $^{14}$C-photosynthate by RNA and protein synthesis inhibitors (actinomycin-D, puromycin and cycloheximide) when applied in combination with IAA, indicate quite clearly that the IAA-induced
mobilization of metabolites is the result of an increased demand for nutrients brought about by the activation of the metabolism at the "sink" region. This view is also supported by the results of experiments showing a reduction of the amount of $^{14}$C-labelled assimilates reaching the IAA-treated tissues by directly supplying a solution of sucrose to this area, and from the different degrees of response elicited by IAA treatment depending on the age of the tissues used.

4. Under the conditions of our experiments, IAA-directed translocation of nutrients cannot be explained only in terms of a senescence delaying effect of the hormone, as indicated by the fact that stolons decapitated for 66 hours before application of auxin were still able to respond to the treatment within 8 hours.

For the reasons mentioned above, it is concluded that the hormone-directed transport of nutrients in stolons of *Saxifraga* does not depend on a direct activation of the pathways of transport, but is rather the result of a local stimulation of metabolic activities at the treated region.

5. As far as the movement of applied IAA is concerned, it is shown that:

a) In intact stolons of *Saxifraga*, applied IAA-$^3$H moves mainly in a basipetal direction. This basipetal movement takes place chiefly in the tissues of the stele and can be stopped by a number of substances, among which are the herbicide endothal, and cycloheximide.
b) In certain circumstances, part of the applied auxin can also be transported acropetally, probably in the phloem, and this movement is not greatly affected by TIBA or morphactin.

c) The metabolism of applied IAA-5-³H is not greatly affected by the application of auxin transport inhibitors, therefore suggesting that the inhibition of the basipetal transport of auxin cannot be accounted for simply by possible changes in its metabolism.


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APPENDIX

Table A

| Int. c react. /mol | Value | Reason for omit.
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<tr>
<td>0.79426 e-24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± 1 S.E.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.79426 ± 0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

App. D

| React. /mol | Value | Reason for omit.
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<th></th>
<th></th>
<th></th>
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<td></td>
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<tr>
<td>Mean ± 1 S.E.</td>
<td></td>
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<tr>
<td>0.79426 ± 0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(For a total of 142, 5 reactions per experiment, a portion of reactions, say 15 reactions)
Results in c.p.m./2 cm of stolon relative to:

**Fig. 2.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA (0.1%)</td>
<td>134180 ± 9687</td>
</tr>
<tr>
<td>IAA + GA₃ (0.1%; n=4)</td>
<td>105108 ± 6700</td>
</tr>
<tr>
<td>IAA + GA₃ + Kin. (0.1%; n=4)</td>
<td>82847 ± 8208</td>
</tr>
<tr>
<td>Lanolin</td>
<td>66300 ± 4550</td>
</tr>
<tr>
<td>n=4</td>
<td>n=4</td>
</tr>
</tbody>
</table>

(n=number of stolons per treatment; Direction of transport from top to bottom).

**Fig. 3.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Int. + TIBA &quot;ring&quot;</td>
<td>63130 ± 2774</td>
</tr>
<tr>
<td>Intact</td>
<td>50162 ± 3277</td>
</tr>
<tr>
<td>Decapit. + Lanol. (0.25%; n=3)</td>
<td>35839 ± 2908</td>
</tr>
<tr>
<td></td>
<td>29555 ± 1416</td>
</tr>
<tr>
<td></td>
<td>20559 ± 2315</td>
</tr>
<tr>
<td></td>
<td>21013 ± 2370</td>
</tr>
<tr>
<td></td>
<td>22622 ± 1744</td>
</tr>
<tr>
<td></td>
<td>28251 ± 5126</td>
</tr>
<tr>
<td></td>
<td>29234 ± 3762</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; direction of transloc., top to bottom)
Results in c.p.m./2 cm of stolon relative to:

**Fig. 4.**

<table>
<thead>
<tr>
<th>IAA (0.1%)</th>
<th>IAA + TIBA &quot;ring&quot;</th>
<th>LANOLIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 4</td>
<td>(0.25%; n= 3)</td>
<td>n= 3</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>45733 ± 7036</td>
<td>40794 ± 8576</td>
<td>31506 ± 2097</td>
</tr>
<tr>
<td>46542 8037</td>
<td>49466 6715</td>
<td>33526 2423</td>
</tr>
<tr>
<td>50675 7399</td>
<td>60041 12532</td>
<td>31189 1107</td>
</tr>
<tr>
<td>57028 12158</td>
<td>40777 6817</td>
<td>37720 6759</td>
</tr>
<tr>
<td>47040 3767</td>
<td>12324 3053</td>
<td>34734 5004</td>
</tr>
<tr>
<td>50030 1163</td>
<td>10713 3275</td>
<td>31342 4677</td>
</tr>
<tr>
<td>45154 8116</td>
<td>17878 2415</td>
<td>24917 3131</td>
</tr>
<tr>
<td>33988 7947</td>
<td>13760 2035</td>
<td>22824 2640</td>
</tr>
<tr>
<td>16490 6474</td>
<td>7776 2417</td>
<td>9413 1079</td>
</tr>
<tr>
<td>4053 661</td>
<td>4903 2274</td>
<td>837 82</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; direction of transport from top to bottom).

Results in c.p.m./mg of fresh weight relative to

**Fig. 5.**

<table>
<thead>
<tr>
<th>IAA 5x10^-5M; n=3</th>
<th>Water n=3</th>
<th>IAA</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stele</td>
<td>Cortex</td>
<td>Stele</td>
<td>Cortex</td>
</tr>
<tr>
<td>Mean/4</td>
<td>Mean/4</td>
<td>Mean/4</td>
<td>Mean/4</td>
</tr>
<tr>
<td>7665 1178</td>
<td>9710 1324</td>
<td>35375 ± 8388</td>
<td>44139 ± 11146</td>
</tr>
<tr>
<td>8259 1244</td>
<td>8599 1016</td>
<td>38018</td>
<td>36464</td>
</tr>
<tr>
<td>6505 954</td>
<td>7760 1079</td>
<td>29838</td>
<td>35360</td>
</tr>
<tr>
<td>4864 629</td>
<td>5475 1043</td>
<td>21975 6311</td>
<td>26075 5676</td>
</tr>
<tr>
<td>3654 377</td>
<td>3054 386</td>
<td>16129</td>
<td>13762</td>
</tr>
<tr>
<td>2983 302</td>
<td>1924 187</td>
<td>13140 5849</td>
<td>8447 3518</td>
</tr>
<tr>
<td>1944 211</td>
<td>1246 112</td>
<td>8622</td>
<td>5346</td>
</tr>
<tr>
<td>841 62</td>
<td>601 59</td>
<td>3614</td>
<td>2645</td>
</tr>
<tr>
<td>369 45</td>
<td>203 19</td>
<td>1737 831</td>
<td>891 649</td>
</tr>
<tr>
<td>112 11</td>
<td>62 8</td>
<td>494 171</td>
<td>280 146</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; direction of transport from top to bottom).
Results in c.p.m./2 cm of stolon relative to:

<table>
<thead>
<tr>
<th></th>
<th>IAA (5x10^{-5}M)</th>
<th>IAA (10^{-5}M)</th>
<th>IAA (10^{-6}M)</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=3</td>
<td>n=3</td>
<td>n=3</td>
<td>n=6</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
<td></td>
</tr>
<tr>
<td>63359 ± 10034</td>
<td>42424 ± 4570</td>
<td>45291 ± 13532</td>
<td>53864 ± 5106</td>
<td></td>
</tr>
<tr>
<td>64562</td>
<td>54067</td>
<td>52963</td>
<td>55301</td>
<td></td>
</tr>
<tr>
<td>67193</td>
<td>69497</td>
<td>59659</td>
<td>63517</td>
<td></td>
</tr>
<tr>
<td>63605</td>
<td>19370</td>
<td>66623</td>
<td>10658</td>
<td>75757</td>
</tr>
<tr>
<td>60671</td>
<td>72713</td>
<td>82548</td>
<td>63000</td>
<td></td>
</tr>
<tr>
<td>59079</td>
<td>4305</td>
<td>74877</td>
<td>6044</td>
<td>73613</td>
</tr>
<tr>
<td>71659</td>
<td>58531</td>
<td>54135</td>
<td>64080</td>
<td>73521</td>
</tr>
<tr>
<td>97537</td>
<td>95203</td>
<td>64470</td>
<td>37612</td>
<td></td>
</tr>
<tr>
<td>179974</td>
<td>31692</td>
<td>175032</td>
<td>36748</td>
<td>88960</td>
</tr>
<tr>
<td>184846</td>
<td>39082</td>
<td>172495</td>
<td>25136</td>
<td>87405</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; direction of transport from top to bottom).

Results in c.p.m./mg of fresh weight relative to:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n=3; 5x10^{-5}M</td>
<td>n=3</td>
<td>5x10^{-5}M</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stèle</td>
<td>Cortex</td>
<td>Stèle</td>
<td>Cortex</td>
</tr>
<tr>
<td>7684 ± 173</td>
<td>342 ± 37</td>
<td>5363 ± 28</td>
<td>678 ± 69</td>
</tr>
<tr>
<td>10625</td>
<td>785</td>
<td>7070</td>
<td>1061</td>
</tr>
<tr>
<td>10693</td>
<td>739</td>
<td>7551</td>
<td>1081</td>
</tr>
<tr>
<td>9382</td>
<td>537</td>
<td>8179</td>
<td>536</td>
</tr>
<tr>
<td>8728</td>
<td>502</td>
<td>8360</td>
<td>1330</td>
</tr>
<tr>
<td>7198</td>
<td>1152</td>
<td>7324</td>
<td>59</td>
</tr>
<tr>
<td>5479</td>
<td>371</td>
<td>7413</td>
<td>1550</td>
</tr>
<tr>
<td>3558</td>
<td>306</td>
<td>6582</td>
<td>2209</td>
</tr>
<tr>
<td>1858</td>
<td>622</td>
<td>9025</td>
<td>1785</td>
</tr>
<tr>
<td>1035</td>
<td>369</td>
<td>8290</td>
<td>669</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4814</td>
<td>1099</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; direction of transport from top to bottom).
**Fig. 7.** (cont.)

Water "ring"; wat. ap. reg.

\[ \text{n = 3} \]

\[ \text{Mean ± S.E.} \]

<table>
<thead>
<tr>
<th>Stele</th>
<th>Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>7105 ± 804</td>
<td>1081 ± 489</td>
</tr>
<tr>
<td>8156</td>
<td>1206</td>
</tr>
<tr>
<td>8567</td>
<td>1003</td>
</tr>
<tr>
<td>7174</td>
<td>878</td>
</tr>
<tr>
<td>7395</td>
<td>947</td>
</tr>
<tr>
<td>5451</td>
<td>930</td>
</tr>
<tr>
<td>4782</td>
<td>1038</td>
</tr>
<tr>
<td>3974</td>
<td>919</td>
</tr>
<tr>
<td>3229</td>
<td>1528</td>
</tr>
<tr>
<td>2995</td>
<td>1646</td>
</tr>
</tbody>
</table>

Results in c.p.m./2 cm of stolon relative to:

**Fig. 8.**

IAA (73 hours)  Wat. (48 h.) - IAA (25 h.)  Wat. (66 h.) - IAA (8 h.)

\( (5 \times 10^{-5} \text{M}; \ n = 6) \)  \( (5 \times 10^{-5} \text{M}; \ n = 5) \)

\[ \text{Mean ± S.E.} \]

| 98225 ± 15873 | 41508 ± 4217 | 37505 ± 8666 |
| 94160  | 40314    | 36479    |
| 99785  | 40940    | 34403    |
| 105152 | 43670    | 5369     | 34772 | 10260 |
| 110622 | 54220    | 33029    |
| 98815  | 52823    | 6960     | 29517 | 7877 |
| 101902 | 59815    | 24758    |
| 98037  | 65819    | 25711    |
| 90615  | 9407     | 97132    | 16526 | 30344 | 11061 |
| 117023 | 12425    | 137172   | 18415 | 44586 | 13741 |

\( \text{(n = number of stolons per treatment; direction of transport from top to bottom).} \)
### Fig. 8. (cont.)

Water (73 hours)

\[ n=5 \]

<table>
<thead>
<tr>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>31261 ± 10251</td>
<td>24181 ± 17293</td>
<td>9105 ± 4033</td>
</tr>
<tr>
<td>24181 ± 17293</td>
<td>9105 ± 4033</td>
<td>3732 ± 457</td>
</tr>
<tr>
<td>17293 ± 9105</td>
<td>4033 ± 3732</td>
<td>1502 ± 457</td>
</tr>
<tr>
<td>9105 ± 3732</td>
<td>4033 ± 1502</td>
<td>222 ± 151</td>
</tr>
<tr>
<td>3732 ± 1502</td>
<td>457 ± 222</td>
<td>62 ± 187</td>
</tr>
<tr>
<td>1502 ± 222</td>
<td>457 ± 62</td>
<td>35 ± 187</td>
</tr>
<tr>
<td>222 ± 151</td>
<td>62 ± 35</td>
<td>45 ± 187</td>
</tr>
</tbody>
</table>

Results in c.p.m./2 cm of stolon relative to:

### Fig. 9.

Gibber. ac. \((10^{-4} M)\)   Kinetin \(2 \times 10^{-4} M\)   Water

\[ n=3 \]

<table>
<thead>
<tr>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>118613 ± 2686</td>
<td>127076 ± 26564</td>
<td>126681 ± 14199</td>
</tr>
<tr>
<td>128254</td>
<td>145679</td>
<td>119596</td>
</tr>
<tr>
<td>143817</td>
<td>160039</td>
<td>127076</td>
</tr>
<tr>
<td>146286</td>
<td>205050</td>
<td>130965</td>
</tr>
<tr>
<td>185117</td>
<td>233144</td>
<td>16778</td>
</tr>
<tr>
<td>255041</td>
<td>227009</td>
<td>167306</td>
</tr>
<tr>
<td>239288</td>
<td>213084</td>
<td>159208</td>
</tr>
<tr>
<td>246583</td>
<td>187364</td>
<td>121920</td>
</tr>
<tr>
<td>173898</td>
<td>124146</td>
<td>84136</td>
</tr>
<tr>
<td>167072</td>
<td>36708</td>
<td>30008</td>
</tr>
</tbody>
</table>

\((n=\text{number of stolons per treatment}; \text{direction of transport from top to bottom}).\)
Results in c.p.m./2 cm of stolon relative to:

**Fig. 10.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA + GA₃ (10⁻⁴M)</td>
<td>3</td>
<td>48501 ± 14684</td>
<td>352121</td>
<td>38889</td>
<td>33633</td>
</tr>
<tr>
<td>IAA + Kin. (10⁻⁴M)</td>
<td>3</td>
<td>53470 ± 12466</td>
<td>46068</td>
<td>55672</td>
<td>5538</td>
</tr>
<tr>
<td>IAA (5x10⁻⁵M)</td>
<td>3</td>
<td>63369 ± 10034</td>
<td>63605</td>
<td>60671</td>
<td>63276</td>
</tr>
<tr>
<td>Water</td>
<td>3</td>
<td>95334 ± 10317</td>
<td>96403</td>
<td>96280</td>
<td>98616</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; direction of transport from top to bottom).

**Fig. 11.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIBA (10⁻⁴M)</td>
<td>5</td>
<td>90299 ± 6134</td>
<td>78534</td>
<td>3347</td>
</tr>
<tr>
<td>NPA (10⁻⁴M)</td>
<td>5</td>
<td>96403</td>
<td>96280</td>
<td>98616</td>
</tr>
<tr>
<td>Water</td>
<td>5</td>
<td>96403</td>
<td>96280</td>
<td>98616</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; direction of transport from top to bottom).
Results in c.p.m./2 cm of stolon relative to:

**Fig. 12.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphactin (10^{-4} M)</td>
<td>69555 ± 2195</td>
<td>57960 ± 7982</td>
</tr>
<tr>
<td>Water</td>
<td>70649</td>
<td>64471</td>
</tr>
<tr>
<td>n=3</td>
<td>n=3</td>
<td></td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; direction of transport from top to bottom).

**Fig. 13.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA + TIBA &quot;ring&quot;</td>
<td>52600 ± 10909</td>
<td>34164 ± 3957</td>
<td>77748 ± 11928</td>
</tr>
<tr>
<td>IAA + TIBA</td>
<td>44411</td>
<td>38466</td>
<td>75218</td>
</tr>
<tr>
<td>IAA (5x10^{-5} M)</td>
<td>36919</td>
<td>35803</td>
<td>80910</td>
</tr>
<tr>
<td>n=10</td>
<td>n=5</td>
<td>n=9</td>
<td></td>
</tr>
<tr>
<td>IAA</td>
<td>36989</td>
<td>3710</td>
<td>82535</td>
</tr>
<tr>
<td>50480</td>
<td>30083</td>
<td>5107</td>
<td>78145</td>
</tr>
<tr>
<td>54773</td>
<td>27671</td>
<td>5654</td>
<td>5803</td>
</tr>
<tr>
<td>64725</td>
<td>24592</td>
<td>79767</td>
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<tr>
<td>99821</td>
<td>23894</td>
<td></td>
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<td>154023</td>
<td>19953</td>
<td>6190</td>
<td></td>
</tr>
<tr>
<td>150594</td>
<td>7557</td>
<td>2449</td>
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</tr>
<tr>
<td>14190</td>
<td>1377</td>
<td>552</td>
<td></td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; direction of transport from top to bottom).
Results in c.p.m./2 cm of stolon relative to:

**Fig. 14.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA + NPA (10^-4 M)</td>
<td>9091 ± 568</td>
<td>11091 ± 2230</td>
<td>6828 ± 1016</td>
</tr>
<tr>
<td>IAA + NPA &quot;ring&quot;</td>
<td>11152</td>
<td>11433</td>
<td>7792</td>
</tr>
<tr>
<td>IAA 5x10^-5 M</td>
<td>10835</td>
<td>12971</td>
<td>11182</td>
</tr>
<tr>
<td></td>
<td>12662</td>
<td>14334</td>
<td>15550</td>
</tr>
<tr>
<td></td>
<td>10425</td>
<td>14670</td>
<td>15277</td>
</tr>
<tr>
<td></td>
<td>9857</td>
<td>17392</td>
<td>12252</td>
</tr>
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<td></td>
<td>14525</td>
<td>27369</td>
<td>18850</td>
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<tr>
<td></td>
<td>32759</td>
<td>28333</td>
<td>29100</td>
</tr>
<tr>
<td></td>
<td>52066</td>
<td>37860</td>
<td>37094</td>
</tr>
<tr>
<td></td>
<td>47916</td>
<td>37932</td>
<td>37784</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; direction of transport from top to bottom).

**Fig. 15.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA + Morph. (10^-4 M)</td>
<td>66836 ± 3858</td>
<td>46988 ± 12727</td>
<td>69946 ± 10710</td>
</tr>
<tr>
<td>IAA + Morph. &quot;ring&quot;</td>
<td>63710</td>
<td>49028</td>
<td>74942</td>
</tr>
<tr>
<td>IAA (5x10^-5 M)</td>
<td>55750</td>
<td>47480</td>
<td>73637</td>
</tr>
<tr>
<td></td>
<td>43439</td>
<td>43149</td>
<td>7620</td>
</tr>
<tr>
<td></td>
<td>33482</td>
<td>46459</td>
<td>62259</td>
</tr>
<tr>
<td></td>
<td>28159</td>
<td>42302</td>
<td>51884</td>
</tr>
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<td></td>
<td>31506</td>
<td>39265</td>
<td>52217</td>
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<td></td>
<td>53795</td>
<td>71709</td>
<td>57049</td>
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<td></td>
<td>100266</td>
<td>93902</td>
<td>78732</td>
</tr>
<tr>
<td></td>
<td>98862</td>
<td>102794</td>
<td>84801</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; direction of transport from top to bottom).
Results in c.p.m./2 cm of stolon relative to:

**Fig. 16.**

IAA + TIBA (10^{-4} M) "ring" IAA (5 \times 10^{-5} M)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stele Mean ± S.E.</th>
<th>cortex Mean ± S.E.</th>
<th>Stele Mean ± S.E.</th>
<th>cortex Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45352 ± 10752</td>
<td>22376 ± 5944</td>
<td>32358 ± 4640</td>
<td>23130 ± 9083</td>
</tr>
<tr>
<td></td>
<td>44050</td>
<td>12935</td>
<td>28879</td>
<td>14087</td>
</tr>
<tr>
<td></td>
<td>36576</td>
<td>7947</td>
<td>30839</td>
<td>18491</td>
</tr>
<tr>
<td></td>
<td>33753</td>
<td>5112</td>
<td>34576</td>
<td>24100</td>
</tr>
<tr>
<td></td>
<td>37828</td>
<td>9145</td>
<td>37527</td>
<td>27099</td>
</tr>
<tr>
<td></td>
<td>37469</td>
<td>12400</td>
<td>36770</td>
<td>28852</td>
</tr>
<tr>
<td></td>
<td>42097</td>
<td>17821</td>
<td>37006</td>
<td>29233</td>
</tr>
<tr>
<td></td>
<td>46631</td>
<td>34617</td>
<td>37515</td>
<td>37620</td>
</tr>
<tr>
<td></td>
<td>58216</td>
<td>16862</td>
<td>47483</td>
<td>66673</td>
</tr>
<tr>
<td></td>
<td>52395</td>
<td>12609</td>
<td>45693</td>
<td>85975</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; direction of transport from top to bottom).

**Fig. 17.**

IAA + Actin. (15 mg/l) IAA (5 \times 10^{-5} M) Water

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>89267 ± 24396</td>
<td>111470 ± 29689</td>
<td>73357 ± 16202</td>
</tr>
<tr>
<td></td>
<td>93648</td>
<td>118287</td>
<td>60650</td>
</tr>
<tr>
<td></td>
<td>88201</td>
<td>113058</td>
<td>55695</td>
</tr>
<tr>
<td></td>
<td>81832</td>
<td>122456</td>
<td>48737</td>
</tr>
<tr>
<td></td>
<td>67331</td>
<td>116772</td>
<td>41180</td>
</tr>
<tr>
<td></td>
<td>47615</td>
<td>102340</td>
<td>31196</td>
</tr>
<tr>
<td></td>
<td>33951</td>
<td>78769</td>
<td>18200</td>
</tr>
<tr>
<td></td>
<td>23676</td>
<td>93344</td>
<td>9897</td>
</tr>
<tr>
<td></td>
<td>17955</td>
<td>144815</td>
<td>3861</td>
</tr>
<tr>
<td></td>
<td>9304</td>
<td>4522</td>
<td>912</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; direction of movement from top to bottom).
Results in c.p.m./2 cm of stolon relative to:

### Fig. 18.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± S.E.</th>
<th>n= 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA + Purom. (2x10^{-4} M)</td>
<td>126918 ± 56085</td>
<td>3</td>
</tr>
<tr>
<td>IAA (5x10^{-5} M)</td>
<td>100035 ± 26982</td>
<td>3</td>
</tr>
<tr>
<td>Water</td>
<td>113798 ± 15466</td>
<td>3</td>
</tr>
</tbody>
</table>

(n = number of stolons per treatment; direction of transport from top to bottom).

### Fig. 19.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± S.E.</th>
<th>n= 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA (18 h.); IAA + CH (3+6 h.)</td>
<td>36584 ± 5066</td>
<td>3</td>
</tr>
<tr>
<td>(CH 10 mg/l; n=3)</td>
<td>42424 ± 4570</td>
<td>3</td>
</tr>
<tr>
<td>IAA (10^{-5} M)</td>
<td>49282 ± 7554</td>
<td>3</td>
</tr>
</tbody>
</table>

(n = number of stolons per treatment; direction of transport from top to bottom).
Results in c.p.m./2 cm of stolon relative to:

**Fig. 20.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n= 3</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA + CH (15 mg/l) &quot;ring&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>198365 ± 48252</td>
</tr>
<tr>
<td></td>
<td></td>
<td>181012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>155473</td>
</tr>
<tr>
<td></td>
<td></td>
<td>133688 ± 8234</td>
</tr>
<tr>
<td></td>
<td></td>
<td>91597</td>
</tr>
<tr>
<td></td>
<td></td>
<td>89061 ± 11104</td>
</tr>
<tr>
<td></td>
<td></td>
<td>91865</td>
</tr>
<tr>
<td></td>
<td></td>
<td>98460</td>
</tr>
<tr>
<td></td>
<td></td>
<td>169534 ± 14948</td>
</tr>
<tr>
<td></td>
<td></td>
<td>147353 ± 7820</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IAA (5x10^-5 M)</th>
<th>n= 3</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>178541 ± 3995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>174770</td>
</tr>
<tr>
<td></td>
<td></td>
<td>196210</td>
</tr>
<tr>
<td></td>
<td></td>
<td>186802 ± 14291</td>
</tr>
<tr>
<td></td>
<td></td>
<td>139563</td>
</tr>
<tr>
<td></td>
<td></td>
<td>127213 ± 15539</td>
</tr>
<tr>
<td></td>
<td></td>
<td>94527</td>
</tr>
<tr>
<td></td>
<td></td>
<td>110253</td>
</tr>
<tr>
<td></td>
<td></td>
<td>164484 ± 11994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>179812 ± 20189</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; CH= cycloheximide; direction of transport from top to bottom).

**Fig. 21.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(Purom. 2x10^-4 M; n=6)</th>
<th>(Actin. 12 mg/l; n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA + Purom. &quot;ring&quot;</td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>IAA + Actin. &quot;ring&quot;</td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>IAA (5x10^-5 M)</td>
<td>(Actin. 5x10^-5 M)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n= 6</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA + Purom. &quot;ring&quot;</td>
<td></td>
<td>18037 ± 1379</td>
</tr>
<tr>
<td>IAA + Actin. &quot;ring&quot;</td>
<td></td>
<td>22741 ± 2401</td>
</tr>
<tr>
<td>IAA (5x10^-5 M)</td>
<td></td>
<td>20556 ± 4297</td>
</tr>
<tr>
<td>(Actin. 12 mg/l; n=6)</td>
<td></td>
<td>19526</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19943</td>
</tr>
<tr>
<td></td>
<td></td>
<td>201281</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19224</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17126 ± 1974</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17458 ± 3293</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18019</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16827</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20152 ± 1672</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21546 ± 3501</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23748</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24640</td>
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<tr>
<td></td>
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<td>35600</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34043</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51626 ± 4523</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52503 ± 6790</td>
</tr>
<tr>
<td></td>
<td></td>
<td>55994 ± 4117</td>
</tr>
<tr>
<td></td>
<td></td>
<td>54085 ± 5757</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; direction of transport from top to bottom).
Results in c.p.m./2 cm of stolon relative to:

**Fig. 22.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA + Sucr. (2x10^-3 M)</td>
<td>152240 ± 44139</td>
</tr>
<tr>
<td>n= 3</td>
<td>151002</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>150356</td>
</tr>
<tr>
<td>151936</td>
<td>141301</td>
</tr>
<tr>
<td>111864</td>
<td>111216</td>
</tr>
<tr>
<td>74001</td>
<td>106680</td>
</tr>
<tr>
<td>70053</td>
<td>107097</td>
</tr>
<tr>
<td>107097</td>
<td>74001</td>
</tr>
<tr>
<td>66026</td>
<td>70053</td>
</tr>
<tr>
<td>27365</td>
<td>111864</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; direction of transport from top to bottom).

**Fig. 23.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA + Mannitol (0.3 M)</td>
<td>117514 ± 31201</td>
</tr>
<tr>
<td>n= 3</td>
<td>133528</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>129516</td>
</tr>
<tr>
<td>130081</td>
<td>117671</td>
</tr>
<tr>
<td>111216</td>
<td>118575</td>
</tr>
<tr>
<td>182741</td>
<td>111936</td>
</tr>
<tr>
<td>106680</td>
<td>56813</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; direction of transport from top to bottom).
Results in c.p.m./2 cm of stolon relative to:

**Fig. 24.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± S.E.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endot. (200 mg/l)</td>
<td>130962 ± 18970</td>
<td>6</td>
</tr>
<tr>
<td>Endot. (500 mg/l)</td>
<td>131311 ± 18970</td>
<td>5</td>
</tr>
<tr>
<td>Endot. (1000 mg/l)</td>
<td>75621 ± 18970</td>
<td>4</td>
</tr>
<tr>
<td>Endot. (200 mg/l)</td>
<td>76667 ± 18970</td>
<td>9</td>
</tr>
<tr>
<td>Endot. (500 mg/l)</td>
<td>70440 ± 18970</td>
<td>4</td>
</tr>
<tr>
<td>Endot. (1000 mg/l)</td>
<td>114993 ± 18970</td>
<td>9</td>
</tr>
<tr>
<td>Water</td>
<td>158303 ± 18970</td>
<td>4</td>
</tr>
</tbody>
</table>

*(n= number of stolons per treatment; intact stolons; direction of transport from top to bottom).*

**Fig. 25.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± S.E.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNP (10⁻⁵ M)</td>
<td>183396 ± 36781</td>
<td>3</td>
</tr>
<tr>
<td>DNP (5x10⁻⁵ M)</td>
<td>166031 ± 36781</td>
<td>3</td>
</tr>
<tr>
<td>Controls</td>
<td>141392 ± 3783</td>
<td>4</td>
</tr>
</tbody>
</table>

*(n= number of stolons per treatment; intact stolons; direction of transport from top to bottom).*
Results in c.p.m./cm of stolon relative to:

<table>
<thead>
<tr>
<th>Fig. 26.</th>
<th>Fig. 27.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIBA &quot;ring&quot; Controls</td>
<td>NPA &quot;ring&quot; Controls</td>
</tr>
<tr>
<td>(10^{-4}M; n=3)</td>
<td>(10^{-4}M; n=3)</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>23378 ± 2916</td>
<td>9327 ± 573</td>
</tr>
<tr>
<td>9998</td>
<td>6164</td>
</tr>
<tr>
<td>4907</td>
<td>4296</td>
</tr>
<tr>
<td>607</td>
<td>3917</td>
</tr>
<tr>
<td>85</td>
<td>2999</td>
</tr>
<tr>
<td>68</td>
<td>2617</td>
</tr>
<tr>
<td>59</td>
<td>2342</td>
</tr>
<tr>
<td>55</td>
<td>2143</td>
</tr>
<tr>
<td>48</td>
<td>2183</td>
</tr>
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<td>50</td>
<td>2461</td>
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<tr>
<td>46</td>
<td>2811</td>
</tr>
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<td>45</td>
<td>3129</td>
</tr>
<tr>
<td>43</td>
<td>2897</td>
</tr>
<tr>
<td>40</td>
<td>1980</td>
</tr>
<tr>
<td>42</td>
<td>1175</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; IAA-5-^3H injected near the apex of intact stolons; direction of transport from top to bottom).
Results in c.p.m./cm of stolon relative to:

**Fig. 28.**

<table>
<thead>
<tr>
<th>Morphactin &quot;ring&quot; (10^{-4} M; n= 3)</th>
<th>Controls (n= 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>63046 ± 3362</td>
<td>24840 ± 2679</td>
</tr>
<tr>
<td>20900</td>
<td>10714</td>
</tr>
<tr>
<td>4544</td>
<td>7828</td>
</tr>
<tr>
<td>1562</td>
<td>727</td>
</tr>
<tr>
<td>391</td>
<td>4770</td>
</tr>
<tr>
<td>67</td>
<td>3620</td>
</tr>
<tr>
<td>84</td>
<td>3620</td>
</tr>
<tr>
<td>59</td>
<td>2561</td>
</tr>
<tr>
<td>67</td>
<td>2201</td>
</tr>
<tr>
<td>53</td>
<td>2258</td>
</tr>
<tr>
<td>61</td>
<td>1737</td>
</tr>
<tr>
<td>53</td>
<td>165</td>
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<tr>
<td>51</td>
<td>1737</td>
</tr>
<tr>
<td>58</td>
<td>106</td>
</tr>
<tr>
<td>51</td>
<td>2201</td>
</tr>
<tr>
<td>50</td>
<td>2258</td>
</tr>
<tr>
<td>53</td>
<td>1737</td>
</tr>
<tr>
<td>(n= number of stolons per treatment; IAA-5-{^3}H injected near the apex of intact stolons; direction of transport from top to bottom).</td>
<td></td>
</tr>
</tbody>
</table>
Results in c.p.m./cm of stolon relative to:

**Fig. 29.**

<table>
<thead>
<tr>
<th>TIBA &quot;rings&quot;</th>
<th>Morphactin &quot;rings&quot;</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>(10⁻⁴M; n= 3)</td>
<td>(10⁻⁴M; n= 3)</td>
<td>n= 3</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>742 ± 34</td>
<td>2320 ± 824</td>
<td>1435 ± 742</td>
</tr>
<tr>
<td>386</td>
<td>2038</td>
<td>1000</td>
</tr>
<tr>
<td>304 ± 32</td>
<td>1338 ± 734</td>
<td>634 ± 144</td>
</tr>
<tr>
<td>221</td>
<td>1197</td>
<td>542</td>
</tr>
<tr>
<td>213 ± 15</td>
<td>979 ± 309</td>
<td>457 ± 119</td>
</tr>
<tr>
<td>213</td>
<td>1091</td>
<td>525</td>
</tr>
<tr>
<td>255 ± 20</td>
<td>1299 ± 307</td>
<td>538 ± 217</td>
</tr>
<tr>
<td>215</td>
<td>1564</td>
<td>632</td>
</tr>
<tr>
<td>218 ± 7</td>
<td>2506 ± 584</td>
<td>783 ± 381</td>
</tr>
<tr>
<td>247</td>
<td>4632</td>
<td>849</td>
</tr>
<tr>
<td>351 ± 45</td>
<td>7700 ± 1350</td>
<td>1078 ± 355</td>
</tr>
<tr>
<td>553</td>
<td>8047</td>
<td>1441</td>
</tr>
<tr>
<td>627 ± 30</td>
<td>5002 ± 1291</td>
<td>2131 ± 740</td>
</tr>
<tr>
<td>1139</td>
<td>6652</td>
<td>4666</td>
</tr>
<tr>
<td>2437 ± 468</td>
<td>13289 ± 7589</td>
<td>14409 ± 9773</td>
</tr>
</tbody>
</table>

Region of IAA-§³H application

| 26227 | 2178 | 5784 | 766 | 13396 | 3801 |
| 19366 |      | 2490 |     | 8399  |      |
| 1711  | 90   | 1147 | 722 | 10309 | 3667 |
| 103   |      | 100  |     | 15028 |      |
| 67    | 4    | 70   | 3   | 16941 | 1874 |
|       |      |      |     | 8300  |      |
|       |      |      |     | 2200  | 458  |

(n= number of stolons per treatment; direction of transport indicated by the harrow).
Results in c.p.m./cm of stolon relative to:

**Fig. 30.**

<table>
<thead>
<tr>
<th>Stolon</th>
<th>Cyclohexim. &quot;ring&quot; (10 mg/l; n = 3)</th>
<th>Controls n = 3</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25231 ± 2490</td>
<td>23151 ± 5367</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7931</td>
<td>6598</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>28160</td>
<td>4082</td>
<td></td>
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<td>13153</td>
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<td>196</td>
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<td></td>
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<tr>
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<td>197</td>
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<tr>
<td></td>
<td>204</td>
<td>204</td>
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</tr>
</tbody>
</table>

(n = number of stolons per treatment; IAA-5−H injected near the apex of intact stolons; direction of transport from top to bottom).
Results in c.p.m./cm of stolon relative to:

**Fig. 31.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Stele Mean ± S.E.</th>
<th>Cortex Mean ± S.E.</th>
<th>Controls Mean ± S.E.</th>
<th>Stele Mean ± S.E.</th>
<th>Cortex Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycloheximide &quot;ring&quot;</td>
<td>5009 ± 2190</td>
<td>20222 ± 4269</td>
<td>599 ± 122</td>
<td>22552 ± 5245</td>
<td></td>
</tr>
<tr>
<td>(10 mg/l; n= 3)</td>
<td>2944</td>
<td>4987</td>
<td>1077</td>
<td>5521</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22090</td>
<td>5149</td>
<td>6070</td>
<td>1633</td>
<td>773</td>
</tr>
<tr>
<td></td>
<td>31547</td>
<td>2903</td>
<td>6717</td>
<td>3241</td>
<td>915</td>
</tr>
<tr>
<td></td>
<td>11002</td>
<td>2151</td>
<td>1723</td>
<td>2730</td>
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<tr>
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<td>3398</td>
<td>1917</td>
<td>850</td>
<td>320</td>
<td>2415</td>
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<tr>
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<td>702</td>
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<td>72</td>
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<tr>
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<td>920</td>
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<td>169</td>
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<td>579</td>
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<td>105</td>
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<td></td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; IAA-5-3H injected near the apex of intact stolons; direction of transport from top to bottom).
Results in c.p.m./cm of stolon relative to:

**Fig. 32.**

<table>
<thead>
<tr>
<th>Puromycin &quot;ring&quot; (2x10^{-4}M; n= 3)</th>
<th>Actinomycin &quot;ring&quot; (20 mg/l; n= 3)</th>
<th>Controls n= 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>9571 ± 1012</td>
<td>12394 ± 1435</td>
<td>13104 ± 2554</td>
</tr>
<tr>
<td>4087</td>
<td>3952</td>
<td>5330</td>
</tr>
<tr>
<td>4177</td>
<td>435</td>
<td>2833</td>
</tr>
<tr>
<td>4918</td>
<td>2338</td>
<td>716</td>
</tr>
<tr>
<td>3160</td>
<td>2418</td>
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<td>2196</td>
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<tr>
<td>1341</td>
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<td>1425</td>
<td>1918</td>
<td>1978</td>
</tr>
<tr>
<td>1600</td>
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</tr>
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<td>1690</td>
<td>3463</td>
<td>140</td>
</tr>
<tr>
<td>690</td>
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<td>1679</td>
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<tr>
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<td>626</td>
<td>2896</td>
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<tr>
<td>27</td>
<td>60</td>
<td>447</td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>1025</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; IAA-5-^3H injected near the apex of intact stolons; direction of transport from top to bottom).
Results in c.p.m./cm of stolon relative to:

**Fig. 33.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNP &quot;ring&quot; (10⁻⁴ M; n=3)</td>
<td>35617 ± 2678</td>
<td>18301</td>
<td>52628</td>
</tr>
<tr>
<td></td>
<td>17416</td>
<td>49569 ± 4262</td>
<td>13303</td>
</tr>
<tr>
<td></td>
<td>3182</td>
<td>29116</td>
<td>29116</td>
</tr>
<tr>
<td></td>
<td>473</td>
<td>203</td>
<td>169</td>
</tr>
<tr>
<td></td>
<td>168</td>
<td>168</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>152</td>
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<td></td>
<td>119</td>
<td>103</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>KCN &quot;ring&quot; (5x10⁻⁴ M; n=3)</td>
<td>18301</td>
<td>38797 ± 4233</td>
<td>5593</td>
</tr>
<tr>
<td></td>
<td>12012</td>
<td>9806</td>
<td>276</td>
</tr>
<tr>
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<td>6980</td>
<td>13303</td>
<td>30</td>
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<tr>
<td></td>
<td>5196</td>
<td>29116</td>
<td>15</td>
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<tr>
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<td>4866</td>
<td>473</td>
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<tr>
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<td>3103</td>
<td>4405</td>
<td>81</td>
</tr>
<tr>
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<td>4405</td>
<td>1268</td>
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<td>3508</td>
<td>916</td>
<td>115</td>
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<tr>
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<td>4290</td>
<td>916</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>5717</td>
<td>1053</td>
<td>115</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; IAA-5-³H injected near the apex of intact stolons; direction of transport from top to bottom).
Results in c.p.m./cm of stolon relative to:

**Fig. 34.**

<table>
<thead>
<tr>
<th></th>
<th>DNP (5x10^{-5}M) &quot;ring&quot;</th>
<th>DNP (10^{-5}M) &quot;ring&quot;</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n= 3</td>
<td>n= 3</td>
<td>n= 3</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
<td></td>
</tr>
<tr>
<td>43870 ± 4832</td>
<td>37314 ± 5518</td>
<td>48012 ± 10589</td>
<td></td>
</tr>
<tr>
<td>19993</td>
<td>22597</td>
<td>21994</td>
<td></td>
</tr>
<tr>
<td>21430</td>
<td>28327</td>
<td>17549</td>
<td>4132</td>
</tr>
<tr>
<td>2390</td>
<td>4886</td>
<td>15746</td>
<td>4754</td>
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<tr>
<td>48223</td>
<td>59740</td>
<td>12359</td>
<td>3745</td>
</tr>
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<td>2606</td>
<td></td>
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<tr>
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<td>5929</td>
<td>569</td>
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<td>102</td>
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<td>187</td>
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<td></td>
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<td>97</td>
<td>336</td>
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<td>81</td>
<td>255</td>
<td>10301</td>
<td>893</td>
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<td>120</td>
<td>7695</td>
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</tbody>
</table>

(n= number of stolons per treatment; IAA-5-^3H injected near the apex of intact stolons; direction of transport from top to bottom).
Results in c.p.m./cm of stolon relative to:

Fig. 35.

<table>
<thead>
<tr>
<th></th>
<th>Endot. 200 mg/l</th>
<th>Endot. 500 mg/l</th>
<th>Endot. 1000 mg/l</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=</td>
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<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>42913 ± 7503</td>
<td>39869 ± 8139</td>
<td>38612 ± 5356</td>
<td>44363 ± 3852</td>
</tr>
<tr>
<td>29928</td>
<td>26355</td>
<td>22566</td>
<td></td>
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</tr>
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<td>18343</td>
<td>19322</td>
<td>23110</td>
<td>6649</td>
<td>12313</td>
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<tr>
<td>25412</td>
<td>37997</td>
<td>35617</td>
<td>7875</td>
<td>1948</td>
</tr>
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<td>7104</td>
<td>3477</td>
<td>1869</td>
<td>11159</td>
<td>5215</td>
</tr>
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<td>1546</td>
<td>453</td>
<td>69</td>
<td>1141</td>
<td>3638</td>
</tr>
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<td>122</td>
<td>191</td>
<td>214</td>
<td>2960</td>
<td>563</td>
</tr>
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<td>86</td>
<td>122</td>
<td>70</td>
<td>2431</td>
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<td>97</td>
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<td>2582</td>
<td>509</td>
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<td>74</td>
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<td>85</td>
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<td>5</td>
<td>84</td>
<td>1052</td>
<td>170</td>
</tr>
</tbody>
</table>

(n = number of stolons per treatment; IAA-5-3H injected near the apex of intact stolons; direction of transport from top to bottom).
Results in c.p.m./cm of stolon relative to:

**Fig. 36.**

<table>
<thead>
<tr>
<th>Endot. (200 mg/l)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>n= 3</td>
<td>n= 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stele</th>
<th>Cortex</th>
<th>Stele</th>
<th>Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>4330 ± 1575</td>
<td>19045 ± 2022</td>
<td>4420 ± 1225</td>
<td>12111 ± 710</td>
</tr>
<tr>
<td>2737</td>
<td>4357</td>
<td>3350</td>
<td>6227</td>
</tr>
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<td>2528</td>
<td>853</td>
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<tr>
<td>2525</td>
<td>2789</td>
<td>3084</td>
<td>3990</td>
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<td>113</td>
<td>261</td>
<td>86</td>
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<td>202</td>
<td>67</td>
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</tr>
</tbody>
</table>

(n= number of stolons per treatment; IAA-5-^3^H injected near the apex of intact stolons; direction of transport from top to bottom).
Results in c.p.m./cm of stolon relative to:

![Table](image)

*(n= number of stolons per treatment; IAA-5-\(^3\)H injected near the apex of intact stolons; direction of transport from top to bottom).*
Results in c.p.m./cm of stolon relative to:

**Fig. 41.**

Cortex and stele separated over a small distance prior to injection of IAA-5⁻³⁵H.  

<table>
<thead>
<tr>
<th>Stele (n= 3)</th>
<th>Cortex (n= 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>14805 ± 1757</td>
<td>31990 ± 3493</td>
</tr>
<tr>
<td>5395</td>
<td>8457</td>
</tr>
<tr>
<td>4121</td>
<td>4737</td>
</tr>
<tr>
<td>962</td>
<td>1072</td>
</tr>
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<td>17064</td>
<td>4625</td>
</tr>
<tr>
<td>7632</td>
<td>539</td>
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<td>11100</td>
<td>987</td>
</tr>
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<td>4119</td>
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<td>682</td>
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<td>17</td>
</tr>
<tr>
<td>17</td>
<td>53</td>
</tr>
</tbody>
</table>

(n= number of stolons per treatment; IAA-5⁻³⁵H injected near the apex of intact stolons; direction of transport from top to bottom).