STUDIES ON AUXIN TRANSPORT

IN

COLEUS AND HELIANTHUS

A thesis submitted for the Degree of
Doctor of Philosophy in the Faculty of
Science of the University of Leicester

by

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Statement

The work embodied in this thesis was carried out by the candidate in the Botanical Laboratories of the University of Leicester, under the supervision of Dr. Elisabeth Wangermann.

The work has not previously been submitted, and is not concurrently being submitted for any other degree.

Signed: D. Garland .....

September 1974.

Acknowledgements

I should like to thank Dr. E. Wangermann for her help and encouragement during the preparation of this thesis.

I should also like to thank Professor H.E. Street for the facilities granted to me in the Botanical Laboratories, University of Leicester.

Thanks are also due to the technical staff in the laboratories and to Mrs. Daphne Young for typing the manuscript.

ABSTRACT

The products formed during transport in excised plant segments of radioactively labelled indolyl-3-acetic acid and 2,4-dichlorophenoxyacetic acid have been investigated. The techniques employed were extraction of the tissue followed by radiochromatography. It was found that indolyl-3-acetic acid is readily converted to indolyl-3-acetyl aspartate in Coleus tissue but not in Helianthus tissue. 2,4-dichlorophenoxyacetic acid appears to undergo little change.

The distribution of radioactive material which results from the transport of labelled auxin in plant segments and small plants was also investigated by means of oxidation and subsequent scintillation counting. It was found that the system has a definite capacity and that much of the transported auxin is immobilised but not necessarily conjugated and that amounts of auxin reaching receiving systems is not a reliable basis for estimating auxin which is transported within tissue.

Auxin transport into root primordia, lateral buds, abscission zones and through developmental transition zones is also briefly considered and it is reported that no barrier to the transport is encountered in these regions.

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GENERAL INTRODUCTION

Auxin transport has been studied for many years but still, surprisingly little is really known about it. The basipolarity of auxin transport has been accepted for a long time but the degree of polarity has often been in dispute. It has been shown that auxins can move, unchanged, through plant segments, but that not all the auxin which is taken up is exported in this way. The fate of the retained auxin is not really understood; neither the tissue in which transport occurs nor the mechanism which is responsible for the polarity in that tissue are clearly defined. It is accepted that indolyl-3-acetic acid is involved in many of the plant's developmental responses and that the concentration of IAA is important in these responses. Presumably the transport system is instrumental in controlling concentration but again the mechanism is not understood.

Some of these problems are considered in this thesis. The question of polarity in Coleus internode segments is discussed in Chapter II. It is found that basipolarity is very strict, even in situations where acropetal transport might be expected to be enhanced. Some suggestions are made as to the cause of the discrepancies on this subject which have appeared in earlier work.

The nature of the metabolites formed from indolyl-3-acetic acid during its transport in Coleus internode segments is discussed in Chapter I and the distribution of that material is discussed in Chapter II. The time course of metabolism and of spatial distribution are compared and it is suggested that the process by which indolyl-3-acetic acid is immobilised and metabolised may be more complex than was previously thought.

The flow of auxin through various anatomical barriers is discussed in Chapter III and it appears that these do not always influence auxin transport. The relevance of the results reported here to the knowledge of the mechanisms of polar transport is also briefly mentioned but no new evidence is offered.

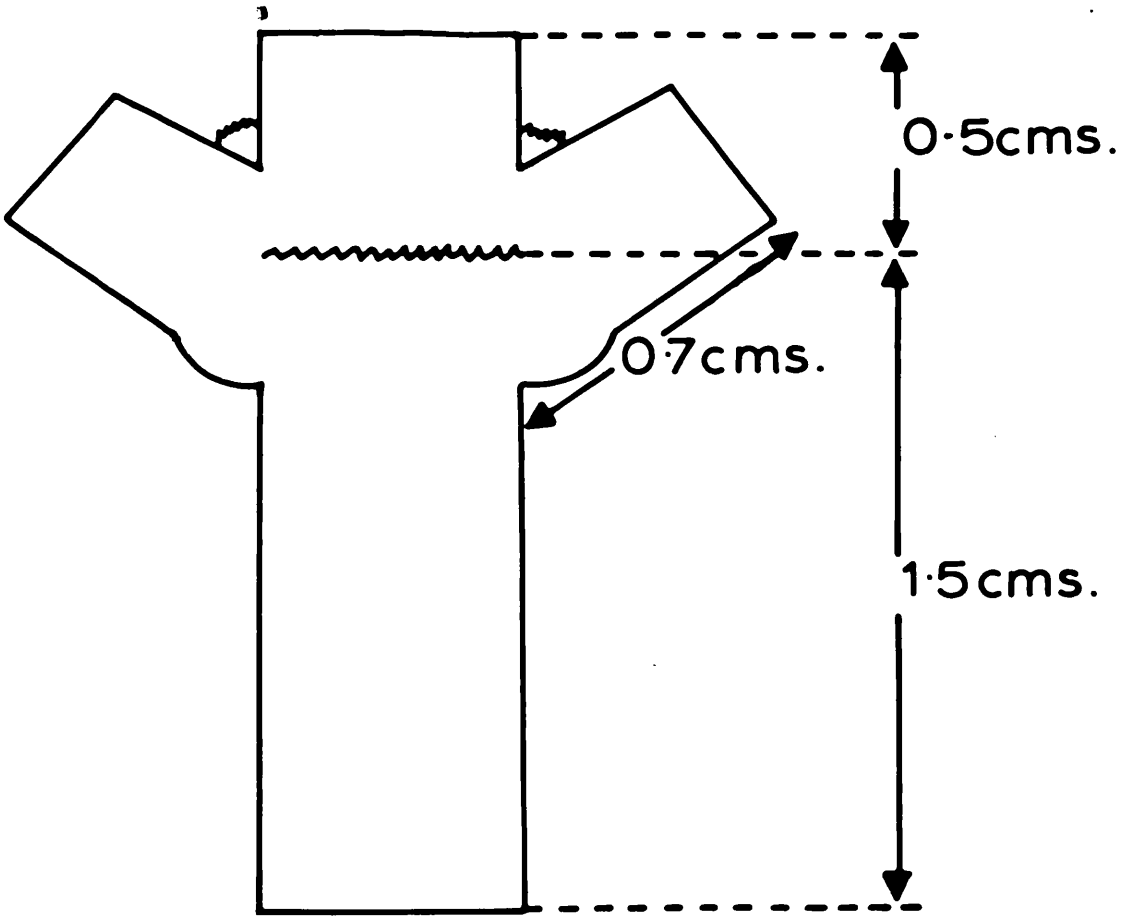
The problem of the removal of auxin from the transport system at its sites of action did not fall within the scope of the investigation reported here although it is an aspect of auxin transport which is still not understood and which, presumably, is important if the auxin which is transported is also to be utilised. It is not known how this removal is effected or how it would influence the aspects of transport which are reported here.

The Oxymat which was used in many of the experiments reported here has made it possible to reliably quantify experimental results and present data not previously available.

MATERIALS

Fig. 1. The form of the nodal explant used in some experiments

The explant consists of the 2nd node of a Coleus plant about 30 cm in height together with part of the internodes above and below and also short petiole stumps and lateral buds. The diagram shows the dimensions of the explant. The measurements shown are made from the ring of hairs which occur on a Coleus stem at about the middle of each node.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Plant material

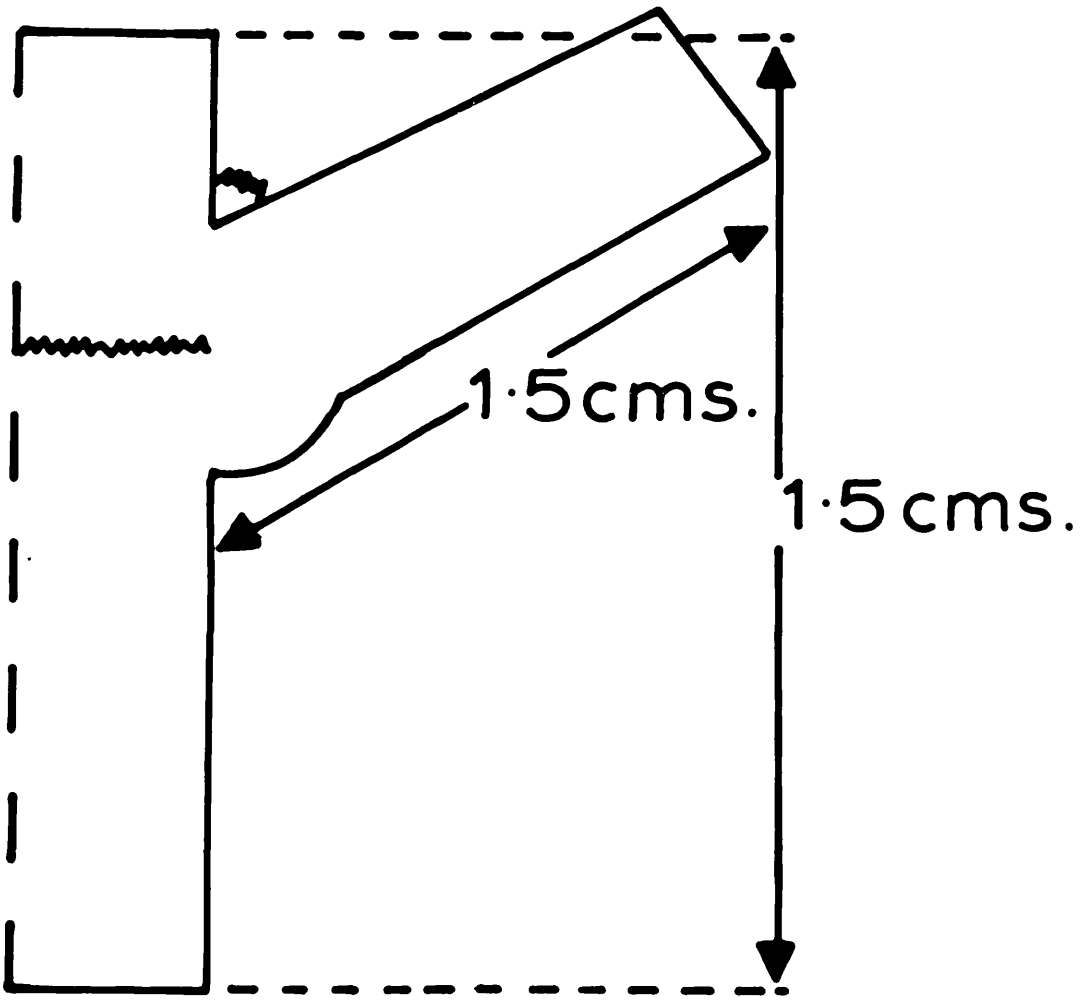
Cuttings were taken from the clone of Coleus blumei Benth. used by Wangermann (1967) and Halliday (1969). They were grown as single-stemmed plants in 5" pots in John Innes no. 1 potting compost in controlled growth rooms. The plants were maintained at a temperature of 25°C and at 70% relative humidity. Illumination was provided by 'daylight' fluorescent tubes. The light intensity was 400 foot-candles and the day length was 20 h. Conditions were designed to prevent flowering but this was not always possible. Unless otherwise stated vegetative plants were always selected.

When segments were to be cut a group of plants was chosen for uniformity of size and pigmentation. Plants were used when about 30 cm high. Segments were cut from the internode below a pair of leaves which were about half expanded (usually the third internode below the apex). Each segment was 15 mm long and cut from the upper part of the internode so that the tissue from the node was just excluded. Only one segment was cut from one internode. Petiole segments were cut from leaves at the third node below the apex. These were also 15 mm long. They were cut from just below the lamina and only one segment was cut from each petiole. Nodal explants were cut from the second node below the apex. Abscission zone explants of the type used by Halliday (1969) were cut from the 6th and 7th leaf pairs below the apex. The form of these explants is shown in Figs. 1 and 2. When whole cuttings were used in experiments they were allowed to root in tubes of water in the controlled growth rooms. They were 8 - 10 cm long.

Helianthus annuus plants were grown from seed. Seedlings were planted out in boxes of John Innes no. 1 potting compost and grown in Saxcil cabinets. The temperature in the cabinets was 14°C at night and 18°C during the day

Fig. 2. The form of the abscission zone explant used in some experiments

The explants are taken from the 6th and 7th nodes. The stem is cut longitudinally mid-way between the two stem surfaces bearing the petioles so two explants are cut from each node.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and the relative humidity was 50%. The day length was 9h. Illumination was by 'warm white' fluorescent tubes at an intensity of 900 foot-candles.

Whole seedlings were used for some experiments but in other cases internode segments were cut. These were taken from the second internode below the apex of 20 cm plants. Only one segment was cut from each internode and it was taken from the upper part of the internode, just below the node.

Chemicals

Indolyl-3-acetic acid (IAA) was obtained from the Sigma Chemical Co., St. Louis, Missouri and indolyl-3-acetyl-L-aspartic acid (IAAsp) was supplied as the di(cyclohexylammonium salt) by Calbiochem, Los Angeles, California. Indolyl-3-acetamide (IAAmd) was supplied by Koch-Light Laboratories Ltd., Colnbrook, Bucks. When these were used as markers for chromatography they were used as 10^{-3}M solutions but when they were applied to tissue they were used as 5×10^{-5}M solutions. The IAAsp was made up directly into the appropriate aqueous solutions but the IAA and the IAAmd had to be dissolved in a small volume of absolute ethanol before being made up to the appropriate concentration with distilled water.

2,4-dichlorophenoxy acetic acid (2,4-D) was obtained from British Drug Houses, Poole, Dorset and used as 10^{-4}M solution. This, too, had to be dissolved in absolute ethanol before being made up into an aqueous solution.

Indolyl-3-(1-^{14}C)acetic acid, indolyl-3-(2-^{14}C)acetic acid and 2,4-dichlorophenoxy-(2-^{14}C)acetic acid (IAA-1-^{14}C, IAA-2-^{14}C and 2,4-D-2-^{14}C respectively) were supplied by the Radiochemical Laboratories, Amersham. The radioactive IAA was supplied as the ammonium salt and therefore made up directly as a 5×10^{-5}M aqueous solution. Specific activities ranged from

52 - 58 mCi/mM. The 2,4-D-^{14}C, specific activity 28 mCi/mM, was supplied in benzene solution. The benzene was evaporated under nitrogen and the resulting solid 2,4-D-2-^{14}C was taken up in the appropriate potassium hydroxide solution to form a 10^{-4}M solution of the potassium salt. This was a rather high concentration, far higher than that used by McCready and Jacobs (1963), but it was chosen because 2,4-D treatments could then be compared directly with IAA treatments, both giving similar counts.

Agar blocks

The agar blocks used as donor and receiver blocks were made from 1.5% Oxoid Ionagar no. 3 or from 2% Lab M agar when the former became unobtainable. The aqueous agar was boiled and while still fluid was drawn up into clean glass tubes of internal diameter 6.5 mm. When the agar had set the resulting cylinders were extruded from the tubes on to a glass sheet and cut into blocks, 4 mm high, with a treble-bladed cutter. If the blocks were to be used as receivers they were used without further treatment. If they were to be used as donors, auxin was added to them after cutting. The application of auxin was performed in a dark-room under a green 'safelight'. Auxin solution was delivered, 5 μl at a time, on to the top surface of the block with an Oxford micro-pipette or a Hamilton syringe. The blocks were left for at least an hour to allow diffusion of the solution before use. When 10 μl of solution were used in the donor the remaining 5 μl were added after the initial dose had been allowed to diffuse into the agar. If the blocks were not required within two or three hours of treatment they were packed in sealed Petri dishes wrapped in damp filter paper and aluminium foil and stored in a refrigerator until needed.

Extraction and chromatographic materials

In most cases plant material was extracted with Analar methanol. When ether extraction was performed Analar ether was rendered peroxide-free by the method of Larsen (1955).

The chromatography solvent systems employed were isopropanol : ammonia : water (80 : 5 : 10 v/v), butanol : acetic acid : water (5 : 1 : 2.2 v/v), chloroform : methanol : 96% acetic acid (90 : 24 : 6 v/v) and chloroform : ethyl acetate : formic acid (50 : 40 : 10 v/v). It was necessary that the chloroform used in these systems contained 1% ethanol. If Merck chloroform was not available it was necessary to purify Analar chloroform (containing 2% ethanol). This was washed 15 times with an equal volume of water and then stored in a deepfreeze for 3 h after which time any remaining ice was filtered out in a chilled funnel. The chloroform was then kept overnight over anhydrous sodium sulphate. The ethanol-free chloroform was then mixed with an equal volume of untreated Analar chloroform (Elliott, personal communication).

In all cases ascending thin-layer chromatography was performed. Silica gel thin-layer plates were prepared in the laboratory from Silica Gel G (from Macherey Nagel and Co.) spread on carefully cleaned glass plates. These plates were activated at 100^{o}C for 30 min before use. Commercially prepared cellulose chromatography plates (Polygram cel 300) were also obtained from Macherey Nagel and Co.

Colour detection of non-radioactive markers was carried out using either Ninhydrin-acetic acid (Fahmy et al., 1961) or 4-dimethylaminobenzaldehyde dissolved in hydrochloric acid and ethanol (van Urk, 1929). Radioactive spots on the chromatograms were detected either by exposure to Kodak Kodirex X-ray film or by scanning with a Panax RTLS-1A scanner fitted with a Servoscribe recorder.

CHAPTER I

EXTRACTION AND CHROMATOGRAPHY OF PLANT SEGMENTS

Introduction

It has been known since the work of Went (1928) that auxin is transported, unchanged, through plant segments but that only a small percentage of the applied auxin is recovered in receiving systems, most of it remaining in the tissue in an immobile form (Gorter and Veen, 1966; Goldsmith, 1967, 1968; Beyer and Morgan, 1969; Newman, 1970).

Over the last twenty years a considerable amount of work has been done on the products of auxins which are formed during its transport in whole plants or excised plant parts. During this time several different products have been found but the one most widely occurring is indoleacetyl aspartate (IAAsp) which has been reported by Andreae and Good (1955), Good et al. (1956), Andreae and Van Ysselstein (1956 & 1960), Fang et al. (1959), Zenk (1963), Geronimo et al. (1964), Winter and Thimann (1966), Winter (1967), Robinson et al. (1968), Veen and Jacobs (1969), Morris et al. (1969), Morris (1970), Aasheim and Iversen (1971), Kendall et al. (1971) and Davies (1972).

Some workers have identified other IAA products in their extracts. Indole-3-acetyl glucose (IAGlu) has been identified by Zenk (1963), Winter and Thimann (1966) and Winter (1967). Indole-3-aldehyde (IAld) has been identified by Tang and Bonner (1947), Wagenknecht and Burris (1950) and Morris et al. (1969). Davies (1972) in an exhaustive study identified indole-3-acetamide (IAamd), IAld, 3-hydroxymethyloxindole, indole-3-methanol and indole-3-carboxylic acid. IAA/protein complexes have also been reported (Bentley, 1961; Bara, 1967; Osborne, 1967; Morris et al., 1969; Osborne and Mullins, 1969; Basu and Tuli, 1972; and Davies, 1972).

The mobility of these products has also been investigated with differing results. As regards the mobility of IAAsp, Morris et al. (1969) found it to be

immobile in pea as did Davies (1972). Lepp and Peel (1971) reported it to be relatively immobile in willow stems but in 1972 Field and Peel demonstrated IAAsp mobility in the sieve tubes of willow. Morris et al. also found that IAld was mobile in the "underground organs" of pea plants.

The time course of IAA metabolism has also been studied and Zenk (1963) found that IAGlu was the first formed product, IAAsp formation undergoing a 2 h lag.

Not all workers have used IAA as their auxin. Some, notably Veen (1966) and Beyer and Morgan (1970), have used naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) has also been widely used. The pattern of 2,4-D movement has been studied by Hay and Thimann (1956), Barrier and Loomis (1957), McCready (1963, 1967), McCready and Jacobs (1963a, b, 1967), Jacobs et al. (1966) and Jacobs and McCready (1966, 1967). The metabolism of 2, 4-D has been studied by Hay and Thimann (1956), Andreae and Good (1957), Bach and Fellig (1959), Bach (1961), Hertel and Flory (1968), Field and Peel (1971) and Hallmen and Eliasson (1972). The subject has been reviewed by Audus (1961), and Newman (1963) has worked on the electrical potentials associated with 2,4-D transport.

Jacobs and McCready (1967) and Field and Peel (1972) studied the transport capacities of different tissues but no references could be found to any investigation into the localisation of metabolites in different tissues following transport of auxins in whole segments.

Most of the work performed on auxin metabolism has involved the use of chromatography in several different solvent systems, many of which are widely quoted in the literature. The systems used in these experiments are isopropanol/ammonia/water (80 : 5 : 10 v/v), (Wangermann, 1970), butanol/acetic acid/water

(5 : 1 : 2.2 v/v), chloroform/ethyl acetate/formic acid (50 : 40 : 10 v/v), (Zenk, 1963), and chloroform/methanol/acetic acid (90 : 24 : 6 v/v), (Elliott, personal communication).

Some of these problems have been considered again in conjunction with quantitative work based on the use of oxidation and scintillation counting and with micro-autoradiography performed in this laboratory by Wangermann (1968, 1970). The starting point for this investigation was the occurrence of IAAsp in Coleus internode segments. As stated previously this is the product of IAA metabolism which is most widely quoted and over the years it has been found in many different species and organs. Andreae and Good (1955) originally isolated it from peas and in 1956 Good et al. found that following IAA treatment it could be extracted from all of the 12 species they investigated. Andreae and Van Ysselstein (1956, 1960) further, extracted it from pea epicotyls and roots. Winter and Thimann (1966), Winter (1968) and Morris et al. (1969) also found IAAsp in pea tissue. Morris (1970) found that more IAAsp was formed in light-grown than in dark-grown pea seedlings. Davies (1972) found IAAsp in IAA-treated pea and bean seedlings and Fang et al. (1959) identified IAAsp in pea and corn tissue but surprisingly found more in corn than in pea although cereals are not usually thought to produce much IAAsp (Good et al., 1956). Geronimo et al. (1964) extracted a substance from plum stem cuttings treated with IAA-2-^{14}C which they suspected to be IAAsp, although they were unable to confirm its identity. Robinson et al. (1968) applied IAA-^{14}C to cotton explants and found IAAsp to be produced. Zenk (1963) treated Hypericum leaves with IAA and reported that IAAsp was formed either directly or from IAGlu which is formed during the 2 - 3 h lag period associated with IAAsp formation. Aasheim and Iversen (1971) concluded that one of the products extracted from IAA-treated

sunflower and cabbage roots was probably IAAsp. Studies on Coleus have also shown IAAsp to occur. Veen and Jacobs (1969) extracted IAAsp from petiole sections of Coleus. In this laboratory Wangermann (1970) tentatively identified IAAsp in Coleus internode segments following IAA treatment. However, when Halliday (1969) treated Coleus nodal explants with IAA during his investigation of foliar abscission he did not always find evidence of IAAsp in his extracts. He found that if he used IAA-2-^{14}C treatment, radioactive IAAsp appeared in his extracts, but when he employed IAA-1-^{14}C treatment no IAAsp could be found.

Since this important difference in results existed it was thought necessary to investigate the discrepancy before further work was attempted.

Demonstration of IAAsp formation in Coleus

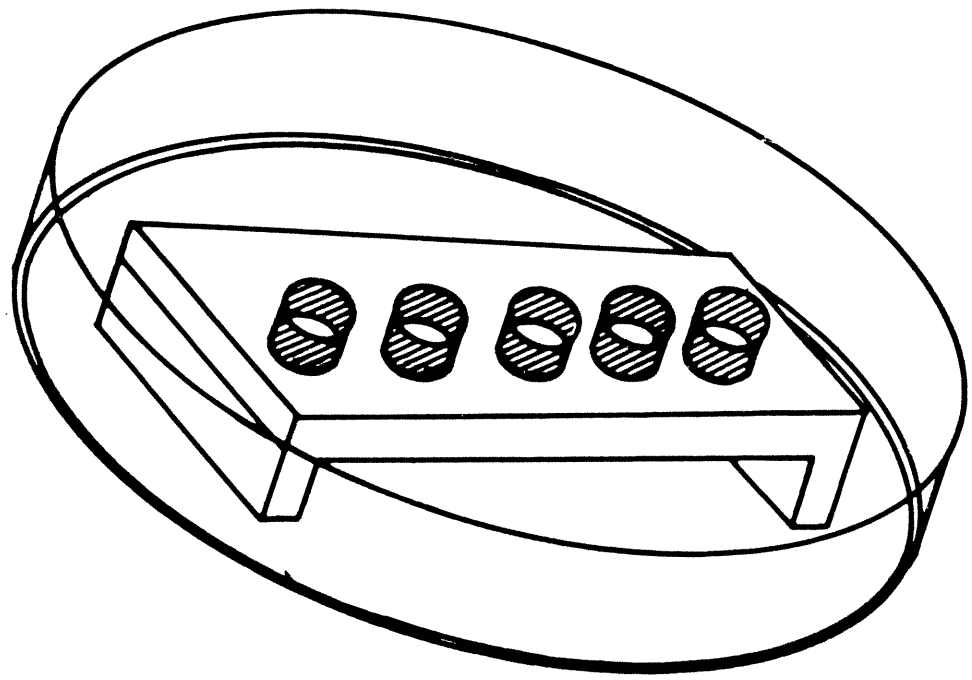
The results obtained by Halliday are surprising in view of the chemistry of the conjugation process. Both the methyl and the carboxyl carbon atoms of the IAA molecule are included in the IAAsp molecule and one would, therefore, expect IAAsp to appear on the radiochromatogram whichever form of the radioactive auxin were employed in the treatment of the tissue.

The main difference between the methods of Wangermann and Halliday was the purification techniques used by Halliday on his plant extracts. There were two such processes and it seemed possible that any IAAsp present in the extracts could have been lost during these purification processes which were designed to remove lipids from the extracts and improve their chromatographic properties. One of his techniques involved partition in ether as one of its steps. This technique was not, therefore, investigated further since Andreae and Good (1955) first characterised IAAsp by its insolubility in ether. The use of ether would explain the loss of IAAsp from an extract but the loss would occur whether IAA-1-^{14}C or IAA-2-^{14}C were used so it does not explain the differing results. The second process was based on dehydration and centrifugation. The techniques of Wangermann and Halliday were repeated in an effort to discover the cause of the discrepancy.

Coleus internode segments were prepared as described previously. They were supported in specially designed perspex 'bridges' which held the segments in the vertical position (see Fig. I.1). The bridges were placed in Petri dishes lined with filter paper soaked in 2% sucrose solution and the bottom cut surfaces of the segments were in contact with this paper. The Petri dishes were placed in a closed translucent plastic tray, lined with damp filter paper.

Fig. I.1 The apparatus in which the internode segments are supported during transport experiments

The perspex bridge holds the segments vertically. It stands in a Petri dish lined with damp filter paper.



IAA-1-^{14}C or IAA-2-^{14}C solution was applied to the upper cut surface of the segments either in agar blocks or in double discs of filter paper. The application of IAA solution was carried out in a dark-room under a green 'safelight' and transport was allowed to proceed under these conditions. After a prescribed transport period the donors were removed and the tissue segments were divided transversely into three sections; the 1 mm section adjacent to the donor, the middle 6 mm section and the basal 8 mm section. The tissue sections and donors were chilled, sliced thinly, placed in small glass vials and just covered with methanol. The donors and tissue were extracted for 48 h in the dark at 0^{o}C.

In some cases the extracts so obtained were separated from the tissues and donors and spotted directly onto silica gel G thin layer chromatography (TLC) plates. Extracts of the donors were chromatographed along with the tissue in order to check that no IAA breakdown occurred in the donors. In other cases the extracts were treated further before being chromatographed. The extract was removed from the solid matter as above, chilled for an hour to cause separation of lipids and then centrifuged for 5 min. The supernatant was separated from the pellet and the pellet was resuspended in methanol. Both fractions were dried in a desiccator over anhydrous $MgSO_4$ for 24 h. The supernatant was chilled and centrifuged again for 10 min. The new supernatant was removed and the pellet resuspended in methanol. In this way the extracts were purified by Halliday's technique but the material he discarded was also retained for chromatography. The particle suspensions were shaken well and kept in the dark at 0^{o}C for at least 24 h before being spotted onto TLC plates. All extracts were stored at 0^{o}C in the dark until required for chromatography.

In the initial experiments the transport time was 24 h. Ten μl of IAA-^{14}C

Fig. I.2 Scan of radiochromatograms of extracts of Coleus internode segments after IAA-2-^{14}C treatment

Both chromatograms were developed in isopropanol/ammonia/water. The top scan represents an IAA marker. It has an R_f value of about 0.50. The lower scan represents the unpurified extract of the middle portion of the segments. It has two peaks, one at 0.50 and one near the origin.

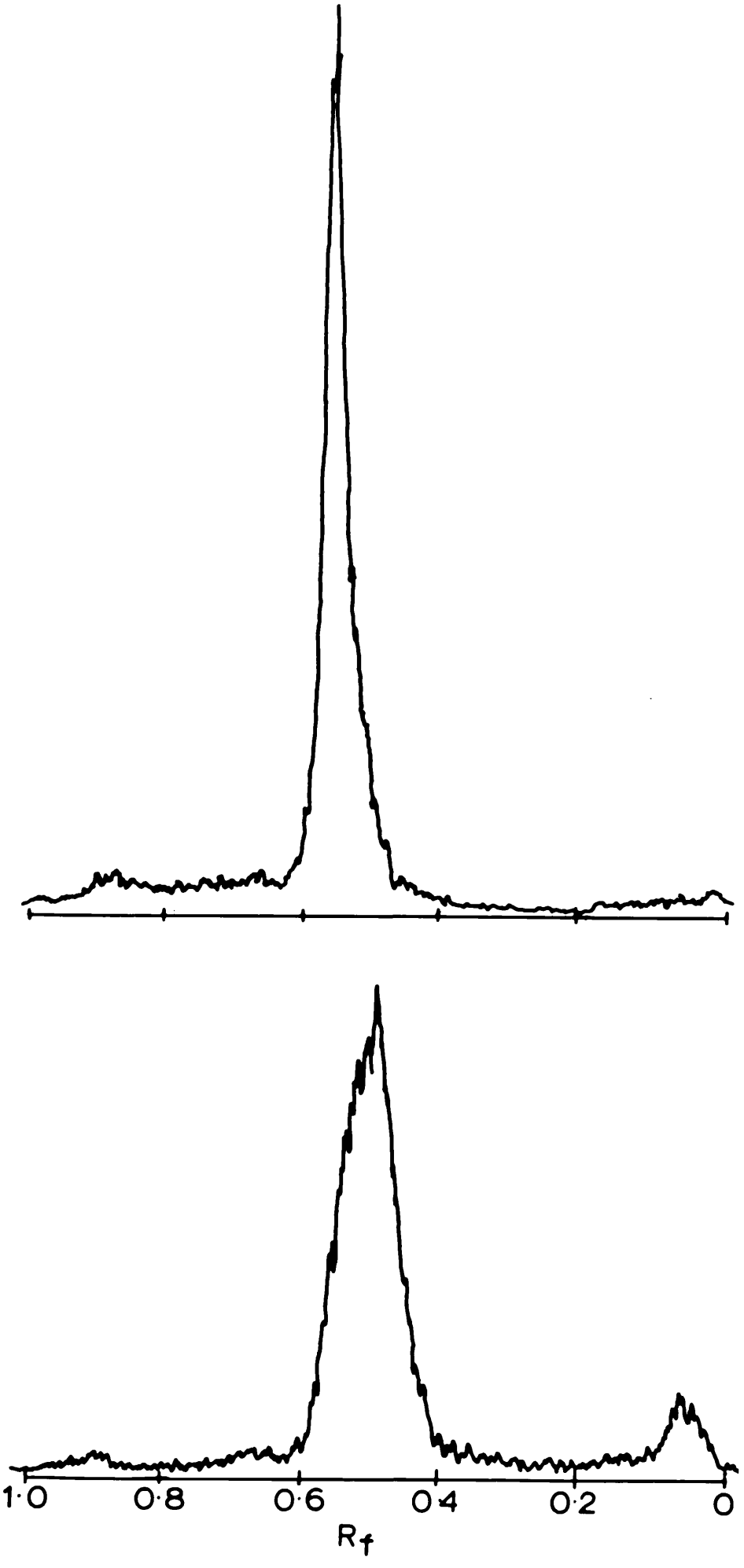

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Fig. I.3 Scan of a radiochromatogram of an IAA-^{14}C marker developed in butanol/acetic acid/water

The R_f value of the IAA in this solvent system is about 0.90-0.95.

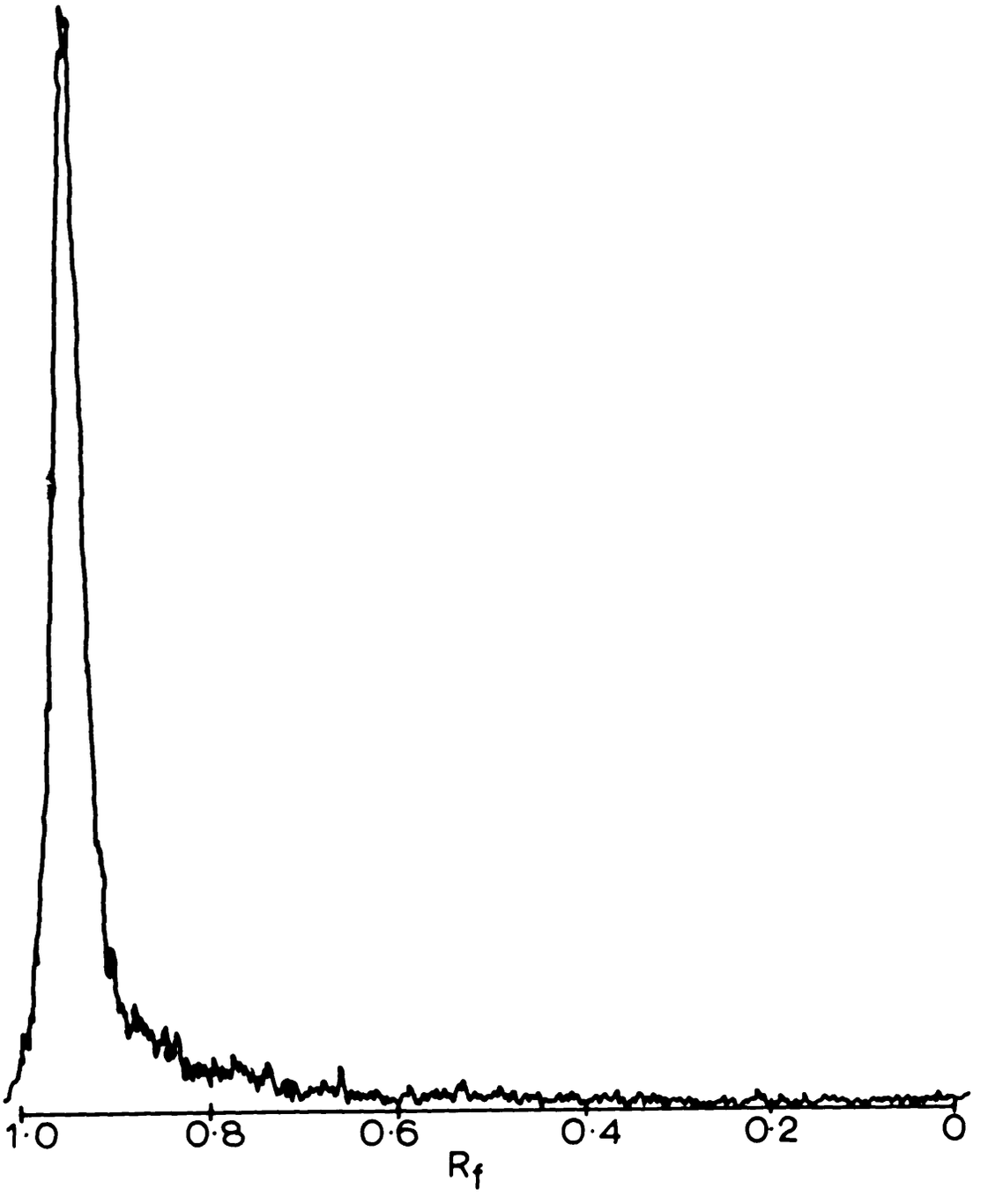
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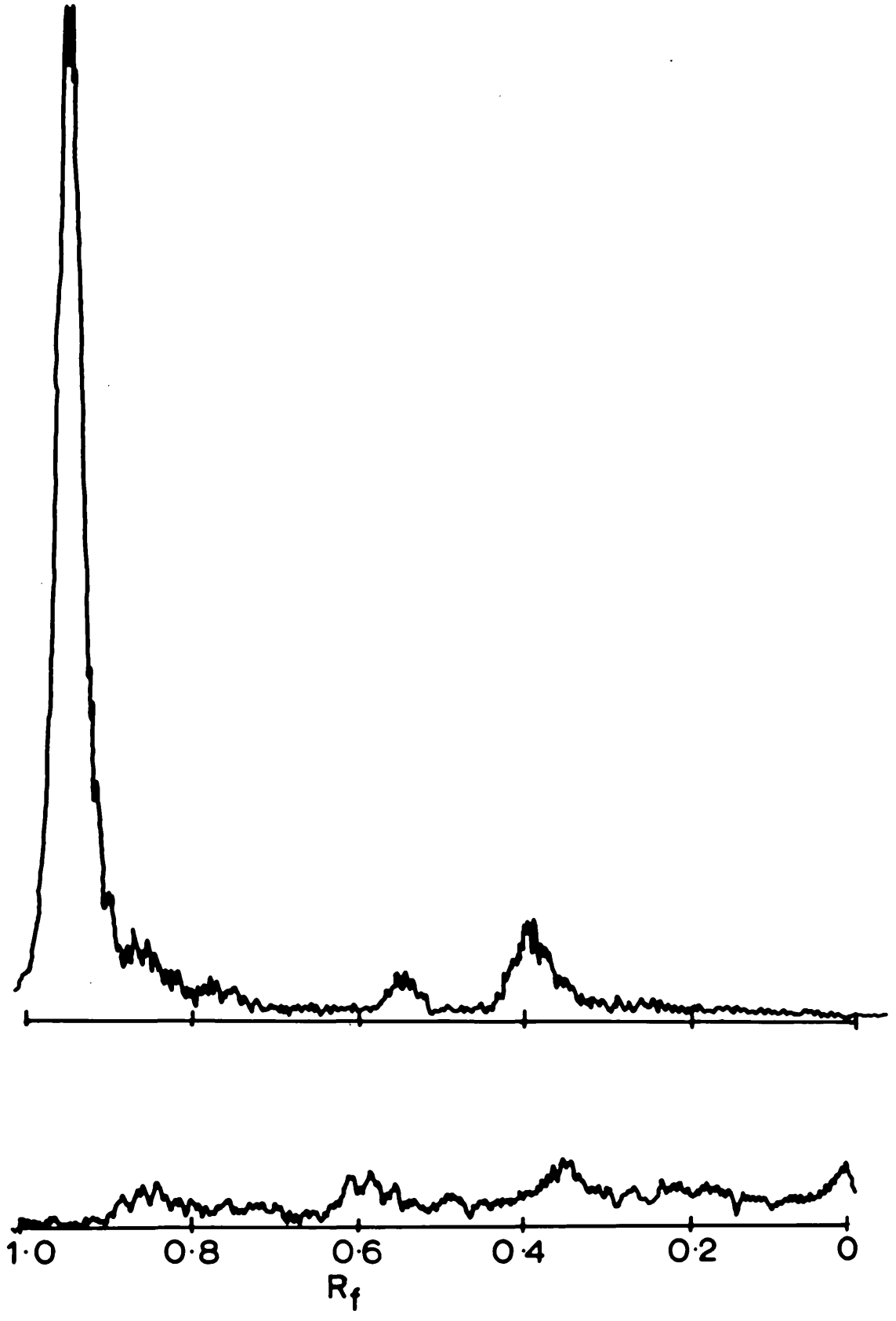
Fig. I.4 Scan of radiochromatograms of Coleus internode extracts after IAA-2-^{14}C treatment

The top scan represents a chromatogram of an extract of the middle portion of internode segments. It is 'spiked' with IAA-2-^{14}C. The lower scan represents a chromatogram of the fraction of the middle extract discarded by Halliday during his purification process. Both extracts have the same peaks at about 0.35 (the 'pigment mark') and at about 0.55-0.60 (the spot thought to represent IAAsp). The IAA in the spiked extract runs at about 0.95. The chromatograms were developed in butanol/acetic acid/water.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solution were pipetted onto the donors at the beginning of the experiment and after 12 h a further 10 μl were added. The chromatography solvents used were isopropanol/ammonia/water and butanol/acetic acid/water. Authentic samples of IAA-^{14}C and IAAsp were also chromatographed with the extracts. Chromatograms were spotted and developed in a dark-room under a green 'safelight'. The resulting chromatograms were first scanned for radioactivity and then exposed to X-ray film for three weeks. The portion of the chromatogram bearing the IAAsp marker was sprayed with Van Urk's reagent and dried in an oven at 50^{o}C. The position of the IAAsp was then marked by a blue/purple spot. These experiments showed that a labelled substance with an R_f value corresponding to that of authentic IAAsp was present in the internode extracts after both IAA-1-^{14}C and IAA-2-^{14}C treatments. It was present in all three sections and could be detected in both solvent systems. Extracts which had been subjected to the purification process were not different from unpurified extracts. Little activity was recovered from the particle suspensions which represented the material discarded by Halliday. When chromatographic results could be obtained, however, the same substances appeared on these chromatograms as on the chromatograms of the extracts. Typical scans and radiographs are shown in Figs. I.2 - I.6 and Plates I.1 - I.4.

IAAsp had an R_f value of 0.05 - 0.15 in isopropanol/ammonia/water and 0.45 - 0.55 in butanol/acetic acid/water. Quite a lot of material was held back at the origin in the alkaline solvent and this confused the picture somewhat since the IAAsp ran very close to the origin and it was not always possible to distinguish between the radioactive material at the origin and the spot thought to be IAAsp.

Some unchanged IAA remained in most extracts. This only appeared to represent a small percentage of the total activity in the extracts and in some

Fig. I.5 Scans of radiochromatograms of extracts of Coleus internode segments after IAA-2-^{14}C treatment

All three chromatograms were developed in butanol/acetic acid/water. The middle scan represents a chromatogram of the extract of the apical portion of the segments and the bottom scan represents the extract of the basal portion of the segments. Both scans have similar peaks. These are the 'pigment mark' at about 0.35, the peak thought to represent IAAsp at 0.50-0.55 and an IAA peak at about 0.90. The top scan is an extract of the donor. It has a large IAA peak and two smaller peaks similar to those found in the extract scans.

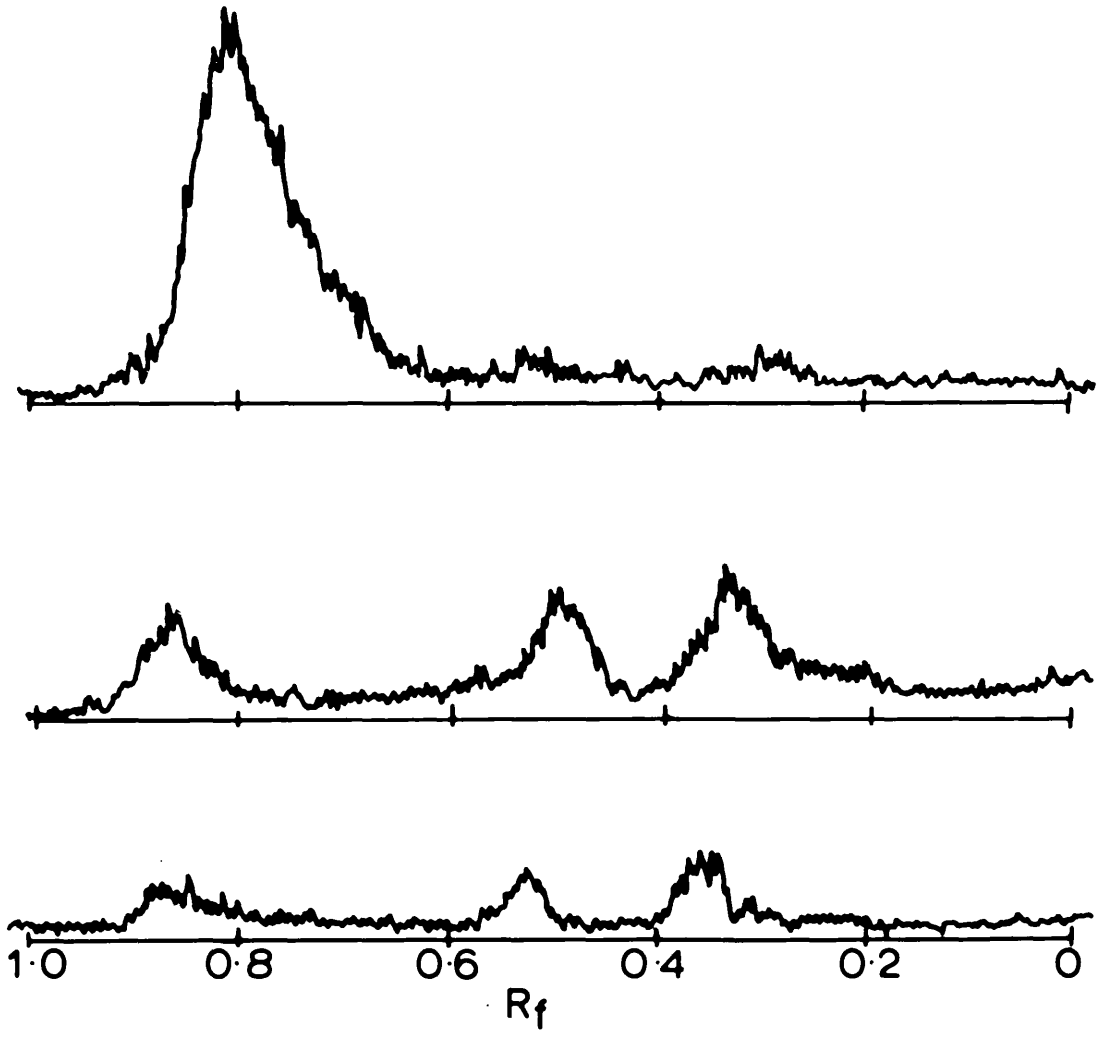
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Fig. I.6 Scans of radiochromatograms of extracts of Coleus internode segments after IAA-2-^{14}C treatment

The chromatograms were developed in butanol/acetic acid/water. The top scan is the extract of the basal portion of the segments and the bottom scan is the 'discarded' fraction produced during the purification of that extract. Both scans have the same two peaks - the 'pigment' peak and the IAAsp peak.

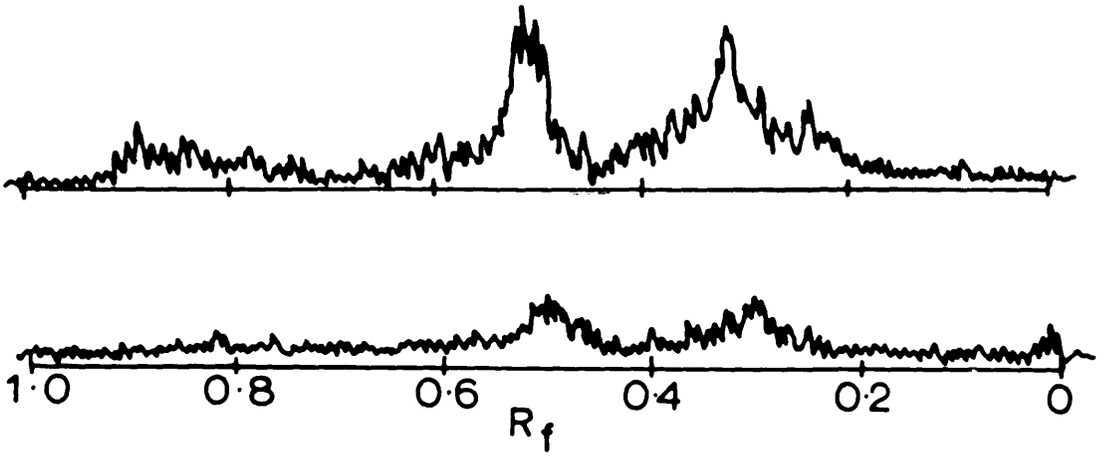
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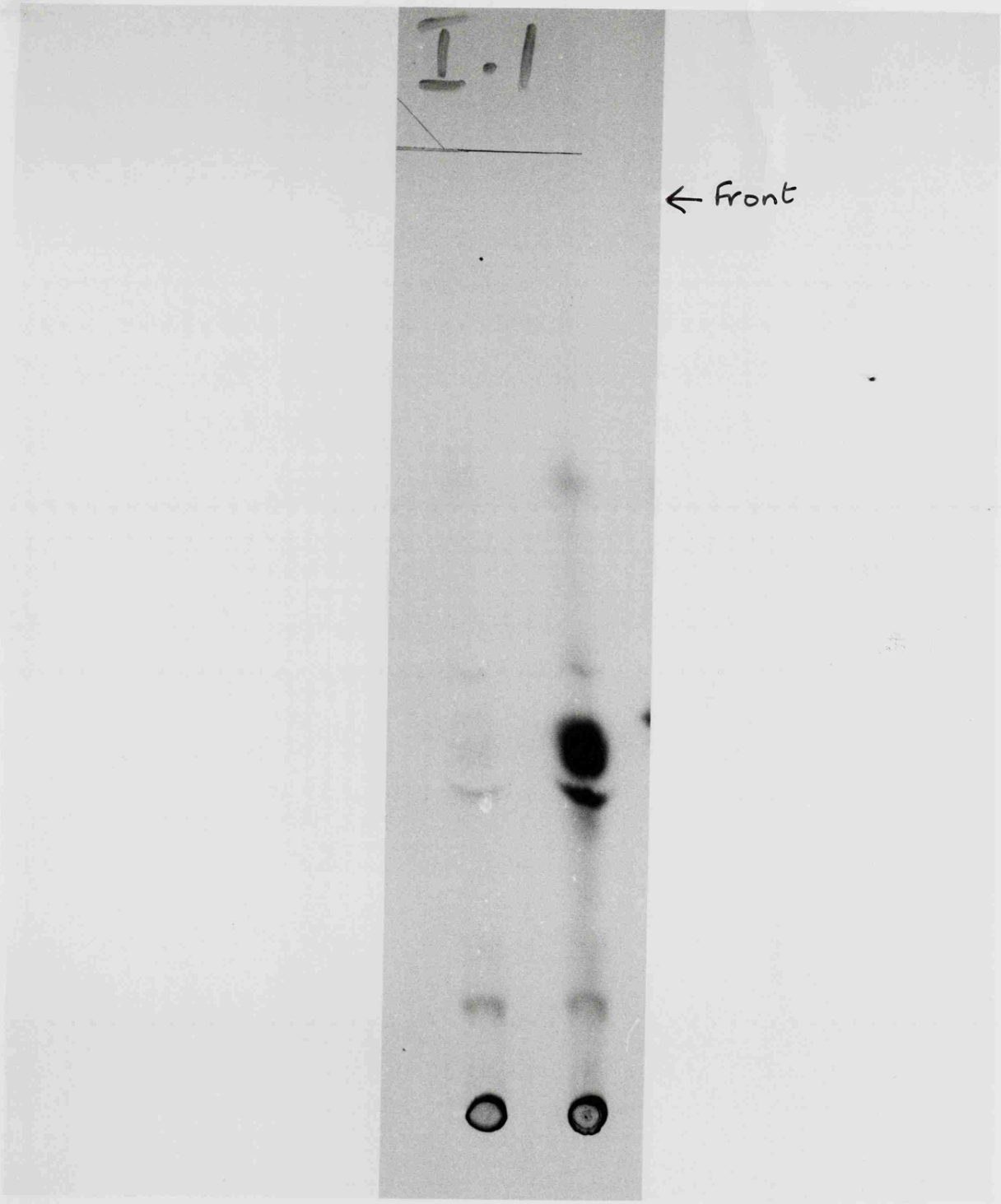
Plate I.1 Radiochromatograms of unpurified Coleus internode extract following 24 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. The left hand chromatogram is the extract of the middle section of the internode segments. The right hand chromatogram is 'spiked' with IAA-2-^{14}C. The spot with the R_f value of 0.15 is thought to be IAAsp. IAA runs at 0.44 and the spots at 0.41, 0.57 and 0.82 are impurities introduced with the IAA.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← Front

cases was barely discernible. IAA ran at 0.45 - 0.55 in isopropanol/ammonia/water and at 0.80 - 0.90 in butanol/acetic acid/water.

One other substance appeared regularly in the butanol/acetic acid/water chromatograms. This ran at 0.30 - 0.35 and corresponded in position and shape with a pigment spot which could be seen on the thin layer plate. This pigment was blue in colour. No separation of pigments occurred in the isopropanol/ammonia/water but the spots at the origin on the silica gel were brown in colour. The pigments were apparently among the materials which did not run in this solvent.

In spite of the precautions taken to maintain the guaranteed purity of the IAA-^{14}C as supplied by the manufacturers there was usually a contaminant in the IAA-^{14}C which was run as a marker. This could be seen in both the solvent systems and had an R_f value slightly lower than IAA in both cases. It appeared to enter the tissue and remain unchanged since it is visible in the radiographs of the chromatograms of all extracts. It is only visible in the radiographs, apparently being masked in the scans.

These results, though not conclusive, suggest that IAAsp is formed from exogenous IAA-^{14}C within 24 h of its application to Coleus internode segments and is present throughout the length of the segments. The presence of IAAsp is indicated whether methylene or carboxyl labelled auxin is used in the treatment and Halliday's purification technique is not responsible for its absence from the extracts.

Contamination of the donor extracts

One problem which arose during these experiments concerned the material extracted from the filter paper donors. The main constituent of the extract was IAA (as applied) but traces of other substances were also present. These

Plate I.2 Radiochromatograms of purified Coleus internode extract following 24 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. Chromatograms, from left to right, are marker IAA, middle extract and 'spiked' middle extract. IAAsp and IAA are indicated in the extract. The R_f value of IAA is depressed by the other constituents of the extract.

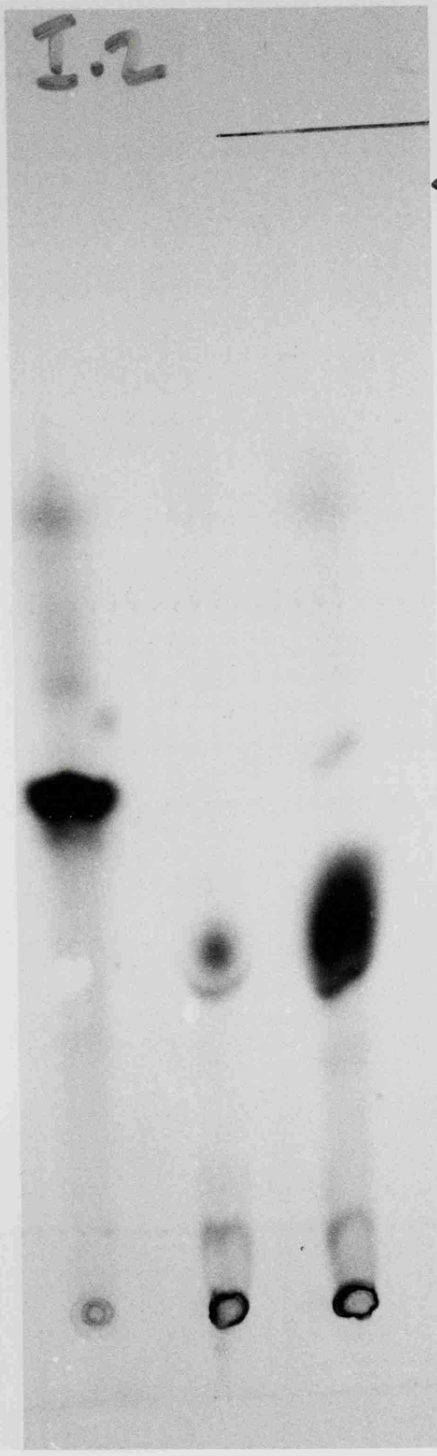
I.2
Front

Plate I. 3 Radiochromatograms of purified Coleus internode extract following 24 h basipetal IAA-2-^{14}C treatment.

The chromatograms were developed in butanol/acetic acid/water. The left hand chromatogram is middle extract. That on the right is 'spiked' with IAA-2-^{14}C. The 'pigment' mark runs at 0.40, IAAsp at 0.56 and IAA at 0.97.

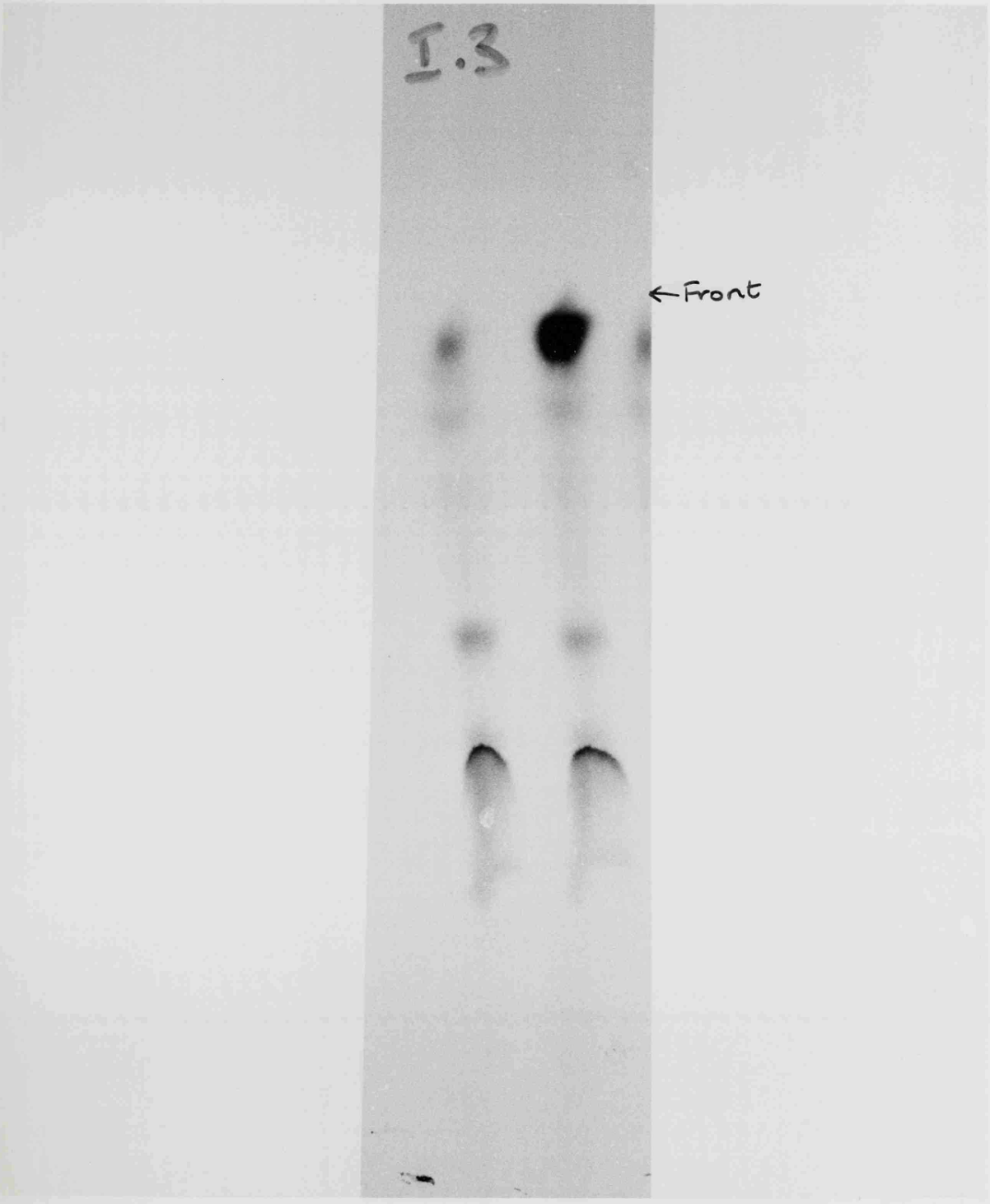
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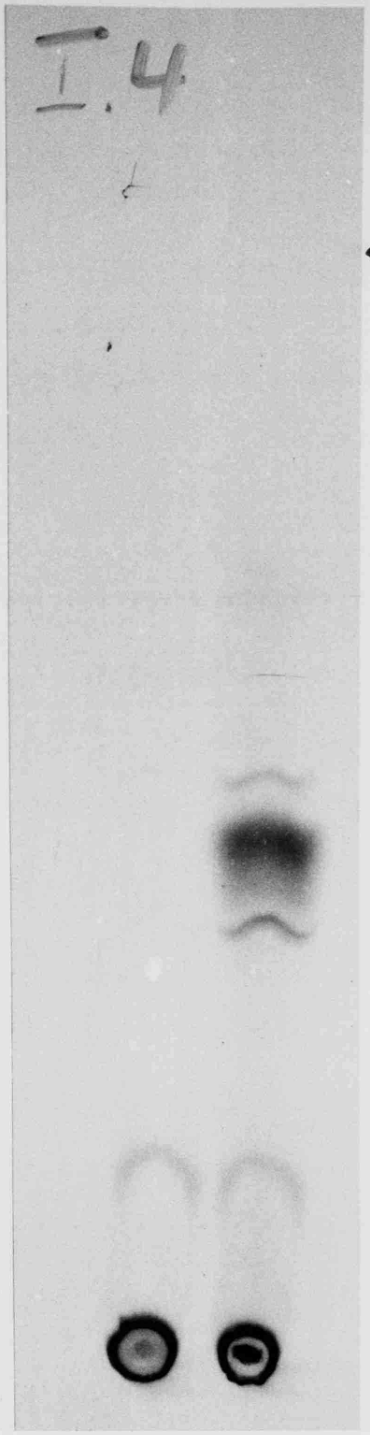
Plate I.4. Radiochromatograms of unpurified Coleus internode extract following 24 h basipetal IAA-1-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. The left hand chromatogram is middle extract and the right chromatogram is 'spiked' with IAA-1-^{14}C. IAAsp ran at 0.20, and IAA at 0.55. The spots above and below IAA are introduced contaminants.


I.4
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had R_f values corresponding to IAAsp and to the blue spot reported above (see Fig. I. 5). There were two possible interpretations of this finding. The first was that IAA had undergone some change while still in the donor, and that this was not, therefore, a physiological process. The second interpretation was that the products of IAA metabolism in the internode tissue had diffused back into the donor system. To check out these possibilities a further experiment was performed in which the internode segments used above were replaced by agar cylinders of similar dimensions. IAA-2-^{14}C solution was applied in a donor and the system was left in a dark-room under a green 'safelight' for 24 h. The two components of the system were extracted separately and chromatographed with an authentic sample of IAA-2-^{14}C.

The results showed that the extracts of the donors and agar cylinders were exactly similar to the IAA-2-^{14}C sample (see Figs. I. 7 and I. 8). IAA does not undergo any significant change during a 24 h period under experimental conditions in the absence of plant material (except for the production of the contaminant which appears in the marker solution as mentioned above). The substances seen in the internode extracts can thus be assumed to be products of metabolism.

Internode segments from flowering plants

Since polarity of transport is thought to be greatly reduced during flowering in plants the possibility existed that the whole transport mechanism could be altered with regard to the tissues involved in transport, the capacity of the system to transport and immobilise material and the products it could form. The experiment described above was thus performed with internode segments taken from Coleus plants which were in bloom. The segments were taken from the usual internode and were again 15 mm in length. Other experimental methods were as previously described. Plates I. 5 and I. 6 show

Fig. I.7 Scans of radiochromatograms of extracts of the components of a non-biological transport system designed to test for decomposition of IAA under experimental conditions

The chromatograms were developed in butanol/acetic acid/water. The top scan is an IAA-2-^{14}C marker. The middle scan is the extract of the agar cylinder through which the IAA-2-^{14}C was allowed to move. The bottom scan is an extract of the donor. In all cases only one peak occurs. It has an R_f value of about 0.90.

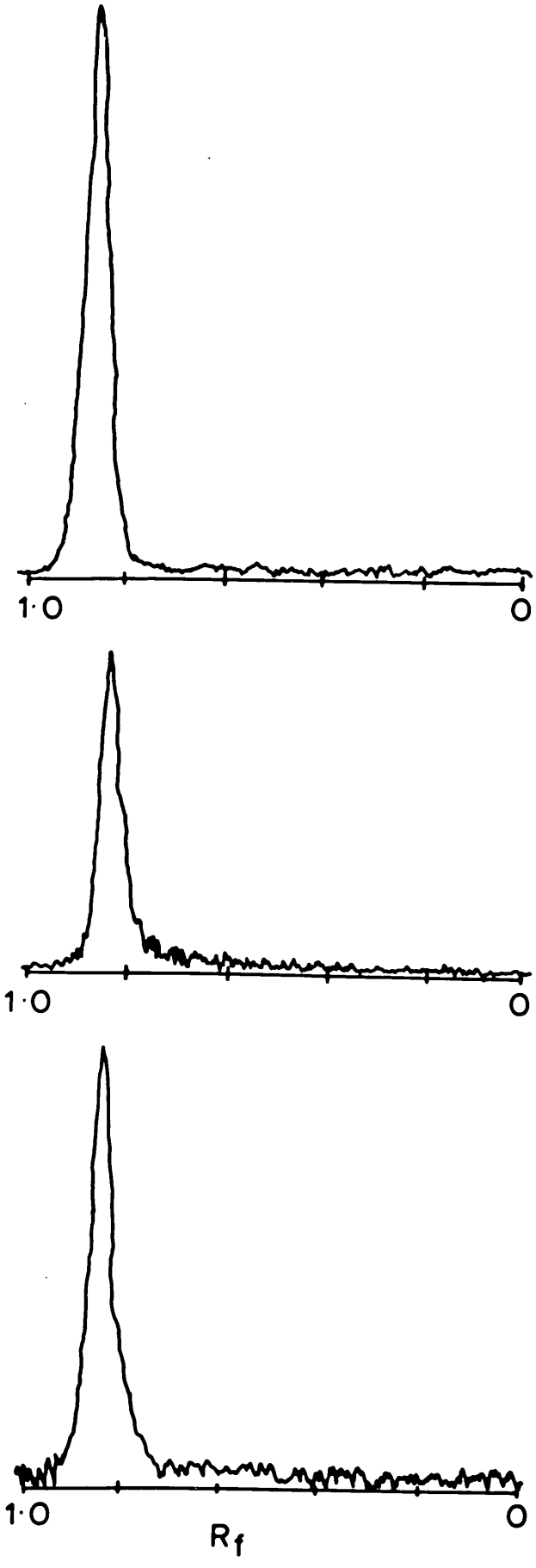
1·0
0
1·0
0
1·0
0
R_f

Fig. I.8 Scans of radiochromatograms of extracts of the components of a non-biological transport system designed to test for decomposition of IAA under experimental conditions

The chromatograms were developed in isopropanol/ammonia/water. The top scan is an IAA marker. The middle scan is the extract of the agar cylinder through which the IAA-2-^{14}C was allowed to move. The bottom scan is an extract of the donor. In all cases only one peak occurs. It has an R_f value of about 0.90.

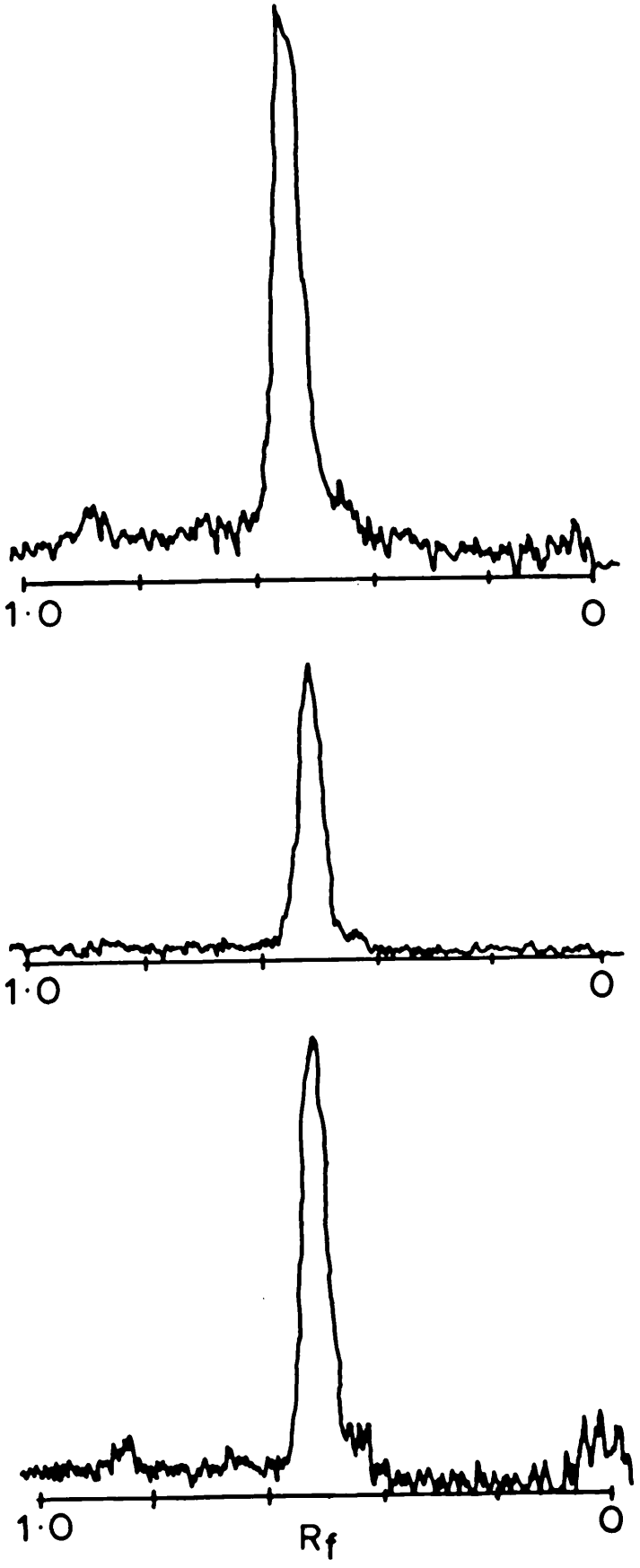
1·0
0
1·0
0
1·0
Rf
0

Plate I. 5 Radiochromatograms of Coleus internode extract following 24 h basipetal IAA-2-^{14}C treatment of segments from flowering plants

The chromatograms were developed in isopropanol/ammonia/water. Chromatograms are, from left to right, internode extract, IAA, 'spiked' extract. IAAsp runs at 0.07/0.10 and IAA runs at 0.35.

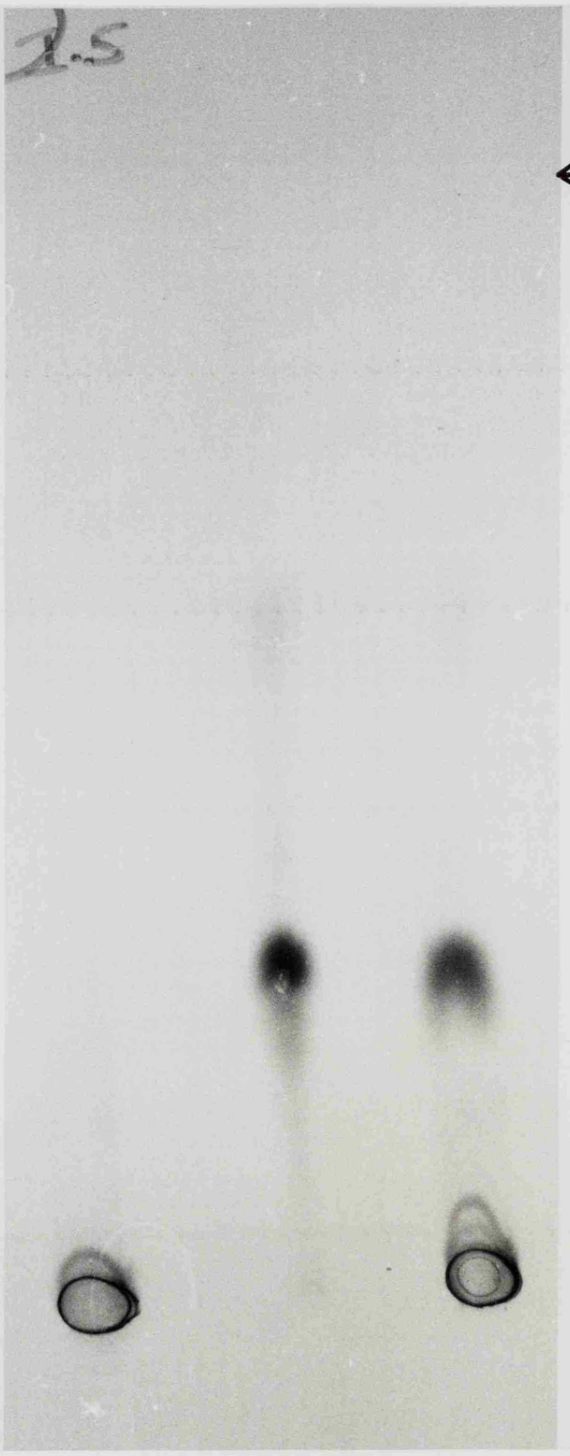
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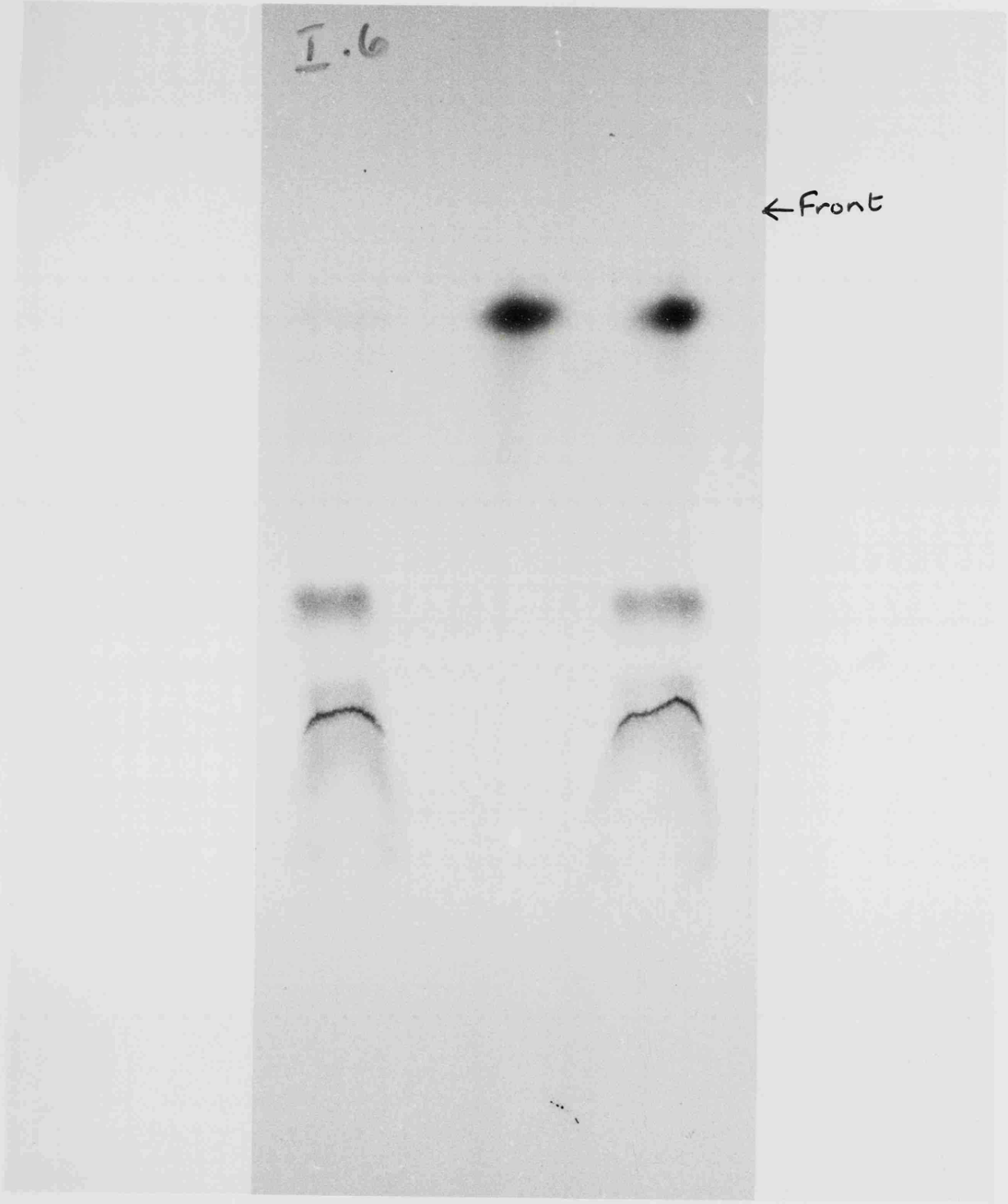
Plate I. 6 Radiochromatograms of Coleus internode extract following 24 h basipetal IAA-2-^{14}C treatment of segments from flowering plants

The chromatograms were developed in butanol/acetic acid/water. Chromatograms are, from left to right, internode extract, IAA, 'spiked' extract. The 'pigment' spot ran at 0.37. IAAsp ran at 0.50 and IAA at 0.89.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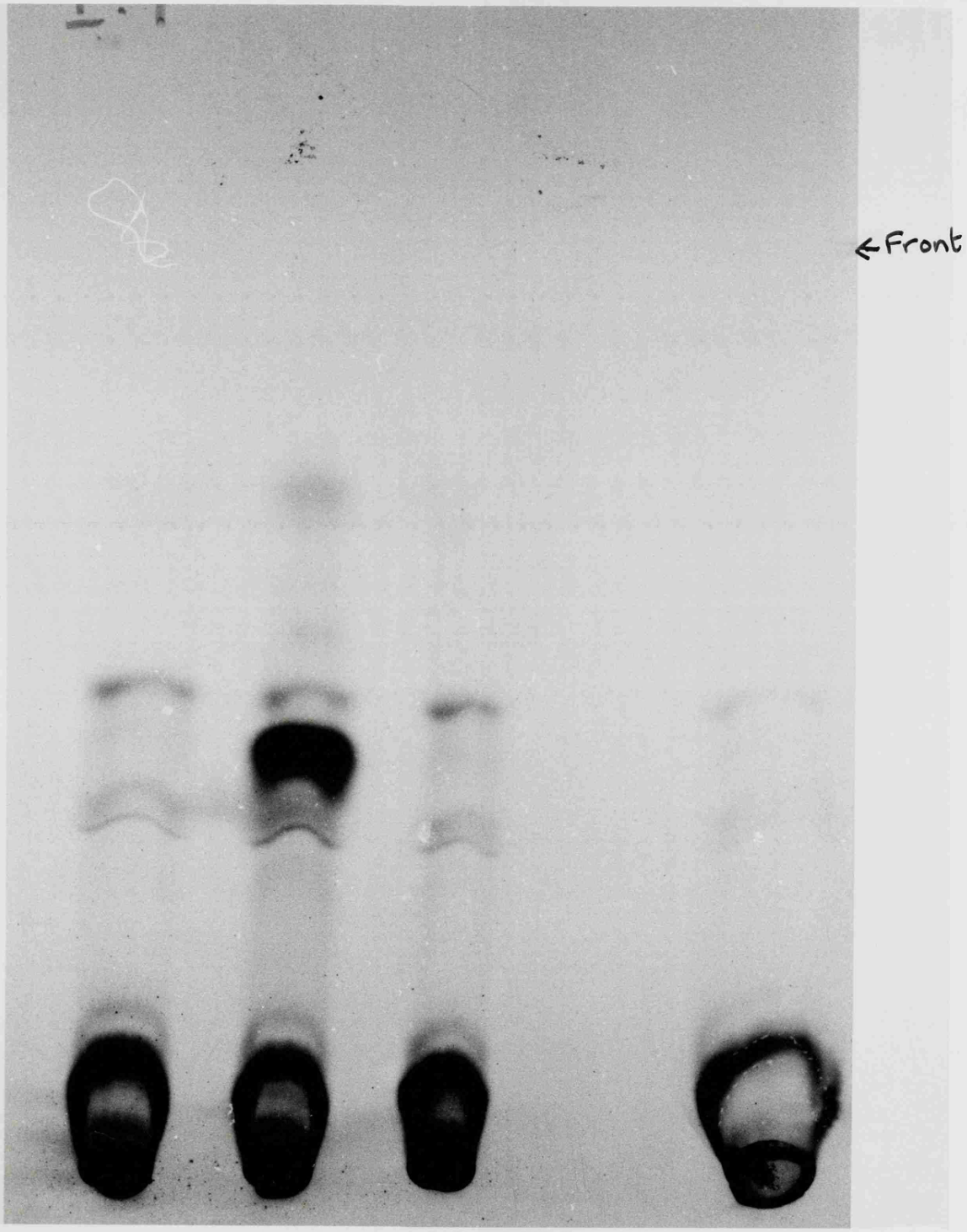
radiographs of the chromatographed extracts of internode segments after a 24 h transport period following apical application of IAA-2-^{14}C. The results of this experiment are very similar to those obtained from earlier experiments in which vegetative plants were selected. Little or no unchanged IAA appears to be present and the IAAsp spots are quite prominent. In the butanol/acetic acid/water system the spot corresponding to the pigment on the silica layer is again present.

Acropetal transport

Different mechanisms are thought to be responsible for acropetal and basipetal transport of auxins. It therefore seemed possible for the reasons stated above that the metabolic products of IAA transport could also differ according to the direction of translocation of the auxin. Internode segments were, therefore, extracted after a period of transport with a basally applied donor. The method of conducting the experiment was as previously described but the segments were inverted in the bridges and the donors were in contact with the basal (upper) cut surfaces. In this experiment one modification was introduced in that one of the extract spots on the chromatogram was spiked with authentic IAAsp solution. The advantage of such a situation is that the authentic IAAsp is more likely to run at the same R_f as any IAAsp in the extract because it, too, will be affected by other constituents of the extract. When that region of the chromatogram was treated with Van Urk's reagent the position of the colour spot which became visible was marked with a mounted needle. A dotted line was made around the edge of the spot. When the chromatogram was exposed to X-ray film this dotted outline was visible on the radiograph (see Plate I. 7). It can be seen to correspond very well in shape and position to the radioactive spot thought to be IAAsp. The radioactive material alone

Plate I. 7 Radiochromatograms of Coleus internode extract following 24 h acropetal IAA-2-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. The second chromatogram from the left is 'spiked' extract. The right hand one shows the outline of the 'cold' IAAsp. colour marker which corresponds in position with the radioactive spot at 0.13/0.15. IAA ran at 0.53.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would not have been concentrated enough to produce a colour reaction and only one colour spot was visible anyway. It therefore seems reasonable to assume that the colour reaction resulted from the presence of the marker spot which coincided in position with a prominent radioactive spot. Two spots were visible on the chromatogram with R_f values just below and just above that of IAA. These probably represented contaminants introduced with the IAA. Again the indications are that little or no unchanged IAA is present in the extracts and that the major constituent is IAAsp.

Use of new chromatography solvents

In a further attempt to investigate IAA metabolism in Coleus internode segments, extracts were prepared and chromatographed with different chromatography solvents (chloroform/ethyl acetate/formic acid and chloroform/methanol/acetic acid). Plate I.8 shows the radiographed chromatogram of an extract resulting from a 24 h period of basipetal transport of IAA-2-^{14}C in internode segments. Plate I.9 shows a radio-chromatogram from a similar experiment employing IAA-1-^{14}C. Again both extracts are seen to be similar. The spot corresponding to the IAAsp marker is seen at 0.30 - 0.40. In both cases there is another dark spot below this (R_f = 0.17 - 0.20) and faint spots occur at a higher R_f value than IAAsp. These are darker in the extracts 'spiked' with IAA and so may be introduced with the IAA. Little or no unchanged IAA is indicated in the extracts. The identity of the material in the other spots is not known. Plate I.10 shows the same extract as Plate I.9 chromatographed in chloroform/methanol/acetic acid. The spot corresponding to the IAAsp marker runs at an R_f value of about 0.25. There is one spot with a higher R_f than the IAAsp and also evidence of the presence of some IAA. Thus in these solvent systems the presence of at least one other metabolite besides IAAsp is indicated.

Plate I. 8 Radiochromatograms of Coleus internode extract following 24 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in chloroform/ethyl acetate/ formic acid. The middle chromatogram is 'spiked' with IAA-2-^{14}C which has an R_f value of 0.67. The spot thought to represent IAAsp runs at 0.30 and another distinct spot runs at 0.17. No IAA can be seen in the extract.


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Plate I.9 Radiochromatograms of Coleus internode extract following 24 h basipetal IAA-1-^{14}C treatment

The chromatograms were developed in chloroform/ethyl acetate/formic acid. The second chromatogram from the left is 'spiked' with IAA-1-^{14}C which has an R_f value of 0.83. The dark spot at 0.37/0.40 coincides with Van Urk colour spot. Another distinct spot runs at 0.20. Very little IAA occurs in the extract.

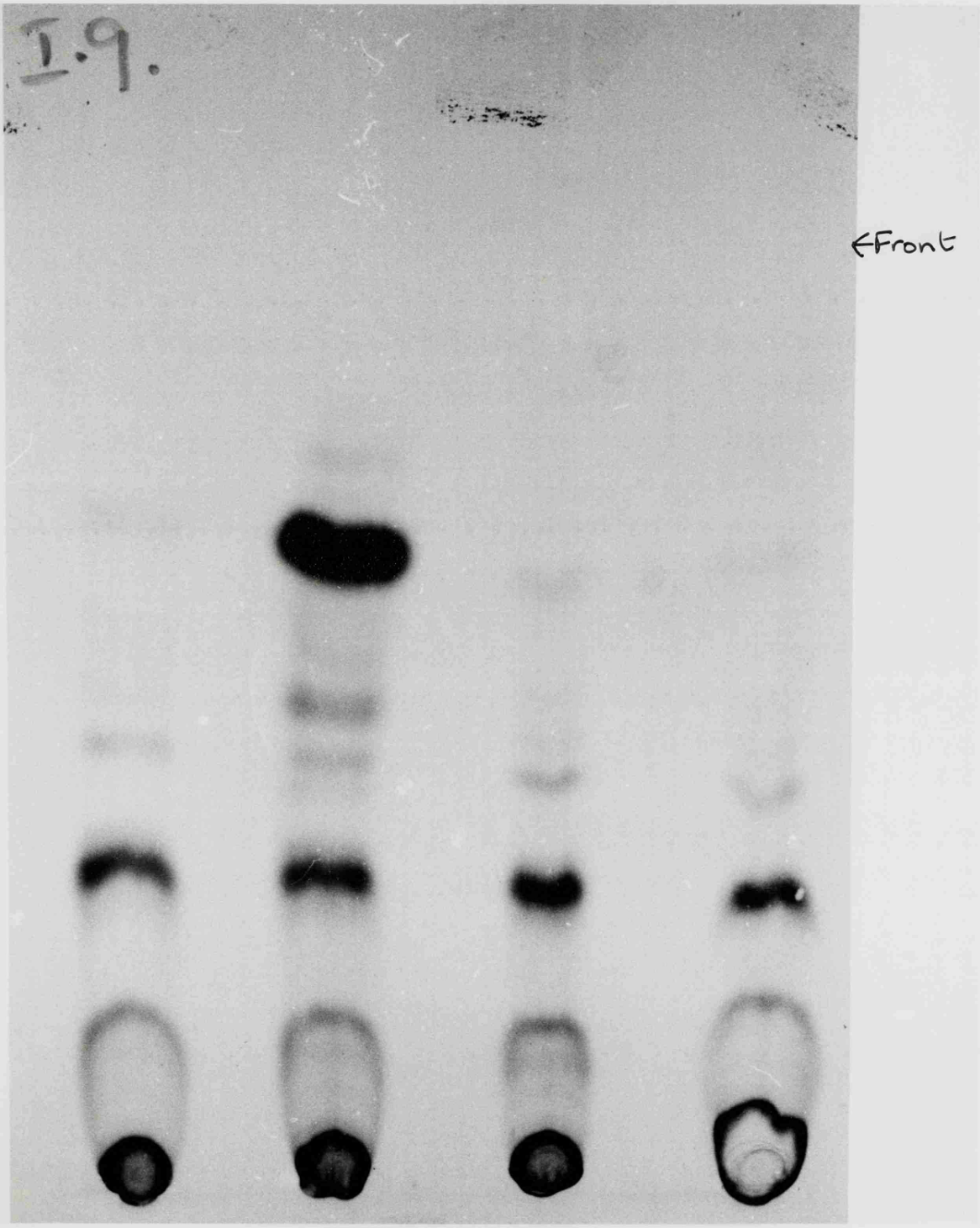
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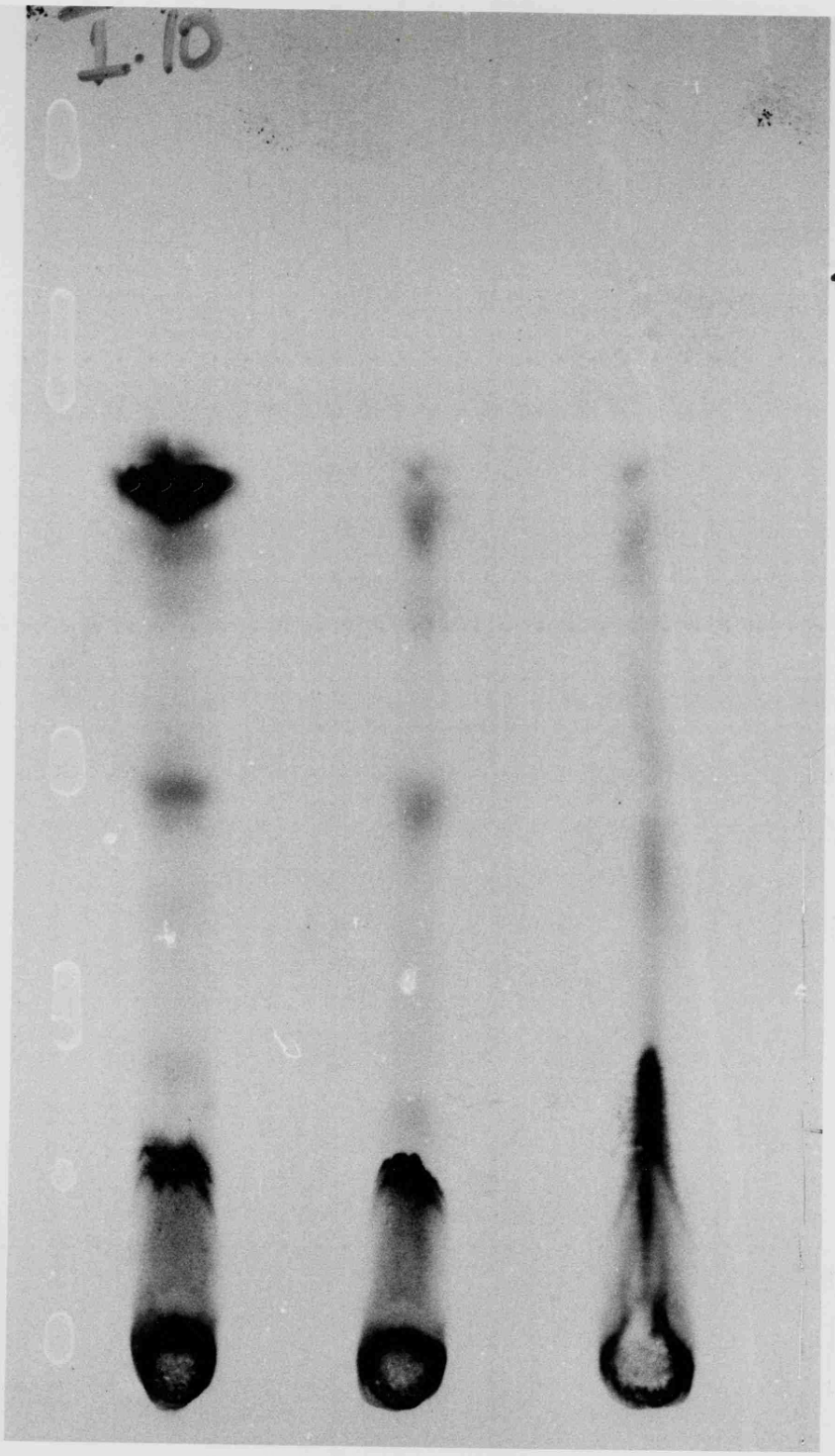
Plate I.10 Radiochromatograms of Coleus internode extract following 24 h basipetal IAA-1-^{14}C treatment

The chromatograms were developed in chloroform/methanol/acetic acid. The first chromatogram on the left is 'spiked' with IAA-1-^{14}C which has an R_f value of 0.97. The spot thought to represent IAAsp because of its correspondence with the Van Urk colour spot runs at 0.23. Little IAA occurs in the extracts.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There is no radioactivity associated with pigmentation in either of these systems.

Modified extraction techniques

The chromatograms obtained in many of the early extraction experiments were not of a very high quality. Halliday's purification technique had not greatly altered the chromatographic properties of the extracts so was not routinely used. It seemed necessary to reduce the volume of the extracts efficiently so rotary film evaporation was used.

After removal of the tissue the extracts were subjected to rotary film evaporation under reduced pressure at 25°C. It did not prove possible to reduce extracts to dryness under these conditions because of the presence of water in the extracts. The use of higher temperatures would probably have adversely affected the extracts. It was therefore decided to remove water from the tissue before extraction, by freeze-drying. After the transport period the tissue was placed in open-topped glass vials and put in an Edwards freeze-drying machine for 24 h. If the segments were to be divided into smaller portions for separate extraction, this was done before the tissue was dried. The dried tissue was then cut into thin longitudinal slices and extracted as previously described. The extract so obtained could then be evaporated to dryness in a rotary film evaporator and taken up in a small volume of methanol. The concentrated extracts were stored in the dark at 0°C until required for chromatography.

Plate I.11 is the radiograph of a chromatogram run in chloroform/ethyl acetate/formic acid. The internode extract was prepared from segments in which basipetal transport of IAA-1-^{14}C had been allowed to proceed for 24 h and it was subjected to rotary film evaporation before being applied to the

Plate I.11 Radiochromatograms of Coleus internode extract and petiole extract following 24 h basipetal IAA-1-^{14}C treatment

The chromatograms were developed in chloroform/ethyl acetate/formic acid. The first and fourth chromatograms are internode extracts and the rest are petiole extracts. The third chromatogram from the left is 'spiked' with IAA-1-^{14}C which has an R_f value of 0.84 and several impurities. The spot corresponding to the 'cold' IAAsp marker runs at 0.41/0.47. The identity of the spots at 0.21 and 0.60 is unknown. No differences exist between the two extracts. No IAA appears to occur in the extracts.

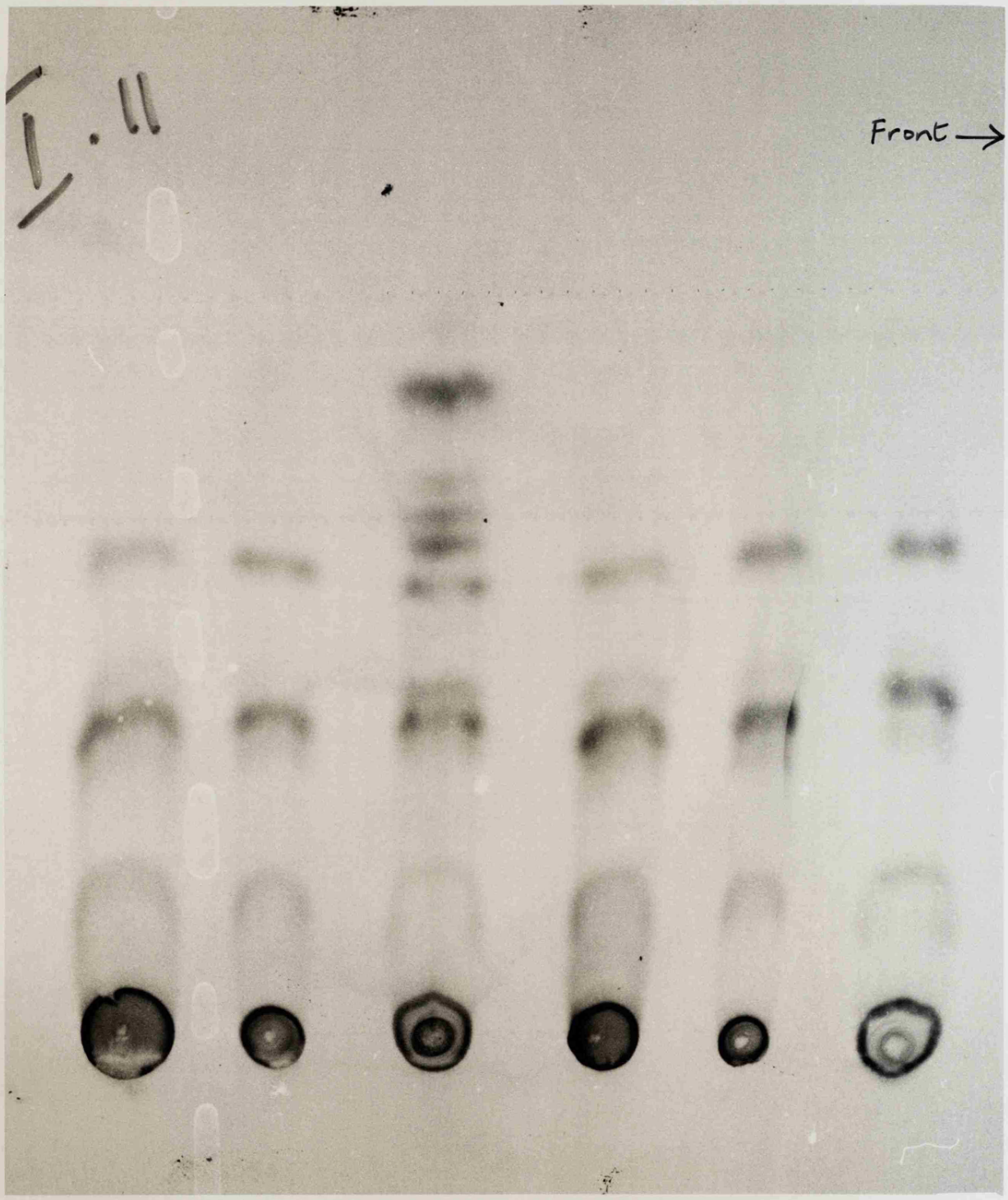
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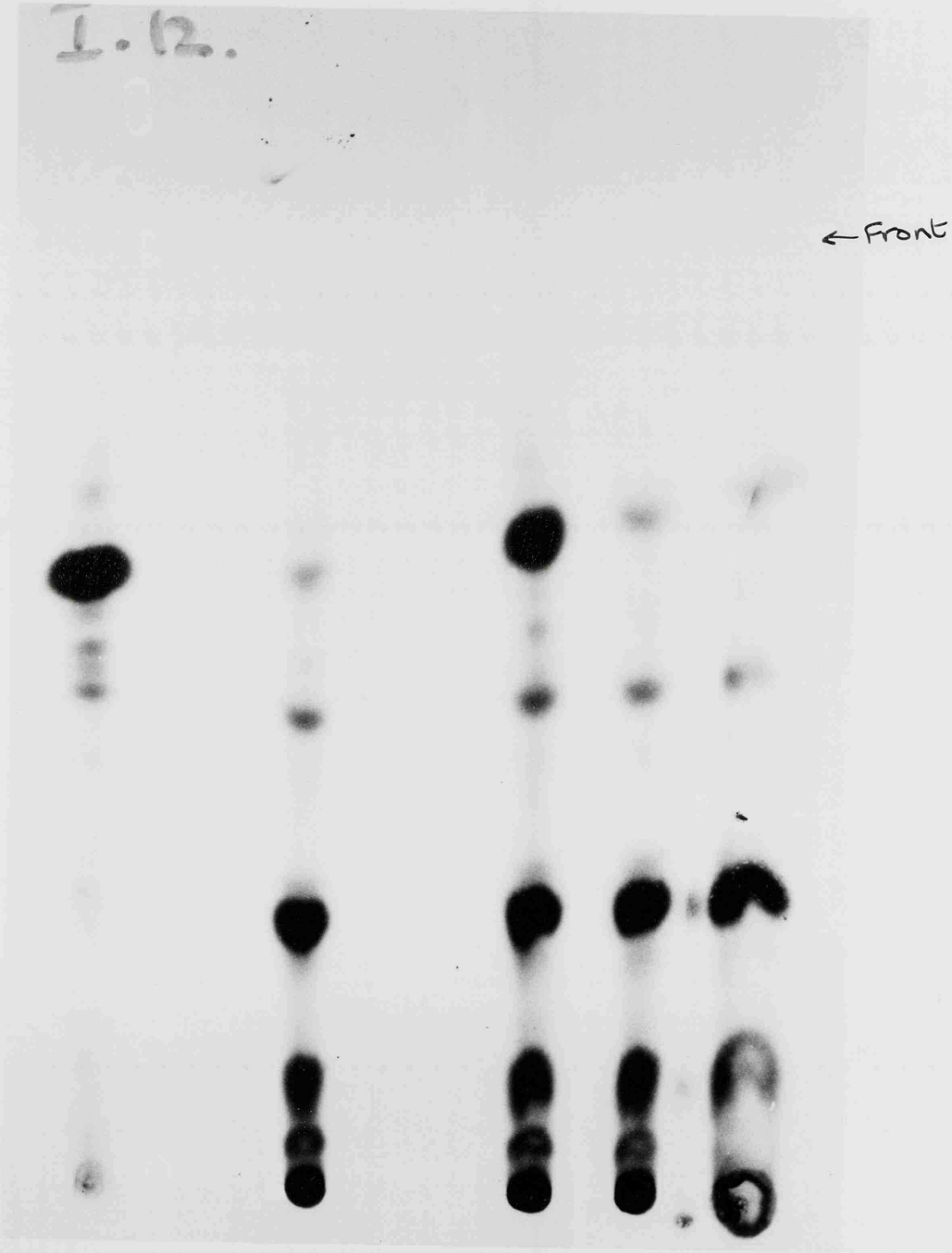
Plate I.12. Radiochromatograms of Coleus internode extracts following 24 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in chloroform/ethyl acetate/formic acid. The chromatogram on the left is an IAA marker with an R_f value of 0.77. Several impurities occur in the IAA but none corresponds with the 2 major spots in the extracts. The third chromatogram is internode extract 'spiked' with IAA. The second and fourth chromatograms are internode extract. The spot at 0.30/0.33 corresponds with the IAAsp marker (see the dotted outline in the right hand chromatogram). Unknown compounds run at 0.07 and 0.13. Little unchanged IAA appears to occur in the extract.

I.12.



chromatography plate. The extract shown in Plate I.12 is from internodes treated apically with IAA-2-^{14}C for 24 h. Freeze-drying and rotary film evaporation were employed in the preparation of the extract. Plates I.11 and I.12 are quite similar. In each case the IAAsp spot is quite dark and as in Plate I.8 there is another obvious spot with an R_f value lower than that of IAAsp. This appears as two spots on Plate I.12. In both Plates I.11 and I.12 there is another distinct spot with an R_f value higher than that of IAAsp but lower than that of IAA. This spot corresponds to the faint spots in a similar position in Plates I.8 and I.9. The identities of the materials in these two spots are not known. Again little or no IAA is indicated.

The techniques of freeze-drying and rotary film evaporation help to improve the clarity of chromatograms and by increasing the concentration of the extracts reduce the exposure time required to produce radiographs. The techniques were routinely used for subsequent experiments.

Petiole segments

Petiole segments were also investigated by extraction. Segments, 15 mm long, were cut from the third leaf pair below the apex. The segments were cut from just below the lamina and only one segment was cut from each petiole. The segments were treated as internode segments were treated above. Plate I.11 shows the radiochromatogram of a petiole extract run alongside an internode extract. In both cases transport was basipetal and the auxin used was carboxyl-labelled. It can be seen that there is no difference between the two extracts. Similar products are formed during acropetal transport of IAA-1-^{14}C in petiole segments (Plate I.13) and during basipetal and acropetal transport of IAA-2-^{14}C in petiole segments (Plates I.14 and I.15). The only difference is that more unchanged IAA appears to be present after the two

Plate I.13. Radiochromatograms of Coleus petiole extracts following 24 h acropetal IAA-1-^{14}C treatment

The chromatograms were developed in chloroform/methanol/acetic acid. The second chromatogram from the left is 'spiked' with IAA-1-^{14}C. The IAA has an R_f value of 0.60. Three impurities also occur in this marker. Three spots occur in the extract chromatograms (the first and third chromatograms), at 0.13, 0.28 and 0.43. That at 0.28 can be seen to be surrounded by a dotted line in the right hand chromatogram. This marks the outline of the IAAsp marker spot with which the extract was 'spiked'. No IAA appears to occur in the extract.

I.13.

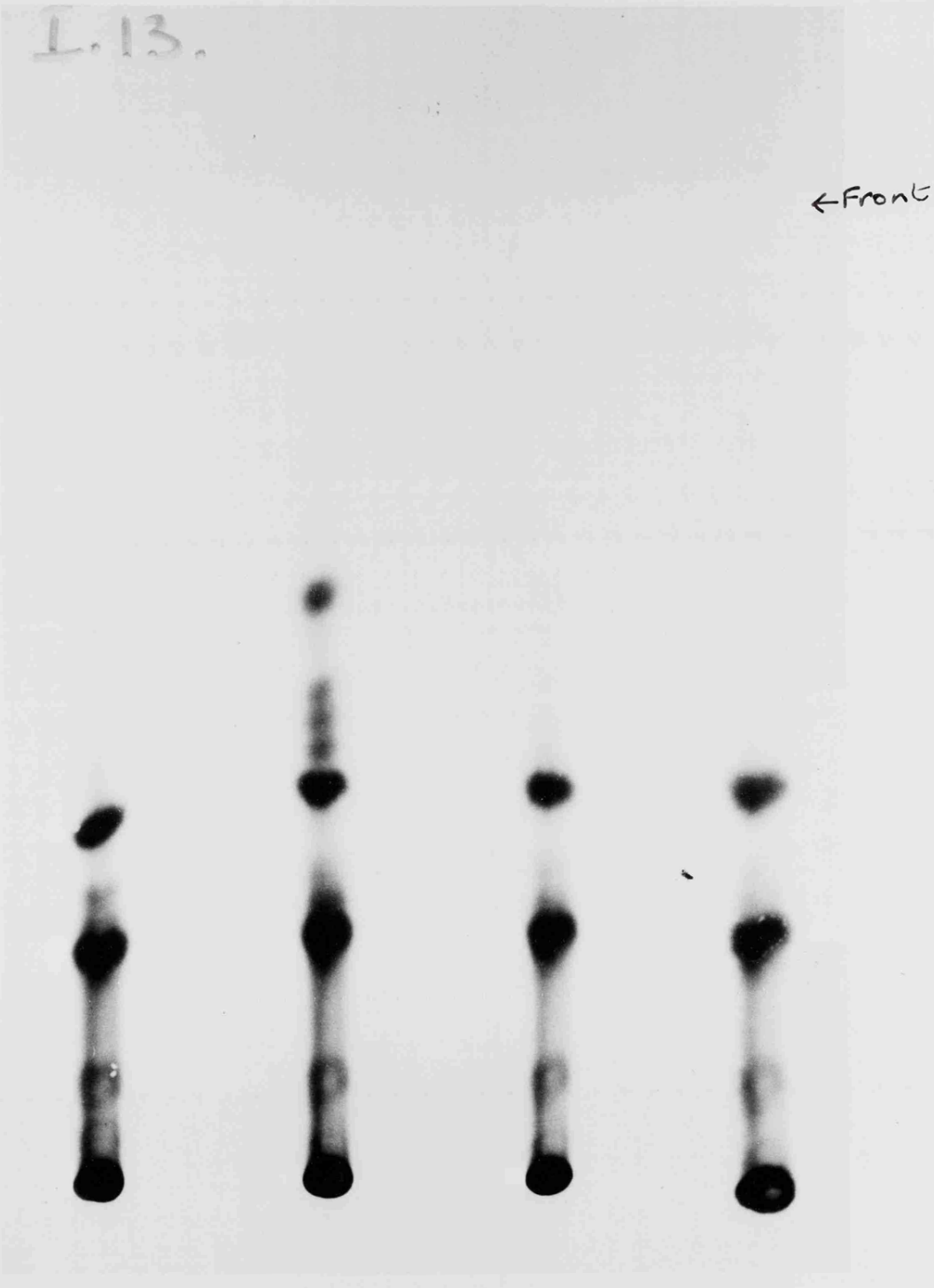


Plate I.14 Radiochromatograms of Coleus petiole extracts following 24 h acropetal and basipetal IAA-2-^{14}C treatment

The chromatograms were developed in chloroform/methanol/acetic acid. The first and fourth chromatograms are the extracts from the petiole segments which had received basipetal treatment. The others are from the acropetally treated segments. The third chromatogram from the left is 'spiked' with IAA-2-^{14}C which has an R_f value of 0.97. The spot which coincides with the IAAsp colour marker runs at 0.35 (see dotted outline of colour marker on the right hand chromatogram). Other faint, unidentified spots run at 0.08, 0.28, 0.51 and 0.90. Some IAA appears to be present in the extracts.

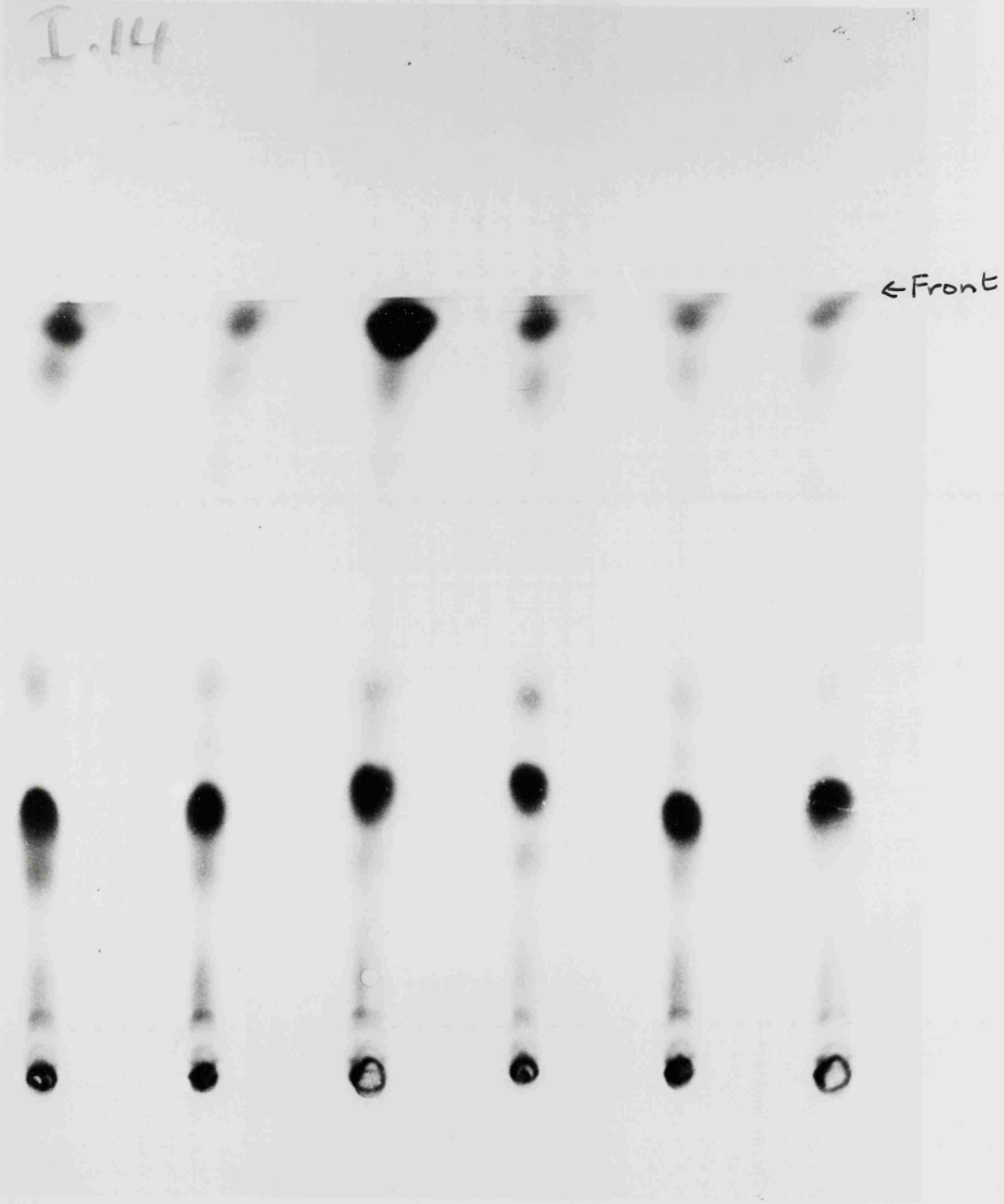

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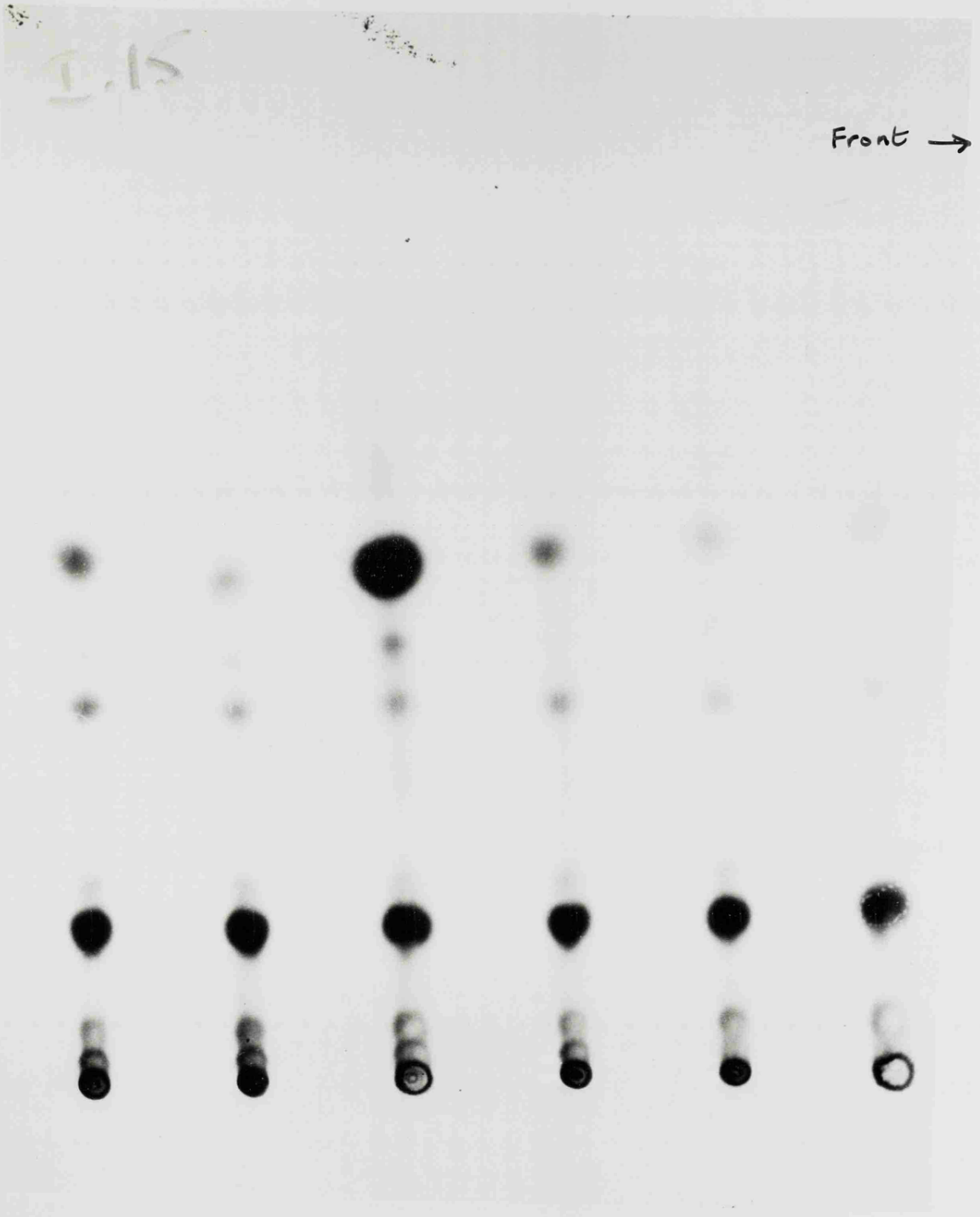
Plate I.15. Radiochromatograms of Coleus petiole extracts following 24 h acropetal and basipetal IAA-2-^{14}C treatment.

The chromatograms were developed in chloroform/ethyl acetate/formic acid. The first and fourth chromatograms are the extracts from the petiole segments which had received basipetal treatment. The others are from the acropetally treated segments. The third chromatogram from the left is 'spiked' with IAA-2-^{14}C which has an R_f value of 0.67. The substance thought to be IAAsp (correspondence with the colour spot can be seen in the right hand chromatogram which was 'spiked' with IAAsp) has an R_f value of 0.18. The spots above and below the IAAsp have not been identified although the latter could be IAGlu. Some IAA appears to be present in the extracts.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IAA-2-^{14}C treatments (Plates I.14 and I.15) than after the acropetal IAA-1-^{14}C treatment (Plate I.13). The presence of IAA was also indicated in Plate I.10 where internode segment extracts were chromatographed.

The use of Ninhydrin

Ninhydrin may be used as a detection agent for IAAsp because it reacts with the amino acid residue (cf. the reaction between Van Urk's reqgent and the indole moiety of the molecule). Internode extracts were, therefore, prepared as described and chromatographed alongside an authentic sample of IAAsp solution. The part of the chromatography plate bearing the IAAsp marker was then sprayed with ninhydrin acetic acid. The colour spot so obtained usually corresponded with a radioactive spot in the extract chromatogram but the marker did not always produce a good colour reaction. Ninhydrin was not found to be as reliable as Van Urk's reagent so its use was discontinued.

Other products of IAA metabolism

There are references in the literature (Davies, 1972) to the production of indole-3-acetamide (IAamd) in IAA-treated tissue. To test this possibility in Coleus internode segments extracts were chromatographed alongside an authentic sample of IAamd. The position of the IAamd spot was detected by use of Van Urk's reagent.

When butanol/acetic acid/water was used as a chromatography solvent there was no radioactive spot corresponding in position to the IAamd marker. In the isopropanol/ammonia/water system the colour spot did not correspond with any of the radioactive spots in the internode extracts but did correspond with a spot in the IAA marker chromatogram (R_f = 0.75 - 0.80). It therefore seems likely that one of the impurities seen in the marker chromatograms is formed

Plate I.16 Radiochromatograms of Coleus internode extract following 30 min basipetal IAA-2-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. Most of the activity is associated with IAA which has an R_f value of 0.62 and has a contaminant running just behind it. A very faint spot runs at 0.17 which could be IAAsp.

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by a reaction between the IAA and the ammonia in the chromatography solvent. This has also been reported by Zenk (1961). The R_f value of IAamd in chloroform/ethyl acetate/formic acid is about 0.60 and spots occur at this R_f value in chromatographed extracts (see Plates I.8, I.9 and I.15). The presence of IAamd has not been confirmed, however. In chloroform/methanol/acetic acid IAamd runs very close to the front as does IAA which would therefore mask its presence.

Another metabolite of IAA which has been extracted from treated tissue is indole-3-acetyl glucose (IAGlu) (Zenk, 1961, 1963; Winter and Thimann, 1966; and Winter, 1967). This is not commercially available and it is not therefore possible to chromatograph an authentic sample. In the chloroform/ethyl acetate/formic acid chromatography system the R_f of IAGlu is quoted as being about 0.10 (Zenk, 1963) and a faint spot with an R_f value similar to this consistently appeared in chromatograms developed in this solvent (see Plates I.8, I.9 and I.15). Winter (1967) quoted the R_f value of IAGlu in butanol/acetic acid to be about 0.60 - 0.70. Such a spot was not visible in chromatograms run in butanol/acetic acid/water in these experiments. The R_f of IAGlu in isopropanol/ammonia/water is in the region of 0.60 and the presence of the glycoside was not indicated in the Coleus extracts examined here.

Thus it would appear that IAamd and IAGlu may be formed from exogenously applied IAA in Coleus internode tissue but that they are probably not major metabolites. In all cases examined IAAsp seems to be the metabolite which is most prominent. The level of IAA present in extracts seemed somewhat variable but usually appeared quite low.

Time course of IAA metabolism

All the experiments described so far were of 24 h duration and the products

Plate I.17 Radiochromatograms of Coleus internode extract following 30 min basipetal IAA-2-^{14}C treatment

The chromatograms were developed in butanol/acetic acid/water. Most of the activity is associated with IAA which has an R_f value of 0.92 and has a contaminant running just behind it. A very faint spot runs at 0.57 which could be IAAsp and a very faint 'pigment' spot runs at 0.35.

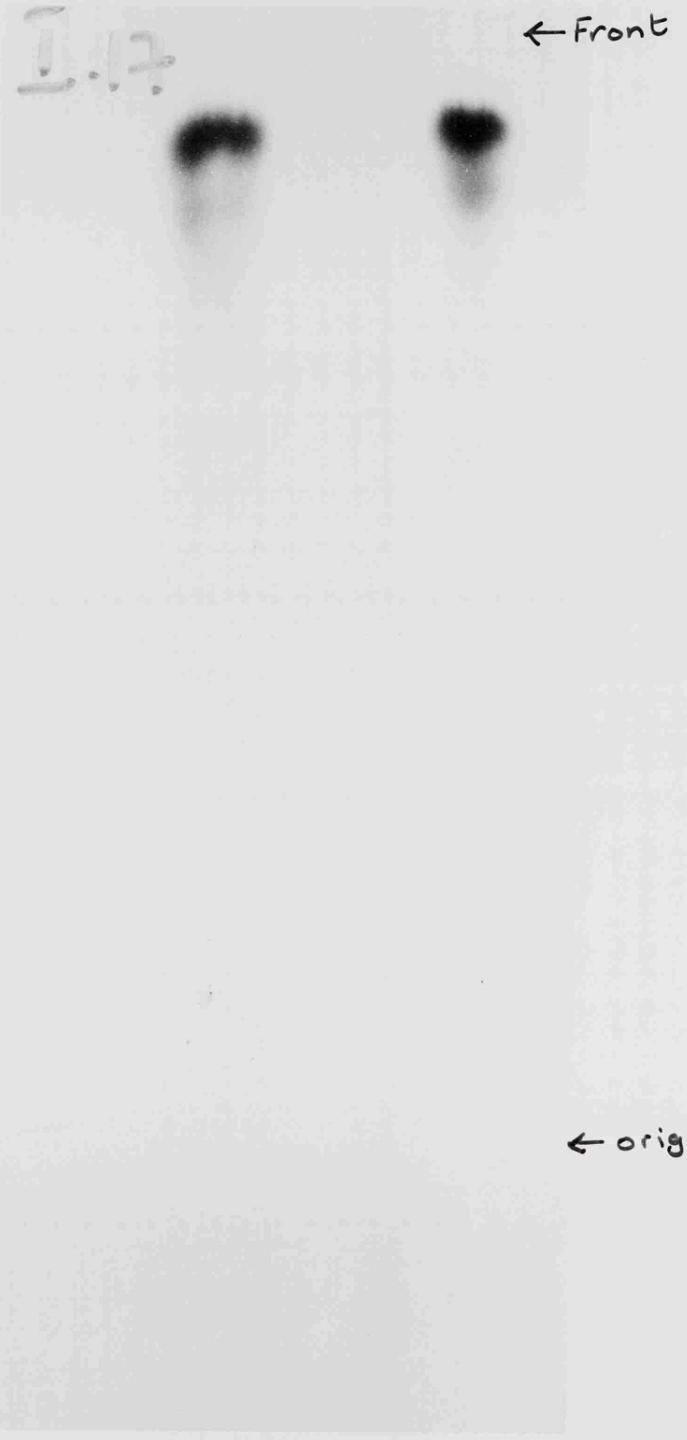
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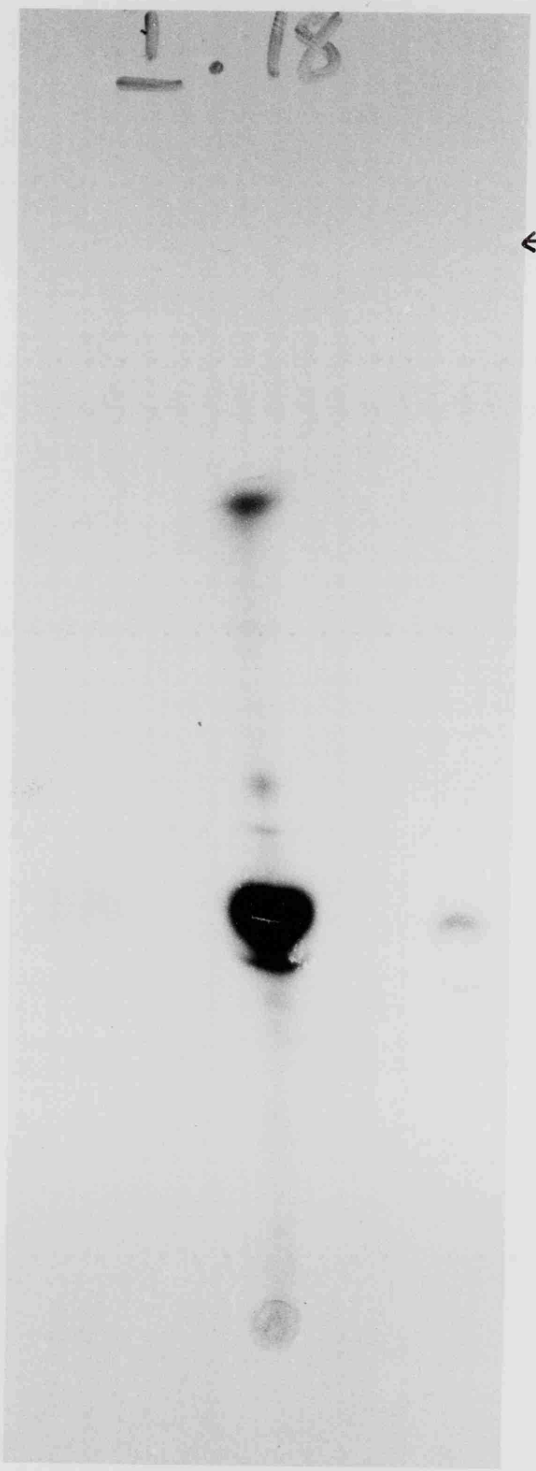
Plate I.18 Radiochromatograms of Coleus internode extracts following 30 min and 1 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. The middle chromatogram is an IAA-2-^{14}C marker. It is not very pure but the R_f value of the IAA is 0.43. The left hand chromatogram is the 30 min extract and the right hand one is the 1 h extract. Only IAA and the contaminant running just behind it are contained in the extract.


1.18
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considered are those which are formed during that time. It was thought important to study the time course of auxin metabolism and observe the first appearances of the metabolites. Basipetal transport experiments utilising IAA-2-^{14}C were thus run for 0.5, 1, 2, 3, 4, 5, 6, and 7 h periods. The radiographs produced from these experiments are shown in Plates I.16 - I.23. It is a little difficult to detect the first appearance of metabolites since this depends on the sensitivity of the detection mechanism. Also the time course of the metabolic processes is probably somewhat variable. In Plates I.16 and I.17 there is a vague indication of the presence of IAAsp (R_f = 0.17 and 0.57 respectively) in an extract from internode segments which had been subjected to experimental conditions for 30 min. The 'pigment' mark is also just visible at 0.35 on the butanol/acetic acid/water radiograph. The bulk of the activity in the 30 min extract is associated with unchanged IAA (0.62 and 0.92). In another 30 min extract which is chromatogrammed alongside an extract from internodes treated for 1 h (Plates I.18 and I.19) only IAA is represented. The IAA spots on the chromatograms are only faint, however, so it would be difficult to detect the presence of IAAsp. In the 2 h and 3 h extracts (Plate I.20) the IAA spot (R_f = 0.48 - 0.50) has become quite pronounced and there is a spot just visible at 0.12 which presumably represents IAAsp. In the 4 h and 5 h extracts (Plates I.21 and I.22) the indications are that IAA uptake is continuing to occur and that little of it has undergone any change. In the 6 h and 7 h extracts (Plate I.23) the IAAsp spot (R_f = 0.07) can be seen to become more prominent while the IAA spot becomes proportionally less prominent. Plate I.24 shows 3 h and 6 h extracts chromatogrammed side by side in butanol/acetic acid/water and demonstrates the proportional decrease in IAA content and accompanying increase in IAAsp content and 'pigment' content. Several other spots are present on this radiograph which are not often seen. Their

Plate I.19 Radiochromatograms of Coleus internode extracts following 30 min and 1 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in butanol/acetic acid/water. The left hand chromatogram is an IAA-2-^{14}C marker which has an R_f value of 0.83. The second and third chromatograms are the 30 min and the 1 h extracts respectively. They contain IAA only.


I. 19
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Plate I.20 Radiochromatograms of Coleus internode extracts following 2 h and 3 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. The left hand chromatogram is an IAA-2-^{14}C marker which has an R_f value of 0.50. The second and third chromatograms are 2 h and 3 h extracts respectively. They contain mostly IAA. There is a faint spot at 0.12 which is probably IAAsp.

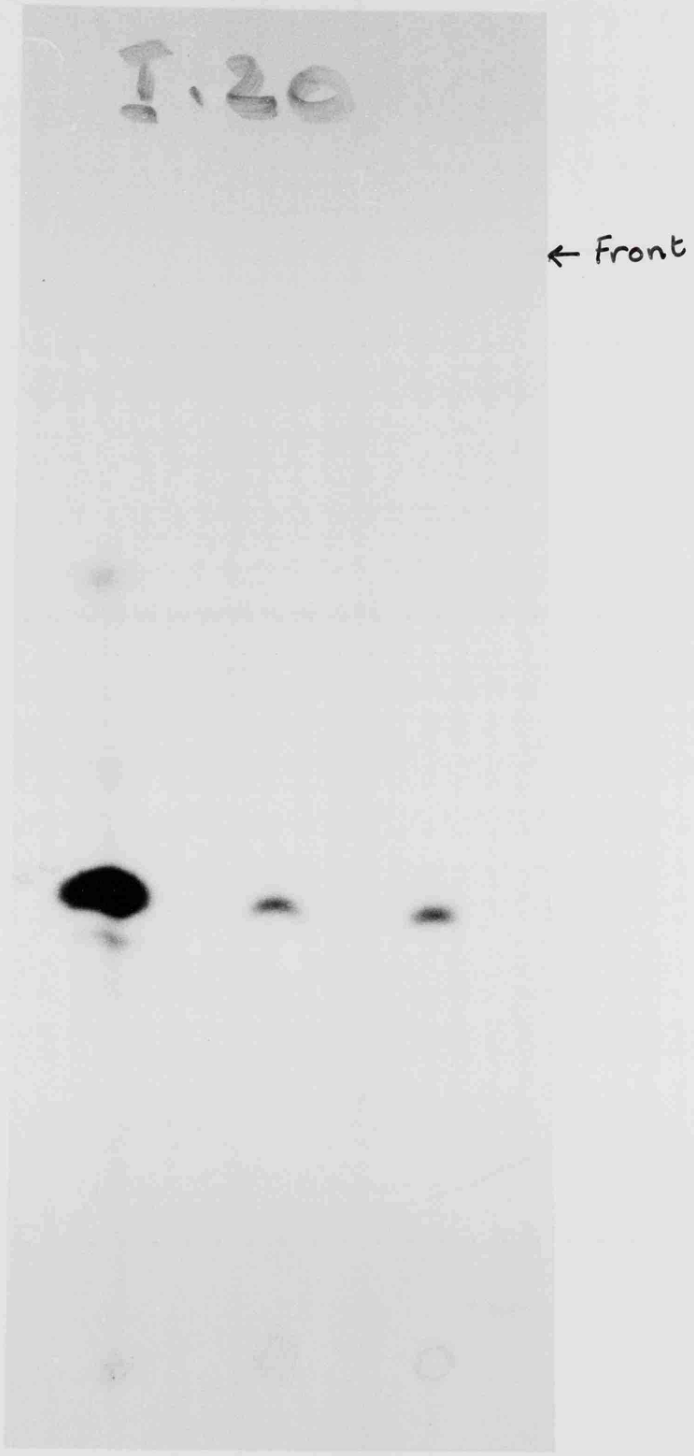
I.20
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Plate I.21 Radiochromatograms of Coleus internode extracts following 4 h and 5 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. The left hand chromatogram is an IAA-2-^{14}C marker. It has an R_f value of 0.40 and two impurities are present. The second and third chromatograms are the 4 h and 5 h extracts respectively, and contain mostly IAA. Some IAAsp formation has probably occurred, there being a faint spot at 0.07. There appears to be more IAA present after 5 h than after 4 h.

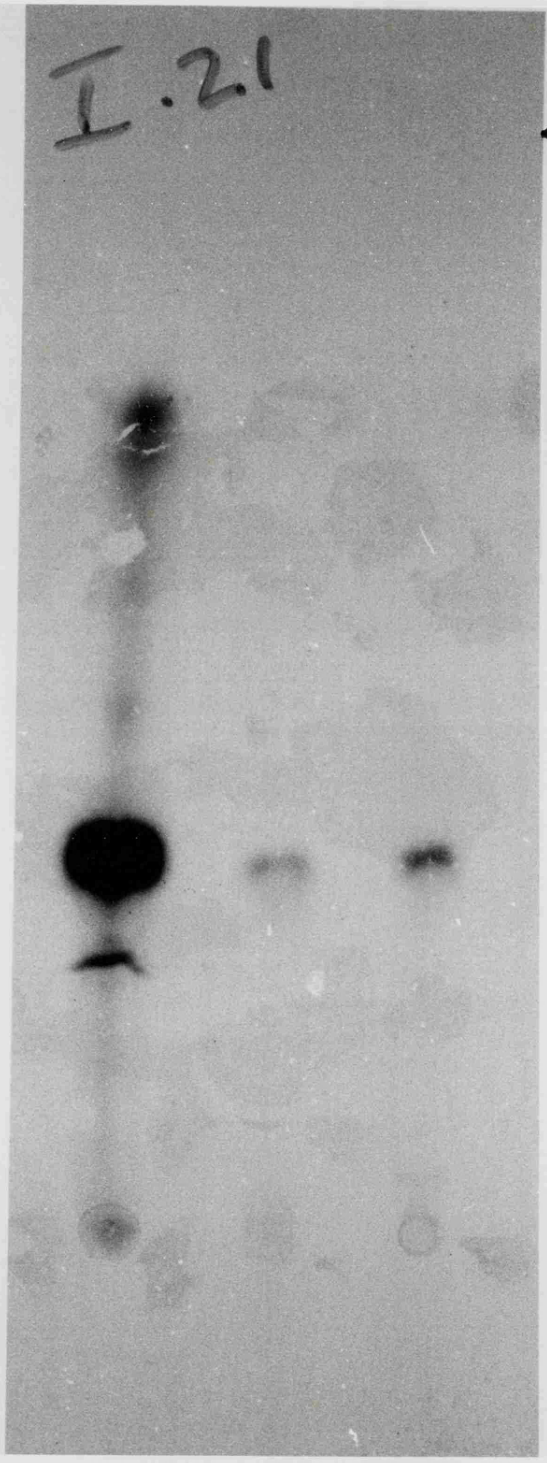
I.21
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Plate I.22 Radiochromatograms of Coleus internode extracts following 4 h and 5 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in butanol/acetic acid/water. The left hand chromatogram is an IAA-2-^{14}C marker which has an R_f value of 0.90. The second and third chromatograms are the 4 h and 5 h extracts respectively. They contain IAA. The 'pigment' spot runs at 0.35 but no IAAsp is indicated on these chromatograms. There appears to be more IAA present after 5 h than after 4 h.

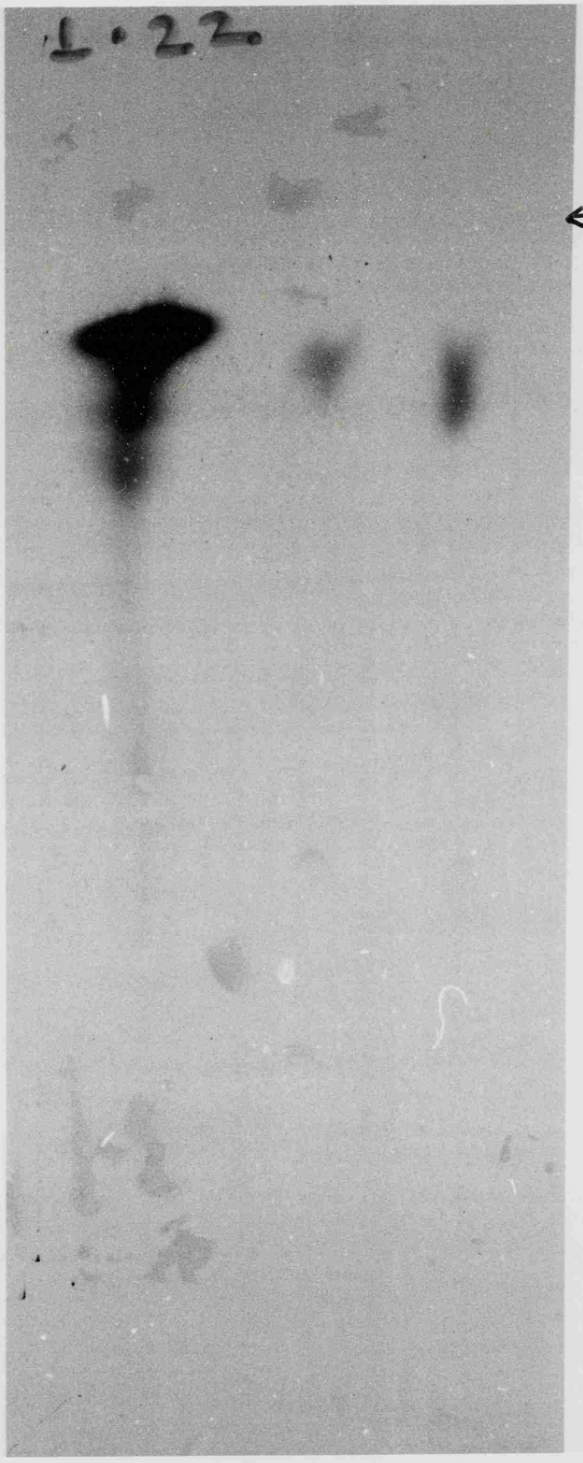
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Plate I.23 Radiochromatograms of Coleus internode extracts following 6 h and 7 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. The left hand chromatogram is an IAA-2-^{14}C marker with an R_f value of 0.38. The second and third chromatograms are the 6 h and the 7 h extracts respectively. Most of the activity in the extracts is associated with IAA. The faint spot at 0.07 is probably IAAsp.

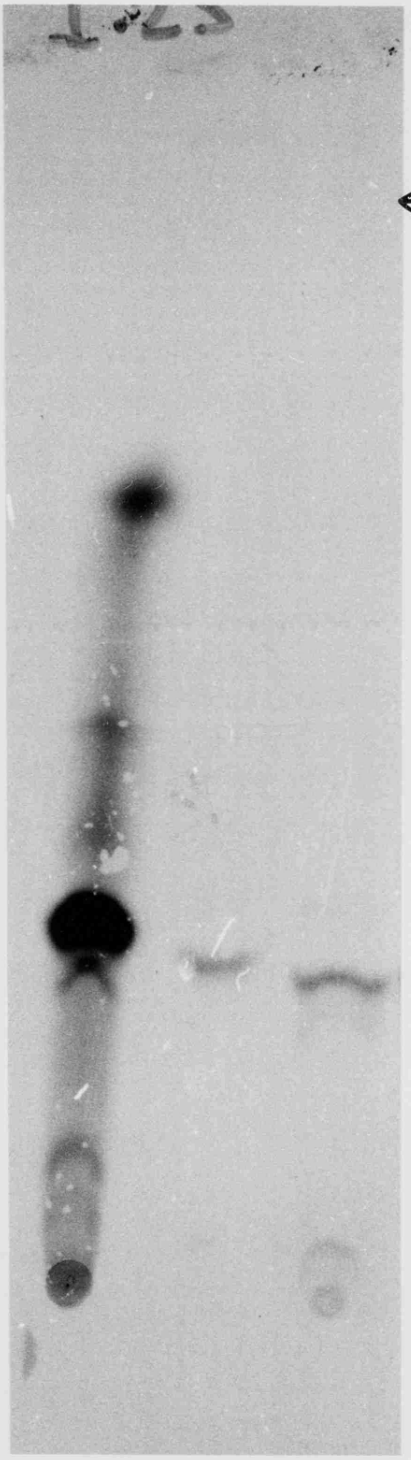
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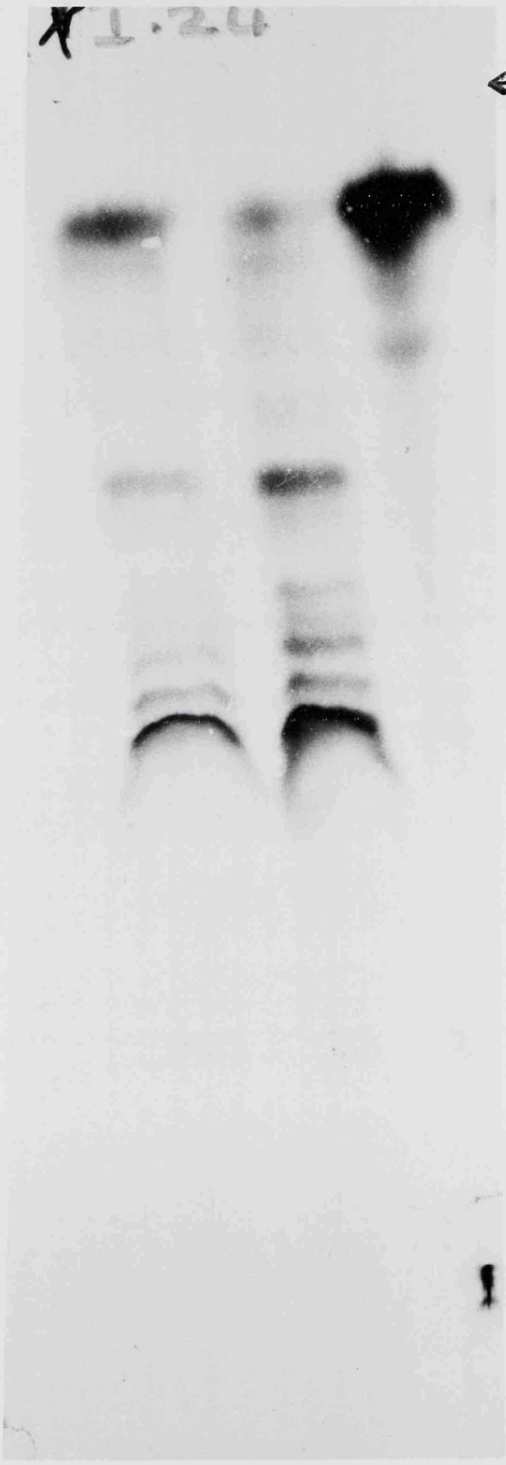
Plate I.24 Radiochromatograms of Coleus internode extracts following 3 h and 6 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in butanol/acetic acid/water. The right hand chromatogram is an IAA-2-^{14}C marker with an R_f value of 0.90. The left hand chromatogram is the 3 h extract. It has a fairly dark IAAspot, an IAAsp spot at 0.60 and a 'pigment' spot at 0.37. Several other unidentified spots are present. In the 6 h extract it can be seen that the IAA level has declined whereas the intensity of the IAAsp spot and the 'pigment' spot has increased.


1.24
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significance is not known. These experiments show that conjugation between IAA-^{14}C and aspartic acid can begin within 30 min of the application of an IAA-^{14}C donor and probably has usually begun within 3 h. The indications are that IAAsp is the first product of IAA metabolism which can be detected and it is the only product which can consistently be shown to be formed within the first six or seven hours. IAA continues to be taken up by the tissues during this time but once IAAsp has started to be produced much of the IAA taken into the tissue is converted into the conjugation product.

Radioactivity associated with pigmentation

As stated earlier it was often noticed that in chromatograms developed in butanol/acetic acid /water a pigmented area with a distinctive shape was visible on the thin-layer plate and a radioactive area as seen on the radiographs corresponded in shape and position with this area. When this phenomenon occurred it was very obvious and its possible significance was considered. No references to it occurred in the literature on auxin transport and no reports could be found regarding the involvement of IAA in anthocyanin synthesis. The short term experiments which investigated the time course of IAA metabolism during transport showed that the radioactive spot gradually increased in intensity as though it represented a product which was being progressively formed or at least as though the pigmented material ran at the same R_f as a product of IAA metabolism. A correspondence between radioactivity and pigmentation was not observed in other chromatography solvent systems and attempts to scrape the areas from the chromatography plate, elute and rechromatogram were not successful. In later experiments neither the pigmented area on the thin-layer plate nor the radioactive spot with that R_f value were seen. Attempts were made to select Coleus plants with highly pigmented stems in order to try and

produce an extract containing the pigment but these attempts were not successful. The fact that no radioactive spot could be seen when the coloured spot was not visible suggests that the colour spot did represent a product of IAA metabolism but since it was not always present it presumably does not represent an important product. It may result from a pathway that is not normally utilised in plant metabolism.

Extraction of dissected tissues

The experiments described so far have investigated extracts of entire excised segments. The localisation within the tissues of the constituents of the extract has not yet been considered. There were two main reasons for approaching this problem. The first was that microautoradiographs produced by Halliday (1969) and Wangermann (1968; 1970) following IAA-^{14}C treatment of excised plant segments had shown a concentration of radioactive material in the vascular tissue. The substances with which this activity was associated were not known. The second reason for the interest in this problem was the persistent appearance of the 'pigment' spot in early chromatograms. The origin of the pigment seen on the TLC plates (which was assumed to be an anthocyanin) was most likely to have been the epidermal tissue of the internode since red/purple colouration was often obvious there. Since this pigment appeared to be associated with some radioactive material it seemed possible that the radioactive metabolite could be concentrated in the epidermis. Micro-autoradiographs had also shown some blackening associated with the epidermis of treated sections (Wangermann, 1968; 1970) which did not occur in the control sections used to investigate bleaching and pressure artefacts. In order to investigate these possibilities dissection of treated internode segments was undertaken. Pith, epidermis and vascular tissue were separated and extracted. This was a delicate and difficult operation. Although the

Plate I.25 Radiochromatograms of the extract of vascular tissue from Coleus internodes following 24 h basipetal IAA-1-^{14}C treatment

The chromatograms were developed in butanol/acetic acid/water. The two middle chromatograms are IAA-1-^{14}C markers. The R_f value of the IAA is 0.91. Several spots appear on the extract chromatograms including one thought to be IAAsp at 0.54 and the 'pigment' spot at 0.40. Some IAA is present in the extract.

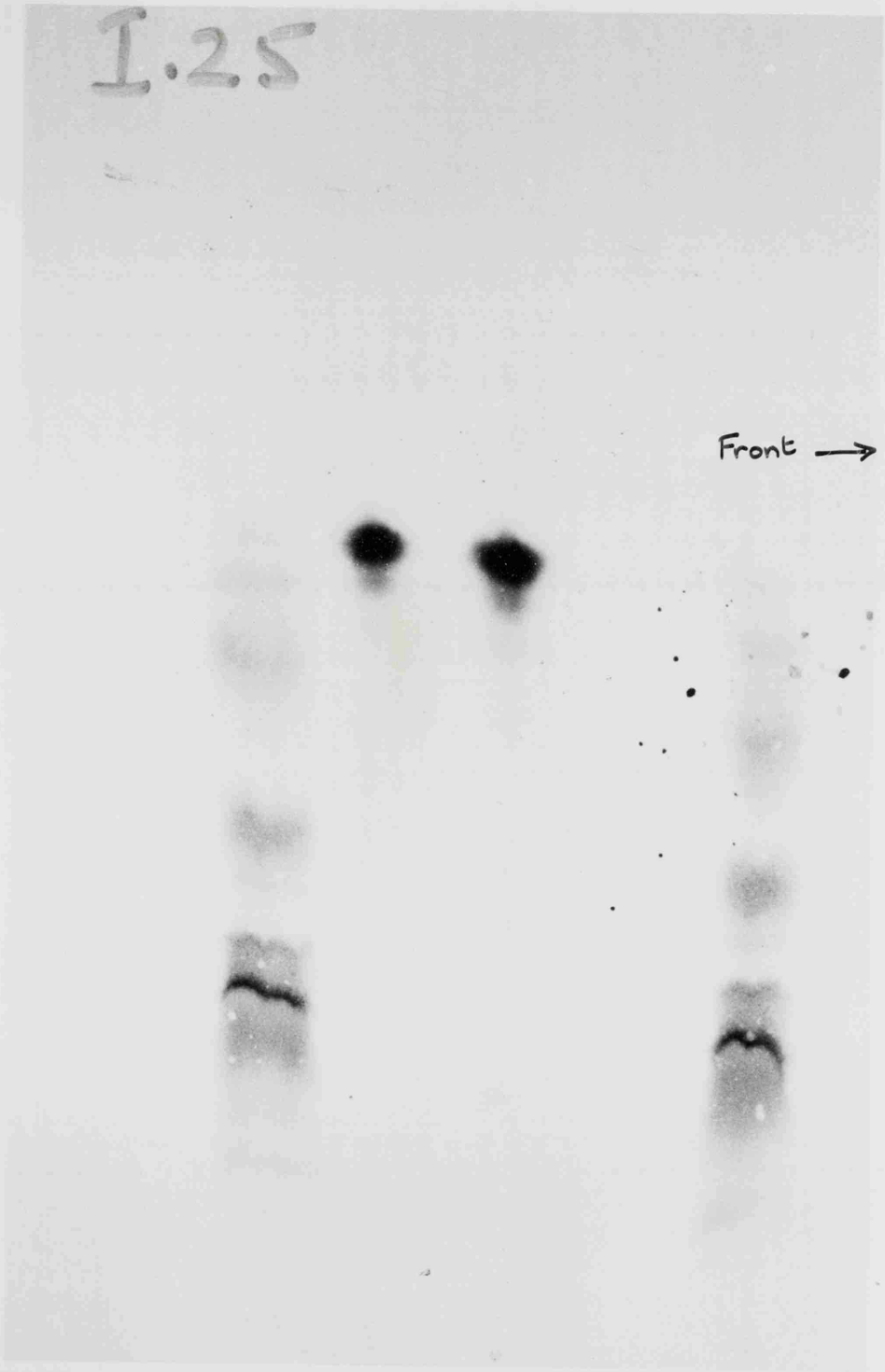
1.25
Front

Plate I.26 Radiochromatograms of the extracts of pith, vascular tissue and epidermis from Coleus internodes following 24 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in butanol/acetic acid/water. The left hand chromatogram is an IAA-2-^{14}C marker with an R_f value of 0.95. The extract chromatograms are very faint and little is to be seen on them. Some IAA occurs in all extracts and IAAsp runs at 0.56. No 'pigment' spot can be seen at all.


I. 26.
← Front

four main vascular bundles of Coleus stems are situated at the 'corners' of the stem there are smaller bundles between them and these were often difficult to see under a binocular microscope. Even those bundles which were easily visible were often difficult to separate from surrounding tissue. Often vascular tissue was ripped off along with epidermal and hypodermal tissue strips and in some cases the exact boundary between the vascular bundles and the pith was difficult to see. Further, the segments were often quite soft after a period of transport and were easily damaged. Early experiments in this series were not, therefore, very successful. Radioactive extracts were obtained from all tissues examined but after chromatography these were all seen to be exactly similar. Apparently leakage and cross-contamination had occurred. The technique was therefore improved. Following the transport period all segments were carefully blotted dry to remove any radioactive material on the surface. Dissection was carried out under a binocular microscope, using a pair of very fine forceps and a sharp cataract knife. Only one tissue component from any one segment was used and care was taken to exclude all unwanted material. Only pith from the centre of the segment, and epidermis from the flat 'sides' of the segment were used and care was taken to remove any tissue which adhered to the vascular tissue after it had been stripped from the segments. Extraction was carried out as for entire segments and extracts chromatographed as usual. Even when great care was taken in the separation of tissues the extracts appeared to contain the same substances. It was, however, possible to see quantitative differences as regards the relative proportions of the constituents. In chromatograms run in butanol/acetic acid/water the 'pigment' mark was seen to appear in all extracts when it was present at all but, as stated above, in later experiments it did not occur in any of the tissue extracts so no information as to its nature was gained in these

Plate I.27 Radiochromatograms of the extract of vascular tissue from Coleus internodes following 24 h basipetal IAA-1-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. The chromatogram on the right hand side is marker IAA-1-^{14}C with an R_f value of 0.51. In the extract chromatogram the dark spot at 0.12 is probably IAAsp. There is a slight indication of the presence of IAA with an unknown compound running just behind it.


1:24
Front

experiments. Plate I. 25 shows an extract of vascular tissue chromatographed alongside an IAA marker. It shows evidence of the presence of IAA (R_f = 0.93), IAAsp (R_f = 0.60) and the 'pigment' mark (R_f = 0.40). Plate I.26 shows all three extracts and no 'pigment' mark is seen in any of them. IAA is indicated at an R_f value of 0.93 and IAAsp runs at 0.56. There is another spot at 0.80 which is not usually seen. Plates I27 and I.28 show chromatograms of the three extracts run in isopropanol/ammonia/water and Plate I.29 shows the same three extracts from a similar experiment performed at a different time. In both experiments there were differences in the relative proportions of IAA and IAAsp between the three extracts. It can be seen from the radiographs of both these experiments that the IAAsp/IAA ratio in the epidermis is higher than that in the pith. From Plate I.29 where all three extracts can be compared directly it can also be seen that the ratio in the vascular tissue is intermediate between the other two. It is almost impossible to estimate actual values for such ratios from observation of radiographs as will be discussed in the next Chapter but relative values can often be reliably estimated. Assuming that IAAsp is an immobilisation product of IAA it can be deduced that some immobilisation occurs in all the tissues investigated but relatively more occurs in the epidermis. Relatively little IAAsp is present in the pith. On the basis of this evidence it is not possible to state the tissue in which the auxin is transported but if the auxin/IAAsp ratio is any indication then it would appear that transport is more likely to occur in the pith and vascular tissue and less likely to occur in the epidermis. It is, however, not necessarily valid to assume that all IAA present is being transported as will be discussed later. Plate I.30 shows the same three extracts chromatographed in chloroform/methanol/acetic acid. Again the pith extract contains little IAAsp (R_f = 0.25) but it is difficult to assess the relative amounts of IAAsp and IAA

Plate I.28 Radiochromatograms of the extracts of epidermis and pith from Coleus internodes following 24 h basipetal IAA-1-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. The chromatogram on the right hand side is marker IAA-1-^{14}C with an R_f value of 0.60 and with two contaminants. The R_f of the IAA is lower in the extract chromatograms because it has been reduced by the other constituents of the extracts. Its value is 0.57. The chromatogram on the left is the epidermis extract. It has a dark spot at 0.17 which is probably IAAsp. There are two faint spots below this and very little IAA appears to be present. The middle chromatogram is the pith extract. It also has the dark spot at 0.17 but has a much more pronounced IAAspot. Both extracts contain a contaminant introduced with the IAA which has an R_f value just lower than that of IAA.

1.28.

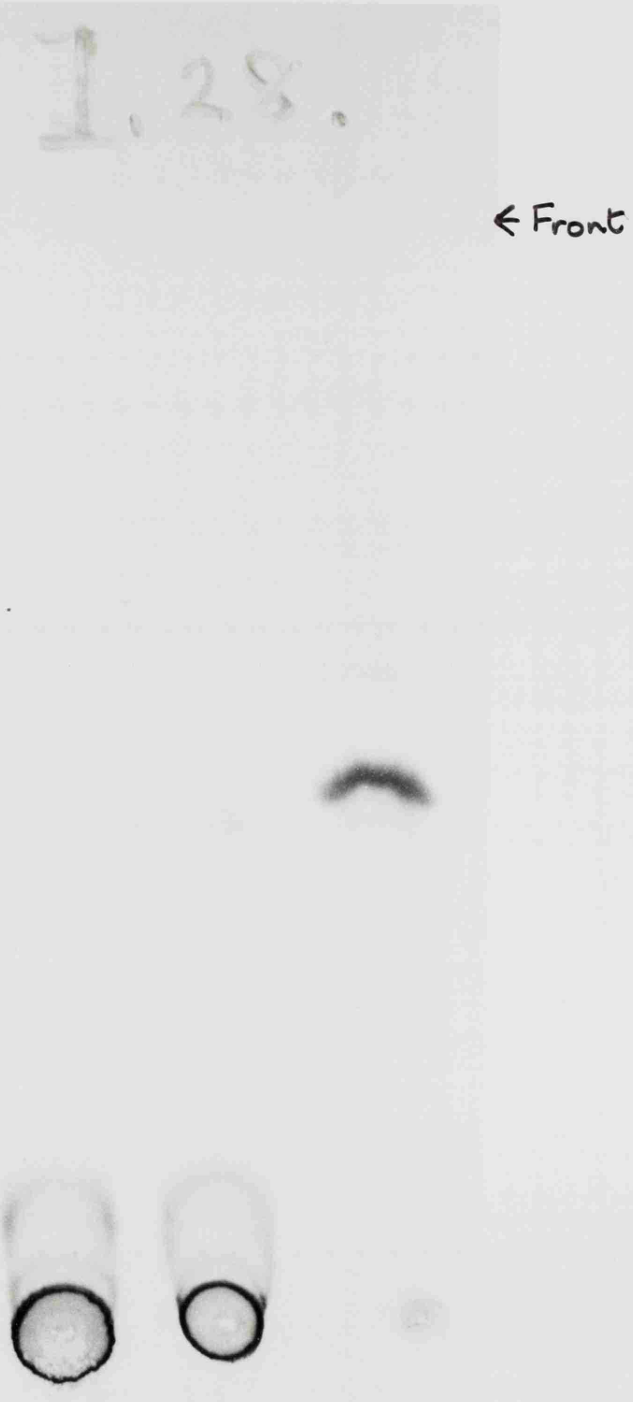

Plate I.29 Radiochromatograms of the extracts of pith, vascular tissue and epidermis from Coleus internodes following 24 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. All spots are very faint. The left hand chromatogram is the pith extract. There is a very faint spot at 0.47 which is probably IAA. The middle chromatogram is the vascular extract and has the spot at 0.47 and also a faint spot at 0.10 which is probably IAAsp. The right hand chromatogram is the epidermal extract. It also has a faint IAA spot but the IAAsp spot is relatively much darker. No other spots are visible.

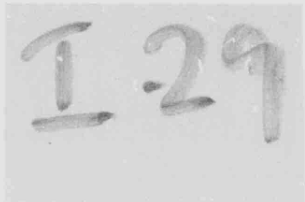

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Plate I.30 Radiochromatograms of the extracts of pith, vascular tissue and epidermis from Coleus internodes following 24 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in chloroform/methanol/acetic acid. IAA runs in all chromatograms at 0.93 and IAAsp runs at 0.25. The left hand chromatogram is the vascular extract and is quite dark, IAA and IAAsp both being prominent. The middle chromatogram is the pith extract and levels of activity are generally lower except for the two spots of low R_f value (below the IAAsp spot). These are relatively quite dark. The right hand chromatogram is the epidermal extract. The spots are also quite dark and large amounts of IAA and IAAsp are indicated.


I.30.
← Front

(0.93) in the three extracts. One point of interest which does emerge from this radiograph is the relative intensity of the two spots with R_f values below that of IAAsp. Their intensity is relatively greater in the pith extract than in the other two extracts.

Whole cuttings

So far only extracts from excised plant segments had been examined in connection with auxin transport and metabolism and no study had been made of the movement of auxin from one organ to another. It was therefore decided to attempt transport experiments on decapitated Coleus cuttings. Cuttings were taken from plants of the usual clone and allowed to root in tap water. They were usually left for about two weeks by which time a good growth of adventitious roots had occurred. The cuttings were then planted in damp vermiculite in plastic cups. Cuttings were decapitated and IAA-^{14}C solution was applied in agar donor blocks to the cut surface. A transport period of 94 h was employed. After that time the donors were removed and the leaves were removed from the cuttings. (Early attempts to extract petioles and laminae were abandoned because little radioactivity could be recovered from the extracts and their chromatographic properties were poor.) The adventitious roots were carefully shaved from the stem which was then cut into sections of convenient length for extraction. The stem and roots were extracted separately. The results of such an experiment may be seen in Plate I.31. The levels of activity in the extracts were not high but IAAsp and IAA are indicated in the shoot extract. IAAsp is indicated in the root extract but IAA does not appear to be present in detectable quantities. The results, therefore, suggest that IAA passes from stem to roots in Coleus cuttings and that IAAsp is formed there.

Plate I. 31 Radiochromatograms of the extracts of the components of whole Coleus cuttings following 94 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. The left hand chromatogram is marker IAA-2-^{14}C which is badly overloaded. The R_f of the IAA itself can be seen to be 0.53. The middle chromatogram is the root extract. The activity seen at an R_f value of 0.13 is probably IAAsp. The right hand chromatogram is stem extract. IAAsp is indicated and there is also some activity corresponding in position to the IAA marker.


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Extraction of Helianthus tissue

Helianthus annuus has been used by Wangermann (1974) in this laboratory for quantitative studies on auxin transport so it was decided to investigate the constituents of Helianthus extracts after IAA-^{14}C treatment. Helianthus plants were grown from seed under the conditions described and plants about 20 cm high were selected for use. Segments were cut from the internode below a leaf pair which was about half expanded, usually the second internode below the apical bud. The segments were cut from just below the node. Each segment was 15 mm in length and only one segment was cut from each internode. Segments were set up in the same way as the Coleus segments and the experimental procedure was as described for Coleus.

Plate I. 32 shows the radio-chromatogram of a Helianthus internode extract chromatographed in isopropanol/ammonia/water alongside an authentic IAA-^{14}C marker. The segments had been treated with apically applied IAA-2-^{14}C solution for 24 h. IAA is seen to be present in the extract but there is little evidence of the presence of IAAsp, or any other product of IAA metabolism. Treated Helianthus internode segments were dissected into the three separate tissue components described for Coleus and these were extracted and chromatographed separately. From Plate I. 33 it can be seen that little activity was recovered from the epidermis and pith extracts. The vascular tissue chromatogram comprised one obvious spot which ran at the same R_f as the marker IAA but again there was no indication of the presence of IAAsp. There was some radioactive material near the origin in the epidermal extract chromatogram which did not appear to be present in the other two chromatograms but this was not very clear. The possibility does exist, however, that there is some IAAsp formed in the epidermis of Helianthus.

Plate I. 32 Radiochromatograms of Helianthus internode extract following 24 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. The first and third chromatograms are IAA-2-^{14}C markers with an R_f value of 0.49 and radioactivity near the front. The middle chromatogram is the Helianthus extract. It has a spot with the R_f value of IAA and two other faint spots.


I.32.
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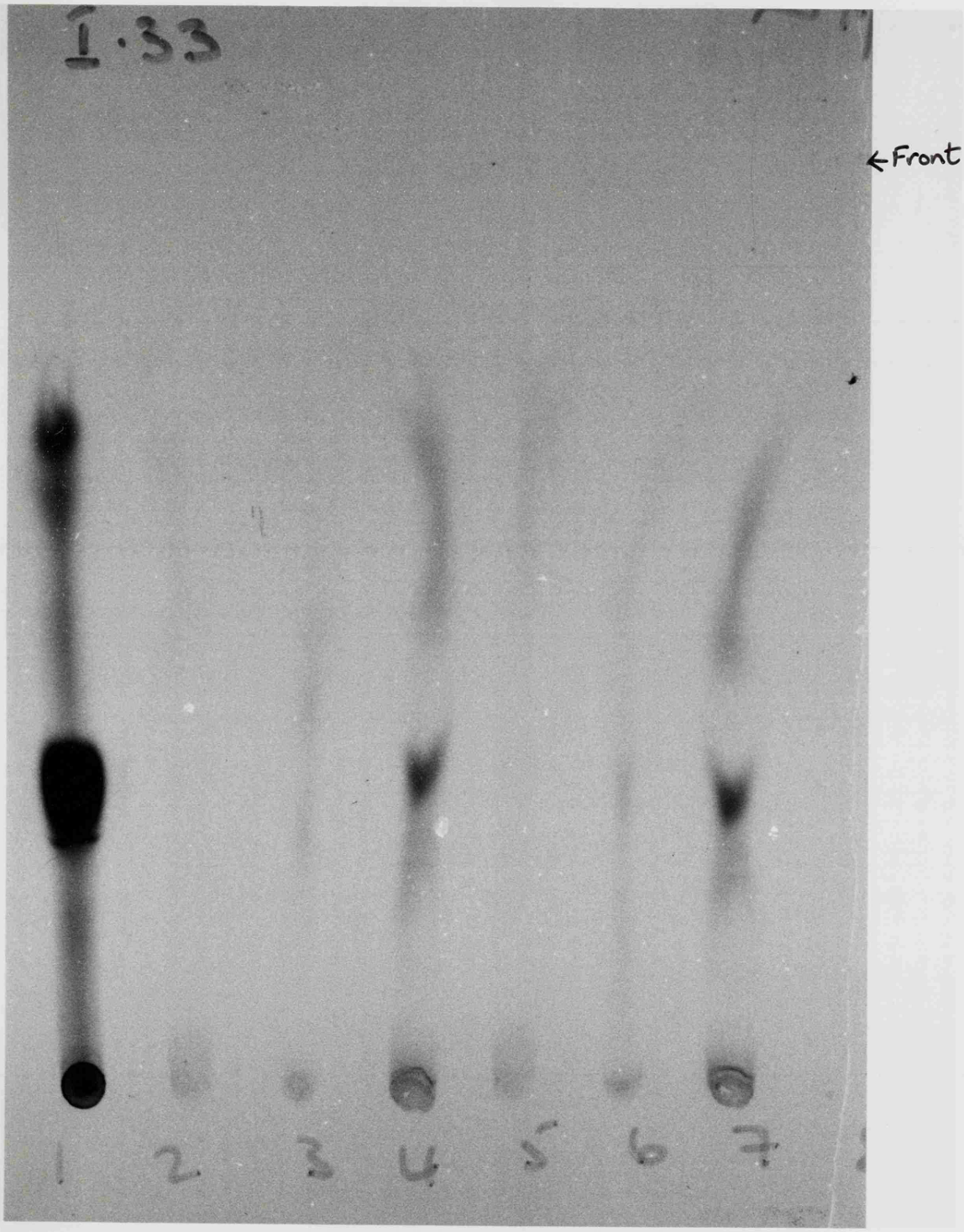
Plate I. 33 Radiochromatograms of extracts of pith, vascular tissue and epidermis from Helianthus internodes following 24 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. The left hand chromatogram is an IAA-2-^{14}C marker with an R_f value of 0.40 and some contaminants. The second and the fifth chromatograms are the epidermal extracts and little activity is apparent. The activity near the origin (R_f = 0.05) could be IAAsp. The third and sixth chromatograms are pith extract. Very little is visible on these chromatograms but there is some indication of the presence of IAA. The fourth and seventh chromatograms are the vascular extract. These are the most distinct chromatograms and activity is apparent at the R_f of IAA. There is some activity close to the front but no distinct spots. There is an indication of the presence of IAAsp.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In an effort to study auxin transport in intact plants small but entire Helianthus seedlings were also investigated and the method of IAA application employed was modified for this experiment. The method of application was that of Morris et al. (1969). A drop of IAA-^{14}C solution was applied directly to the apical bud of the seedling with a Hamilton syringe. The seedlings selected had hypocotyls about 5 cm in length and the apical bud was protected by the cotyledons. For the duration of the experiment the seedlings were planted in plastic beakers filled with damp vermiculite and the beakers were kept in a plastic bowl lined with damp filter paper. A 2 μl drop of IAA-2-^{14}C solution was delivered onto the bud. It was held in place by its own surface tension and the first pair of leaves. A further 2 μl drop was added after 24 h and the transport period was 48 h. The seedlings were then rinsed, dried with tissue paper and divided into cotyledons, hypocotyl, and root. The root/hypocotyl transition point was estimated by eye. The three components were extracted and chromatographed separately. Little radioactivity was recovered from the cotyledon or the root system. Most of the activity recovered was from the hypocotyl and the chromatogram of the hypocotyl extract (see Plate I.34) resembles that of the internode segment and the vascular tissue extracts (Plates I.32 and I.33 respectively) in comprising IAA with no IAAsp or any other clearly defined substance.

Extraction of different regions of internode segments

In the earliest experiments in this series some consideration was given to the nature of the extracts from different regions of treated Coleus internode segments. The products of metabolism appeared to be the same in all of them and it was concluded that all extracts were similar. However, experiments with the Intertechnique Oxymat (see Chapter II) gave cause to believe that even if

Plate I. 34 Radiochromatograms of the components of whole Helianthus seedlings following 48 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. The left hand chromatogram is an IAA-2-^{14}C marker with an R_f value of 0.40 and some contaminants. The chromatograms are only very faint and no real spots can be seen in the cotyledon extract chromatograms (second and fifth from the left) or the root extract chromatograms (third and sixth from the left). Only IAA is indicated in the stem extract chromatograms (fourth and seventh from the left).

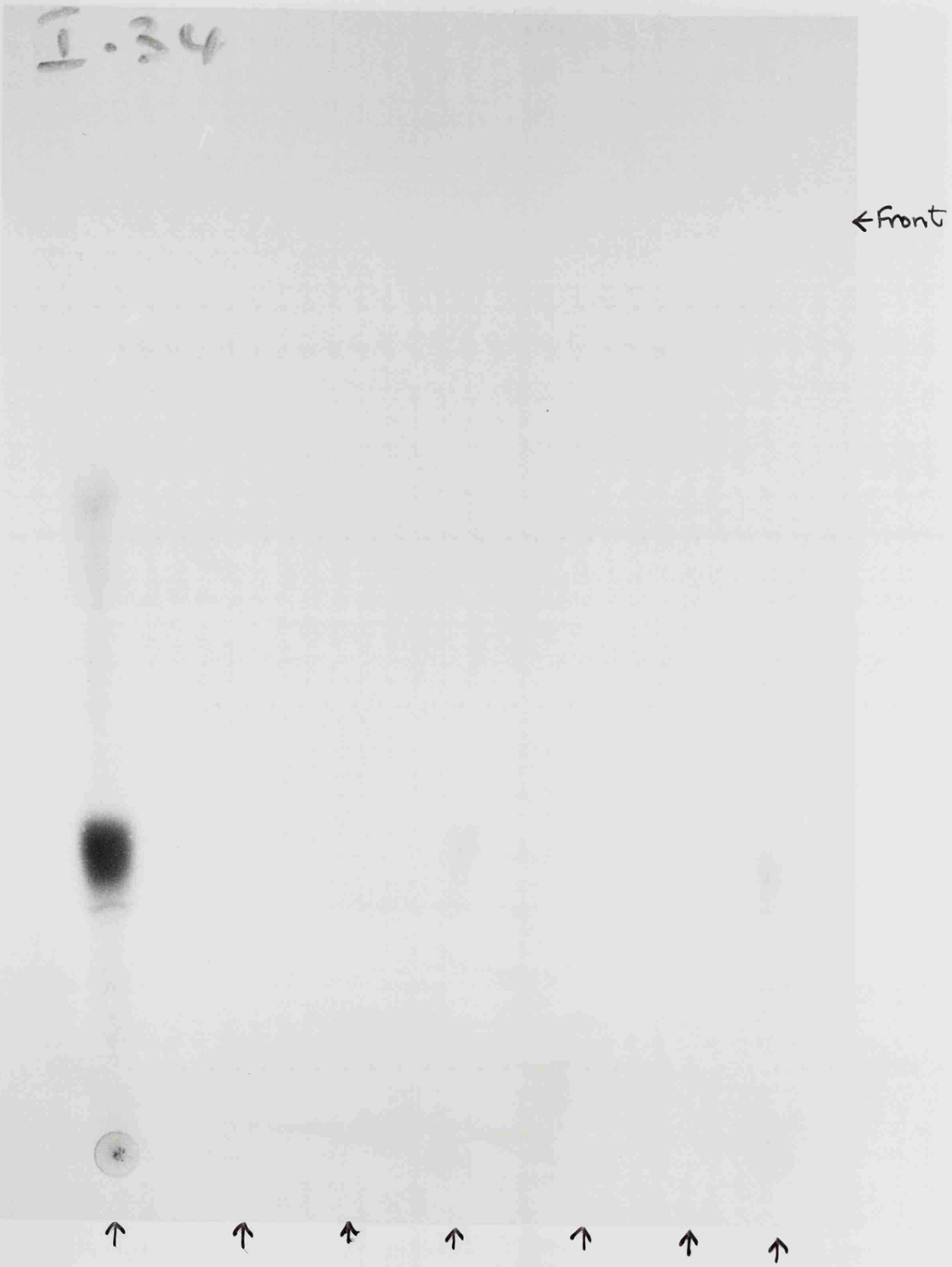
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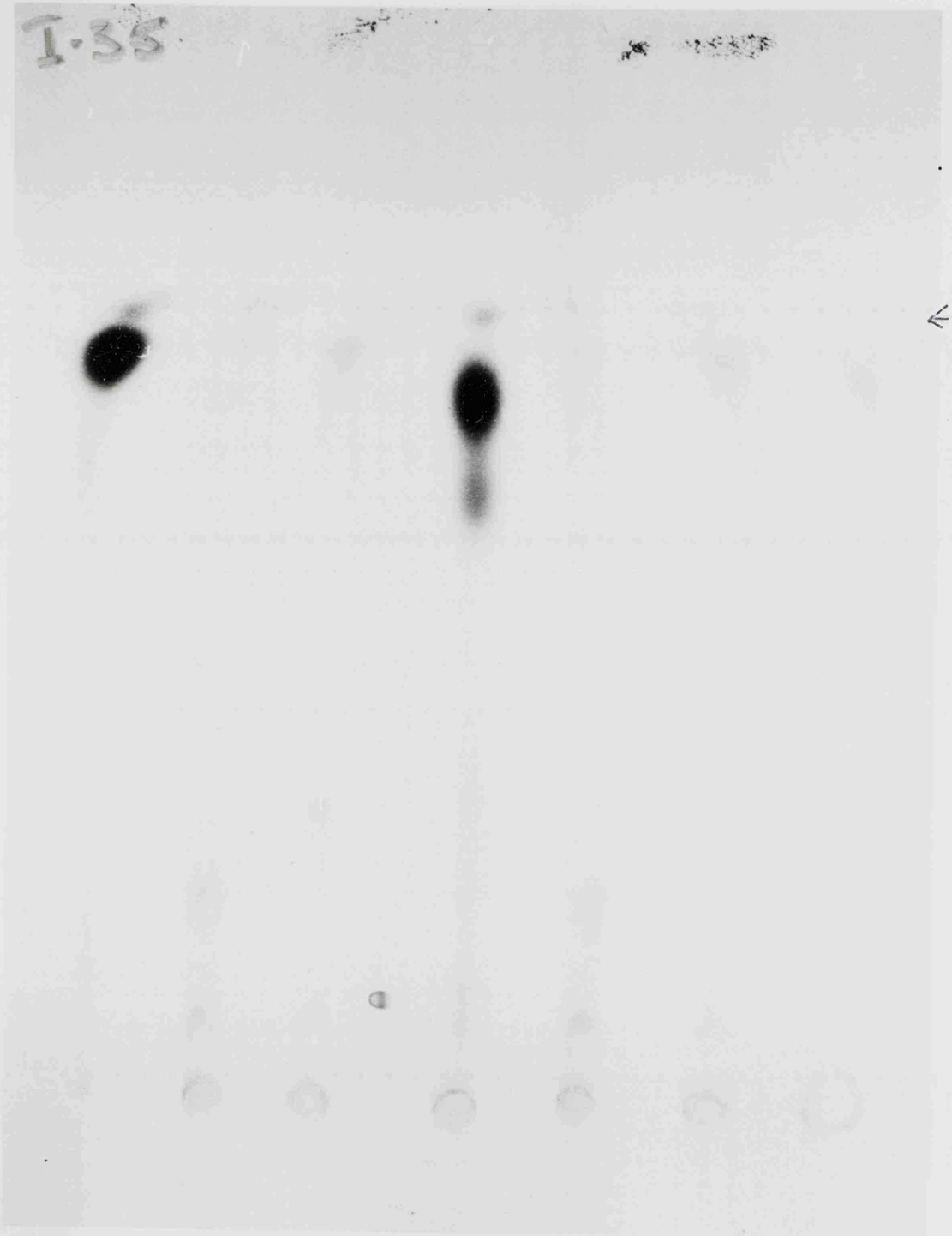
Plate I. 35 Radiochromatograms of extracts of different regions of Coleus internode segments following 24 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in chloroform/methanol/acetic acid. The left hand chromatogram is IAA-2-^{14}C marker with an R_f value of 0.90. The second and fifth chromatograms are the extract of the apical 1 mm of the segment. Little IAA appears to be present and the faint spot at 0.36 is probably IAAsp. There are two spots with R_f values lower than that of IAAsp. The third, sixth and seventh chromatograms are the extract of the remaining 14 mm of the segment. The IAA spot is relatively more dense than in the apical extract chromatograms. There is only one spot with an R_f value lower than that of IAAsp. The middle chromatogram is the extract of the lower portion of the segment 'spiked' with IAA-2-^{14}C.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qualitative differences between these extracts did not exist then there should at least be quantitative ones. Therefore internode segments were prepared and treated in the usual way. After the transport period the donors were removed and the segments were divided into two or three smaller sections, which were extracted separately and chromatographed. The choice of the sizes of the subsections was based on information gained from micro-autoradiographs and from the Oxymat experiments described in Chapter II.

Plates I.35 and I.36 show the result of an experiment in which internode segments were treated for 24 h with IAA-2-^{14}C solution and then divided into two sections for extraction, the apical 1 mm and the remaining 14 mm. The reason for this division was that the apical 1 mm of a treated segment had been seen in micro-autoradiographs to contain a great deal of radioactive material, presumably as a result of rapid uptake and it was though likely that metabolism of IAA could be different in this apical region. Chromatograms run in chloroform/ethyl acetate/formic acid and chloroform/methanol/acetic acid both show that there is less IAA relative to the other constituents of the extract in the apical 1 mm than in the rest of the segment. In the chloroform/ethyl acetate/formic acid chromatogram there also appears to be a relatively smaller amount of the substances of low R_f in the basal extract than in the apical extract. It has previously been thought that one of these spots could represent IAGlu. In the chloroform/methanol/acetic acid chromatograms there is one spot (R_f = 0.25) not represented at all in the basal extract.

In another experiment the segments were treated as above but divided into the apical 11 mm and the basal 4 mm. (This division was based on differences in activity profiles drawn up as a result of Oxymat experiments). The results of this experiment are shown in Plate I.37. This time it is the basal extract

which has a lower IAA concentration. It also has a higher concentration of the material thought to be IAGlu.

In other experiments the internode segments were divided into three sections. Plate I.38 shows the results of an experiment in which internode segments received a 24 h treatment and were divided into the apical 1 mm, the middle 10 mm and the basal 4 mm. From this radiograph it appears that the IAA/IAAsp ratio is higher in the middle section than in the apical or basal sections. Plate I.39 shows the results of a similar experiment, but the transport period had only been 5 h and the three sections were the apical 5 mm, the middle 7 mm and the basal 3 mm (the divisions again being based on Oxymat results for a similar transport period). The indications in the resulting chromatogram are similar to those from the above experiment (Plate I.38).

'Chase' experiments

Some experiments were performed (see Chapter II) to discover the extent to which transported IAA-^{14}C and its metabolic products could be 'chased' from internode segments by unlabelled IAA or by removal of the source of IAA altogether. During the course of these experiments it was found that little of the activity could be 'chased' and that a small amount of activity could be detected in the agar block which replaced the original donor. The level of activity in the replacement donor block was similar whether the block contained 'cold' IAA or whether it was a plain agar block. This suggested that IAAsp which is considered to be immobile within the tissues could move out of the tissues into the agar block. Such a movement of IAAsp was encountered earlier when extracts of donors were found to contain traces of IAAsp. It was decided, however, to extract the components of the experimental system after an experiment in which a 5 h basipetal transport period had been followed by a 16 h period in which the donor blocks were replaced by plain agar

Plate I. 36 Radiochromatograms of extracts of different regions of Coleus internode segments following 24 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in chloroform/ethyl acetate/ formic acid. The left hand chromatogram is an IAA-2-^{14}C marker with an R_f value of 0.87. The second and fifth chromatograms are the extract of the apical 1 mm of the segment. There is hardly any IAA indicated. The spot at 0.43 coincides with the IAAsp colour marker and is quite prominent. There are two spots with R_f values below this. The third, sixth and seventh chromatograms are the extract of the remaining 14 mm of the segment. The IAA spot is more prominent and IAAsp is also present. There is only one spot with a lower R_f value than the IAAsp spot. The middle chromatogram is the extract of the lower portion of the segment 'spiked' with IAA-2-^{14}C.

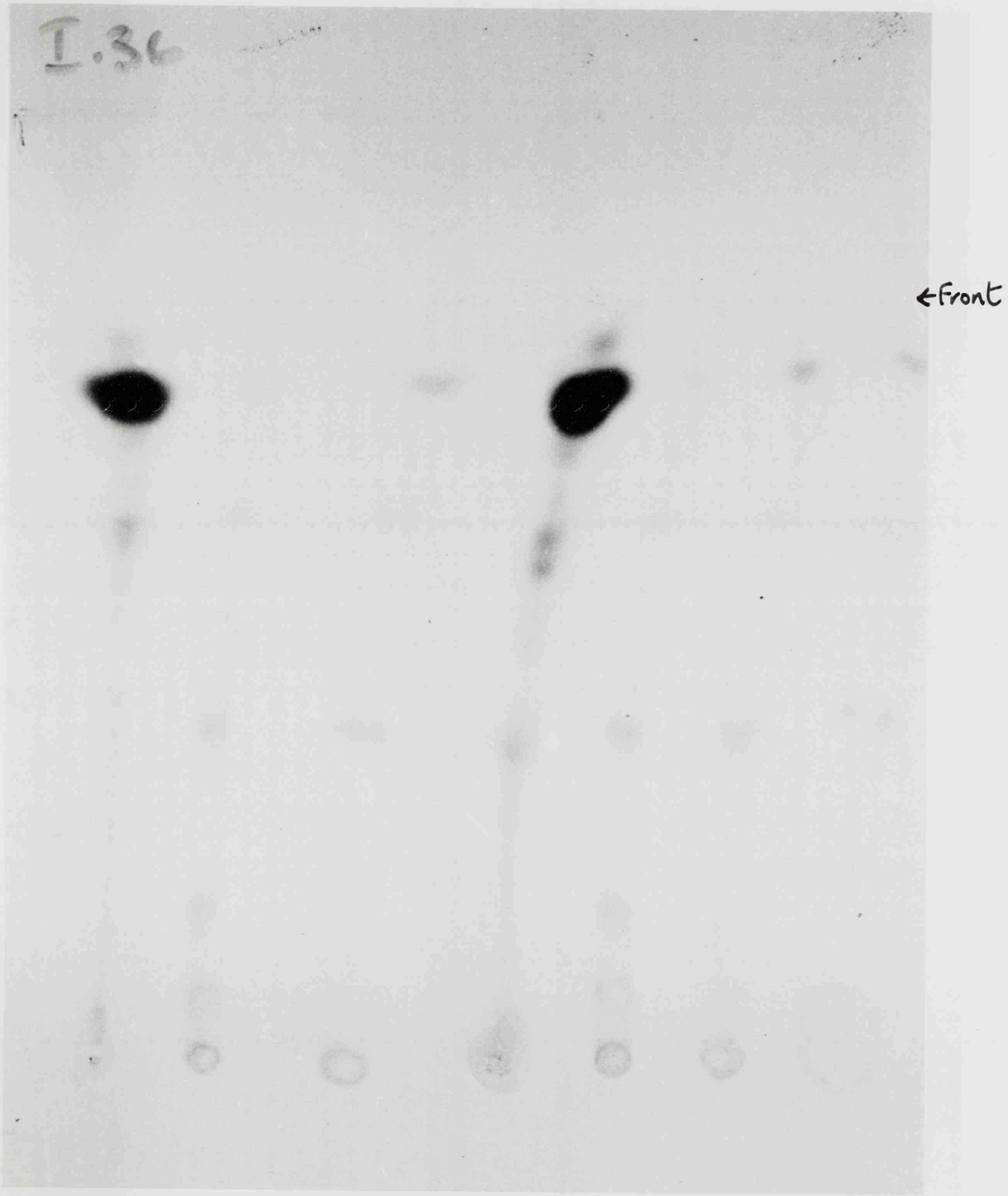
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Plate I. 37 Radiochromatograms of extracts of different regions of Coleus internode segments following 24 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in chloroform/ethyl acetate/ formic acid. The left hand chromatogram is an IAA-2-^{14}C marker with an R_f value of 0. 83 and some contaminants. The second, fourth, fifth and seventh chromatograms are the extract of the upper 11 mm of the segment. The fourth chromatogram is 'spiked' with IAA-2-^{14}C and the seventh one is 'spiked' with IAAsp. The dotted outline of the colour spot is visible around the radioactive spot at 0. 40 which is therefore presumably IAAsp. The IAA spot in the chromatogram is fairly prominent. There is one other spot with an R_f value below that of IAAsp. The third and sixth chromatograms are the extract of the basal 4 mm of the segment. The IAA spot is hardly visible and the IAAsp spot is very prominent. The unknown spot is also present.

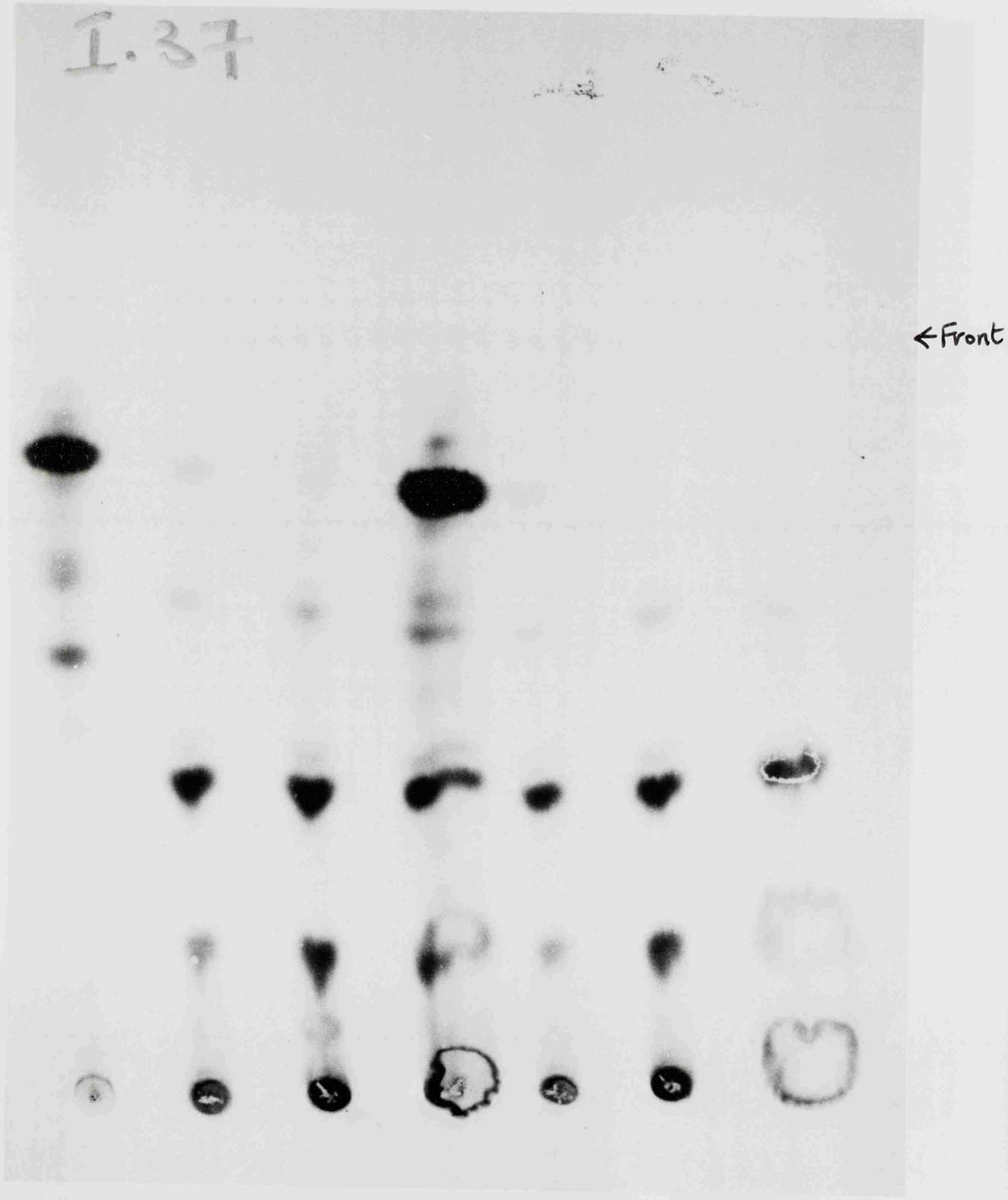
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Plate I. 38 Radiochromatograms of extracts of different regions of Coleus internode segments following 24 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. The left hand chromatogram is an IAA-2-^{14}C marker with an R_f value of 0.43. There is a contaminant running just behind the IAA and probably some IAamd near the front. The second and sixth chromatograms are the extract of the apical 1 mm of the segment. The dark spot at 0.07 is probably IAAsp and there is a faint IAA spot. The third and seventh chromatograms are the extract of the middle 10 mm of the segment. The IAAsp spot is quite faint and the IAA spot is quite dark. The fourth and eighth chromatograms are the extract of the basal 4 mm of the segment. The IAA and IAAsp spots are both quite dark. It would appear that there is less IAAsp than there is in the apical extract and more IAA. The fifth chromatogram is the apical extract 'spiked' with IAA-2-^{14}C.

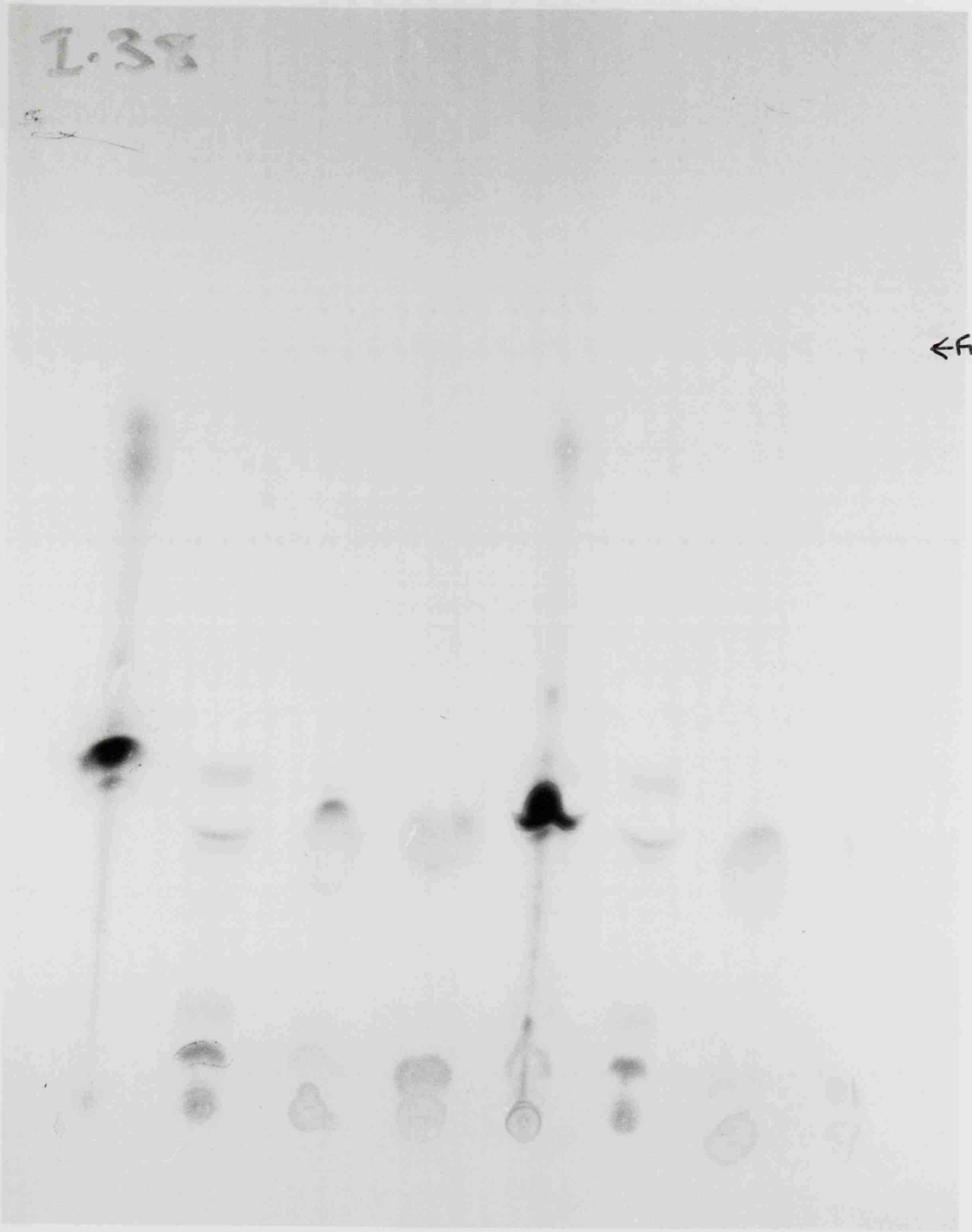
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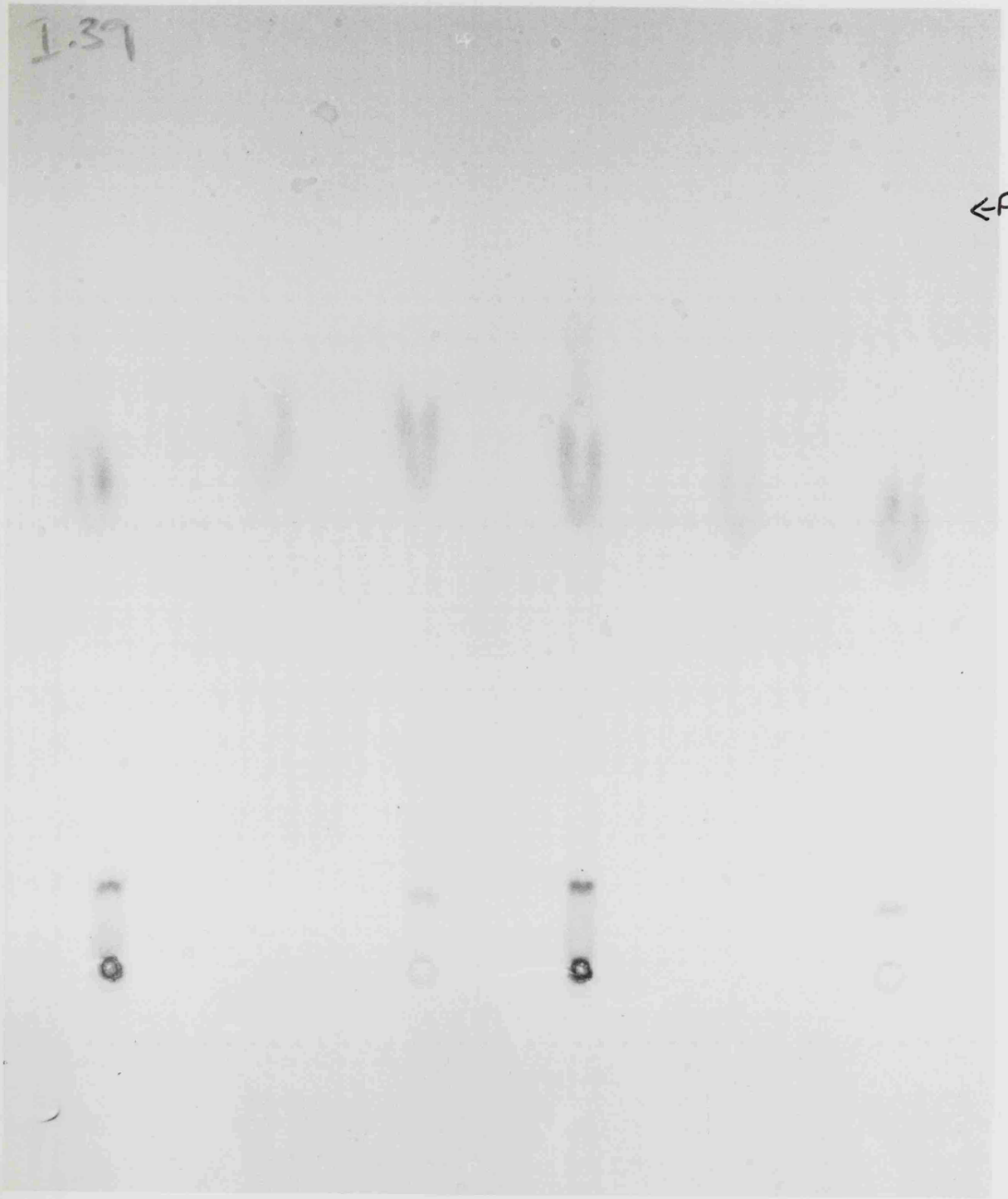
Plate I. 39 Radiochromatograms of extracts of different regions of Coleus internode segments following 5 h basipetal IAA-2-^{14}C treatment

The chromatograms were developed in isopropanol/ammonia/water. All three extracts have the same two constituents, IAA running at 0.60 - 0.70 and IAAsp running at 0.11. The relative proportions appear to be different. The first and fourth chromatograms are the extract of the apical 5 mm of the segment. The IAAsp spot is very dark and the IAA spot which is large and more diffuse is also quite prominent. The second and fifth chromatograms are the extract of the middle 7 mm of the segment. The IAAsp spot is not visible. Only IAA can be seen. The third and sixth chromatograms are the extract of the basal 3 mm of the segment. Neither spot is as dark as the apical extract spots. Both are quite distinct.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blocks. After the 21 h period the tissue and both sets of agar blocks were extracted and chromatographed. Plate I.40 shows a chromatogram of these extracts developed in isopropanol/ammonia/water. In fact the level of activity in the replacement donor was so low that it could not be detected on this chromatogram but other points of interest do emerge. A trace of IAAsp is present in the extract of the original donor although the bulk of the activity is associated with IAA as would be expected. This shows that IAAsp can move by diffusion at the cut surface into an agar block, even though it is immobile within the tissue. In an experiment where receiver extract was chromatographed, however, there was no sign of IAAsp in the extract (see Plate I.41). The receiver contained only IAA and the impurities which were included in the IAA-^{14}C solution. Another feature of the 'chase' experiment is the apparent lack of IAA in the tissue extract. The IAAsp spot is very obvious. It would appear that during the chase period all unchanged IAA is either moved from the tissues or metabolised to IAAsp.

Investigation of protein-bound IAA

Reports exist in the literature that IAA can form protein complexes (Bentley, 1961; Bara, 1967; Osborne, 1967; Morris et al., 1969; Osborne and Mullins, 1969; Basu and Tuli, 1972; and Davies, 1972). Hallmen and Eliasson (1973) reported a 2,4-D/protein complex. Andreae and Van Ysselstein (1960) found no evidence of protein/IAA complexes in pea roots which had been allowed to take up IAA and Osborne (1967) and Morris et al. (1969) found that no protein/IAA complex could be found in tissue which had ceased to elongate. The Coleus internode segments used in these experiments were tested for IAA/protein complexing. The method of releasing IAA from a complex by hydrolysis was similar to the methods used by Morris et al. (1969) and Davies (1972) but no radioactive IAA was recovered from the resulting extract. This finding was not unexpected in the

Plate I.40 Radiochromatograms of the extract of Coleus internode segments following a 5 h basipetal IAA-2-^{14}C treatment and a 16 h chase period

The chromatograms were developed in isopropanol/ammonia/water. The left hand chromatogram is an IAA-2-^{14}C marker with an R_f value of 0.55 and with some activity at the front. The second chromatogram is the extract of the donor block. Most of the activity is associated with IAA. There is a very faint spot at 0.06. The third chromatogram is tissue extract and there is only one dark spot at 0.06 which is probably IAAsp.


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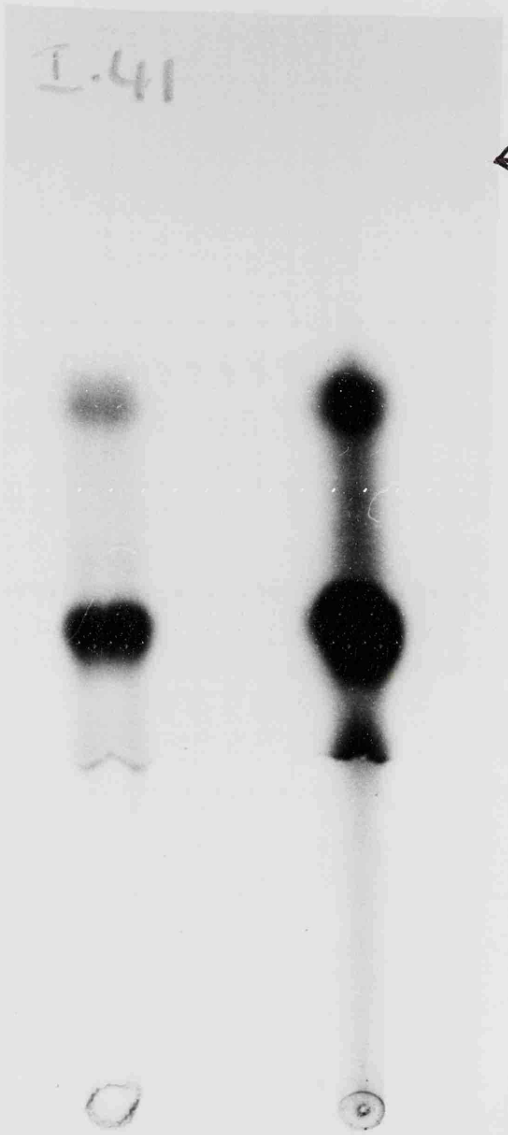
Plate I.41 Radiochromatograms of the extract of receiver blocks following 24 h basipetal IAA-2-^{14}C treatment of Coleus internode segments

The first spot is the receiver extract and the second is an IAA-2-^{14}C marker. The marker had an R_f value of 0.50 and there are contaminants present one of which is possibly IAamd. Only constituents of the marker are present in the receiver extract.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light of reports in the literature since the segments were cut from expanded internodes. No evidence of IAA/protein complexes was found even in segments which had been treated for 24 h.

2,4-D

Quantitative experiments (see Chapter II) showed certain differences in transport characteristics between 2,4-D and IAA which suggested that 2,4-D does not undergo immobilisation. Extraction experiments following 2,4-D treatment were, therefore, performed to investigate its metabolism during transport. The experimental procedure was that used in the IAA experiments but 5 μl of 10^{-4}M 2,4-D solution were applied in the donor block to the apical cut surface of each internode segment. Plates I.42 and I.43 show the results of such an experiment. It appears that little or no 2,4-D metabolism occurs during transport in Coleus internode segments. The contaminant present in the marker solution and in the donor do not appear to enter the tissue.

Plate I.42 Radiochromatograms of Coleus internode extract following 24 h basipetal 2,4-D-^{14}C transport

The chromatograms were developed in butanol/acetic acid/water. The left hand chromatogram is 2,4-4-^{14}C marker, and the middle chromatogram is an extract of the donor. Both show the dark 2,4-D spot at 0.80 and small amounts of contaminants at 0.10 and 0.52. The right had chromatogram is the tissue extract. Only one spot is present and this has an R_f value of 0.63. This is lower than the R_f value of 2,4-D in the other two chromatograms but the value is probably affected by the other constituents of the extract.

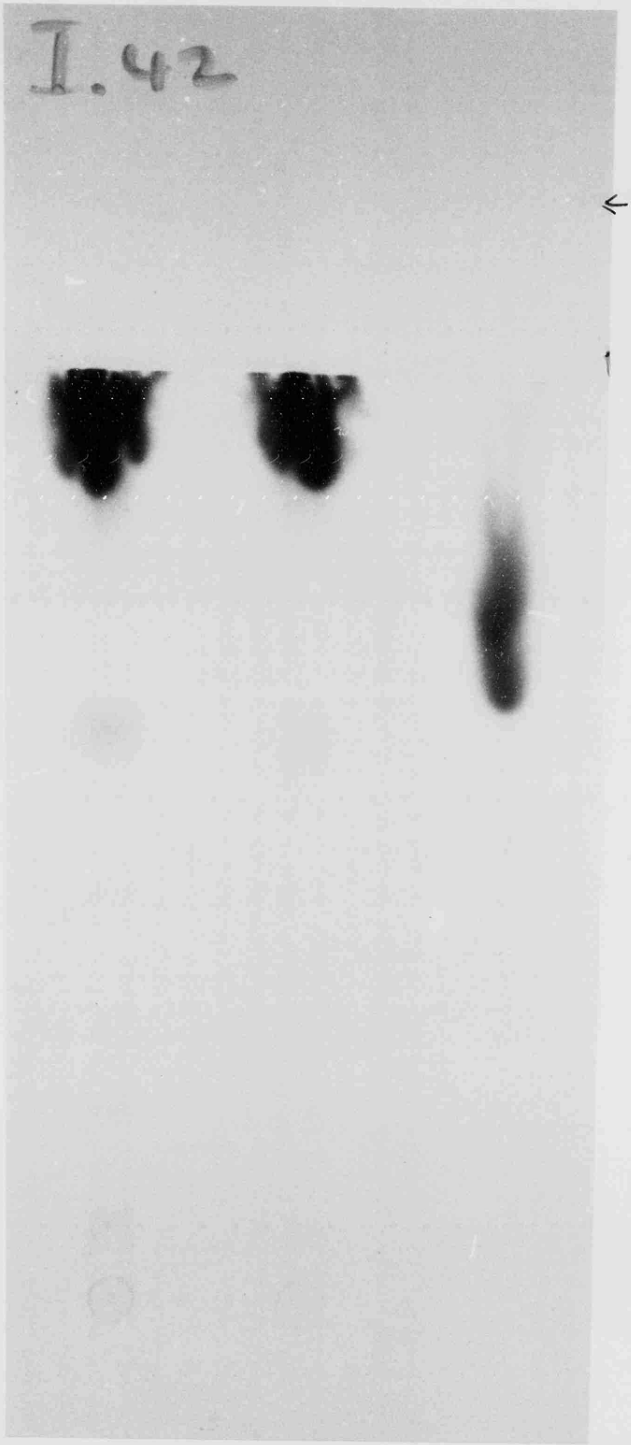
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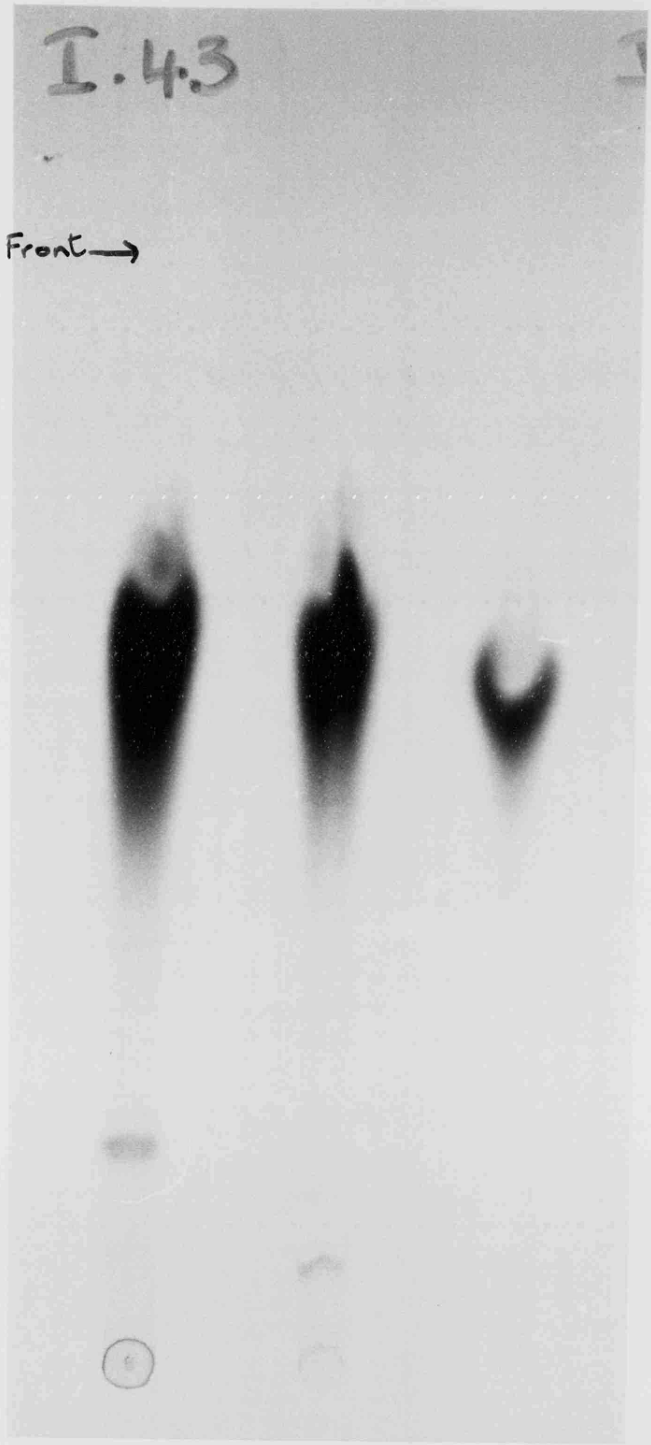
Plate I.43 Radiochromatograms of Coleus internode extract following 24 h basipetal transport of 2,4-D-^{14}C.

The chromatograms were developed in isopropanol/ammonia/water. The left hand chromatogram is 2,4-D-^{14}C marker, and the middle chromatogram is an extract of the donor. Both show the 2,4-D spot at 0.60 and a contaminant with a low R_f value. The right hand chromatogram is the tissue extract. It has only one spot with an R_f value which corresponds with that of the 2,4-D spot in the other two chromatograms.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Discussion

In this chapter inferences are drawn largely from correspondence between radioactive spots in chromatograms of extracts and colour spots resulting from Van Urk or Ninhydrin detection of authentic samples of indole compounds chromatographed in parallel; and from a knowledge of expected chromatographic behaviour based on the literature. Such indications are not, in themselves, conclusive and it would have been desirable to obtain confirmation of the indications using more sophisticated techniques. Such procedures do not fall within the scope of this project and therefore it would not be wise to attempt to draw firm conclusions from these data. Some of the points which arose, e.g. the relative amounts of IAA and IAAsp in an extract, are explored further in the next chapter, as are the time course of IAA-^{14}C uptake and transport and transport patterns in segments from flowering plants. In other cases, however, complementary and confirmatory experiments have unfortunately had to be omitted.

It would appear from the results recorded in Chapter I that IAAsp is the main product of IAA metabolism in Coleus tissue. It was not always the only product present but it was usually the most prominent one and its presence was indicated in all the situations investigated. Thus IAAsp appears to be produced in internodes, petioles and roots. It occurs whether the plant from which the segments were cut is in the vegetative or the floral condition, and it is formed during acropetal and basipetal transport. The different treatments of the plant extracts which were employed did not seem to have any effect on its presence. Its presence is indicated in four different chromatography solvent systems. It is first formed quite soon after the application of IAA and it is probably formed in several tissues within an internode.

It is not surprising that IAAsp should feature so prominently in these results since frequent reports of its formation exist in the literature as quoted in the introduction to this chapter. In many of these reports it is shown to be the only product or the major product of IAA metabolism. Veen (1966) found that radioactive naphthaleneacetyl aspartate (NAAsp) accounted for 40% of the activity extractable from Coleus explants after 24 h treatment with NAA-^{14}C. Robinson et al. (1968) reported that in cotton explants nearly all the activity recovered after 4 h was associated with IAAsp. Veen and Jacobs (1969) found IAAsp and a glucoside derivative in Coleus petiole segments and noted a correlation between the age of the petiole and the amount of IAAsp formed. They reported that 'in extracts of older petiole segments most of the radioactivity was found at the R_f typical of the IAA-aspartate complex'. Morris et al. (1969) found that 'in all organs a considerable portion of the applied IAA was converted to IAAsp'. Kendall et al. (1971) found IAAsp to be the only noticeable product of IAA to be produced in etiolated pea plants, with perhaps a little IAld. Iversen et al. (1971) stated that the major part of the activity recovered from Phaseolus roots was associated with IAAsp. Of the several metabolites of pea and bean identified by Davies (1972) IAAsp was the one present in the greatest amounts. One rather surprising report of the occurrence of IAAsp is that of Winter and Thimann (1966) who identified IAAsp in an ether extract of pea stem sections, since IAAsp is not generally regarded as being ether-soluble.

While the reports in the literature support the results from these experiments with Coleus they make it even more surprising that little evidence could be found for the formation of an aspartic acid conjugate in Helianthus. Good et al. (1956) found IAAsp in sunflower hypocotyls and Aasheim and Iversen (1971) tentatively identified IAAsp in extracts of treated Helianthus roots. Also it has

been shown that the activity distribution profiles (see Chapter II) are similar for Coleus and Helianthus suggesting that their immobilisation patterns should be similar. However, other results discussed in the next chapter suggest that even in Coleus not all the immobilised IAA exists as the aspartate conjugate, so it could be that the auxin in Helianthus is immobilised and yet not chemically changed. Also, Wangermann (unpublished data) in her experiments with wounded Helianthus internode segments found that some of the accumulated auxin could be 'chased' out. The only slight indication of the presence of IAAsp in Helianthus tissue was in the epidermal extract. As stated earlier there is some reason to believe that products of auxin metabolism are to be found in the epidermis of internodes but the presence of IAAsp in the epidermis of sunflower segments has not been confirmed.

As regards the presence of other metabolites in the extracts the situation is not very clear. The most puzzling substance was the 'pigment' spot on the chromatograms. Its occurrence was both regular and prominent in early experiments and it seemed surprising that no references to its formation could be found. Its sudden failure to appear was even more surprising. The clone of Coleus used in these experiments has been used in this laboratory for some years during which time the plants have always been maintained under the same conditions. It seems unlikely that the plants should suddenly fail to undergo a metabolic process when no apparent change had occurred in the clone and when the conditions under which the plants were grown and the experiments were conducted had not been altered. Neither did the failure of the pigmented material to separate coincide with any of the changes in extraction procedure which were reported. Such a coincidence would have been consistent with a chromatographic artefact. It may be significant that the intensity of the radioactive spot increased with time. This could suggest a

gradual accumulation of the material. Alternatively, it could be that all the radioactive substances were simply carried along with the pigmented material and that after a short period of uptake and transport not much activity would be available to be carried along with the pigment. If this were the case it would explain the failure of the radioactive spot to appear in the absence of the pigmented spot on the TLC plate. At the time when the pigment and its associated radioactivity were routinely noticed in butanol/acetic acid/water chromatograms attempts were made to find another chromatography solvent in which the material would run. In the preliminary attempts of this nature darkly pigmented Coleus stems were selected and attempts were made to extract them without first applying any exogenous auxin. No pigment spot could be seen on chromatograms of extracts of untreated Coleus stems. It is therefore possible that even when the substance could be separated from other constituents of the extracts it could only be separated in the presence of exogenous auxin or one of its products. Even so this does not explain the later failure of the pigment to appear at all.

The presence of IAamd has been explained by Zenk (1963) as the product of a reaction between the exogenous IAA and the ammonia in the chromatography solvent and this could well be the case in these experiments. However, Good et al. (1956) found IAamd in all the 12 species they studied. It was particularly abundant in cereal extracts but traces were present in the extracts of dicotyledonous plants they tested. The solvent system they used was isopropanol/ammonia/water so in the light of Zenk's findings the possibility cannot be excluded that, at least in those cases where IAamd was not abundant, it was formed during the chromatographic procedure. Davies (1972) identified traces of IAamd in pea and bean segments, however, and in his case it appeared in extracts chromatographed

in solvent systems which did not contain ammonia. Iversen et al. (1971) found an unknown compound in bean root extracts which had a high R_f value in isopropanol/ammonia/water. They did not think the unknown compound was indole acetaldehyde (IAAld). They did not discuss the possibility that it could be IAamd. In these experiments there is an unconfirmed indication of the presence of IAamd in extracts chromatographed in chloroform/ethyl acetate/ formic acid but its failure to appear in isopropanol/ammonia/water chromatographs except in marker solutions does not support this indication.

The data regarding IAGlu are also confusing, especially since no authentic sample was available for chromatography. Using R_f values as quoted in the literature its presence is only indicated in chromatograms developed in chloroform/ethyl acetate/formic acid and there is no evidence for its occurrence in the other systems. Indole acetyl glucoside has been identified by several workers. Zenk (1961, 1963) found it in Hypericum and Colchicum leaves. Winter and Thimann (1966) identified it in Avena coleoptiles and Winter (1967) found it in Pisum stem. Veen and Jacobs (1969) found IAGlu in Coleus petiole segments. Davies (1972) extracted it from Pisum and Phaseolus epicotyls. Hertel and Flory (1968) extracted naphthalene acetyl glucoside (NAGlu) from coleoptiles treated with NAA.

It is possible that the length of the transport period could be critical in experiments of this kind because reports exist of metabolites which are formed and then broken down again. Thus Iversen et al. (1971) found their unknown compound only after a 5 h treatment. It was not present after 20 h. Zenk (1963) reports that IAGlu is an early product of IAA which is formed before the IAAsp synthesis system comes into force and that later IAGlu, though not a metabolic intermediate, is converted to IAAsp. Hertel and Flory (1968), on the other

hand, report that in coleoptiles NAGlu accumulates in the tissue and is not turned over. Veen and Jacobs (1969) found that the metabolites they identified depended on the age of the tissue: within a 5 h transport period they identified IAAsp in older petioles but IAGlu in their 'petiole no. 2''. The failure to find IAA/protein complexes in the internode tissue investigated in these experiments is not surprising as explained earlier.

When the time course of auxin metabolism was studied no turnover of products such as those mentioned above was noticed. During the first 7 h period there was an increase in the overall amount of radioactivity in the segments (also confirmed in Chapter II). At first most of the activity was associated with IAA and the amount of this appeared to increase. When the IAAsp started to form it then gradually increased and the relative amount of unchanged IAA present decreased. During the 7 h period no other products of IAA (except the material or materials associated with the pigment spot which is discussed above) were observed. Certainly in these experiments the IAAsp was the first identifiable metabolite formed. This is not in agreement with Zenk (1963) who found IAGlu to be formed during the 2-3 h lag associated with IAAsp synthesis. The actual length of that lag seemed to be variable in these experiments. In one set of experiments IAAsp appeared to be produced within 30 min but in another a 3 h lag was noticed. A breakdown of IAAsp after 6 h as reported by Morris et al. (1969) was not noticed in these experiments. Most experiments ran for a 24 h transport period and IAAsp was always present in large amounts after such experiments, suggesting that it does continue to accumulate. However, quantitative data on IAAsp accumulation over long periods have not been gathered. Some data are presented in the next chapter but these refer only to 5 h and 24 h periods. From these results it is not possible to state that IAAsp is not further metabolised,

but no indications of such a breakdown were observed.

No evidence was obtained for different transport mechanisms although it had been thought that acropetal and basipetal transport could occur by mechanisms which differed to the extent that metabolites were different; or that a different mechanism could exist when basipolarity was reduced. The products of IAA were the same in stems and petioles (the evidence for roots was not conclusive) and acropetal transport produced the same metabolites as basipetal transport. In the tissue from flowering plants where polarity might be expected to be reduced (Leopold and Guernsey, 1953; Naqvi and Gordon, 1965) the same products are again seen. Apparently even where polarity is reduced the mechanism is not altered to the extent where the products are different. However, data presented in the next chapter do not support the idea that polarity is, in fact, reduced in stems from flowering plants. The results of the experiments on whole cuttings suggest that transport from the shoot continues into the root as reported by Morris et al. (1969) although no different metabolites could be identified.

In the dissection experiments only quantitative differences were noticed and not qualitative ones as had been thought possible. It seemed that relatively more free IAA was present in the pith than in the other tissues and that relatively more IAAsp was present in the epidermis than in the pith or the vascular tissue. There were also indications that IAAsp might be present in the epidermis of Helianthus, so the possibility exists that immobilised IAA could be stored in epidermal tissue. Autoradiographs published by Wangermann (1968, 1970) have shown a concentration of radioactivity in the vascular tissue. Wangermann (1974) in her wounding experiments found that damaging the pith of a Helianthus internode segment did not produce much change in the transport pattern whereas damage

to the vascular tissue had a marked effect. These dissection experiments have not attempted to compare overall levels of activity in the three tissue components, only the relative levels of the two main constituents of the extracts. Therefore they do not disagree with the results of Wangermann (1968, 1970, 1974). They merely suggest that little of the enzyme required for the synthesis of IAAsp is present in the pith and that some unchanged IAA is there. It could be that this unchanged IAA is moving simply by diffusion and not by any active process.

Experiments designed to 'chase' activity from a segment are dealt with more thoroughly in Chapter II, but the experiments considered in the present chapter suggest that after a 'chase' period only IAAsp and no free IAA is present in Coleus segments. Any free IAA which had been present is apparently exported or conjugated during the period following the removal of the donor.

The experiments with 2,4-D that are reported in this chapter are not extensive. They were performed largely to complement the quantitative experiments in Chapter II. They do suggest, however, that 2,4-D is not broken down in Coleus segments. This is in agreement with Andreae and Good (1957) and Field and Peel (1971) who found no metabolites of 2,4-D in pea and willow respectively. However, several other workers have reported finding metabolites of 2,4-D in transport experiments (Hay and Thimann, 1956; Bach and Fellig, 1959; Bach, 1961; Hertel and Flory, 1968; and Hallmén and Eliasson, 1972). All these studies were on either bean or wheat. Coleus has not been extenstively studied with regard to 2,4-D metabolism.

CHAPTER II

EXPERIMENTS INVOLVING OXIDATION OF PLANT MATERIAL

The distribution of radioactivity along treated Coleus internode segments was investigated using the Intertechnique Oxymat (see Appendix).

Internode segments were prepared in the usual way. They were supported in perspex racks with the lower cut surfaces in contact with plain agar blocks (receiver blocks). Each receiver block stood on a double rectangle of aluminium foil (1 cm x 0.5 cm) which was placed on the damp filter paper lining the Petri dish. The radioactive IAA solution was applied in agar donor blocks. Except where otherwise stated 5 μl of solution were used in each block. As in the extraction experiments the Petri dishes containing the internode segments were kept in a closed, translucent plastic tray, the bottom of which was lined with damp filter paper.

After the initial preparation of the segments the whole assembly was transferred to a dark room illuminated by a green 'safelight'. The donors were applied there and transport was allowed to proceed under these conditions. Following the transport period the donors were removed from the internode segments and placed in the polycarbonate combustion vessels designed for use in the Oxymat. Usually 2 donors were placed in 1 vessel. The segments were then carefully divided transversely into sections 1 mm thick. At first these sections were cut by hand but subsequently a Mickel Gel Slicer was used. This made cutting more accurate and also was quicker so that it minimised tissue deterioration and any redistribution of activity which followed removal of the donor blocks. Before the slicer was used it was often possible to reliably cut only 14 sections from a segment. Where this was the case it is indicated in the table of results. The sections of each segment are designated A - O or A - N in the circumstances described above. Similar sections from different segments were pooled in combustion vessels. Usually 5 sections were put into 1 vessel.

This practice of pooling samples was intended to give reliable results particularly in the case of low activity samples. Receiver blocks were also placed in combustion vessels, usually 5 blocks to a vessel. All the vessels and their contents were then oxidised in the Oxymat. Where possible the samples were oxidised in the estimated ascending order of radioactivity so that the 'memory' of the Oxymat introduced as small an error as possible. Several 'blank' samples were oxidised during the course of each experiment in order to calculate a value for background radiation and this value was used to correct all samples.

When all samples had been oxidised they were allowed to equilibrate for 2 - 3 h before being counted in a Beckmann LS 100 scintillation counter. Samples were counted for 10 min each or to a preset error of 3%. Mean values for individual samples were calculated from corrected values for ten pooled samples, and it is the mean values which are recorded in the tables of results below. Figures in the 'Counts' column represent counts/min.

Basipetal transport

In the course of the development of the above technique preliminary experiments were performed. These used filter paper discs as donors and involved the division of the segments into 4 large sections. These experiments served to investigate suitable volumes of IAA solution to apply to each segment. The use of filter paper donors was discontinued because they seemed to restrict uptake of auxin into the tissue and also caused some leakage down the surfaces of the segments. The preliminary experiments showed that after basipetal transport levels of activity declined with distance from the donor only over the first three quarters of the segment. More radioactivity could be detected in the distal

section than in the third section. These experiments also served to demonstrate the degree of recovery provided by the Oxymat and the reproducibility of its results.

In the first experiment basipetal transport was allowed to continue for 12 h and 10 μl of IAA solution were applied to each segment. The results are shown in Table II.1.

Table II.1 Activity distribution after 12 h basipetal transport

Component	Count	
Donor	31,402	
A	8,994	
B	2,313	
C	963	
D	584	
E	373	
F	266	
G	198	
H	149	
I	134	
J	138	
K	150	
L	407	
M	564	
N	2,019	
Receiver	338	
Total	48,992	(10 μl)

The basal accumulation of activity was again apparent and a more accurate picture of the activity profile was obtained. The range of activity levels was so great that it was necessary to use a log scale to plot the activities. All profiles subsequently shown are semi-log plots. The level of activity in the section proximal to the donor was shown to be very high. This was not unexpected since micro-autoradiographs produced in this laboratory (Wangermann,1968; 1970) showed dense blackening of the film exposed to the mm adjacent to the donor. This suggests that uptake occurs more readily than transport. This

high level fell off rapidly at first and then more slowly over 11 mm. It began to rise again over the distal 3 mm until the level of activity in section N of the segment was similar to that in section B.

When the treated internode segments were divided into sections after a 5 h treatment the log profile was seen to be a basically similar shape to that constructed for the 12 h treatment. The results are in Table II.2. Again the level of activity fell from sections A - I. In this shorter experiment, however, the log profile flattened a little over sections I - L before rising rapidly over the last three sections. As recorded in the 12 h experiment the level of activity in section O was similar to that in section B. Thus the basal accumulation noted in these experiments had established its form within the first 5 h of transport.

Table II.2 Activity distribution after 5 h basipetal transport

Component	Count
Donor	22,268
A	4,120
B	1,038
C	439
D	247
E	155
F	130
G	107
H	94
I	88
J	83
K	81
L	83
M	99
N	168
O	1,141
Receiver	181
Total	30,523

Acropetal transport

Acropetal transport in Coleus internode segments was studied next. As in the case of the first experiment on basipetal transport the donor carried 10 μl of IAA solution. The experiments ran for 12 h (Table II.3) and 5 h (Table II.4).

Table II.3 Activity distribution after 12 h acropetal transport

Component	Count
Donor	27,900
A	9,543
B	2,708
C	985
D	408
E	182
F	116
G	63
H	45
I	35
J	26
K	30
L	24
M	33
N	33
Receiver	89
Total	42,220(10 μl)

Table II.4 Activity distribution after 5 h acropetal transport

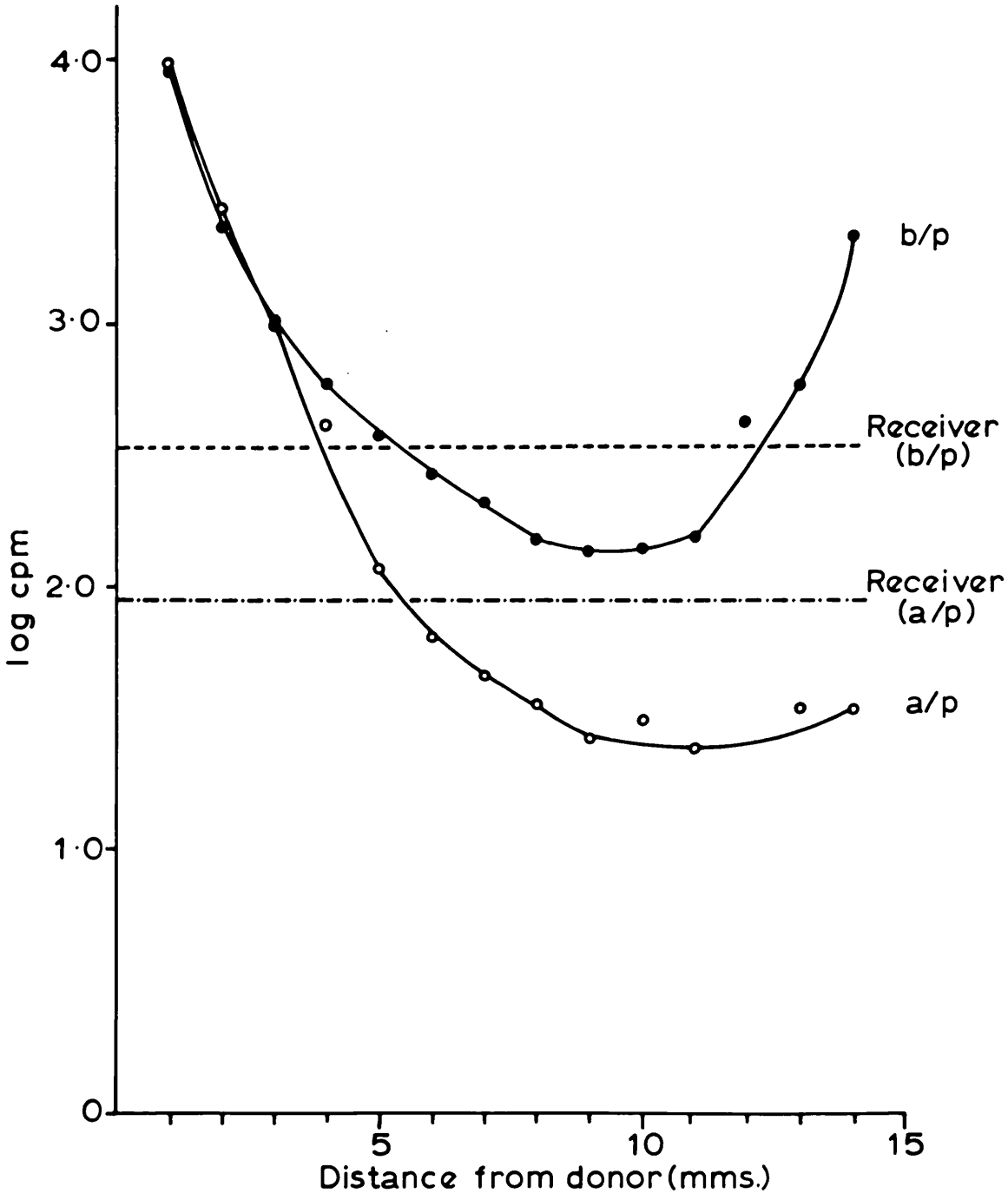
Component	Count
Donor	43,164
A	5,047
B	917
C	256
D	140
E	67
F	55
G	41
H	31
I	20
J	14
K	11
L	12
M	8
N	5
Receiver	89
Total	49,912(10 μl)

It can be seen from the results that the basal uptake of radioactive material from the donor into the proximal section over 12 h is comparable with apical uptake over the same period. In the two corresponding experiments the mean level of activity recorded in section A after apical uptake was approximately 9,000 counts/min and the mean level recorded in section A after basal uptake was 9,500 counts/min. Likewise, counts in sections B, C and D are comparable. However, from section E onwards the profiles for acropetal and basipetal transport are quite different.

In both the acropetal experiments the activity levels declined steadily

Fig. II.1 Log profiles of the distribution of radioactivity in Coleus internode segments after 12 h basipetal and acropetal transport of IAA-2-^{14}C

The distribution of radioactive material after 12 h basipetal transport is very different from that after a similar period of acropetal transport. A large accumulation of radioactivity occurs at the base of the segment after basipetal transport, the level in the distal section being similar to that in the second section. After acropetal transport the levels of activity decline with distance from the donor and then level off. More material passes into the basal donor than into the apical donor.


4·0
3·0
2·0
1·0
0
log cpm
b/p
Receiver (b/p)
Receiver (a/p)
a/p
5
10
15
Distance from donor (mms.)

from sections A - I and then either levelled off (12 h experiment), or became too low to be reliable (5 h experiment). There was no basal accumulation of the type seen after basipetal transport and counts in the receiver were low. The log activity profile after acropetal transport closely resembles a straight line. The log profiles for these experiments are shown in Figs II.1 and II.2.

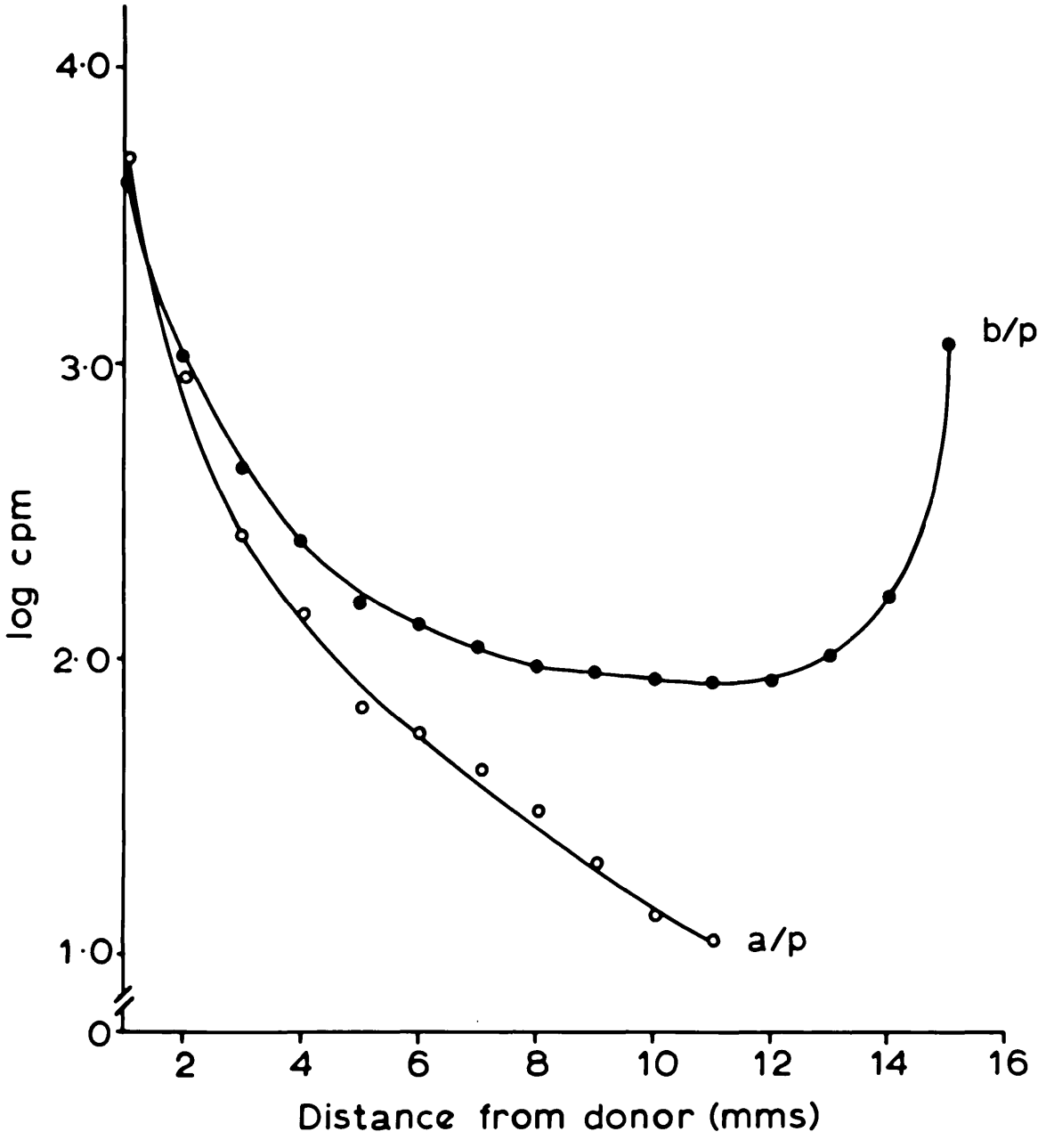
Investigation of the accumulation of activity at the base of segments

Since the basal accumulation of activity was so pronounced it was further investigated in order to confirm that it was not caused by the experimental procedure. Experiments involving plant segments are often criticised because of the limitations imposed by the cut surfaces. Therefore, although the good contact between agar and receiver, essential in this kind of experiment, had not previously been in any doubt, the possibility that the accumulation could be caused by the inability of the material to cross the cut surface and enter the agar block had to be considered.

Making the assumption that the contact between agar and tissue at the top of the segments was good since it usually allowed uptake of material from the donor and could be easily visually examined, the experiment was performed again but with the segments inverted. The transport time was 5 h. The donor block was thus at the bottom of the assembly and the receiver at the top. Good contact between tissue and receiver was confirmed visually and care was taken as usual to ensure the good contact at the bottom of the assembly which would be confirmed later by good uptake from the donor. The technique was modified slightly when the donor was at the bottom of the assembly. A plain agar block was placed below the donor block to absorb any IAA-^{14}C which might otherwise have leaked out into the bottom of the Petri dish.

Fig. II.2 Log profiles of the distribution of radioactivity in Coleus internode segments after 5 h basipetal and acropetal transport of IAA-2-^{14}C

These two curves are not directly comparable because the donor concentration was greater in the acropetal experiment than in the basipetal experiment. The curves show that the basal accumulation of radioactive material seen after 12 h of basipetal transport is already established after 5 h and again the level of radioactivity in the distal section is similar to that in the second section. In contrast, detectable levels of radioactive material have only penetrated 11mm acropetally.


4·0
3·0
2·0
1·0
0
log cpm
b/p
a/p
2
4
6
8
10
12
14
16
Distance from donor (mms)

The results of such an experiment and the results of a control experiment performed concurrently in which the segments were placed apical end uppermost are shown in Table II.5.

Table II.5 Activity distribution after 5 h basipetal transport

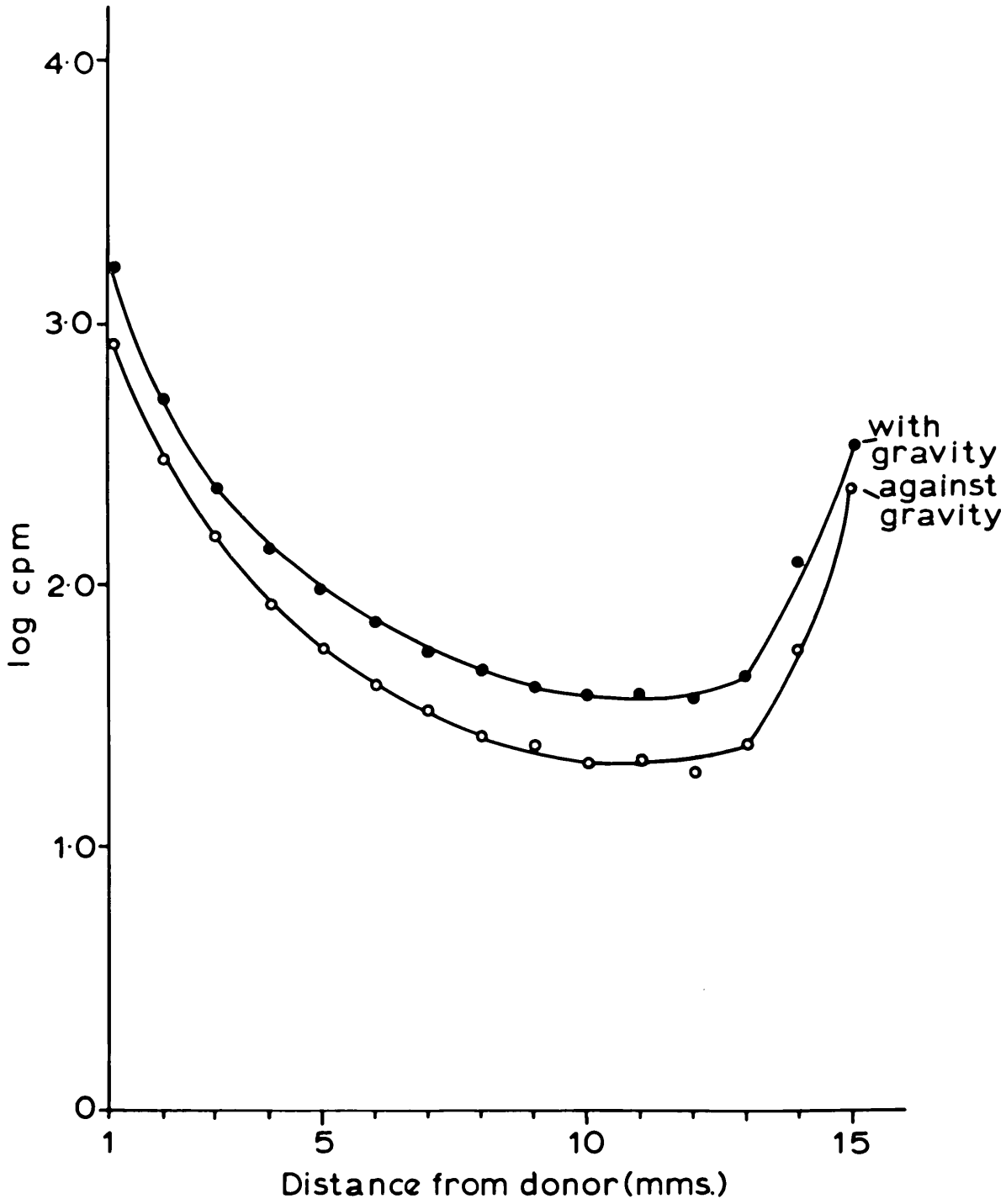
	Segments inverted	Control segments
Component	Count	Count
Donor	20,734	20,401
A	833	1,598
B	294	506
C	150	233
D	84	139
E	56	94
F	41	73
G	32	56
H	26	46
I	24	40
J	20	37
K	22	38
L	19	35
M	24	43
N	57	121
O	231	339
Receiver	31	25
Total	22,678	23,824

The log profiles are shown in Fig. II.3. It can be seen that the profiles are similar in both situations. The basal accumulation occurs in both cases and the level of activity recorded in section O is close to that recorded in section B in both situations.

Another point which emerges from these results is that more activity is recorded in sections from the control segments than from the inverted segments. If movement of radioactive material is considered in the two experiments it can be shown that when the segments were orientated normally 14.4% of the available activity moved into the segment, of which 46.7% remained in section A. When the segments were inverted only 8.6% of

Fig. II.3 Log profiles of the distribution of radioactivity in Coleus internode segments after 5 h basipetal transport of IAA-2-^{14}C with and against gravity

The curves are of the same general shape and the basal accumulation of activity occurs in both cases. Uptake and transport are apparently somewhat reduced in the inverted segments.


4·0
3·0
2·0
1·0
0
log cpm
1
5
10
15
Distance from donor (mms.)
with gravity
against gravity

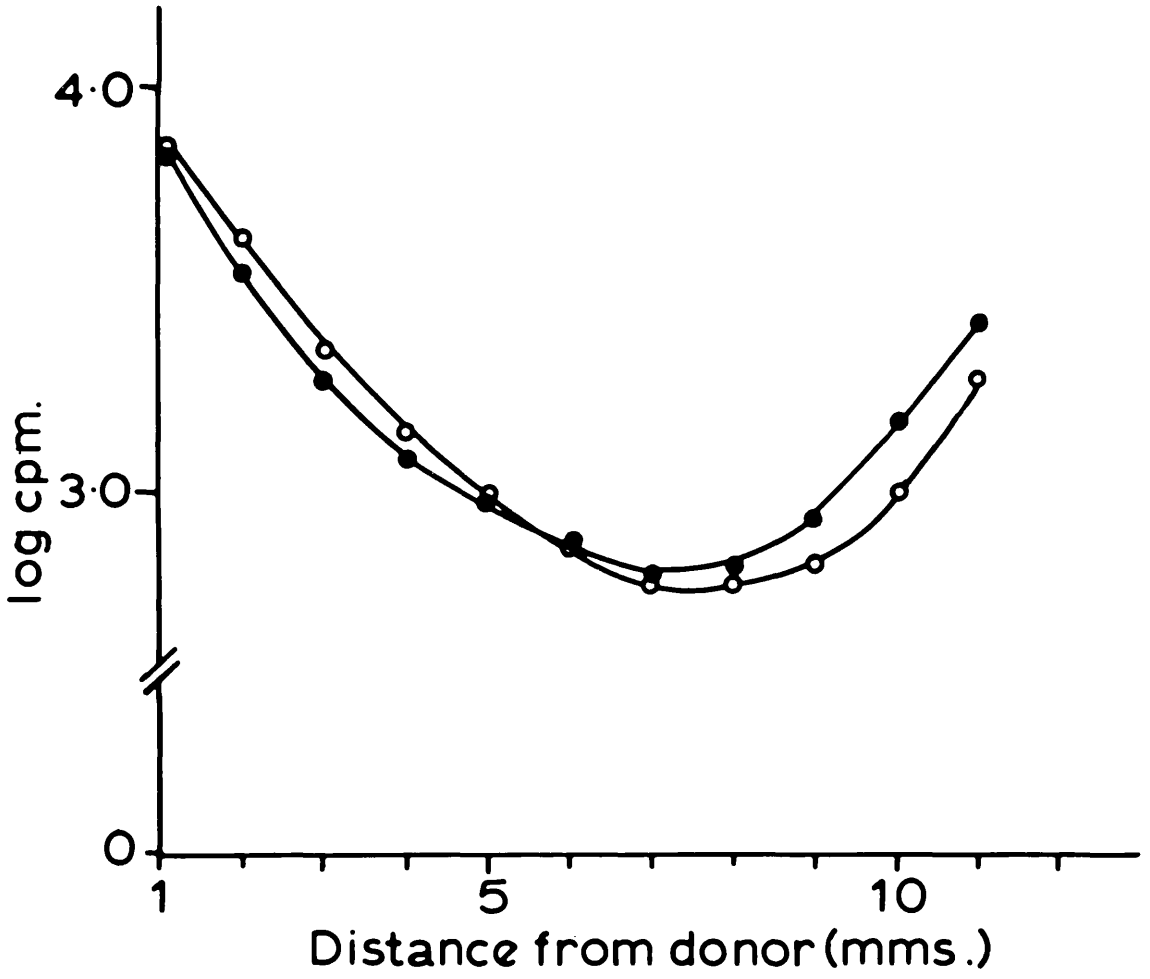
available activity moved into the tissue, of which 42.9% remained in section A. In both these experiments uptake is low compared with that shown in Table II.2 but that experiment was performed at a different time.

Thus the uptake of radioactive material and its subsequent transport are reduced when the segments are inverted but the resulting distribution of activity in the tissue follows the same pattern. The basal accumulation of activity still occurs even when the physiological base of the segments is presumed to be in good contact with the receiver. These results suggest that the labelled material is not prevented from leaving the tissue by a physical discontinuity at the cut surface. This experiment does not preclude the possibility of chemical interference at the cut surface as described by Steeves et al. (1953), Beyer and Morgan (1970) and Iversen and Aasheim (1970), but this will be dealt with later. The reduced uptake could suggest inadequate contact at the bottom of the assembly but it could also suggest that uptake and transport of IAA are directly influenced by gravity. Such an effect has been reported by Hertel and Leopold (1963), Lyon (1965) and Naqvi and Gordon (1966), but Jacobs (1950) and Gillespie and Thimann (1963) found transport to be unaffected by gravity. As stated above, the occurrence of the basal accumulation in the inverted segments indicates that poor contact is not responsible for that phenomenon.

In this laboratory Wangermann (1974) investigated the accumulation of activity at the base of Helianthus internode segments. In order to insure good contact she substituted a solid/liquid interface between the tissue and the receiver for the solid/solid interface described here, by using a small volume of water as a receiving system. The log profiles for this experiment and for a control experiment with agar receivers are shown in Fig. II.4. The two

Fig. II.4 Log profiles of the distribution of radioactivity in Helianthus internode segments after basipetal transport of IAA-2-^{14}C, with solid and liquid receiving systems.

These data are from experiments performed by Dr. E. Wangermann. They show that there is little difference in the form of the basal accumulation of activity whether agar receiver blocks were used (open circles) or whether water receivers were used (solid circles). Receiver counts were almost identical in both situations.


4·0
3·0
0
log cpm.
1
5
10
Distance from donor (mms.)

curves are very similar and the basal accumulation of activity is very pronounced in both cases. The counts are far higher than those obtained from experiments with Coleus internode segments (partly because of a longer transport period) but the profiles have the same shape. Thus the basal accumulation of activity does not appear to result from poor contact with the receiver and occurs in both species investigated.

Petiole segments

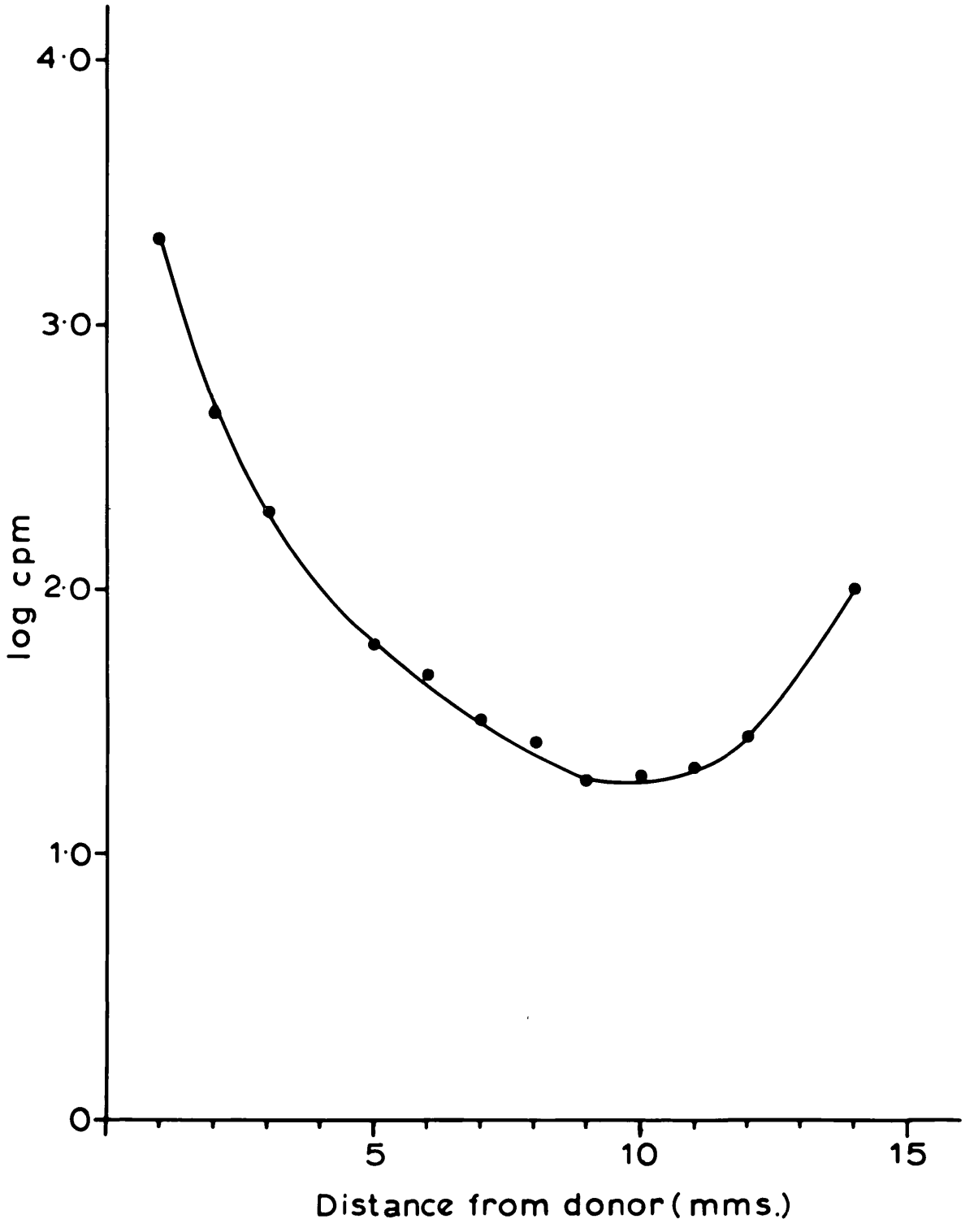
The distribution of radioactivity in petiole segments was investigated next. Segments were cut from the petioles at the third node below the apical bud. Each segment was cut from just below the lamina and was 15 mm in length. The segments were treated in the same way as the internode segments. Five μl of IAA-2-^{14}C solution were applied to the apical cut surface of each segment and the transport period was 5 h. The results were calculated in the usual way and are shown in Table II.6 and the log profile is shown in Fig. II.5.

Table II.6 Activity distribution in petiole segments after 5 h basipetal transport

Component	Count
Donor	25,599
A	2,084
B	457
C	197
D	89
E	61
F	47
G	32
H	26
I	19
J	20
K	21
L	27
M	66
N	99
Receiver	46
Total	28,886

Fig. II.5 Log profile of the distribution of radioactivity in Coleus petiole segments after 5 h basipetal transport of IAA-2-^{14}C

The distribution of radioactive material in petiole segments is similar to that in internode segments.


4·0
3·0
2·0
1·0
0
log cpm
5
10
15
Distance from donor (mms.)

It can be seen from the results that the distribution of activity in petiole segments closely resembles that in internode segments. The profile follows the usual form. Individual counts are lower than in a corresponding experiment with internode segments (see Table II.2), probably because of the lower cross-sectional diameter of a petiole segment.

Carboxyl-labelled auxin

All the experiments described so far have involved the use of methylene-labelled auxin. It was, therefore, decided to run experiments using carboxyl-labelled IAA to see if the distribution of activity was different, and to measure any decarboxylation which might occur during transport. The segments were prepared as usual and set up in the same apparatus. However, particular care was taken to seal the lid of the plastic tray with silicone grease and CO_2 traps were included in the tray. A small strip of filter paper (1 cm x 4 cm) was soaked in a 2% solution of KOH. The strip was folded at one end so that it could be made to stand up in a 5 ml beaker. Such a beaker with a KOH wick was placed in each Petri dish, alongside the bridge supporting the segments. Donors, each containing 5 μl of 5×10^{-5}M IAA-1-^{14}C solution, were placed on the apical cut ends of the segments. In the first experiment the transport period was 5 h and in the second it was 24 h. After the transport period the KOH wicks were put into Oxymat capsules as were the usual components of the experimental system. All samples were oxidised and counted. The results of the 5 h experiment are shown in Table II.7 (see overleaf) and those of the 24 h experiment in Table II.8 (see overleaf). The log profiles are shown in Figs II.6 and II.7.

These total counts (see Tables II.7 and II.8) are unexpectedly low but a standard unburned sample of 5 μl of IAA-1-^{14}C solution gave a similar count. The experiments were repeated with a completely new batch of IAA-1-^{14}C and the results obtained were quite comparable. It was later discovered that

Fig. II.6 Log profile of the distribution of radioactivity in Coleus internode segments after 5 h basipetal transport of IAA-1-^{14}C

The distribution of radioactive material after basipetal transport of IAA-1-^{14}C is very similar to that after basipetal transport of IAA-2-^{14}C. The basal accumulation is still marked. Little $^{14}CO_2$ was evolved during the transport period.

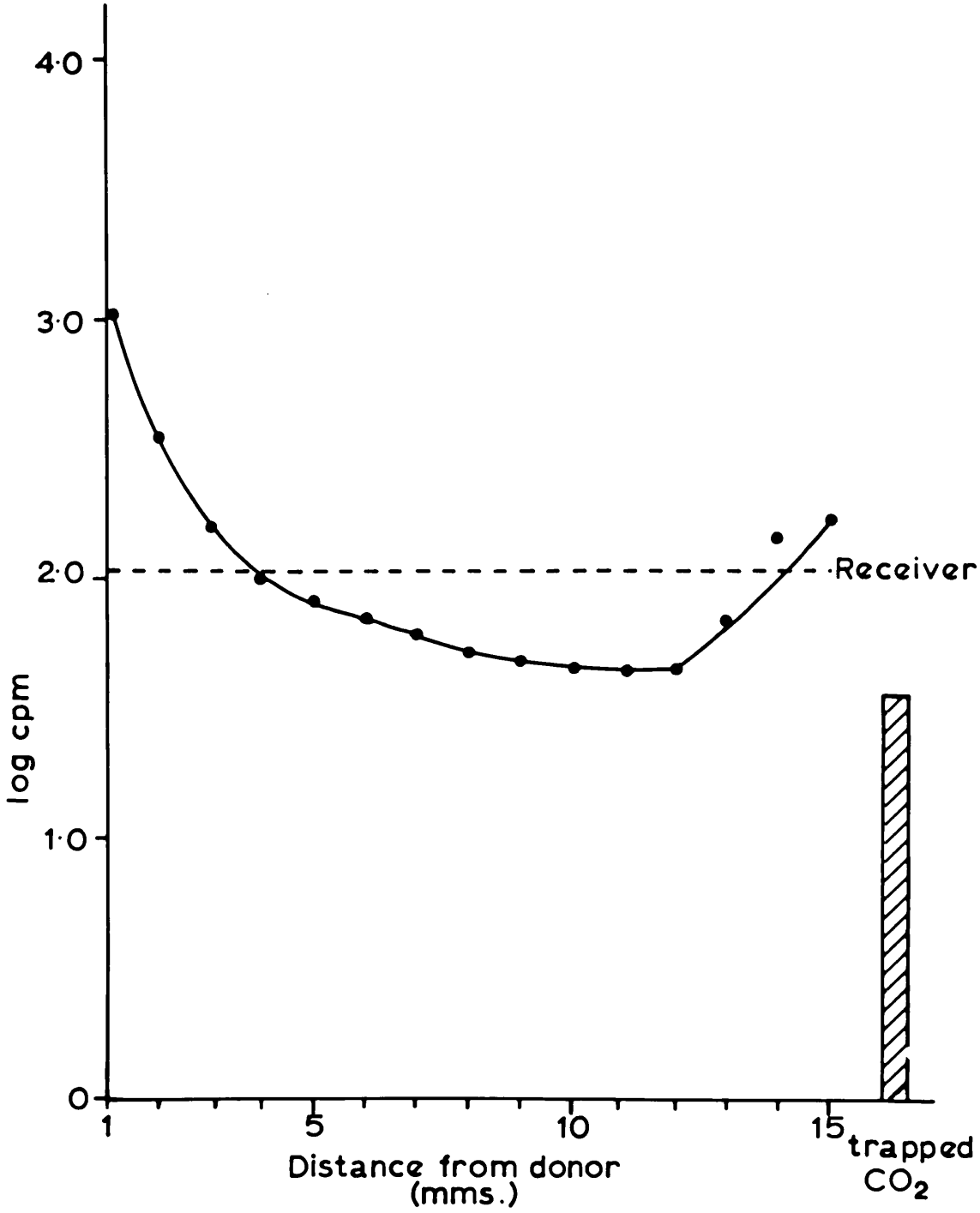

4·0
3·0
2·0
1·0
0
log cpm
Receiver
1
5
10
15
Distance from donor
(mms.)
trapped
CO_2

Fig. II. 7 Log profile of the distribution of radioactivity in Coleus internode segments after 24 h basipetal transport of IAA-1-^{14}C

The distribution of radioactivity after a 24 h transport period is similar to that after shorter periods and individual sections all contain higher levels of radioactive material. The amount of decarboxylation which has occurred is only a little more than that which occurs during a 5 h transport period.

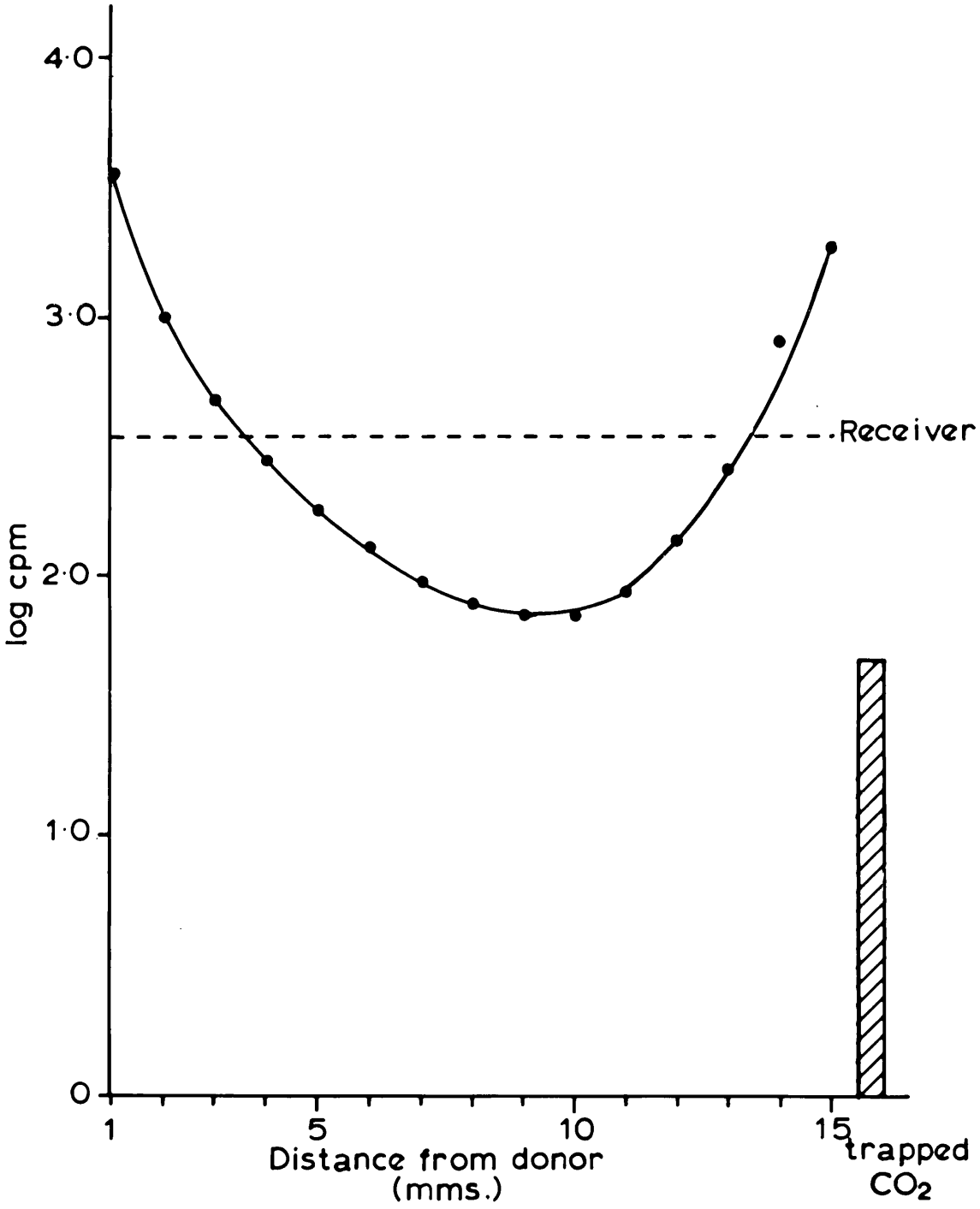

4·0
3·0
2·0
1·0
0
log cpm
Receiver
1
5
10
15
Distance from donor
(mms.)
trapped
CO_2

Table II.7 Activity distribution after 5 h basipetal transport of IAA-1-^{14}C

Component	Count
Donor	16,072
A	1,022
B	342
C	158
D	99
E	82
F	71
G	60
H	52
I	48
J	45
K	43
L	44
M	70
N	144
O	162
Receiver	108
KOH wick	36
Total	18,658

Table II.8 Activity distribution after 24 h basipetal transport of IAA-1-^{14}C

Component	Count
Donor	8,375
A	3,462
B	972
C	470
D	273
E	178
F	128
G	96
H	77
I	70
J	71
K	89
L	137
M	260
N	799
O	1,852
Receiver	348
KOH wick	47
Total	17,704

counting efficiency was reduced in these experiments by the use of a sub-standard batch of PBD. However, it can still be seen that very little decarboxylation occurs when internode segments are treated with IAA-1-^{14}C. This can be seen not only from the low level of activity recovered in the CO_2 trapping system but also from the fact that the total activity recovered from the experimental system corresponds with the level of activity from a standard 5 μl sample of IAA-1-^{14}C solution. The results of this experiment also demonstrate that the shape of the 5 h profile is similar to that obtained from a similar experiment with methylene-labelled auxin. The only slight difference was that the levelling off of the counts noticed over sections G - L in the methylene-label experiments was not so obvious here. In the carboxyl label situation the levels in these sections continue to fall but at a reduced rate. Levels in sections M - O rise markedly as usual. Counts

in the 24 h experiment are very high in spite of the reduced efficiency, demonstrating that uptake and transport continue to occur in these excised segments between 12 and 24 h after auxin application.

Estimation of polarity

In the past estimates of polarity of auxin transport have often been based on the amount of auxin transported into receiver blocks (Hertel and Leopold, 1963; Leopold, 1963; McCready, 1963, 1967; McCready and Jacobs, 1963a, b;, 1967; Naqvi and Gordon, 1965; Pilet, 1965, 1967; Gorter and Veen, 1966; Werblin and Jacobs, 1966; Jacobs and McCready, 1967; Whitehouse and Zalik, 1967; Horton and Fletcher, 1968; Kirk and Jacobs, 1968; Harel, 1969; Chang and Jacobs, 1972; Koevenig and Jacobs, 1972; Sheldrake, 1972; Cande, Goldsmith and Ray, 1973 and Wochock and Sussex, 1973). The results quoted here show that such estimations may not accurately illustrate the situation. In Coleus the amount of activity which passes into the receiver is very variable and only represents a very small proportion of the activity which is transported to the base of the segment. IAA must have been transported over several millimetres to reach the basal end of the segment even though it is not then exported. The accumulation continues to increase over many hours (see Fig. II.11 and the long term experiments in Chapter III) and although export also continues it does so at a much lower rate. The variability in the amount of exported material though important compared with the size of the receiver counts becomes less important when the activity in the basal accumulation is also considered. It is essential therefore to consider this material in calculations to determine polarity. Since no accumulation occurs as a result of acropetal transport, auxin transport in Coleus can be shown to be far more strictly polar than is often supposed. Jacobs (1954) quoted a value

of 3 : 1 for Coleus internode tissue. Naqvi and Cordon (1965) quoted a similar value for vegetative Coleus internode tissue and a value of 1.3 : 1 for segments from flowering plants. Leopold (1963) discovered a reversal of polarity in older segments from Coleus plants. De la Fuente and Leopold (1966), however, published the results of experiments in which they took account of radioactivity in the tissue of segments of several species and also considered the effect of the length of the segments used on the value calculated for polarity. This work utilised the mathematical model of Leopold and Hall (1966) and their results indicated that in a 4 mm internode segment of Coleus the polarity ratio was 12.

An example of the effect that the basal accumulation can have on polarity calculations can be seen from the data quoted in Tables II.1 and II.3. These two tables show the distribution of activity in internode segments after 12 h basipetal and acropetal transport respectively. In each case the donors contained 10 μl of IAA-2-^{14}C solution so the two sets of data are directly comparable. If activity recovered from the receiver block alone is considered the polarity ratioappears to be 4 : 1 (material being transported acropetally representing 26.3% of material transported basipetally). However, if the basal accumulation which occurs in sections L-N after basipetal transport is also taken into account (see also Fig. II.1) the polarity ratio works out at 20 : 1 (material transported acropetally being only 5.4% of material transported basipetally). This method of estimating transport polarity is more valid because it is based on a measure of material which is transported within the tissue rather than material which is exported from the tissue. Basing polarity calculations on the radioactivity recovered in sections L-N and the receiver effectively utilises an 11 mm segment. If polarity is calculated for a 4 mm

segment from the above data then the value becomes 7 : 1. The increase in polarity with increased segment length agrees with the findings of de la Fuente and Leopold (1966) and the value of 7 : 1 is in reasonable agreement with their value of 12 : 1.

Polarity and the age of plant material

There are reports in the literature of polarity of auxin transport being reduced with increasing age of plant organs. McCready and Jacobs (1963b) reported that in Phaseolus petioles polarity decreased with increasing age of the petiole because of increased acropetal transport. They also found, however, that this was not the case with Coleus petioles in which polarity was unaltered with age. Werblin and Jacobs (1966) and Veen and Jacobs (1969), on the other hand, found that there was no significant acropetal transport in Coleus petiole segments and that the amount of auxin transported basipetally declined with increasing age. Horton and Fletcher (1968) using the auxin picloram found that transport polarity declined with age in both Coleus and bean petiole sections. In the case of Coleus the polarity declined by virtue of decreased basipetal transport whereas in the case of bean it declined because of increased acropetal transport. Kaldewey (1966) commented on the effect of age of fruit stalks of Fritillaria on basipetal transport of IAA. He found that younger organs took up more auxin but secreted less into receivers than the older ones. He did not discuss the effect of age on acropetal transport. The problem was therefore investigated with regard to Coleus internode segments.

Plants were selected for uniformity and segments were cut from the usual internode as described previously. Segments were also cut from two internodes below the usual one. These segments were also cut from just below the node and were 15 mm in length. Only one segment was cut from each internode. 'Young' and 'old' segments were each divided into two batches,

one for acropetal transport and the other for basipetal transport. Each donor block contained 5 μl of IAA-2-^{14}C. The first experiment ran for 24 h and the four sets of data obtained are presented in Tables II.9 and II.10

Table II.9 Distribution of activity in 'young' internode segments after 24 h basipetal and acropetal transport of IAA-2-^{14}C

Component	Count	
	basipetal	acropetal
Donor	10,389	15,064
A	5,613	7,550
B	1,880	1,578
C	984	617
D	629	336
E	423	197
F	296	119
G	216	60
H	168	43
I	136	28
J	126	15
K	116	14
L	138	8
M	196	6
N	300	5
O	3,783	18
Receiver	954	6
Total	26,347	25,664

Polarity is very strict in both situations. This is probably because acropetal transport declines over very long transport periods whereas basipetal transport continues. Since the level of acropetal transport is relatively very low it is not surprising that some workers (Werblin and Jacobs, 1966; Veen and Jacobs, 1969) have found no evidence of acropetal transport using older and less reliable counting methods. The polarity in the young tissue is 125 : 1 and in the older tissue it is 56 : 1. This decline in polarity with age appears to be due largely to increased acropetal transport. It is not strictly valid to compare intensity of transport between old and young tissue because of the differences in cross-sectional area of the segments (the

Table II.10 Distribution of activity in 'old' internode segments after 24 h basipetal and acropetal transport of IAA-2-^{14}C

Component	Count	
	basipetal	acropetal
Donor	7,413	12,255
A	6,107	7,832
B	2,205	2,342
C	1,151	885
D	738	466
E	539	274
F	389	209
G	282	85
H	251	63
I	222	41
J	214	28
K	243	18
L	281	15
M	405	13
N	743	10
O	3,447	34
Receiver	832	31
Total	25,462	24,601

polarity values quoted are calculated from transport within segments of the same type and so the width of the internode need not be considered). However, some inferences can still be drawn. Whereas the material transported basipetally in the older tissue represents 106% of that transported basipetally in the young tissue, the material transported acropetally in the old tissue represents 240% of that transported acropetally in the young tissue. Stated another way, the radioactivity transported basipetally in the young tissue (5371 cpm) represents 20% of activity available in the donor and that transported acropetally (43 cpm) represents 0.2% of available activity. In the old tissue the corresponding figures are (5708) 22% and (103) 0.4%. It is not surprising that acropetal transport should be greater in older tissue since increased cross-sectional area and larger tissue spaces such as are found in older tissue are both conducive to increased diffusion.

These results are different from those of McCready and Jacobs (1963) who found that in petiole segments of Coleus polarity did not alter with age (cf. Phaseolus). They also differ from those of Werblin and Jacobs (1966) and Veen and Jacobs (1969) who found no detectable acropetal transport and who noted a decline in basipetal transport with age. Horton and Fletcher (1968) used Picloram in their studies with Coleus petiole segments and they also noted a decline in basipetal transport with age. In these experiments there is a slight increase in basipetal transport in the older segments. This increase is not great (6%) and the girth of the older segments is considerably greater than that of the younger segments. It is, therefore, possible that the efficiency of transport actually decreases with age since there is likely to be more tissue in older segments through which active transport can take place. Unless the tissue responsible for active transport and its relative proportion in segments of different ages can be reliably stated, however, this efficiency of transport cannot be actually calculated. Certainly no overall decline in basipetal transport was noticed.

It is significant that the workers quoted above used petiole segments, not internode segments. Petioles do not undergo much anatomical change with age, unlike stems, and the age of a petiole would not be expected to have such an effect on polarity of auxin transport.

It must also be remembered that the transport period of 24 h in these experiments is considerably longer than most of those used in other work quoted. Horton and Fletcher (1968) did, in fact, run some of their experiments for as long as 24 h but in general shorter transport times have been preferred. McCready and Jacobs (1963b) ran their experiments for up to 5 h. Naqvi and Gordon (1965) ran their experiments for 3 h. Werblin and Jacobs (1966) and Veen and Jacobs (1969) used maximum transport times of 8 h and 5 h respectively.

In most cases segments were also considerably shorter so long transport times were not necessary to produce a measurable flux. However, de la Fuente and Leopold (1966) have pointed out the importance of the length of segments in polarity determinations. Although there are objections to long transport periods (Jacobs, 1954; McCready and Jacobs, 1963a) it can be argued that if the material which is transported but not exported is to be considered in polarity determination then longer transport periods must be used (see Discussion). However, the above experiment was repeated with a shorter transport time (5 h) for purposes of comparison. The results are given in Tables II.11 and II.12.

Table II.11 Distribution of activity in 'young' internode segments after 5 h basipetal and acropetal transport of IAA-1-^{14}C

Component	Count	
	basipetal	acropetal
Donor	20,347	21,205
A	1,605	1,614
B	499	286
C	232	100
D	136	39
E	86	14
F	59	7
G	49	2
H	40	3
I	33	3
J	29	2
K	27	2
L	29	3
M	36	3
N	73	4
O	239	3
Receiver	7	3
Total	23,526	23,293

In a 5 h transport period acropetal transport has not proceeded far enough to calculate transported material from the counts in segments L-O as has been done in previous determinations. Thus activity in sections E-O

Table II.12 Distribution of activity in 'old' internode segments after 5 h basipetal and acropetal transport of IAA-2-^{14}C

Component	Count	
	basipetal	acropetal
Donor	19,813	19,728
A	1,786	2,193
B	558	462
C	277	166
D	165	66
E	107	30
F	74	12
G	57	4
H	49	4
I	46	3
J	41	1
K	41	2
L	42	1
M	49	2
N	86	1
O	310	5
Receiver	12	9
Total	23,513	22,689

inclusive is added to that in the receiver so that the segments over which polarity is estimated are effectively 4 mm long (cf. the effective length of 11 mm used in the 24 h experiment). Polarity in the young tissue is 14 : 1 and that in the old tissue is 12 : 1. Again polarity is seen to decline with age. As would be expected with short segments the polarity values are quite low (cf. 125 : 1 and 56 : 1 in the 24 h experiment). Again the decline in polarity appears to be due to a proportional increase in acropetal transport. In the young tissue the material transported basipetally represents 3% of available material and that transported acropetally represents 0.2% of available material. The corresponding values for the old tissue are 4% and 0.3% respectively. (Increases of one third and one half respectively).

The apparent decline in polarity with age is more marked after the 24 h experiment than after the 5 h experiment. Although comparisons of

values calculated from experiments with different transport periods and effective segment lengths are of doubtful use it appears that the decline in polarity with age is more marked after the 24 h experiment than after the 5 h experiment. In both cases however, it is shown that polarity does decline with age in Coleus internode segments but that even the reduced polarity is very strict.

It is unlikely that ageing of segments during the transport period would come into effect since IAA was applied to the segments soon after their excision. [A decline in the transport capacity with age of a segment is thought to occur (de la Fuente and Leopold, 1969) but this is thought to be overcome by the early application of IAA (Osborne and Mullins, 1969)].

Polarity in segments from flowering plants

Polarity has been said to be reduced in flowering plants (Leopold and Guernsey, 1953; Naqvi and Gordon, 1965) and in Chapter I transport in internode segments cut from flowering plants was considered and the products formed during IAA transport were investigated. It was found that the products formed in internode segments were the same whether the plants from which the segments had been excised were in the vegetative or the flowering condition. In view of the effect of the basal accumulation of activity on polarity estimations, it was decided to investigate polarity in segments from flowering plants. It was hoped to find out to what extent it was, in fact, reduced and whether it was reduced by a decrease in basipetal transport or an increase in acropetal or both. Internode segments were cut from Coleus plants which had been grown under the usual conditions but which were flowering nevertheless. The segments were prepared as described previously and set up in the usual apparatus. Five μl of IAA-2-^{14}C solution were applied to the apical ends of the segments in one batch

and to the basal ends of the segments in a second batch. The transport period in both cases was 12 h. The results obtained from the two experiments are shown in Table II.13.

Table II.13 Activity distribution in internode segments from flowering plants after 12 h basipetal and acropetal transport of IAA-2-^{14}C

Component	Count	
	basipetal	acropetal
Donor	17,773	20,495
A	7,579	6,322
B	1,494	1,335
C	606	441
D	320	164
E	172	71
F	105	30
G	73	18
H	54	8
I	47	6
J	60	5
K	84	3
L	132	11
M	349	12
N	992	11
Receiver	372	38
Total	30,212	28,970

A similar experiment was performed with a 5 h transport period and the results of that experiment are shown in Table II.14.

The polarity values which may be calculated from Tables II.13 and II.14 are 25 : 1 (based on a segment 11 mm in length) and 9 : 1 (based on a segment 4 mm in length) respectively. The increase in polarity with the length of the segment is again apparent. The polarity values are of the order to those calculated previously for internode segments from vegetative plants. It does not appear that polarity is reduced to any significant extent when the plant is flowering. Certainly the polarity of auxin transport is still very strict.

Table II.14 Activity distribution in internode segments from flowering plants after 5 h basipetal and acropetal transport of IAA-2-^{14}C

Component	Count	
	basipetal	acropetal
Donor	20,976	21,948
A	1,328	1,705
B	430	456
C	205	144
D	125	52
E	77	22
F	58	13
G	45	11
H	38	5
I	33	5
J	28	6
K	29	5
L	31	5
M	34	3
N	76	6
O	437	15
Receiver	34	5
Total	23,984	24,406

The time course of basipetal transport

To discover how the activity profiles in internode segments are built up with increasing transport times experiments were carried out over a series of short transport periods. Internode segments were cut in the usual fashion and set up, apical end uppermost, in the usual bridges. Donor blocks, each containing 5 μl of IAA-2-^{14}C, were applied to the segments for transport periods of 1, 1.5, 2, 2.5, 3, 4, 5 and 6 h duration. Preliminary experiments had shown that early in the course of transport the profile could change quite markedly over the course of an hour which was the reason for the inclusion of the two intermediate transport times. The results are shown in Tables II.15 and II.16 and the log profiles are shown in Fig. II.8.

The figures for the 2.5 h experiment do not fall into the general pattern, uptake and transport appearing to be greatly reduced. However, the results are

Plate II. 8 Log profiles of the distribution of activity in Coleus internode segments after short periods of IAA-2-^{14}C transport

A. shows the 1 h, the 1. 5 h and the 2 h log profiles. The 1 h profile (solid squares) is a straight line. The gradient of the curve changes abruptly over the next 0. 5 h. The direction of the 1. 5 h profile (dotted line) changes abruptly 10 mm from the donor as though active transport is only effective along part of the segment. After 2 h (solid line with circles) the overall pattern is similar but activity levels in some sections have fallen.

B. shows the 2. 5 h curve. A steady level of activity has been established over much of the segment and the basal accumulation is just becoming apparent.

C. shows the 3 h (dotted line) and 4 h (solid line) curves.

D. shows the 5 h (dotted line) and 6 h (solid line) curves. They demonstrate the steady increase of levels of radio-activity in all sections of the segment and the maintenance of the familiar shape of the log profile once it has been established.

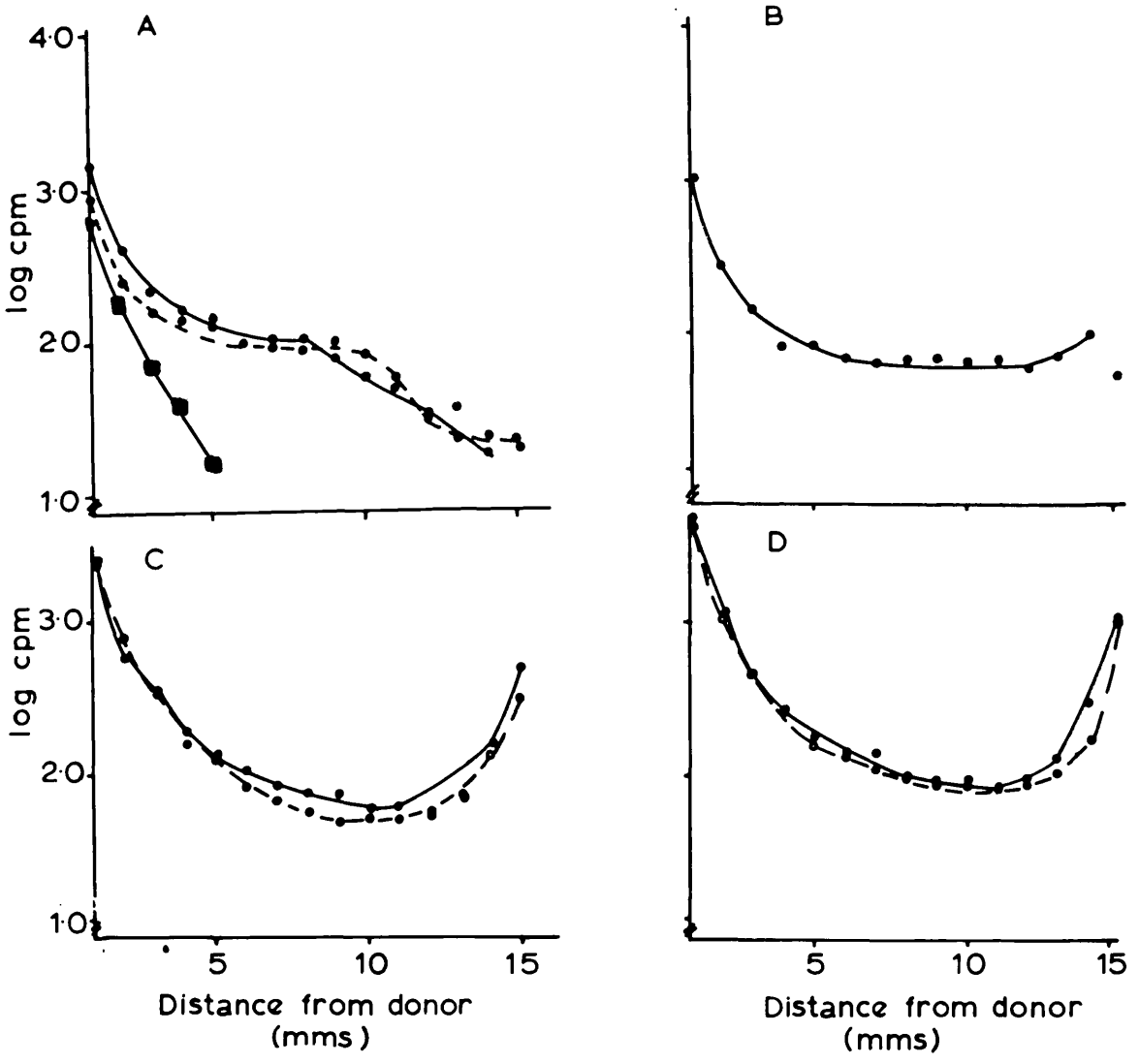

A
B
C
D
4·0
3·0
2·0
1·0
log cpm
5
10
15
Distance from donor
(mms)

Table II.15 Activity distribution in internode segments after 1, 1.5, 2 and 2.5 h basipetal transport of IAA-2-^{14}C

Component	Count			
	1 h	1.5 h	2 h	2.5 h
Donor	29,348	28,240	26,768	29,246
A	621	876	1,445	1,078
B	177	243	400	267
C	67	160	220	140
D	38	134	161	80
E	16	142	128	79
F	-	99	132	64
G	-	94	104	61
H	-	90	100	64
I	-	96	77	64
J	-	83	58	61
K	-	55	49	62
L	-	29	33	56
M	-	23	36	64
N	-	23	18	89
O	-	21	20	46
Receiver	-	9	12	10
Total	30,267	30,417	29,761	31,467

Table II.16 Activity distribution in internode segments after 3, 4, 5 and 6 h basipetal transport of IAA-2-^{14}C

Component	Count			
	3 h	4 h	5 h	6 h
Donor	24,116	25,151	22,268	21,552
A	2,327	2,582	4,120	4,814
B	776	581	1,038	1,071
C	313	335	439	425
D	156	180	247	253
E	139	125	155	171
F	82	104	130	132
G	67	83	107	138
H	55	73	94	97
I	49	74	88	82
J	51	60	83	87
K	51	61	81	82
L	52	56	83	90
M	70	74	99	127
N	131	153	168	292
O	299	482	1,141	1,187
Receiver	36	85	181	390
Total	28,770	30,259	30,522	30,990

included here because although the counts are low they are uniformly low and the profile is still of interest. (The data for the 2.5 h experiment are not, however, included in Table II.17 or in Figs II.9 - II.12).

The log profile for the 1 h experiment is not unlike that for the 1 h acropetal transport experiment (compare Figs II.8A and II.14). It approximates to a straight line and radioactivity has only penetrated into the proximal 5 mm of the segment. Half an hour later the radioactivity has penetrated to the distal end of the segment and some activity can also be recovered from the receiver block. The log profile no longer takes the form of a straight line (Fig. II.8A) but comprises three distinct regions, a descending portion, a horizontal portion and a second descending portion. After a 2 h transport period there are only two distinct regions on the log profile, there being no horizontal region. The apparent irregularity of the curves is somewhat surprising and on first inspection it may seem that the irregularities could be due to experimental error. However, in all the experiments in which the Oxymat has been used the data obtained have been very reliable in that the replicates have always been very close and all points plotted have fitted smooth curves. Thus it seems reasonable to give credibility to these log profiles, especially since there are two similar sets of data (for 1.5 and 2 h) and since they fall between the two different but smooth curves of the 1 h and 2.5 h log profiles. After 2.5 h there is a decline in activity over the proximal 5 mm and beyond that levels of radioactivity are similar in all sections except the most distal where levels are slightly higher (Fig. II.8B). After 3 h (see Fig. II.8C) the basal accumulation of activity is apparent over the distal 3 mm and after 4 h it has increased in intensity although the distribution over the basal three sections remains the same. The 5 and 6 h log profiles

(Fig. II.8D) are of the usual form and are similar in shape to the 3 and 4 h curves. There is little difference between the 5 h and 6 h curves but they do show the way in which the basal accumulation builds up from the basal cut surface back over the distal 3 mm. Auxin continues to be steadily taken up from the donor during this time. The progress of this uptake from the donor, expressed as a percentage of the activity initially available in the donor, is presented in Table II.17.

Table II.17 The time course of uptake of auxin at the apical cut surface of internode segments

Transport period (h)	% of available activity taken from the donor into the tissue
1	3.0
1.5	7.2
2	10.1
3	16.2
4	16.9
5	27.0
6	30.5

In view of the apparently irregular movement of auxin and the fact that the activity levels in certain sections decline over some time intervals (see Fig. II.8A), the auxin levels in individual sections were plotted against time. The data from Tables II.15 and II.16 were used and the resulting graphs are shown in Figs. II.9 to II.12 (N.B. these graphs are not log plots). It can be seen that over the first (apical) four sections of the segment the level of radioactivity rises with time, although not at a constant rate. In the fifth section, however, the level rises for the first 1.5 h, drops slightly, rises, drops and then continues to rise. In the sixth and subsequent sections the level rises for 2 h, drops after 3 h and then continues to rise again. This trend is most obvious

Fig. II.9 Profiles of radioactivity in individual sections of Coleus internode segments over a 6 h period

A, B and C are the first, second and third sections respectively. Levels of radioactivity increase with time in the three sections and the increase is most rapid in the first section (curve A).

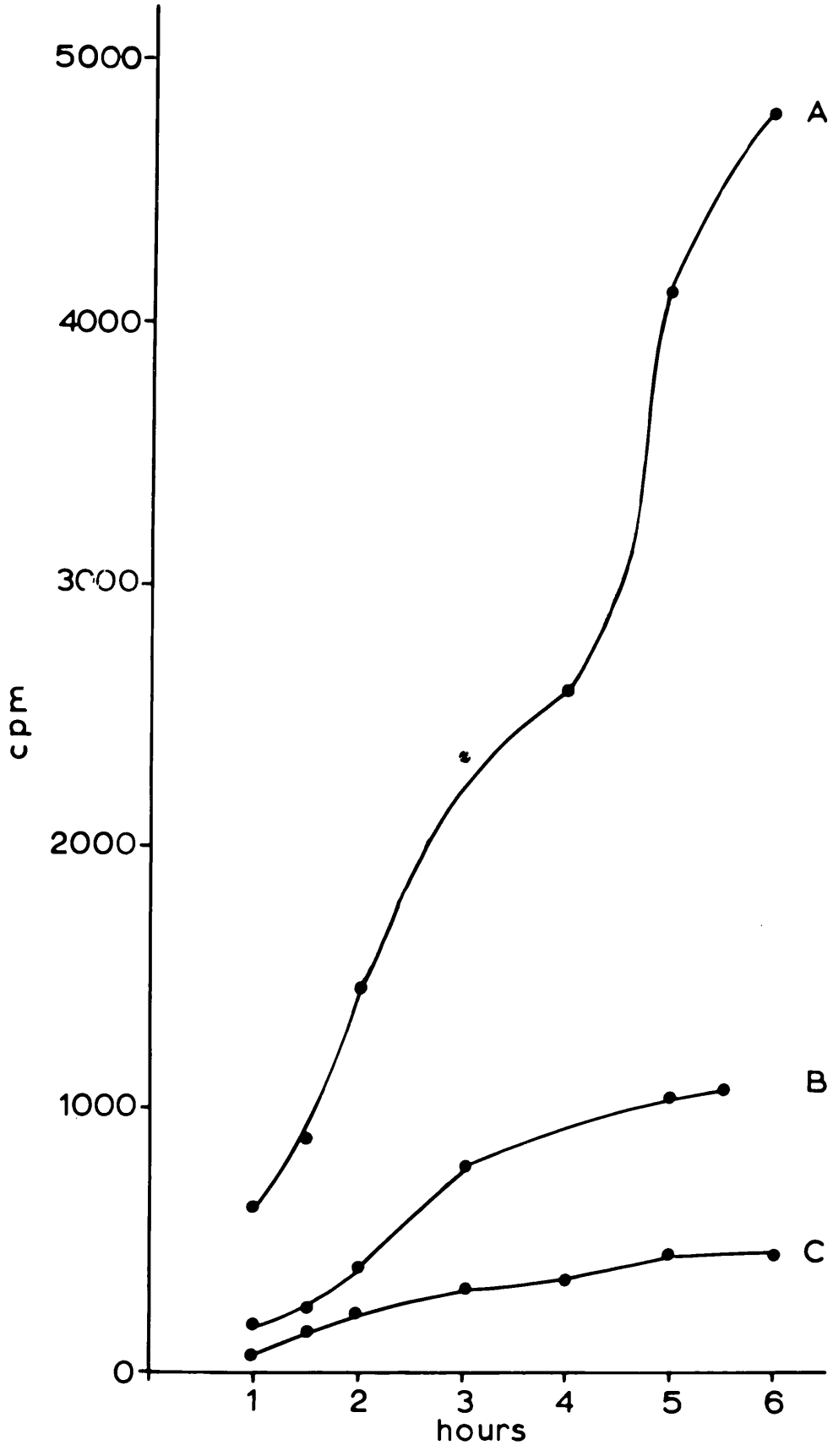

5000
4000
3000
2000
1000
0
cpm
A
B
C
1
2
3
4
5
6
hours

Fig. II.10 Profiles of radioactivity in individual sections of Coleus internode segments over a 6 h period

Curves D and E represent the fourth and fifth sections respectively and levels of radioactivity rise or remain stationary over all the time intervals plotted. Curves F and G represent the sixth and seventh sections and it can be seen that levels of activity fall between the second and third hours of transport before rising steadily.

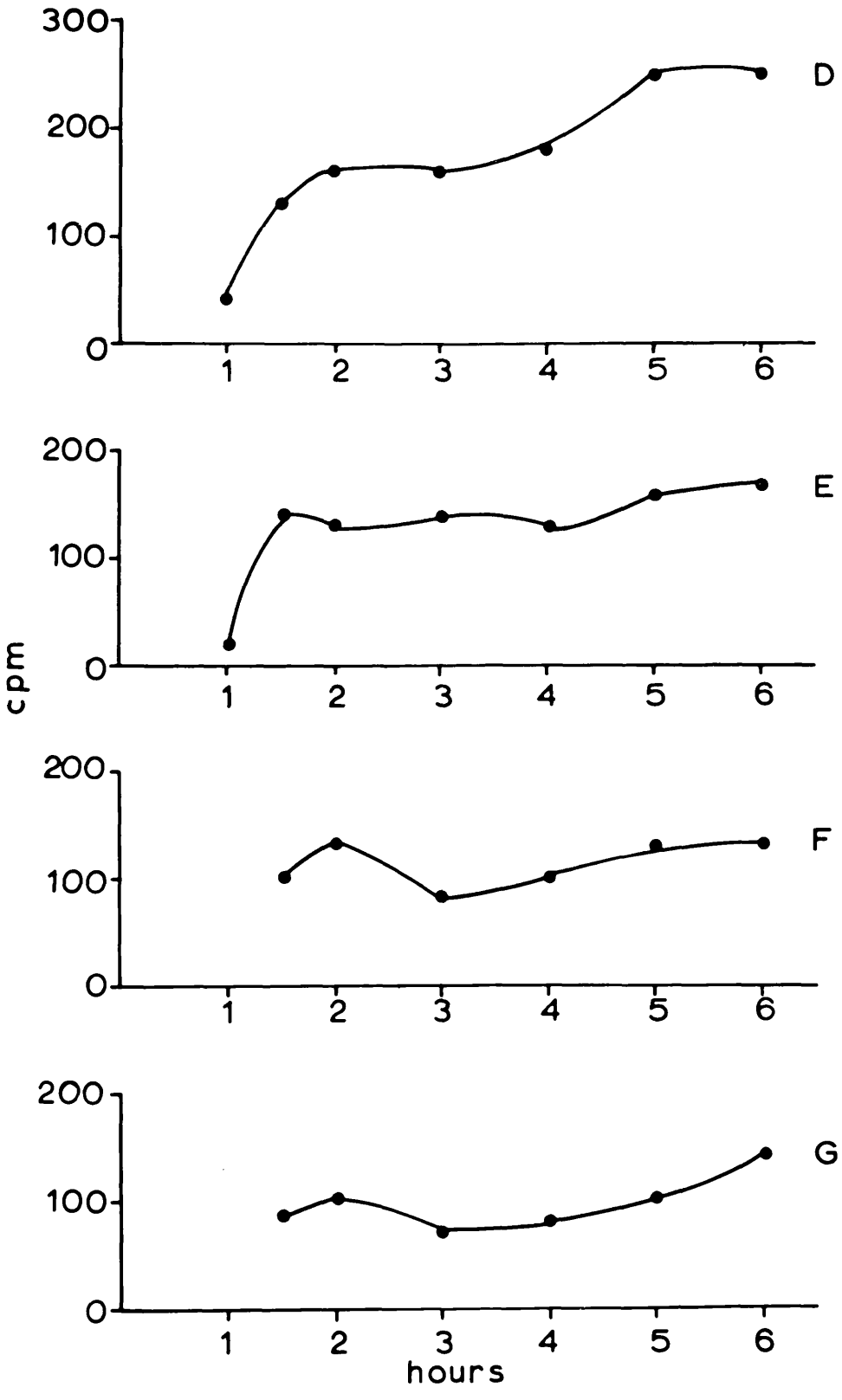

300
200
100
0
D
200
100
0
E
cpm
200
100
0
F
200
100
0
G
1
2
3
4
5
6
hours

Fig. II.11 Profiles of radioactivity in individual sections of Coleus internode segments over a 6 h period

The decline of activity levels noted in Fig. II.10 is seen in curve H (eighth section). In the next two sections (curves I and J) the decline occurs between 1.5 and 3 h. In the more proximal sections (K-M) levels of activity rise with time.

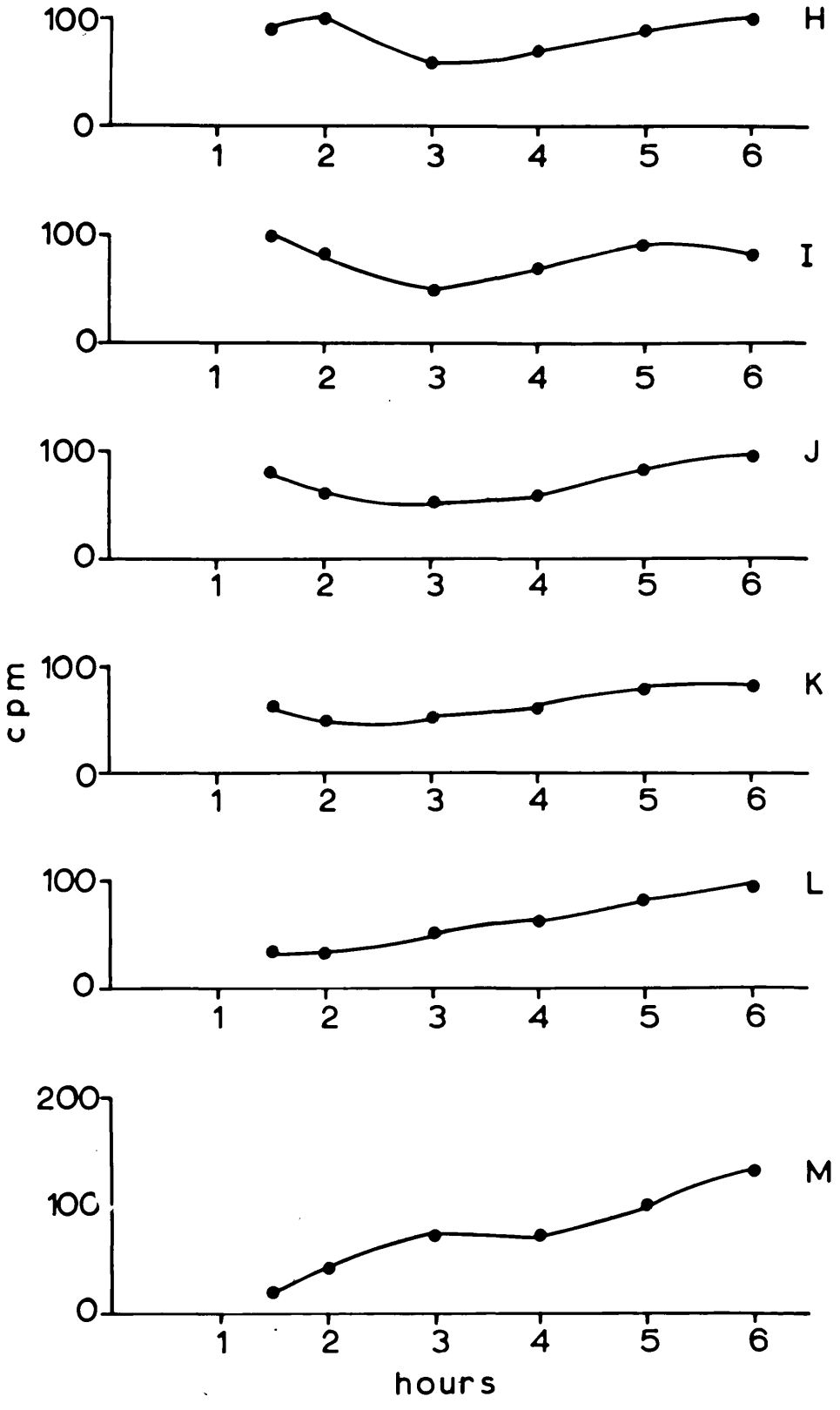
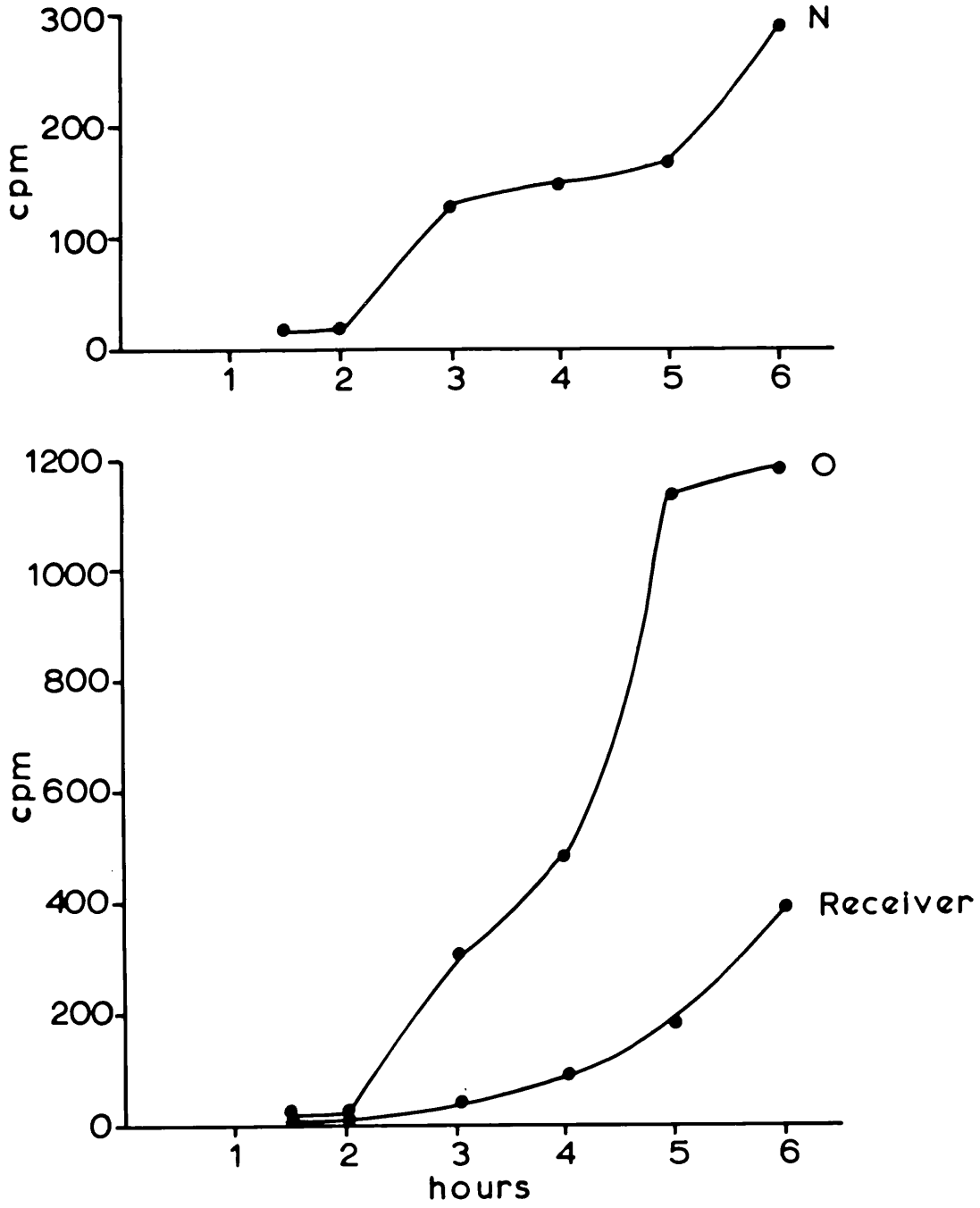

100
0
H
1
2
3
4
5
6
100
0
I
1
2
3
4
5
6
100
0
J
1
2
3
4
5
6
cpm
100
0
K
1
2
3
4
5
6
100
0
L
1
2
3
4
5
6
200
100
0
M
1
2
3
4
5
6
hours

Fig. II. 12 Profiles of radioactivity in individual sections of Coleus internode segments over a 6 h period

Levels of radioactivity in the two distal sections (N and O) and in the receiver rise with time over the 6 h period.


300
200
100
0
cpm
N
1
2
3
4
5
6
1200
1000
800
600
400
200
0
cpm
O
Receiver
1
2
3
4
5
6
hours

in the sixth to eighth sections (F-H). In the ninth and tenth sections the activity falls between 1 h and 1.5 h from its initially high level. In the distal sections the level of activity rises with time and no decline is seen. In the last section, particularly, the increase in activity level is quite rapid. The level of activity in the receiver rises steadily, describing a concave curve.

The time intervals in this experiment were not short enough to construct a detailed picture of the sequence of events but they do illustrate some interesting features and show that the transport of exogenous auxin in short experiments is not a simple continuous process. The distribution of activity after 1 h, as will be shown in the next section of this chapter, resembles that after 1 h of acropetal transport with regard to the gradient of the log decline and the penetration into the segment.

The distribution of activity in the segment changes markedly during the subsequent half hour and the different regions of the log profile suggest that the flow of auxin is not regular since a plateau is followed by a decline (see the 1.5 and 2 h curves in Fig. II.8A).

The graphs of levels of activity in individual segments support this idea of irregularity of flow because in the sections in the middle region of the segment levels of activity fall after transport has been operational for 2.5 - 3 h. This means that a certain amount of material must move into a section and then over a short period the material moves out of the section more quickly than it is replaced by new material which enters the section.

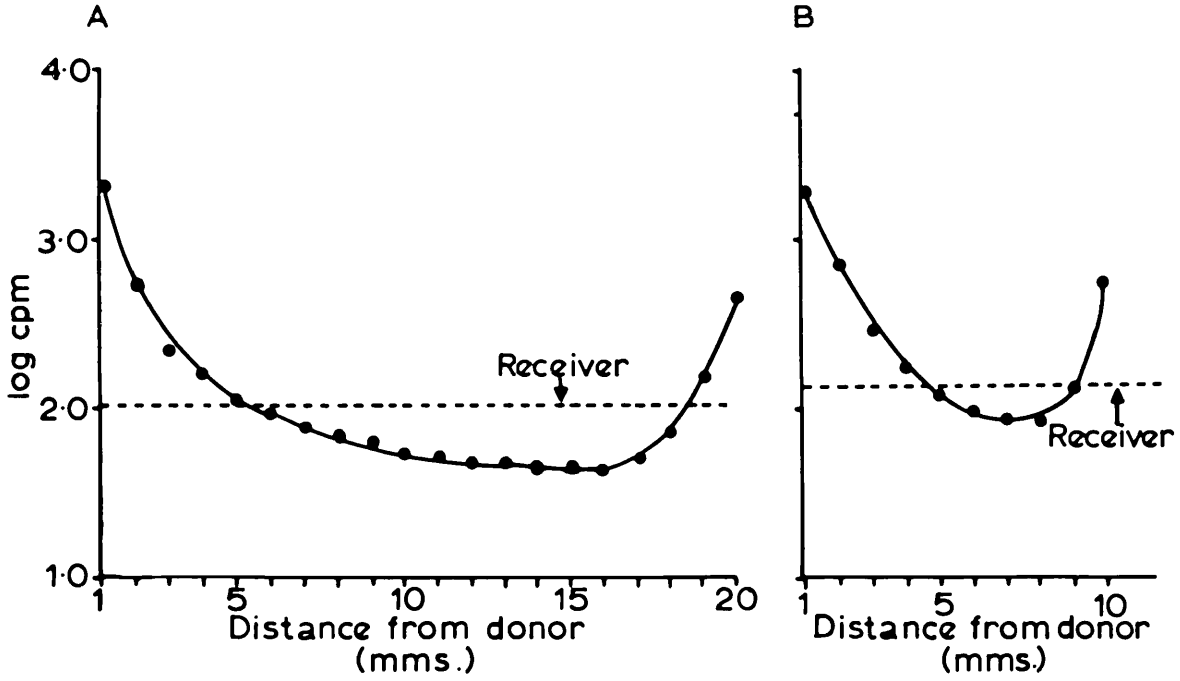
Since the construction of the profile was being investigated in these experiments it was decided that for purposes of comparison it would be interesting to see to what extent the profile has developed over 5 h in segments of different lengths. The log profiles for segments 10 mm and 20 mm in length

Fig. II.13 Log profiles of the distribution of radioactivity in Coleus internode segments of different lengths after 5 h basipetal transport of IAA -2-^{14}C

A. shows the distribution of radioactive material in a segment 20 mm in length.

B. shows the distribution in a segment 10 mm in length.

In both cases the level of radioactive material in the distal section is nearly as high as that in the second section from the donor. The basal accumulation is distributed over 4 mm in the long segment and over 2 mm in the short segment. Levels of activity in the proximal section are similar in both cases.


A
4·0
3·0
2·0
1·0
log cpm
Receiver
1
5
10
15
20
Distance from donor
(mms.)
B
Receiver
1
5
10
Distance from donor
(mms.)

are shown in Fig. II.13. The log profiles are similar to those for 15 mm segments after a 5 h transport period in that there is a decline in activity over the apical region and an accumulation at the base. The accumulation is spread over 2 mm in the short segment and over 4 mm in the long segment. Of activity available in the donor, 16% entered the long segment and 13.7% entered the short segment. There is a long trough in the 20 mm profile and there was more radioactive material in the middle sections of the short segment than in the middle sections of the long segment.

The time course of acropetal transport

A similar series of experiments was conducted to investigate acropetal transport. Transport periods of 1, 2, 3, 4, 6 and 8 h were employed and the results of the experiment are shown in Tables II.18 and II.19. The log profiles are shown in Fig. II.14.

Table II.18 Activity distribution in internode segments after 1, 2 and 3 h acropetal transport of IAA-2-^{14}C

Component	Count		
	1 h	2 h	3 h
Donor	28,395	27,761	28,024
A	1,052	1,556	1,525
B	256	268	349
C	59	67	100
D	16	25	43
E	8	11	27
F	-	8	14
G	-	5	11
H	-	-	7
I	-	-	6
J	-	-	-
K	-	-	-
L	-	-	-
M	-	-	-
N	-	-	-
O	-	-	-
Receiver	-	-	-
Total	29,786	29,702	30,106

Fig. II.14 Log profiles of the distribution of radioactivity in Coleus internode segments after short periods of acropetal IAA-2-^{14}C transport

The profiles in A - F are established after 1, 2, 3, 4, 6 and 8 h respectively. In all cases the log profile approximates to a straight lines. The gradient of the line and the penetration into the tissue remain similar throughout the 8 h period. Levels of radioactive material in individual sections rise with time.

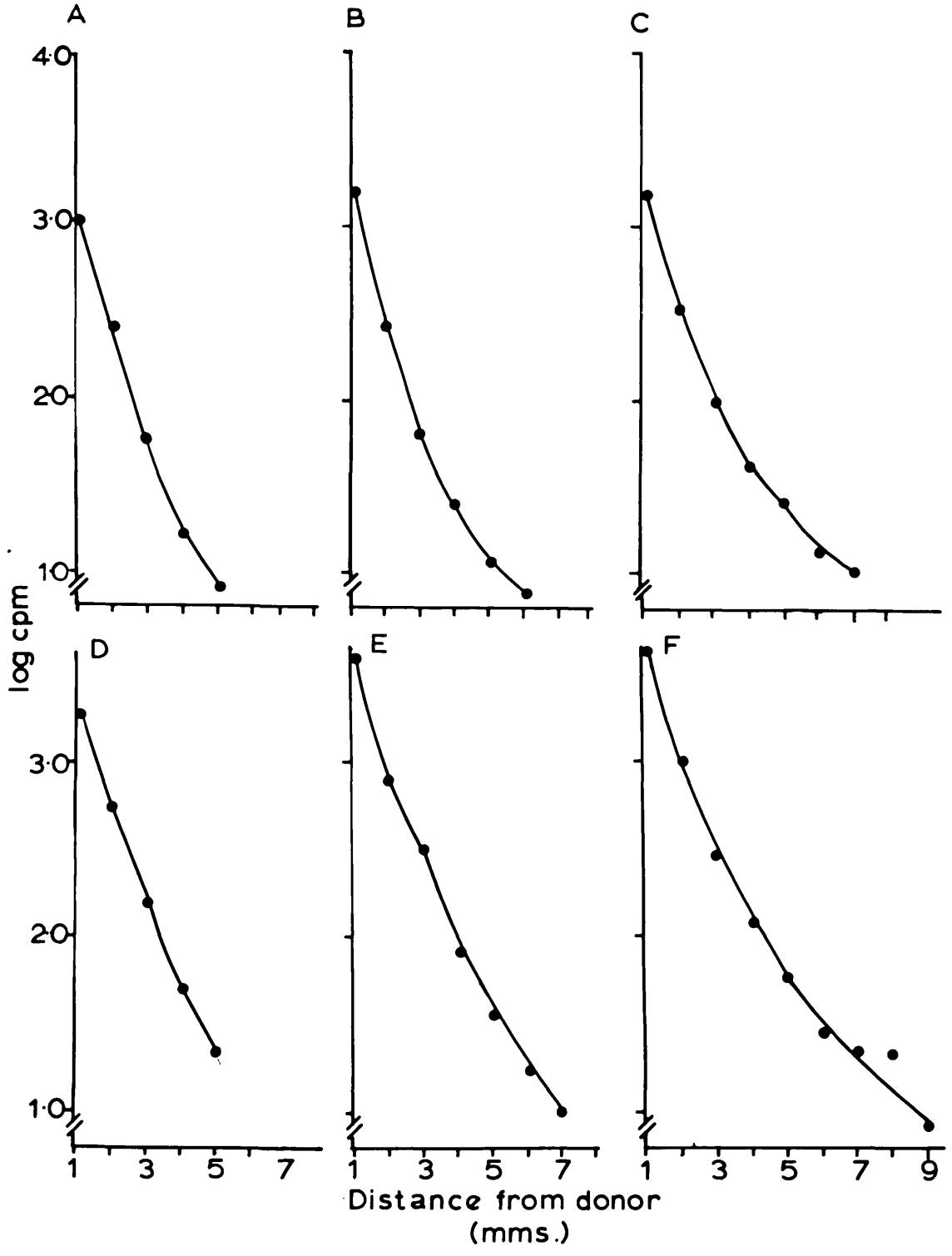

A
B
C
D
E
F
4·0
3·0
2·0
1·0
log cpm
1
3
5
7
9
Distance from donor
(mms.)

Table II.19 Activity distribution in internode segments after 4, 6 and 8 h acropetal transport of IAA-2-^{14}C

Component	Count		
	4 h	6 h	8 h
Donor	27,747	26,273	23,422
A	1,827	3,690	4,278
B	540	785	973
C	150	312	285
D	49	81	117
E	22	35	55
F	12	17	27
G	6	10	21
H	-	5	20
I	-	-	8
J	-	-	6
K	-	-	-
L	-	-	-
M	-	-	-
N	-	-	-
O	-	-	-
Receiver	-	-	-
Total	30,353	31,208	29,212

It can be seen from Fig. II.14 that all the profiles approximate to straight lines. It can also be seen that the penetration of radioactive material into the segment does not progress much over the 8 h period. Radioactivity can be detected in the proximal 5 sections after 1 h and it can only reliably be detected in the proximal 8 or 9 sections after 8 h. During this time, however, auxin continues to be taken up (see Table II.20). The log profiles form an ascending series of nearly parallel straight lines.

Table II.20 Time course of auxin uptake at the basal cut surface of internode segments. Uptake is expressed as a percentage of the activity initially available in the donor.

Transport period (h)	% of available activity taken from donor into tissue
1	4.7
2	5.5
3	6.9
4	8.6
5	15.8
6	19.8

'Chase' experiments

It has been shown that when radioactively labelled auxin is applied to the apical cut surface of a Coleus internode segment the excised segment will take up auxin from the donor and transport much of it through its tissues. It will retain most of the radioactive material and export some at the basal end in the form of unchanged IAA. It has also been shown that IAA is metabolised in the tissue and that IAAsp and possibly some other products are present as well as some unchanged IAA. IAAsp is generally thought to be immobile (Morris et al., 1969; Lepp and Peel, 1971 and Davies, 1972) but in order to find out the degree of mobility of the material in Coleus internode segments and the extent to which the profile could be changed after the removal of the donor 'chase' experiments were performed. In this way it was hoped to find out if the IAA was permanently immobilised or if it could still be transported to sites of activity.

Internode segments were cut as usual and set up in the usual apparatus. Thirty internodes were used and randomly divided into three batches of ten. An agar donor was applied to the upper (apical) cut surface of each segment and transport was allowed to proceed for 5 h. After this time all donors were removed and twenty of them were replaced by plain agar blocks. The remaining ten segments were cut into fifteen sections which were then pooled, oxidised and counted. After a further 2 h period the second batch of segments was sectioned and the sections, donors (donor 1) and replacement donors (donor 2) were oxidised and counted as usual. The third batch of segments was allowed to remain with the replacement donors in position for 18 h, after which time all components of the system were oxidised and counted. The results of the experiment are shown in Table II.21 (see overleaf).

Table II.21 Activity distribution in internode segments after 5 h basipetal transport and with a 2 h and an 18 h 'chase' period

Component	Count		
	5 h	5/2 h	5/18 h
Donor 1	21,174	21,007	21,565
A	1,453	1,092	682
B	708	575	429
C	321	282	262
D	199	174	180
E	143	118	132
F	116	88	104
G	94	75	81
H	80	67	68
I	70	55	62
J	63	55	53
K	57	51	46
L	58	56	53
M	60	65	61
N	97	124	85
O	453	612	580
Receiver	26	101	131
Donor 2	-	114	87
Total	25,172	24,711	24,661

It can be seen from Table II.21 that most of the radioactivity in the segment after a 5 h transport period remains there after the 'chase' periods. The profiles and the log profiles for the 5 h experiment and the 5/2 h experiment are shown in Figs II.15 and II.16. The profiles for the 5/18 h experiment are not included because they are so similar to the 5/2 h profiles that they only confuse the graphs.

It can be seen from the profiles that after a 2 h 'chase' period the levels of radioactivity have dropped in the first 10 sections. The drop in level is most marked in the first two sections and after that the drop is only very slight. Levels in the distal five sections are similar or slightly higher than those in the corresponding sections after the 5 h transport period and counts in the receiver are somewhat higher after the 'chase' period. The overall shape of the profile remains unaltered, however. It would appear that when the source of radioactive

Fig. II.15 Profiles of radioactivity in Coleus internode segments after 5 h basipetal transport of IAA-2-^{14}C with and without a 2 h 'chase' period

It can be seen that very little of the radioactive material is moved from the segment during the 'chase' period. Some material does move from the proximal and middle regions into the distal region.

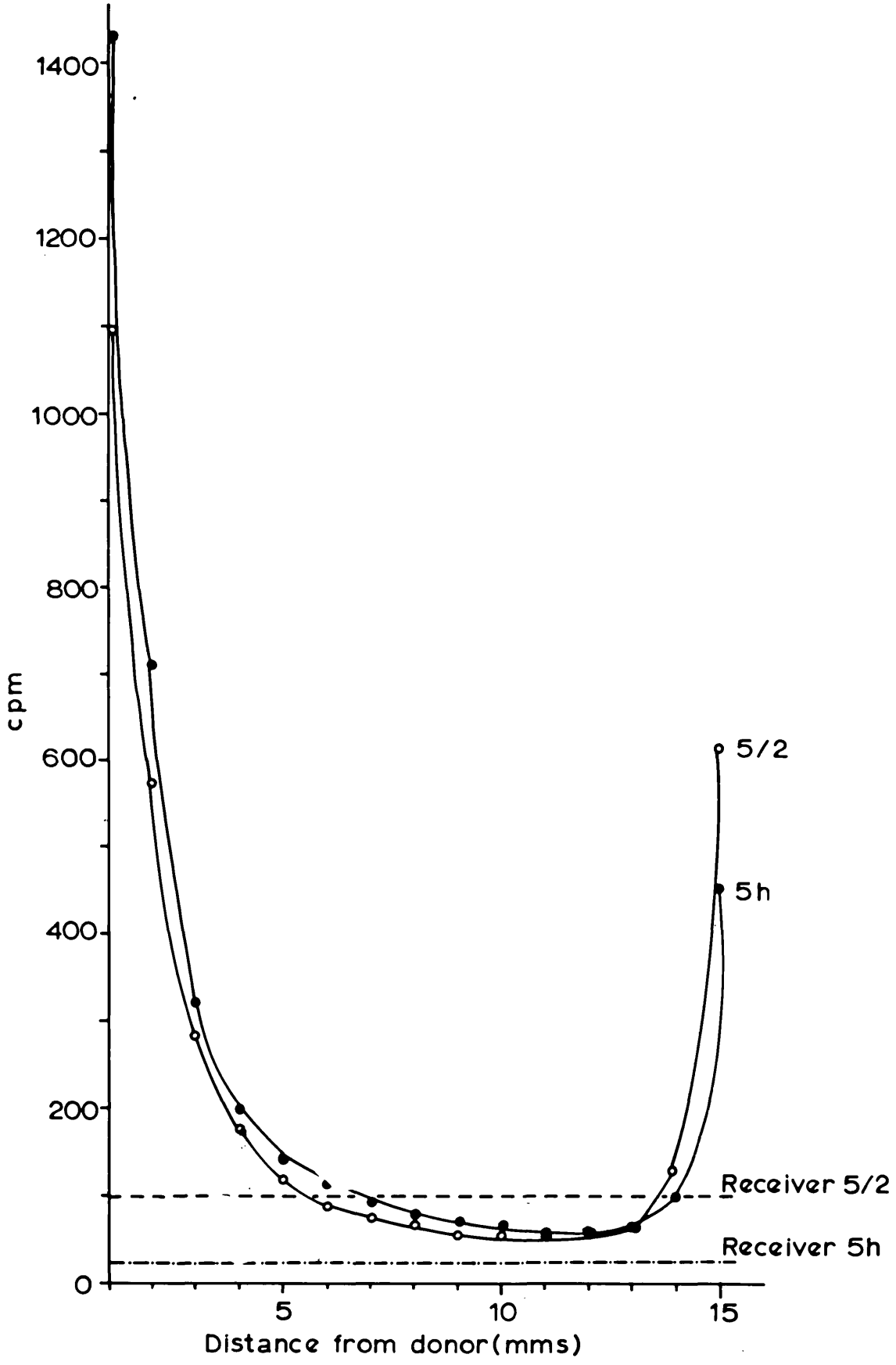

1400
1200
1000
800
600
400
200
0
cpm
5/2
5h
Receiver 5/2
Receiver 5h
5
10
15
Distance from donor(mms)

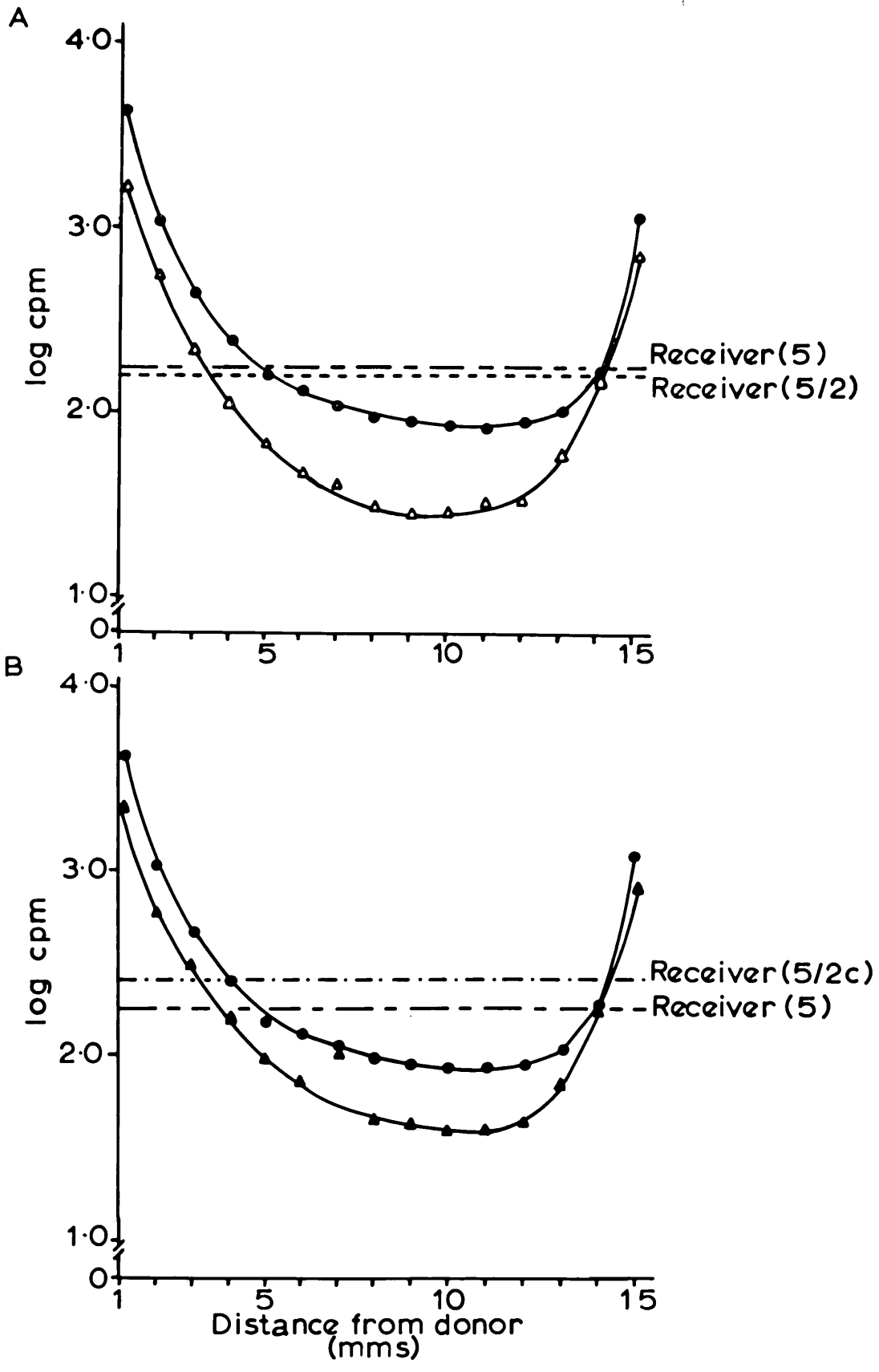
Fig. II.16 Log profiles of radioactivity in Coleus internode segments after 5 h basipetal transport of IAA-2-^{14}C with and without a 2 h 'chase' period

Little radioactive material is moved during the 'chase' period.

A. shows the profiles for a 5 h transport period (circles), and a 5 h transport period with a 2 h 'blank chase' period (triangles).

B. shows the profiles for a 5 h transport period(circles), and a 5 h transport period with a 2 h 'cold chase' period (triangles).

In both cases the 'chase' period makes little difference to the profile.


A
4·0
3·0
2·0
1·0
0
log cpm
Receiver(5)
Receiver(5/2)
1
5
10
15
B
4·0
3·0
2·0
1·0
0
log cpm
Receiver(5/2c)
Receiver(5)
1
5
10
15
Distance from donor
(mms)

IAA is removed from an excised segment some of the material in the proximal region of the segment moves basipetally. Of the material which moves during the 'chase' period some is exported into the receiver and much is retained at the base of the segment. All the material which is mobile appears to move soon after the removal of the donor because little change occurs in the profile during the extra 16 h 'chase' period except in the first section. It can also be seen that a certain amount of activity moves back into the replacement donor during the 'chase' period.

This experiment shows that most of the radioactive material in an internode segment after a transport period is immobile and does not move within the segment when the source of radioactive auxin is removed. In order to see if this material could be 'pushed' down the segment by the application of more auxin similar experiments were performed but instead of replacement of the donor block with a plain agar block it was replaced with an agar block containing 5 μl of 'cold' IAA solution. 'Chase' periods of 2 h and 18 h were again employed and experiments utilising plain agar replacement donors were performed concurrently. The results are shown in Tables II.22 and II.23 (see overleaf).

It can be seen in both cases that the data are very similar whether more IAA is applied to the transport system or not. In other words the material retained within an internode segment during a 5 h transport period is not turned over but is irreversibly immobilised. In both experiments the levels of radioactivity recovered from the replacement donors are similar whether the replacement donors contain IAA solution or not. It is, therefore, unlikely that the activity is associated with IAA because this would involve movement against a concentration gradient in the case of the 'cold chase' experiment. Experiments recorded in Chapter I were not successful in determining the nature of the radio-

Table II.22 Comparison of activity distribution in internode segments subjected to a 5 h transport period with a 2 h 'plain chase' (5/2) and a 2 h 'cold chase' (5/2C)

Component	Count	
	5/2	5/2C
Donor 1	26,195	26,043
Donor 2	141	130
A	1,666	2,138
B	520	580
C	215	285
D	115	156
E	66	93
F	44	69
G	39	99
H	30	43
I	27	41
J	27	37
K	31	38
L	33	42
M	57	66
N	149	184
O	691	774
Receiver	167	251
Total	30,213	31,069

Table II.23 Comparison of activity distribution in internode segments subjected to a 5 h transport period with an 18 h 'plain chase' (5/18) and an 18 h 'cold chase' (5/18C)

Component	Count	
	5/18	5/18C
Donor 1	24,620	23,569
Donor 2	117	121
A	2,250	2,267
B	965	910
C	453	444
D	251	261
E	179	168
F	116	119
G	83	91
H	63	67
I	54	57
J	54	53
K	66	60
L	83	79
M	151	133
N	450	302
O	1,055	1,171
Receiver	389	321
Total	31,399	30,193

active material in the replacement donors but in earlier experiments traces of IAAsp had been found in donor blocks and since IAAsp is produced in the apical region of the segment it is not unreasonable to suppose that some might diffuse across the cut surface although it is immobile in the tissue.

Results recorded in Chapter I (see Plate I.40) suggest that after a 'chase' period all the ^{14}C retained in the internode segments is associated with IAAsp, although the 'time course' experiments in Chapter I (see Plate I.21 and I.22) suggested that immediately after a 5 h transport period much unchanged IAA remained in the segments.

IAA/IAAsp Ratios

The experiments involving chromatography (see Chapter I) gave an indication of the substances present in the tissue as a result of IAA transport and experiments reported earlier in the present chapter have shown the overall distribution of radioactivity in internode segments after various transport periods. Some of the experiments in Chapter I (see Plates I.35 - I.39) had indicated that the relative levels of IAA and IAAsp differed in different regions of the segment. The techniques of chromatography and oxidation were used together to calculate these relative levels and correlate the information gathered. The method chosen to measure relative amounts of IAA and IAAsp in extracts was to scrape areas from chromatograms and oxidise them in the Oxymat. Since the chromatograms had not been prepared quantitatively this method could only be used to discover relative and not absolute amounts of IAA and IAAsp.

The experiment was performed first with the chromatogram used to produce Plate I.39. The radiograph shown in the plate was superimposed on the original thin layer chromatography plate. The boundaries of the IAA and IAAsp spots in the chromatograms were traced on the radiograph with a ball-point pen and the

outline was thus transcribed through the radiograph onto the TLC plate. The areas so marked were carefully scraped off the plate and the cellulose dust was transferred into a polycarbonate combustion vessel and oxidised in the Oxymat. Care was taken to remove all the material within the circumscribed areas without including any cellulose from outside the area. The dust from one spot was transferred to one vessel. Since duplicate chromatograms were run on the TLC plate it was possible to calculate the ratios from the duplicate counts. The counts obtained from the different spots are shown in Table II.24.

Table II.24 Radioactivity recovered from the IAA and IAAsp spots on chromatograms of extracts of different regions of Coleus internode segments. Apical, middle and basal denote the three regions; '1' and '2' denote the two replicates of each extract.

Spot	Corrected Count		Mean Count		Ratio
	IAA	IAAsp	IAA	IAAsp	IAA/IAAsp
Apical 1	430	210	425	232	1.8 : 1
2	420	253			
Middle 1	191	0	176	0	infinite
2	161	0			
Basal 1	342	72	347	69	5.0 : 1
2	352	66			

As can be seen from Table II.24 replicate samples gave similar counts so the mean values quoted are representative. From the data in the table the IAA/IAAsp ratio is calculated for each region of the segment. These numerical data support the visual observations recorded in Chapter I as regards the relatively high level of IAA in the middle region of the segments. No measureable activity could be recovered from the IAAsp area of the middle extract chromatogram. Relative IAA levels were much lower in the apical and basal regions, particularly the apical region where the ratio was only one third that in the basal region. In all regions there was more IAA than IAAsp. Experiments recorded in Chapter I

(see Plates I.21 and I.22) had shown that after a 5 h transport period most of the radioactivity in internode segment extracts was associated with IAA and this experiment supported this visual indication.

The next stage in the investigation was to calculate ratios for segments after a 24 h transport period. The overall ratio in an entire segment was calculated first. The data were obtained by scraping the appropriate areas from chromatograms of the extract of entire internode segments. There were two such chromatograms on the plate and the data are presented in Table II.25.

Table II.25 Radioactivity recovered from spots on chromatograms of extract of *Coleus* internode segments after 24 h basipetal IAA treatment; '1' and '2' denote the two replicates of each extract.

Spot	Corrected Count		Mean Count		Ratio
	IAA	IAAsp	IAA	IAAsp	
1	568	243	580	264	2.2 : 1
2	591	285			

Again there is more IAA than IAAsp present in the segments. Data reported earlier in this chapter show that most of the radioactivity found in a segment is immobile and cannot be chased out. These chase experiments were based on a 5 h transport period and although immobility of the radioactive material was indicated, chromatography suggested the presence of large amounts of IAA and little IAAsp. After 24 h there is apparently still more IAA than IAAsp present. It therefore appears that IAA can be immobilised without first undergoing metabolic change.

To further investigate the situation the experiment was performed with extracts of different regions of internode segments after a 24 h transport period. After the transport period segments were divided into the apical 5 mm, the middle 7 mm and the basal 3 mm sections and the three sections were extracted

and chromatographed separately and areas scraped from the chromatogram as described. Four replicate chromatograms were used in this experiment and the data in Table II.26 represent the mean ratios calculated from these data.

Table II.26 IAA/IAAsp ratios in different regions of internode segments after 24 h IAA-2-^{14}C treatment

Region	Ratio
Apical	1.6 : 1
Middle	1.7 : 1
Basal	1.4 : 1
Mean overall	1.6 : 1

In this experiment the relative level of IAA appeared to be somewhat lower but there was still more IAA than IAAsp present. Again the ratio was greatest in the middle region but in this case the lowest ratio was calculated for the basal region (cf. the situation after 5 h as quoted in Table II.24) and the variation in ratios was only small.

The IAA/IAAsp ratio decreases between 5 h and 24 h and it is known that IAA continues to be taken up from the donor during this time. Therefore the relative rate of conversion of IAA to IAAsp must increase as more IAA becomes available. Also the relative amount of IAAsp tends to be higher in areas where overall activity levels are high. These findings suggest that IAA is more readily converted to IAAsp when the IAA concentration is high. To understand the situation fully it is necessary to know whether there is a maximum IAA level which the tissue can accommodate before conversion in which case absolute IAA levels in the different regions should be similar.

All the experiments quoted so far in this section have utilised a 5 μl volume of IAA-2-^{14}C solution in the donor block. A further experiment was set up in which one set of internode segments had 5 μl of IAA-2-^{14}C solution in each donor

and another set (each set comprising 20 replicates) had 10 μl of solution in each donor block. All donors were applied to the apical cut surfaces of the segments and the experiment ran for 24 h. After this time all segments were transversely divided into three sections as above. The sections from half of each set of segments were extracted and chromatographed in the usual way. The other sections were pooled in groups of two and subjected to oxidation in the Oxymat and subsequent scintillation counting. The chromatograms were then scraped as described above and the different areas from the plate were also oxidised and counted. In this way the IAA/IAAsp ratios calculated from the scraped chromatograms could be related to the actual level of activity recovered from the appropriate region of the internode segment and the activity associated with IAA could be calculated in each case. Data obtained for the two different donor concentrations were used to compare actual IAA levels under the two sets of experimental conditions. The results are shown in Table II.27 overleaf.

The data in Table II.27 show that over 24 h there is very little difference in the levels of activity in the two sets of segments and there is even slightly more labelled material in the segments which had the smaller amount of IAA-2-^{14}C available to them.

Both the total amount of labelled material in the segments and its distribution are very similar in the two situations so it would seem that there is a certain capacity for IAA uptake and transport which cannot be exceeded. The IAA/IAAsp ratios in the three regions are also quite similar in both cases. As in all the estimations made so far there is more IAA than IAAsp in all parts of the segment, and as in the other 24 h estimation (see Table II.26) the relative amount of IAA is greatest in the middle section and least in the basal section. If the ratio

Table II.27 Distribution of activity in different regions of internode segments after 24 h basipetal transport of IAA with two different donor concentrations.

Region	Total Count (cpm)		IAA/IAAsp Ratio		Calculated IAA Count (cpm)	
	5 µl	10 µl	5 µl	10 µl	5 µl	10 µl
Apical	5,096	4,809	1.8 : 1	1.7 : 1	3,276	3,027
Middle	466	314	2.3 : 1	2.8 : 1	325	231
Basal	765	658	1.4 : 1	1.6 : 1	446	405
(Donor)	17,170	40,940				
(Total)	23,497	46,721				

calculated from oxidation of the chromatographs is used to calculate the amount of the total activity which is associated with IAA-^{14}C in a section, it can be shown that the level of IAA present varies considerably between the three regions but that corresponding regions from segments with different donor concentrations have very similar IAA levels. Thus there is not a simple maximum amount of IAA that can remain unchanged in the tissue because the actual levels of activity associated with IAA are vastly different. If the values calculated are corrected for the different lengths of the three sections the difference becomes even greater (see Table II.28).

Table II.28 Radioactivity (in cpm) associated with IAA-^{14}C per mm length of internode in the three regions and with the two donor concentrations.

Region	Activity associated with IAA (counts min^{-1} mm^{-1})	
	5 μl	10 μl
Apical	655	605
Middle	46	33
Basal	149	135

Again the values obtained for the two sets of segments are quite closely comparable.

At first these results do not seem to be in agreement with data presented earlier (see Chapter I and earlier sections of this chapter) but replicates have been good in all experiments and all calculated values have been quite comparable which suggest that this method of scraping chromatograms, although technically difficult, is dependable. From chromatograms shown in Chapter I and from the 'chase' experiments it was thought that very little IAA would be found in the segments after long transport periods, particularly in the apical and basal regions. In fact, there is more IAA than IAAsp in all the situations investigated. This

shows the dangers of attempting to make quantitative estimates from visual inspection of radiochromatograms. In all cases where IAA appeared to be present in an extract the spot on the chromatogram tended to be large and diffuse, whereas IAAsp spots were usually small and dark. It is very difficult to compare such different spots and false impressions can easily be gained.

It is not surprising (and in fact this was indicated in the chromatograms) that after 5 h transport there is a great deal of unchanged IAA still present, particularly in the middle region of the segment. After 24 h the relative IAA level drops to about 1.5 : 1 or 2 : 1 and although the middle region is still the part of the segment to have the largest IAA/IAAsp ratio the basal region has become the region with the lowest ratio.

Even if the inconsistency between the visual and quantitative evidence of the chromatograms can be attributed to errors of interpretation, the discrepancy between these latest quantitative data and the Oxymat data from which the profiles have been constructed cannot be dismissed so easily. All profiles constructed after basipetal transport have exhibited a form which suggests that auxin is immobilised in the tissue and since these profiles could not be substantially altered by a 'chase' period this suggestion was upheld. It now appears that most of the radioactivity, though immobile, is associated with IAA. It therefore seems that immobilisation of IAA is not necessarily to be equated with metabolism of IAA to IAAsp. The internode segments extracted after a 'chase' period appeared to contain no IAA at all. No quantitative data are available for the chromatograms, but since segments extracted after transport periods of similar lengths but without the 'chase' periods (see Plates I.21 and I.22) appeared to contain a large amount of IAA it may be that IAA can be immobilised without change of chromatographic behaviour and at a later stage it can be metabolised to IAAsp in situ. Also the

possibility that some free IAA still exists in the segments after a 'chase' period but that it was not detected by visual inspection of the chromatograms cannot be excluded in view of the problems discussed above.

Although the values calculated here do not support the idea of a fixed maximum amount of IAA which can be present in the tissue it could be that the native IAA presumed to be present in the segment already occupies many of the available sites and that the figures obtained are merely a reflection of the number of sites still available to exogenous auxin. This is somewhat unlikely, however, since it would mean that the profile of native IAA distribution would have its peak in the middle of the segment which would not be consistent with the idea of the auxin gradient which it thought to exist initially along plant segments, nor with the expected pattern of movement of the endogenous auxin down the segment which would lead to remove this gradient. It is more likely that the IAA values calculated here represent combined mobile and immobile IAA and that if mobile IAA could be calculated on its own then there would be a fixed maximum amount of it present throughout the segment.

2,4-D Transport

The experiments reported so far have shown that IAA transport is very closely associated with immobilisation, and the profiles of radioactivity established in the segments due to the transport of radioactive IAA illustrate immobilisation patterns rather than transport patterns. Other substances, including 2,4-D, are thought to be transported in the same transport system as IAA without undergoing immobilisation. 2,4-D transport was therefore studied to investigate the distribution of radioactivity resulting from auxin transport alone.

2,4-D-2-^{14}C was used in the experiments in a 10^{-4}M solution. This

high concentration was selected largely to make the results comparable with those of experiments utilising IAA-^{14}C which had specific activities twice as high as that of the 2,4-D-^{14}C. The experimental technique was the same as that used for the previous experiments and 5 μl of 2,4-D-^{14}C solution were used in each donor block. Preliminary experiments showed that 2,4-D transport was much slower than IAA transport and that 24 h transport periods were required for 2,4-D-^{14}C to be distributed along the whole segment.

Twenty four hour transport periods were therefore used in all experiments. In the first set of experiments acropetal transport and basipetal transport were measured and one batch of segments was subjected to a 24 h 'chase' period with 'cold' 2,4-D after the 24 h transport period. The results of these experiments are shown in Table II.29 and the log profiles are shown in Figs II.17 and II.18.

It can be seen from the table that 2,4-D transport in Coleus internodes is highly polar. Uptake is greater at the basal cut surface but very little of the material taken up is transported acropetally. Most of the radioactive material remains in the first 3 mm of the segment and reliably measureable levels of activity can only be detected over 8 mm. On the other hand much basipetal transport occurs. No basal accumulation of activity occurs. The log distribution approximately takes the form of a straight line but with a much less steep gradient than that seen for acropetal transport. There is a slight increase in the level of activity in the distal 2 mm but this bears no resemblance to the large accumulation seen after IAA transport which represents mostly immobile material. Quite a lot of radioactive material is exported into the receiver, far more than is present in the distal 1 mm section.

The situation after a 'chase' period also provides a marked contrast to the

Fig. II.17 Log profiles of radioactivity in Coleus internode segments after 24 h basipetal and acropetal transport of 2,4-D-^{14}C

Both the log profiles approximate to straight lines although after basipetal transport there is some deviation from a log decline in the distal part of the segment. The radioactive material has only penetrated 9 mm into the tissue and the gradient of the acropetal line is much steeper than that of the basipetal line.

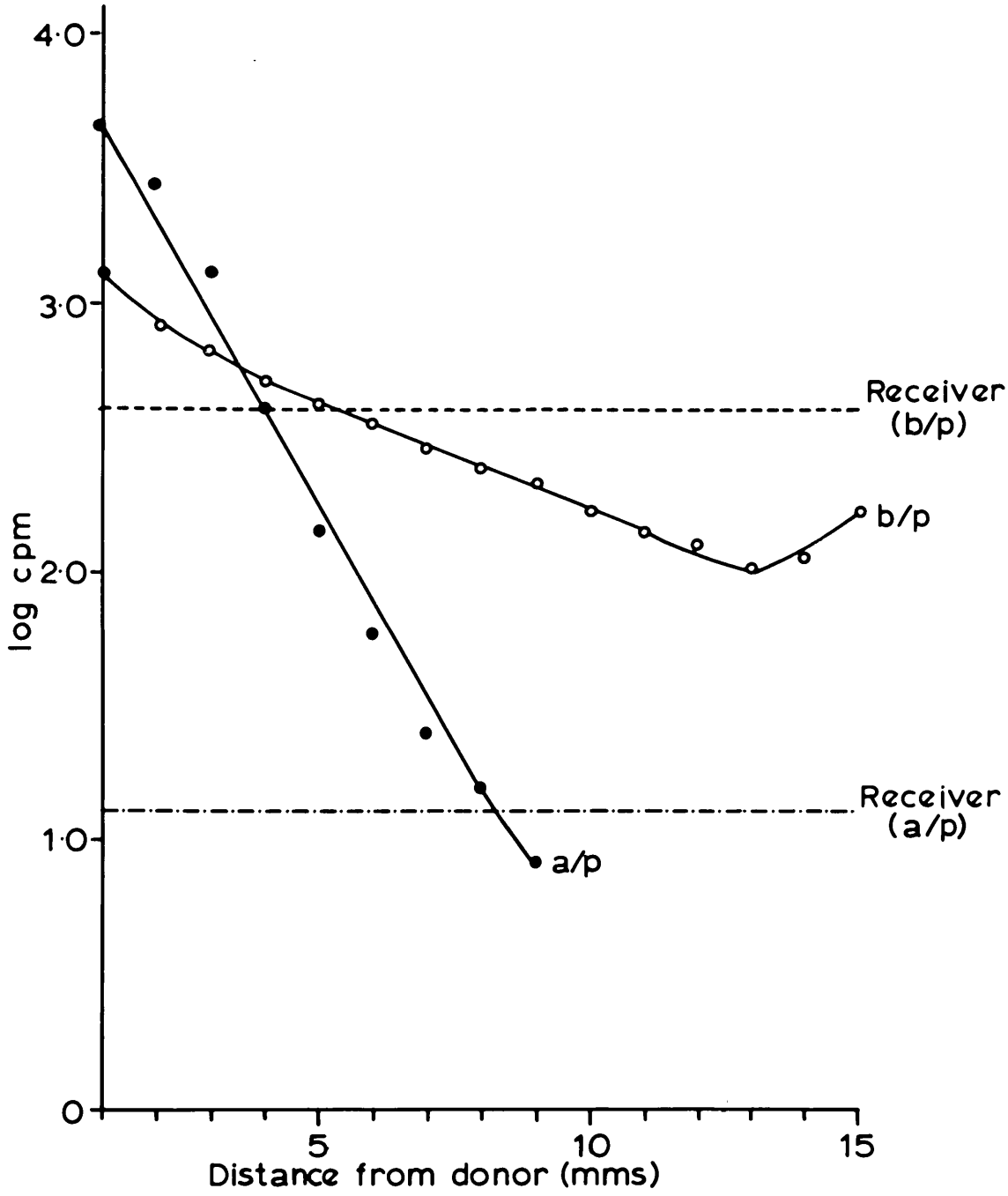

4·0
3·0
2·0
1·0
0
log cpm
Receiver
(b/p)
b/p
Receiver
(a/p)
a/p
5
10
15
Distance from donor (mms)

Fig. II.18 Log profiles of radioactivity in Coleus internode segments after 24 h basipetal transport of 2,4-D-^{14}C with and without 24 h 'chase' periods

A is the log profile established after 24 h basipetal transport of 2,4-D-^{14}C. B shows the results of a 'cold' IAA 'chase'. C shows the results of a 'blank chase' and D shows the results of a 'cold' 2,4-D 'chase'. All three 'chase' experiments produced similar results and during the 'chase' periods a considerable movement of radioactive material occurred within the tissue. No further export into the receiver occurred during the 'chase' periods.

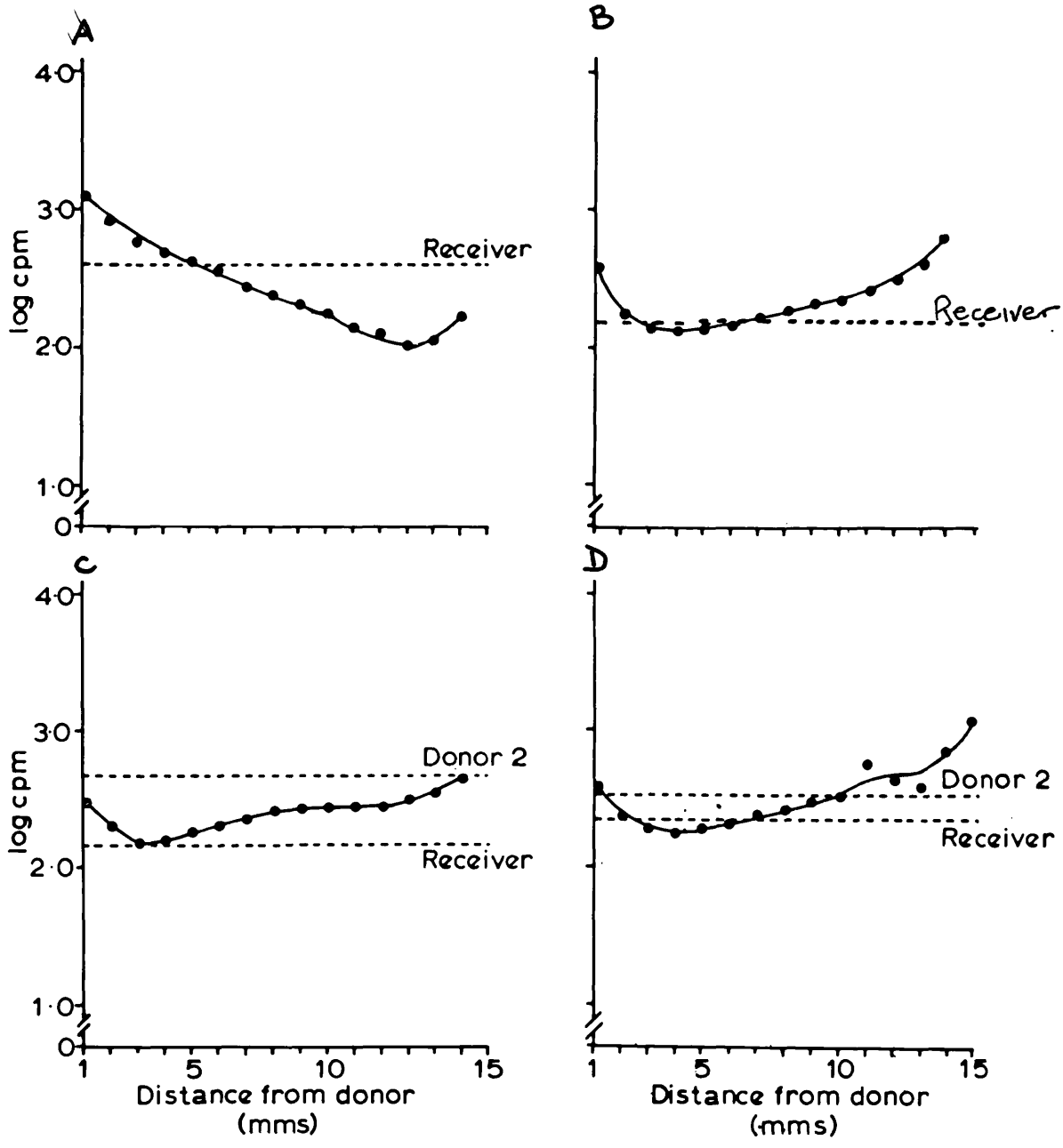

A
B
C
D
4·0
3·0
2·0
1·0
0
log cpm
Receiver
Donor 2
1
5
10
15
Distance from donor
(mms)

Table II.29 Distribution of activity in Coleus internode segments after 24 h 2,4-D-2-^{14}C treatment

Component	Count		
	24 h a/p	24 h b/p	24/24 chase
Donor 1	17,293	19,776	19,166
Donor 2	-	-	341
A	4,595	1,275	382
B	2,844	830	237
C	1,325	661	188
D	408	506	176
E	140	414	190
F	57	352	206
G	24	282	235
H	15	240	265
I	8	211	302
J	11	174	340
K	4	141	571
L	2	128	441
M	3	102	392
N	8	113	721
O	8	170	1,216
Receiver	13	405	225
Total	26,758	25,780	25,594

IAA transport experiments as can be seen from the greatly altered log profile (see Fig. II.18). A great deal of the activity present in the segment after 24 h transport can be moved after the removal of the source of radioactive 2,4-D. The radioactive material is not exported at the base of the segments, (indeed it appears that radioactive material may be taken up again from the receiver) and so the 'chased' material tends to accumulate at the basal end of the segment. This accumulation does not resemble the accumulation which results from IAA transport because it is not apparent during the transport period and because it is distributed over 12 mm. During the 'chase' period the profile is thus reversed over most of the segment. It would appear that 2,4-D cannot be exported at the basal cut surface indefinitely. Some change must occur as the segment ages. There is a slight basal rise in the log profile of the 24 h experiment so it is

possible that the inability to export 2,4-D had already come into effect before the 'chase' period began. It is thus possible that material was taken back into the segment from the receiver during the original 24 h transport period. Some radioactive material moves into the replacement donor during the 'chase' period.

The mobility of the transported 2,4-D was further investigated. Since a considerable amount of material could be 'chased' by 'cold' 2,4-D experiments were performed in which donor 2 contained 'cold' IAA or was a plain agar block. The purpose of the experiments was to see if a 'head' of IAA had the same effect as a 'head' of 2,4-D, since both are thought to move in the system, and to find out if the 2,4-D in the segment would move down the segment without being 'pushed' by new material entering the transport system. The IAA was in 5×10^{-5} M solution. This concentration was the same as that used in the IAA transport experiments. The results of the two experiments are shown in Table II. 30 and the log profiles are shown in Fig. II. 18 B.

Table II. 30 Activity distribution in internode segments after 24 h 2,4-D treatment and different 24 h 'chase' periods

Component	Count	
	Plain 'chase'	IAA 'chase'
Donor 1	21,993	21,246
Donor 2	465	308
A	285	383
B	192	173
C	150	138
D	152	133
E	178	140
F	195	149
G	216	171
H	249	185
I	257	211
J	261	225
K	270	266
L	260	319
M	311	417
N	340	628
O	451	535
Receiver	143	191
Total	26,368	25,818

The profiles are very similar to that for the experiment with the 2,4-D 'chase' in that radioactive material moves from the tissue into the replacement donor, from the receiver back into the tissue and basipetally within the tissue to the extent that the profile is reversed except for the apical 3 or 4 mm. In all the 'chase' experiments the levels of activity in the apical 3 or 4 mm are much lower than previously but the levels still decline from the apical surface. This is probably because further movement from that region is blocked by the accumulation of material extending back from the base of the segment. An experiment recorded in Chapter I (see Plates I.42 and I.43) suggests that 2,4-D remains largely unchanged during transport. These experiments suggest that as well as remaining chemically unchanged the 2,4-D also remains mobile. Its mobility is not influenced by the continued presence of a source of auxin since it continued to move during the 'blank' chase.

These experiments illustrate the differences between IAA and 2,4-D as regards their behaviour during transport in the same system.

Diffusion

Acropetal movement is often thought to be a purely physical, non-active process (Goldsmith, 1966a and b; Leopold and de la Fuente, 1967; McCready, 1967; and Wilkins and Shyte, 1967). This was not found to be the case by Keitt and Baker (1967), however, who reported that TIBA and reduced temperature both reduced acropetal transport.

In order to draw up a profile of straightforward diffusion in a non-biological system an agar model was set up. Agar donor and receiver blocks were prepared as usual and in addition agar cylinders, 15 mm in length and with the same cross section as the blocks, were cut. These cylinders were set up in the perspex bridges used for the plant segments. An agar donor containing

5 μl of IAA-2-^{14}C solution was applied to the upper cut surface of each agar cylinder and the lower cut surface was in contact with a receiver block. The experimental assembly was placed in the same sealed plastic tray used for the plant segments in order to minimise shrinkage of the agar. The tray was kept in a dark room under a green light to prevent IAA degradation. The transport period was 5 h and after this time the donors were removed and the agar cylinders were sectioned in the same way that plant segments were sectioned. It was not possible to cut more than 13 sections from each agar segment because some shrinkage had occurred. All samples were pooled, oxidised and counted as usual. The profile and the log profile are shown in Figs II.19 and II.20.

It can be seen that the log profile for the cylinder describes a straight line. The donor and receiver were not sectioned and so would not be expected to fit the straight line.

Since movement of radioactive auxin in an agar cylinder can be assumed to be the result of simple diffusion this experiment has verified that movement of IAA through a medium by diffusion results in a distribution with a log decline. In the biological transport considered in the present experiments the basipetal transport of IAA results in a distribution which bears no resemblance to the distribution caused by diffusion. The basipetal transport of 2,4-D establishes a straight line log distribution but its gradient is far less steep than that for acropetal transport of 2,4-D. The gradient of a log profile is a characteristic of diffusion of a substance in a medium and it can be seen that the gradient of the log profile for acropetal transport of IAA in internode segments does not alter appreciably over several hours of transport (see Fig. II.14).

The gradients of the log profiles are of interest in the consideration of the rates of diffusion encountered in the experiments. 2,4-D diffuses more readily

Fig. II.19 Profile of radioactivity in agar cylinders after 5 h diffusion of IAA-2-^{14}C

The curve as expected is a rectangular hyperbola.

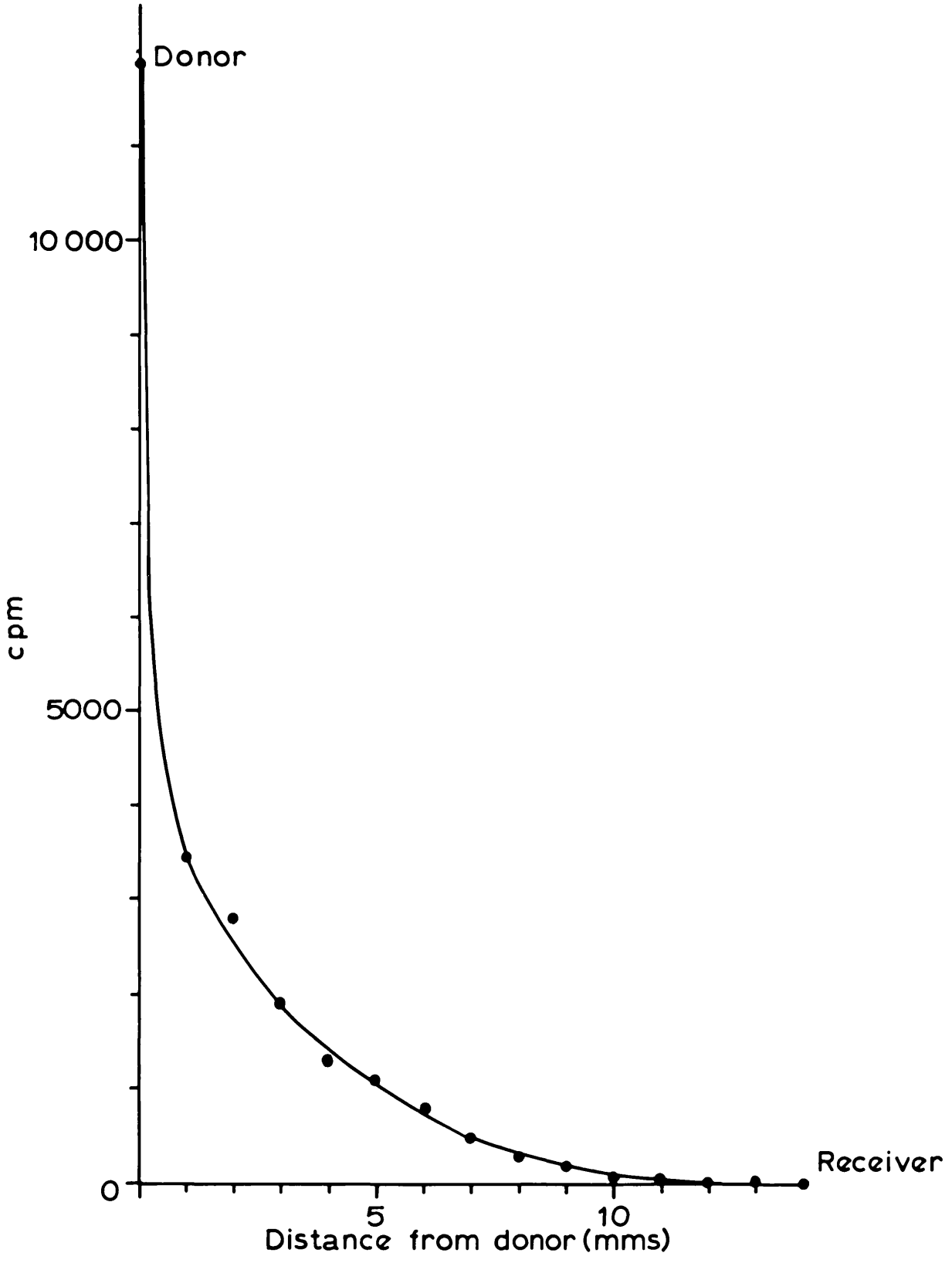
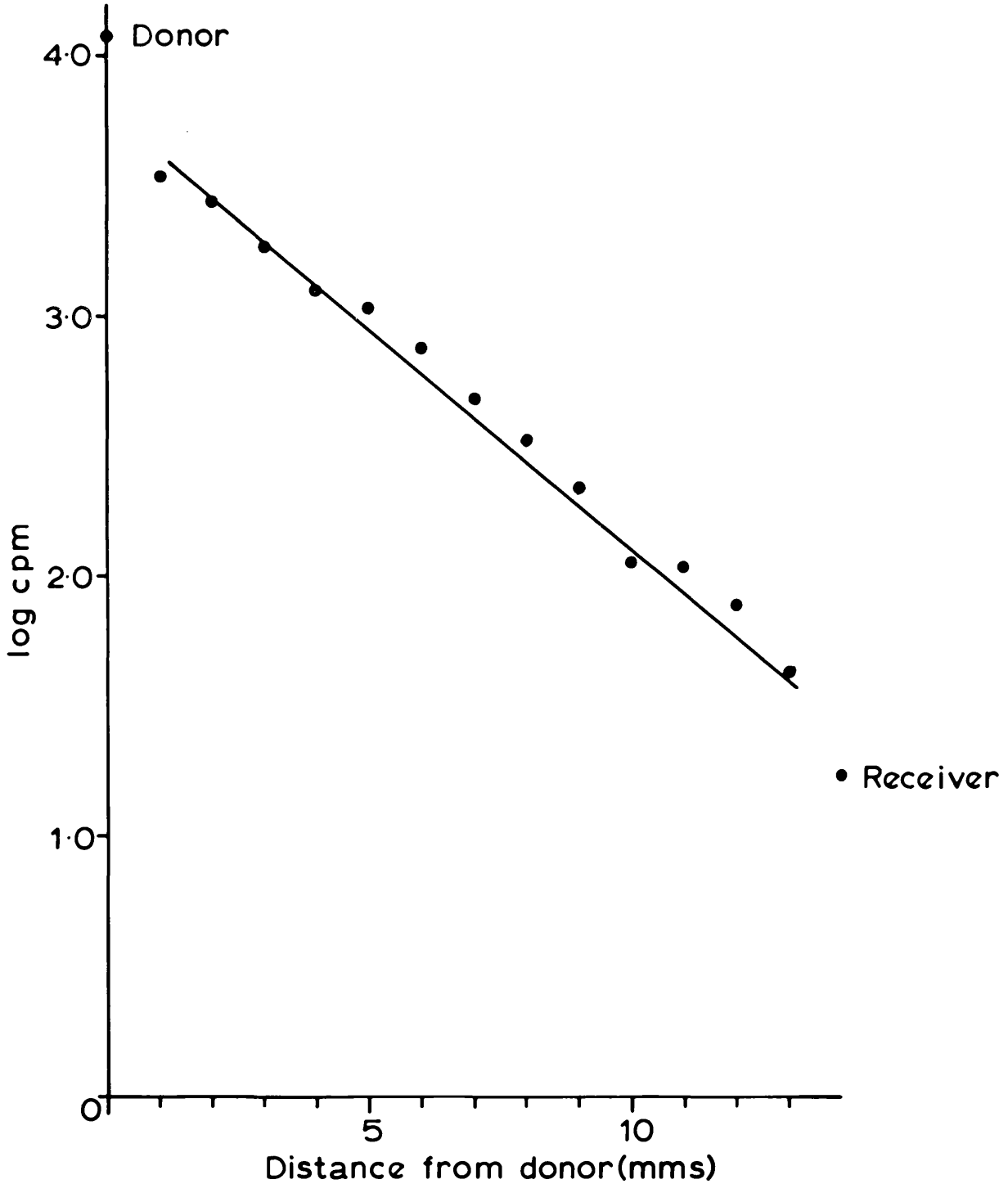

Donor
10 000
5000
0
cpm
Receiver
5
10
Distance from donor (mms)

Fig. II.20 Log profile of radioactivity in agar cylinders after 5 h diffusion of IAA-2-^{14}C

The distribution shows a logarithmic decline.


Donor
4·0
3·0
2·0
1·0
0
log cpm
5
10
Distance from donor(mms)
• Receiver

than IAA in Coleus segments and IAA flux through agar is far greater than that through tissue segments from base to apex. In the agar much activity can be seen to have travelled more than 4 mm, whereas over a similar period only 70 cpm travel that distance in a Coleus internode segment. One would expect this to be the case since a biological system would offer far greater resistance to diffusion than a simple agar system. Experiments involving the movement of IAA through different living and dead systems were performed by Chang and Jacobs (1972) who concluded that polarity was the result of an active suppression of acropetal transport rather than an active component in basipetal transport. This was not indicated in the present experiments where basipetal transport appears to be an active process.

Discussion

The early experiments in this series demonstrate a marked polarity of auxin transport in Coleus segments, but not a polarity of uptake. Uptake from the donor occurs rapidly at the apical and basal cut surfaces. It occurs so rapidly, in fact, that transport cannot immediately keep pace with it and proximal sections of the segment always contain considerably more radioactive material than the subsequent sections. There appears to be a maximum capacity for IAA in the tissue because after a given period of acropetal or basipetal transport the levels of radioactivity in the proximal section are often similar, although the total amounts of IAA which have been taken into the segments in the two situations are quite different (see Tables II.1 and II.3 where section A contains approximately 9000 and 9500 cpm respectively, although total counts in the segments are very different). Auxin seems to be taken up by the tissue until the capacity of the region proximal to the cut surface is reached. As material is transported away from that region, to a greater or lesser extent depending on the orientation of the tissue with respect to the donor, more auxin is taken up to keep the tissues of the proximal region saturated. This is also demonstrated in the experiments utilising segments of different lengths, because more auxin entered the longer segments than the shorter ones. It has been accepted by many workers that uptake and transport are separate processes. Andreae and Van Ysselstein (1960) found that uptake of IAA by pea epicotyls was initially physical and only later metabolic, a change not mirrored in transport. Winter (1967) found that TIBA had no effect on auxin uptake in pea at concentrations which inhibited transport. Wangermann (1974) found TIBA actually increased uptake when it inhibited transport in Coleus. Koevenig and Sillix (1973) reported that uptake was not polar in anther filament segments of Cleome hassleriana. Some workers have, however, found differences in apcial and basal uptake. Goldsmith (1967) found that the effects of nitrogen on apical and basal uptake in coleoptiles were different. Wilkins et al (1971) found uptake to be polarised in Zea root segments, and Bridges and Wilkins (1973) found that morphactin had different effects on apical and basal uptake.

The accumulation of radioactive material at the base of segments after basipetal transport of IAA-^{14}C is a very prominent feature of the experiments reported in this chapter. It is not the first time it has been noted. Goldsmith

and Thimann (1962) noticed some departure from the logarithmic distribution at the base of the segments. This was noticed in coleoptile segments and was not an accumulation as such, but a change in gradient of the profile which did not persist in longer experiments. Leopold and de la Fuente (1967) showed an accumulation at the base of bean petiole segments and remarked that this accumulation was suppressed by TIBA and DNP. Other workers have published, without comment, data which suggest such an accumulation. Thimann and Wardlaw (1963) divided treated pea internode segments into three sections and counted them. There was a slightly higher level of activity in the third section than in the second section. A similar result was obtained by Thompson (1967) with peanut internode segments, but in that case the first and third sections included the donor and receiver respectively, so no firm conclusion could be drawn.

However, the accumulation was considered worthy of further investigation because of its relevance to estimations of polarity, and because of its possible physiological significance. Experiments reported in this chapter on petiole segments showed that the distribution of radioactivity after basipetal transport was exactly similar to that in internode segments, and experiments which will be reported in Chapter III show that the accumulation can also occur in root apices where there is no cut surface. Other experiments were performed to eliminate the possibility that the accumulation was caused simply by the inability of the IAA-^{14}C to cross the interface between the tissue and the receiver block; these experiments showed that this was not the case. Since one of the experiments quoted (Wangermann, 1974) employed Helianthus internode segments it also served to show that the phenomenon occurs in more than one species. The profile obtained in the experiments is similar whether IAA-1-^{14}C or IAA-2-^{14}C is used in the donor, so the material which comprises the accumulation is apparently not a decarboxylation product. In fact, $^{14}CO_2$ production was measured by trapping and oxidising; the results of the experiment indicate that very little decarboxylation occurs during IAA transport in Coleus. This finding was not based purely on low recovery of radioactivity from the $^{14}CO_2$ trapping device (which could merely have been indicative of inefficient trapping), but was also based on the fact that total counts recovered from the experimental system were the same as those recovered from an unburned 5μl sample of IAA-1-^{14}C solution. These results are consistent with results reported in Chapter I, where it was found that the radioactive products formed during transport of IAA-1-^{14}C were no different from those formed during

IAA-2-^{14}C transport. Other workers have reported decarboxylation to occur in various species during transport. Decarboxylation has been found to occur in peas by Andreae et al (1961), Winter and Thimann (1966), Andreae (1967), Winter (1967) and Morris (1970). Iversen and Aasheim (1970) reported decarboxylation in sunflower and cabbage roots, andde la Fuente and Leopold (1972) also noted decarboxylation in sunflower hypocotyl segments. Kerstetter and Keitt (1966) found decarboxylation in tobacco; Field and Peel (1971) reported it in willow; Wilkins et al (1971) in Zea, and Eliasson (1972) in aspen. Veen (1966), however, found decarboxylation of NAA to be negligible in Coleus. The difference that the accumulation of activity makes to estimations of polarity, and the consequent importance of including it in such estimations, have been illustrated in the present chapter. It has been shown that estimations based on receiver counts alone can be unreliable. The widely differing values for polarity in Coleus which have been reported in the literature (Jacobs, 1964; Leopold, 1963; Naqvi and Gordon, 1965; de la Fuente and Leopold, 1966) may be partly explained on this basis. De la Fuente and Leopold (1966), however, pointed out another reason for the different polarity values which have been quoted, and this is the length of segment used. They based their calculations on the mathematical model of Leopold and Hall (1966) and performed experiments which confirmed these calculations. According to their idea, the polarity observed in a tissue segment is due to a very small polarity at the cellular level; the greater the number of cells the auxin has to pass through, the greater will be the amplification of this effect. The results quoted do show this tendency: when transport has proceeded long enough to calculate polarity over segments of different effective lengths, the polarity value has been shown to increase with the length of the segment. It is probale, therefore, that a cell in the auxin transport system has a higher capacity to export auxin from itself at the basal end than at the apical end. All the present experiments certainly demonstrated a very marked polarity in Coleus internode tissue. Acropetal flux is very low compared to basipetal flux and even in situations where acropetal transport is potentially enhanced, e g in old tissue or tissue from flowering plants, polarity is only slightly reduced. This suggests that the ability of a cell to export auxin from its apical end is very low.

Experiments were run over a variety of transport periods, but generally speaking these were longer in the present experiments than in many others.

Long transport periods were criticised by Jacobs (1954) and McCready and Jacobs (1963a). They found that polarity was evident after 3 h but not after 24 h. These findings were based on receiver counts. Long transport periods were considered necessary here for two reasons. In the first place if short transport periods are used, short segments must be used, and it has been shown that estimates of polarity are lower when short segments are used. Secondly, it was indicated in the present experiments that for several hours acropetal and basipetal flux continue to proceed at their respective rates, and that the differential between them (over a set distance) remains reasonably constant. However, over a 24 h transport period the indications are that basipetal transport continues whereas acropetal transport begins to tail off (see Table II.9), again resulting in high values for polarity over long periods. Work with isolated segments can only be cautiously regarded as an indication of the situation in whole plants, but in the whole plant transport occurs over long distances and for long periods and it seems to be imposing unnecessary limitations on experimental work to use short segments and short transport times. The tissue did not appear to undergo any obvious deterioration during the experiments; experiments reported in Chapter III utilised very long transport periods and the results obtained offer some useful information on auxin transport. According to Osborne and Mullins (1969) ageing does not cause reduced auxin transport if IAA is applied to the segments soon after excision as was the case in the present experiments.

The basal accumulation of radioactivity, in addition to being considered with regard to the estimation of polarity, was also investigated as regards its formation, its distribution in segments of different lengths, and its metabolic constitution and significance. During the time course experiments it was shown that the basal accumulation begins to occur as soon as the radioactive material reaches the base of the segments. It begins to occur at the extreme basal region and extends back from there. The distance over which it can extend is governed by the length of the segment; once the accumulation has established itself the profile retains the same general shape, while levels of radioactivity in all sections of the segment continue to rise with time. There is no tendency for the curve to flatten out and the central trough between the apical and basal accumulations is maintained over very long periods.

The constituents of the basal accumulation of radioactivity were considered in Chapter I. It was indicated that IAA and IAAsp were both present and that IAAsp was more abundant than in the middle region of the segment. Quantitative experiments reported in this chapter showed that there was more IAA than IAAsp present in all regions after both the transport times investigated, and that the relative IAAsp level was lowest in the middle region in both situations. They also showed that the relative IAA levels declined markedly between the 5 h and 12 h periods, even though more IAA was being taken into the tissue. Veen (1966) in his transport experiments with NAA-^{14}C through nodal explants of Coleus estimated relative quantities of NAA and NAAsp in different parts of his explants. His actual ratios are different from those reported here, but the trends which they illustrate are similar. He reports approximately five times as much NAAsp as NAA in the apical part of his explants after 24 h transport. The total counts in this region are quite high. In the basal region of his explants the activity after 24 h is approximately 60 times lower than in the apical region, and he reports finding three times as much NAA as NAAsp. Veen probably had no basal accumulation, although his sections are too thick to reliably state this to be the case. His caculations do show, however, that NAAsp tends to be formed in regions where levels of radioactivity are high, which is broadly what is reported here.

In spite of the high IAA levels in the tissue it was found that most of the radioactive material which constituted the profile was immobile and could not be 'chased' out. It therefore follows that unchanged IAA (or at least some material which had the chromatographic properties of IAA after methanol extraction) remains immobile in the tissue. Other workers have reported that 'chasing' did not produce a noticeable effect on IAA distribution. Wilkins, Cane and McCorquodale (1972) performed 'chase' experiments with Zea root sections and reported them to have little effect. They also utilised 'cold chases' and 'blank chases' and found the results to be similar in both cases. Wangermann (1970) 'chased' IAA-2-^{14}C with 'cold' IAA in Coleus internode segments and then prepared micro-autoradiographs. These radiographs showed a distribution of radioactivity exactly similar to that in a segment which had undergone the transport period without the 'chase' period. However radiographs of internode sections which had been soaked in water or ethanol after the transport period demonstrated an almost complete loss of radioactive

material in the tissue. Wangermann's success in leaching activity from the tissues, and the lack of radioactive material which could be extracted by hydrolysis in the present experiments (see Chapter I), suggest that methanol extraction removes almost all the radioactive material from the tissue. Davies (1972) found this to be true for ethanol extraction. It therefore seems that estimations based on alcohol extracts give a true representation of the material present in the tissue and can be used as a basis on which to explain the metabolism of IAA. The indications from the present experiments are that when IAA is taken rapidly into the tissue at the apical end of the segment, uptake exceeds the transport capacity and much IAA accumulates and is immobilised. Some IAA remains mobile and moves along the transport system. The amount of IAA which can move in the transport system is thus regulated and does not undergo a great deal of immobilisation in the middle region of the segment. When the mobile IAA reaches the cut surface it is held back to some extent even though contact with the receiver at the distal cut surface has been shown to be good. It appears that the cut surface can export auxin, but not as readily as the active transport system can deliver it at the cut surface, so the IAA concentration in adjacent tissues increases and immobilisation occurs. Since the immobilisation does not appear to be reversible, the material remains bound at the basal end of the segment, even though the cut surface retains the capacity to export the mobile material slowly. Thus the reason for the shape of the radioactivity profile would be that the IAA binding sites (the biochemical nature of which is not known) are activated by the arrival of high concentrations of IAA. Since the flow is regulated so that the concentrations of IAA which arrive are different in different regions of the segment, immobilisation rates are also different in different regions of the segment. Thus, according to this reasoning, at any time there is only a relatively small amount of mobile IAA present in the tissue of a segment; and this has been demonstrated experimentally by the inability of the radioactive material to be 'chased'. It is likely that the actual number of binding sites present before treatment in any section of a segment is similar (because the profile obtained is similar in all situations examined even though the different segments were cut from different parts of the plant, suggesting that there is not a 'gradient' of sites within the plant), and that the induction of these sites by the concentration of

IAA building up in the section is responsible for the degree of binding that occurs. If this is so, it is possible to visualise a situation after a long period of transport in which all available sites are occupied, and in which the profile is no longer a curve but a horizontal straight line. This is not the case. Data from experiments which have run for 10 days have still produced profiles of the usual shape. The binding sites must therefore be vacated after they have been occupied. It is proposed that this occurs when the immobile IAA is conjugated with aspartic acid to form IAAsp, which is not held at the binding sites but which still remains immobile in the same region of the segment.

In the past it has been assumed that immobilisation and conjugation are the same process. The present experiments have shown, however, that although most of the radioactive material in the segments after a short transport period is immobile (as shown by the inability of the material to move during the 'chase' experiments), little of it is IAAsp; most of it chromatographed as unchanged IAA (see Tables II. 24-28). Further, there was not found to be an absolute fixed amount of IAA present in any unit length of segment after a transport period. One would expect to find such a fixed amount if all the IAA present in the segments was in the transport system, and if the IAA which was unable to be accommodated in the transport system was immediately conjugated. Under these circumstances the fixed amount that would be present would represent the capacity of the transport system. The experiments performed on Helianthus suggested that little or no IAAsp was formed in that species, even though Wangermann's (1974) work showed that the basal accumulation occurs in Helianthus segments. According to the hypothesis, the lag phase noticed in association with the formation of IAAsp would be the time during which the binding sites were taking up IAA. IAAsp-synthetase would not be formed in a region until all the binding sites were occupied. It is suggested that IAAsp is an immobilisation product of IAA but that it represents a second stage of immobilisation. This two-stage immobilisation system could be a mechanism whereby excess IAA can be immobilised without blocking the system by irreversible occupation of binding sites. In this way IAA flow is maintained, as is the potential to immobilise IAA at a later time when the flow could again be too high for the system.

The concentration of IAA used (5×10^{-5}M) is reduced to about 5×10^{-6}M by its inclusion in the agar block. This is equivalent to 0.9 mg/l. This is within

the range of concentrations used by Goldsmith and Thimann (1962) and according to McCready and Jacobs (1963a) is within the lower regions of the physiological range. The degree of immobilisation observed here could, therefore, occur in a whole plant.

The profiles obtained for basipetal transport of IAA are largely a reflection of immobilisation rather than transport. In the agar model system discussed in this chapter it was seen that levels of radioactivity due to diffusion through the agar followed a log decline. Similarly acropetal transport profiles approximated to log declines, and the gradient of the profiles did not differ significantly over the 8 h in which the transport was studied at intervals. The log profiles established after acropetal transport, therefore, seem to indicate movement through the segments by diffusion with a fixed diffusion coefficient. The profiles (according to results recorded in Chapter I - see Plate I.7) represent ^{14}C which is associated with IAA and also with IAAsp, so apparently some conjugation occurs during the diffusion of IAA. Since quantitative data for relative concentrations are not available for acropetal transport experiments, it may be that only a little IAAsp is present and that this could have been formed in the basal region as a result of the rapid uptake. 'Chase' experiments as well as quantitative experiments of the type described in this chapter would help to clarify the situation.

The log profile obtained after 1 h of basipetal transport resembles the acropetal transport log profiles just discussed (see Figs. II.8 and II.13) as regards gradient and penetration into the segment. It appears that when a source of IAA is applied to the apical end of a segment the active transport system cannot immediately deal with that IAA and there is an induction period for the system, during which time the IAA can only move by diffusion. The system apparently comes into action during the following 30 min period, having been generated by the supply of IAA, and the profile quickly changes. The gradient becomes less steep and is no longer characteristic of diffusion alone, although the diffusion presumably still occurs and contributes to the shape of the profile.

The apparent irregularity in the flow of auxin during the early period of active transport is not easy to explain. The trough in the activity curves observed after 3 h persists through the middle sections of the segment and cannot be explained by a low overall uptake during one experimental period. Indeed, in some sections (see Fig. II.8A, 1.5 and 2 h curves and Fig. II.13,

curves for sections H, I and J) the decline can be seen over two time intervals. The distinct regions on the 1.5 and 2 h log profiles suggest that the active transport system has only moved radioactive material part of the way along the segment (about 7-10 mm) in this time. Since levels are very low in these sections at these times it is probable that the immobilisation system has not been activated. Slight differences in the efficiency of the transport system as it comes into effect in neighbouring regions of the segment would not be masked by high auxin levels and consequent immobilisation. It could be, therefore, that such differences in efficiency could be responsible for the fluctuation in auxin levels noticed in these experiments. Fluctuation in auxin levels in coleoptile segments have been reported by Shen-Miller (1973), but in his experiments these resulted from waves of acropetal transport following removal of the donor in pulse experiments.

2,4-D shows polarity of transport, but its basipetal transport is much slower than that of IAA. If acropetal transport of 2,4-D through Coleus internode segments is considered it can be shown that the log profile is a straight line. The gradient is less steep than that of the IAA profiles, indicating faster diffusion, probably because of the smaller size of the molecule. Basipetal transport of 2,4-D also produces a straight line log profile but it is indicative of much more rapid transport and greater flux than the acropetal movement and must, therefore, contain an active component. Assuming that 2,4-D travels in the same system as IAA, it is probably true to say that the system has a lower affinity for 2,4-D which would account for its slower transport. The non-active component of basipetal transport which is relatively unimportant in IAA transport plays a greater part in the transport of 2,4-D, and the difference between the basipetal and acropetal profiles of 2,4-D transport are not so marked as that between the two corresponding profiles for IAA transport. Also the 2,4-D profiles only reflect transport and not immobilisation. The transport of 2,4-D to the cut surface is slow enough for the cut surface to export it into the receiver, at least for a time, and quite a lot of radioactivity can be recovered from the receiver after 2,4-D transport. This export apparently cannot continue for very long periods and the inability of the segment to continue exporting 2,4-D is probably an effect of the 2,4-D itself, which was used here in quite high concentrations, rather than an effect of the passage of time, because experiments reported in Chapter III show that

IAA can continue to be exported for long periods. The lack of immobilisation associated with 2,4-D transport is demonstrated by the fact that 2,4-D can be 'chased' after the removal of the donor from the segment. In the experiments reported here it did not prove possible to move the 2,4-D out of the tissue and into the receiver for the reason discussed above. It would probably prove useful to cut a small portion off the basal end of the segment before the 'chase' period so that if the damage assumed to prevent export of the 2,4-D were localised near the cut surface export could start again, and 2,4-D might be 'chased' out of the tissue.

Goldsmith and Thimann (1962) suggested that when the donor concentration was increased IAA became immobilised in Avena coleoptile sections and a smaller proportion moved into basal receivers. Scott and Jacobs (1963) reported that the movement of exogenous IAA through Coleus tissue into a receiver did not continue to increase with increasing donor concentration, but reached a maximum. Veen considered it unlikely that Goldsmith and Thimann's immobilisation could account for Scott and Jacob's saturation phenomenon, because their experiments ran for only 2.5 h, by which time most of the ^{14}C recovered by acetonitrile extraction in his experiments was associated with unchanged NAA. However, Veen suggested that the saturation of the transport system reported by Scott and Jacobs was a consequence of the occupation of ' all sites in the secretion unit'. He further suggested that if the secretion unit is occupied ' the concentration in the tissue next to the receiver block will increase and pass a hypothetical threshold value required for enzyme induction'. The adaptive enzyme system to which he referred is that necessary for the IAA/IAAsp conjugation process to occur. Basically his theory fits in with the hypothesis presented here, in that it involves the induction of an immobilisation system by high concentrations of IAA which cannot be carried in the transport system, although he only seems to consider his mechanism to function in the distal region of the segment. The hypothesis arising from the data presented here does not attempt to define the spatial distribution of the transport and immobilisation systems, but it would overcome the objection of Veen to Goldsmith and Thimann's theory. Itsuggests that even if the transport system becomes saturated during the induction period of the enzyme system responsible for IAAsp formation, immobilisation can still occur by the use of the IAA-binding sites, leaving the transport system to

function normally, and that it is saturation of the binding sites that forms the "threshold" for the induction of the conjugating enzyme. Veen, however, found that the export of NAA into the receiver ceased after 10 h, and he attributed this to complete immobilisation. Such a decline in export of IAA was not noticed here and complete immobilisation is not envisaged in the hypothesis. These results do not seem to agree with de la Fuente's (1969) suggestion of a 'transportable pool of auxin which is not entirely located at transport sites'. Such a small amount of auxin is apparently transportable in these experiments that it is hard to believe that it is not all located in the transport system.

Veen's idea of the 'secretion unit' is in broad agreement with the work of Hertel and Leopold (1963), Christie and Leopold (1965 a and b), Cande et al (1973) and Goldsmith and Ray (1973). All these workers have shown that it is secretion from a cell that forms the active component of transport, while uptake into a cell and movement through a cell are caused by a passive process, probably diffusion. The actual mechanism of transport has not been investigated in this study but the results obtained agree with the facts reported by the above workers. Uptake has been seen to be a separate process from polar transport. If the active component of transport is the uptake into cells one would expect to see polarity of uptake; this has not been indicated here. These results also agree with de la Fuente and Leopold (1966), who showed that the differential in transport capacities must exist at the cellular level and be amplified over large numbers of cells. If this is the case then each cell in the transport system must be capable of taking up auxin passively at its apical and basal ends, and also capable of exporting auxin passively at its apical end. At its basal end, however, it can export auxin passively and by some active, metabolic process. Presumably all cells, and not just the cells in the active transport system, are capable of transporting auxin passively.

The results reported in this chapter, therefore, indicate that IAA transport is an active process carried out by a system with a definite capacity. The capacity of the system and the associated immobilisation system ensure a continued but limited flow of auxin which is presumably important in maintaining the delivery of the appropriate concentrations of auxin to sites of action. Auxin which is withdrawn from the transportsystem and immobilised does not appear to be available for further use. Its immobilisation could not be reversed in the present experiments. It is probable, however, that the IAAsp

which is formed is broken down in time, either in response to an auxin shortage or for disposal as a waste product. This was not seen to occur during the time course of the experiment reported here.

CHAPTER III

SOME EXPERIMENTS CONCERNING THE PHYSIOLOGICAL SIGNIFICANCE OF AUXIN TRANSPORT

Introduction

In the preceding chapters some aspects of the transport patterns of auxin and its associated metabolism have been considered. Auxin transport is important in a plant not only because it is the means by which the growth hormone is distributed to the other parts of the plant, but also because the capacity of the system regulates the concentrations of auxin which reach the various sites of action.

It is accepted that root initiation in cuttings and stem segments occurs as a result of a basal accumulation of auxin. The basal accumulation was shown in the present experiments to occur even when a receiver was present, and was also quantified. It was thought that it would be of interest to investigate the location of roots on excised segments, during labelled auxin treatment, in relation to the activity profile obtained for those segments.

Halliday's (1969) work on abscission had suggested that an accumulation of IAA occurs in the cortical region of the abscission zones of older Coleus petioles. During the course of his work he often noticed that some radioactive material appeared to move into the lateral bud. The latter finding could have significance in connection with apical dominance.

In Chapter I the products formed during the course of auxin transport in whole plants were briefly considered. In this chapter auxin transport in whole plants is again considered, as are the topics mentioned above.

Rooting internodes

The accumulation of radioactive material which occurs at the base of a plant segment as a result of basipetal transport of ^{14}C-labelled IAA has been reported and discussed in Chapter II. The distribution of the accumulation is a function of the length of the segment. It seemed reasonable to assume that this accumulation of auxin could be connected with the formation of adventitious roots.

Twenty internode segments were cut as usual and set up apical end uppermost in perspex bridges in Petri dishes. No receiver blocks were used but the filter paper lining the Petri dishes was soaked in 2% sucrose solution to provide nutrition for the segments. Agar donor blocks were applied to the apical cut surface of the segments. Each donor contained 5 μl of IAA-2-^{14}C solution. The Petri dishes were placed in a covered plastic tray lined with damp filter paper and the assembly was placed in a dark room under a green safelight. The duration of the experiment was 10 days. During this time more IAA-2-^{14}C solution was applied to the segments at intervals. In all, seven further applications, each of 5 μl of solution, were made. They were made directly on to the existing donor block if this was still in good condition but if the agar was dehydrated or infected then the solution was applied in a new donor block. The filter papers in the Petri dishes and the tray were also moistened daily with sucrose solution and water, respectively. After the 10 day period all the segments displayed substantial growth of adventitious roots. In all cases the roots arose in a ring 3 - 4 mm from the basal cut surface. The donors were removed from all the internodes and the roots were cut from them. Half the internode segments and their roots were separately extracted in the usual way and chromatographed. The other internodes were cut into 15 sections which were pooled in combustion vessels. The roots were also pooled in these vessels and all samples were

oxidised and counted. A similar experiment was performed with the segments inverted and with the donor in contact with the basal cut surface of the segment. Further applications of IAA-2-^{14}C solution were made as above. The experiment ran for 15 days, by which time very little root formation had occurred. A few roots were present on four of the segments in a ring 3 - 4 mm from the physiologically basal cut surface. It was decided to terminate the experiment before the tissue degenerated. The four internode segments with roots and six others were used for oxidation. The remaining 10 segments were extracted and chromatographed. Radiographs of the chromatograms produced in the two parts of the experiment are shown in Plates III.1 and III.2. The distribution of activity in both situations is shown in Table III.1 and the log profiles are shown in Fig. III.1.

Table III.1 Activity distribution in internode segments and their adventitious roots after 10 days basipetal transport and 15 days acropetal transport

Component	Count	
	basipetal	acropetal
A	66,814	47,870
B	15,189	22,390
C	6,645	7,760
D	4,293	5,624
E	2,878	5,888
F	2,377	2,089
G	1,736	1,479
H	1,509	933
I	1,498	692
J	1,169	1,259
K	1,626	891
L	2,449	501
M	3,102	490
N	6,841	813
O	9,968	891
Roots from one cutting	909	851

As would be expected from the Oxymat results very little radioactivity could be recovered in the root extract from the basipetal transport experiment

Plate III.1 Radiochromatograms of Coleus internode and root extract following 10 days basipetal transport of IAA-2-^{14}C

The chromatograms were developed in isopropanol/ammonia/water. The left hand chromatogram is the adventitious root extract. Very little radioactive material is indicated although there is a very faint spot at 0.09. The right hand chromatogram is the internode extract. Several spots are present on this chromatogram. That at about 0.40 could be IAA and that at 0.08 could be IAAsp.


.1
← Front

Plate III.2 Radiochromatograms of Coleus internode extract following 15 days acropetal transport of IAA-2-^{14}

The chromatograms were developed in isopropanol/ammonia/water. The left hand chromatogram is an IAA-2-^{14}C marker. The right hand chromatogram is the internode extract. It is only faint but a spot can be seen at 0.13 (probably IAAsp) and a faint spot runs at 0.53 which corresponds to the marker IAA. Other, indistinct spots can also be seen.

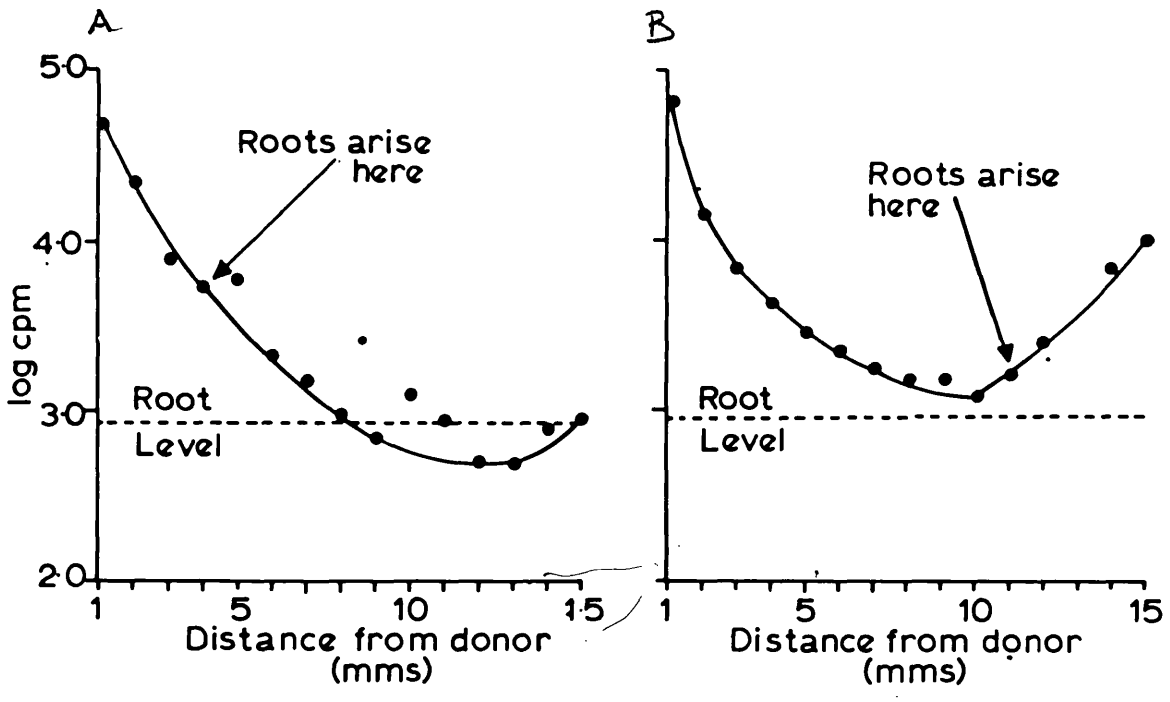

III.2
← Front

Fig. III.1 Log profiles of the distribution of radioactivity in Coleus internode segments after 15 days acropetal transport and 10 days basipetal transport.

A. shows the log profile for the acropetal transport.

B. shows the log profile for the basipetal transport.

In both cases the section from which the adventitious roots arose is indicated by an arrow. The mean levels of activity recovered from the roots on one segment are indicated by the dotted lines and can be seen to be similar. Curve B has the familiar shape for basipetal transport. Curve A only shows a very slight distal accumulation in spite of the extended transport period.


A
B
5·0
4·0
3·0
2·0
log cpm
Roots arise here
Root Level
1
5
10
15
Distance from donor (mms)
Roots arise here
Root Level
1
5
10
15
Distance from donor (mms)

and since all the roots produced in the second experiment were oxidised none was available for extraction. However, it can be seen from the radiochromatogram in Plate III.1 that some radioactivity remained at the origin of the root extract chromatogram and that there was a faint spot at 0.09 which could have been IAAsp. A great deal of radioactive material was extracted from the internode segments in both cases and the ^{14}C appeared to be associated with several products (see Plates III.1 and III.2). The presence of IAAsp was indicated in both cases. IAA was also indicated but did not appear to be abundant. The nature of the other substances was not known but over such long periods metabolism would have proceeded in many directions and the substances present would not reflect the metabolism directly associated with transport. There was some indication that after 15 days some ^{14}C was bound to protein since some radioactive material was extracted from the tissue by hydrolysis.

It appears from the results of this experiment that root formation which was abundant in the first part of the experiment was inhibited in the second part. Root formation would normally occur at the physiologically basal end of the segment but when IAA was applied to that region at the experimental concentration it apparently inhibited root initiation. Auxin was the only factor involved in root initiation which was considered here but others are also involved. Hess (1969) names four co-factors involved in rooting and none of these was considered. It is not therefore possible to say definitely that IAA concentration was the only factor responsible for the results observed. The roots that were initiated in the second part of the experiment arose, like those initiated in the first part, in a circle about 4 mm distant from the basal cut surface. It can be seen from Table III.1 and Fig. III.1 that the mean level of radioactivity which passes into the roots produced by one segment is very similar in each case although the levels

of activity in the sections from which they arose are very different. This demonstrates the fine control of IAA concentration which is exercised within the plant in order to produce the physiological effects of the auxin. In the case of the apically-treated segments it can be seen that the roots arise in the region of the segment near the proximal limit of the basal accumulation.

The general shape of the profiles are also of interest here because of the length of the transport periods involved. The basipetal transport profile has the familiar form even though the actual counts are very high indeed, and the count in the distal section approaches that in the second section from the donor as has been observed to be the case in earlier, shorter experiments. As stated in the discussion of Chapter II the profile shows no tendency to flatten out in a way that suggests saturation of sites of immobilisation. The acropetal transport profile shows a very slight accumulation in the distal region but, even after such a long time when the levels of radioactivity in individual sections are very high, it does not approach the distribution associated with basipetal transport. The levels in the sections are far higher than those recorded after short basipetal experiments when the accumulation was noticed. This suggests again that the accumulation does not occur directly as a result of the presence of a high concentration of radioactive material but as the result of the rapid arrival of that material.

These results are in agreement with those of Gorter (1968) who stated that roots are not formed where most auxin is present but at some distance from there, i.e. if bound auxin is involved its concentration must not be too high. In the same paper she also stated that for roots to be produced on a cutting, leaves or a carbohydrate source must be present. It could be that in the second part of this experiment, apart from the fact that the IAA was at a

supra-optimal concentration, the carbohydrate source which was distant from the root-bearing region of the segment was therefore inadequate. Strydom and Hartmann (1960) produced roots on plum stem cuttings by a basal application of IAA-^{14}C but were unable to produce the effect by an apical application. The concentration they used was 4,000 ppm. They also noted radioactive material in the newly formed root. It seems, therefore, that auxin (at a carefully controlled concentration) passes into the newly formed root and is probably metabolised there.

Lateral Buds

Much work has been performed on suggested mechanisms of apical dominance but little has been done to actually establish whether IAA does move into apical buds from the basipetal transport stream. Halliday's (1969) results indicated movement of radioactive material from the transport stream of the petiole into the lateral bud.

It seemed useful to investigate this problem briefly in relation to more general features of IAA transport.

Nodal explants similar to the type used by Gorter (1964), Gorter and Veen (1966) and Veen (1966), but with different dimensions, were used for this experiment (see Fig. 1). Lateral buds which were normally removed from the experimental plants had been allowed to grow on the plants for the purposes of this experiment and in the plants selected the buds were just opening. The explants were supported in the usual perspex bridges. IAA-2-^{14}C was applied to the apical cut surface of each stem in an agar donor block. Each donor block contained 10 μl of IAA-^{14}C solution. The transport period was 24 h. After this time all the donors were removed and the explants were divided into two groups of 15. Explants from both groups were divided into stem, lateral buds

and petioles. The components from the first group were extracted in the usual manner and chromatographed and radiographed. The resulting radiochromatogram is shown in Plate III.3. The components of the explants from the second group were placed into previously weighed polycarbonate combustion vessels. The stem segments had to be cut in order for this to be done. All the vessels and their contents were then weighed and the nett weight of the contents calculated. The calculated mean weights for each component of each explant are shown in Table III.2. The samples were then oxidised and counted and the count for each component of one explant was calculated. From the mean count and the mean weight the mean count/g fresh weight was calculated (see Table III.2).

Table III.2 Fresh weights and radioactive content of the three components of nodal explants after 24 h basipetal transport of IAA-2-^{14}C

Component	Mean weight (g)	Count (cpm)	Count/g
Donor	-	22,045	-
Stem	0.3377	30,397	90,008
Bud	0.0533	176	3,298
Petiole	0.1089	151	1,388
Receiver	-	484	-
Total	0.4999	53,253	

The chromatogram (Plate III.3) does not show much except that there are products of IAA in all three compounds. IAA and IAAsp are indicated in all three extracts and there is possibly an unknown metabolite which is not represented in the bud extract. The chromatogram does serve to show that some auxin does enter the bud and petiole by acropetal transport and is presumably metabolised there.

The Oxymat results are rather more interesting. They confirm that

Plate III.3 Radiochromatograms of Coleus lateral bud, petiole and stem extracts after 24 h basipetal transport of IAA-2-^{14}C in a nodal explant

The chromatograms were developed in chloroform/methanol/acetic acid. The left hand chromatogram is the bud extract, the middle one is the petiole extract and the right hand one is the stem extract. All three have a spot at 0.80 which is probably IAA. The bud and stem extracts also have a spot at 0.20-0.30 (probably IAAsp) but this is not so obvious in the petiole extract.

III.3

some acropetal transport does occur (although the material in the bud and petiole represent a small percentage of the material in the explant as a whole) and suggest that the auxin could move preferentially into the axillary bud. Actual counts recovered from a bud are slightly higher than those recovered from a petiole segment and if this activity is calculated per g of fresh weight the difference becomes quite marked, and the effective concentrations of auxin are seen to be quite different. It is probable that the radioactive material recovered from the buds and petioles moves into those areas from the transport stream by diffusion but one might expect to recover more activity from the petiole since diffusion into it would probably be less restricted, unless the IAA was being metabolised more quickly in the bud. The difference in the levels of activity recovered from the two components is not great so it would not be wise to attach too much significance to it, but the concentration difference in the two components definitely exists and this finding does have some relevance to work on apical dominance. This experiment can only be taken to show that IAA can move acropetally into lateral buds, probably more readily than it moves into petioles. Apical dominance is not very strong in Coleus so it cannot be compared with other species. Thimann, Sachs and Mathur (1971) caused inhibition of lateral bud development in Coleus by direct auxin application. Werblin and Jacobs (1966) suggested that their observed lack of acropetal transport in Coleus petiole segments could account for its low level of apical dominance. There is probably far more to apical dominance than a direct inhibition of lateral bud development by high auxin concentrations but it has now been shown that auxin can move into lateral buds from the transport stream in the stem.

Plate III.4 Radiochromatograms of Coleus abscission zone extract and petiole extract following 24 h basipetal transport of IAA-2-^{14}C in abscission zone explants

The chromatograms were developed in isopropanol/ammonia/water. The left hand chromatogram is the abscission zone extract and the middle chromatogram is an extract from discs cut from the petiole stumps 5 mm distal to the abscission zones. Both extracts contain IAAsp (R_f = 0.10). Neither chromatogram has a spot at 0.50 which would correspond to the position of the IAA marker in the chromatogram on the right.


←Front

Plate III.5 Radiochromatograms of Coleus abscission zone extracts following 24 h basipetal transport of IAA-2-^{14}C in abscission zone explants

The chromatograms were developed in butanol/acetic acid/water. The right hand chromatogram is IAA-2-^{14}C marker and the R_f value of the IAA is 0.90. The first chromatogram is an extract of the cortical tissue of the abscission zone and the second chromatogram is an extract of the vascular tissue. Both contain IAA and there would appear to be more of it in the vascular tissue extract than in the cortical tissue extract. The cortical tissue appears to contain some products not found in the vascular tissue. The third chromatogram is the cortical extract 'spiked' with IAA-2-^{14}C.

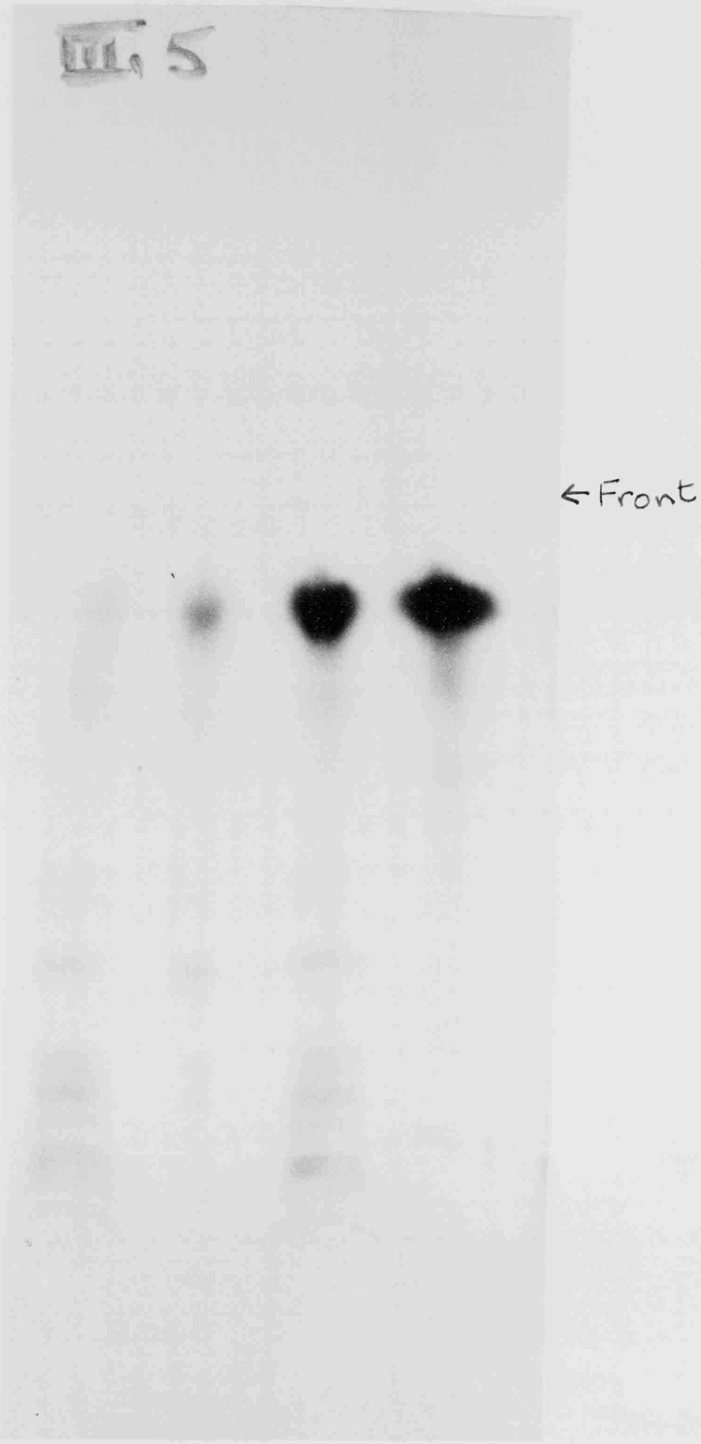


Fig. 5
← Front

Abscission Zones

Halliday (1969) found that IAA-^{14}C applied to petiole stumps on abscission zone explants (see fig. 2) travelled basipetally in that stump to the abscission zone and tended to accumulate there. His micro-autoradiographs showed the accumulation to occur across the entire zone although he thought that most of the radioactive material was still to be found in the vascular tissue, and that if the activity in the abscission zone were measured the accumulation would be masked unless the vascular tissue were first removed. His findings were further investigated here by extraction and chromatography, and by scintillation counting of the tissue.

Thirty abscission zone explants were cut (see Fig. 2) and set up as described by Halliday. Two explants were placed in each closed Petri dish lined with damp filter paper. The explant stood with its longitudinal cut surface downwards on a filter paper disc soaked in 2% sucrose solution, standing on a small rectangle of polythene sheet. The cut surface of the petiole stump was thus uppermost and an agar donor block containing IAA-^{14}C solution could easily be applied. In the first set of experiments 10μl of IAA-2-^{14}C solution were applied in each donor. After 24 h two discs, each 1 mm thick, were cut from the petiole stump, the first (which contained the abscission zone) about 1 mm from the junction with the stem, the second 5 mm up from the abscission zone. (Although the abscission zone is externally visible in Coleus, it was not, in fact, found to be quite as easy to determine visually as Halliday reported, but the disc could still be considered to contain the zone). The two sets of discs were extracted separately and chromatographed. The chromatogram obtained is shown in Plate III.4. Little difference was observed between the chromatograms of the two extracts. Little IAA appeared to be present in either case. Although visual inspection of chromatograms can be misleading, there was certainly no indication that IAA had accumulated in the abscission zone. IAAsp was indicated in both extracts. To find whether accumulation in the abscission zone was being masked by the radioactive material in the vascular tissue, the experiment was set up again using 60 explants, but the vascular tissue was removed from each disc and extracted separately. The results of the experiment can be seen in Plates III.5, III.6 and III.7. Generally speaking the chromatograms revealed little difference between any of the extracts investigated, but if anything the indication was that the non-vascular tissue of the abscission zone

Plate III.6 Radiochromatograms of Coleus abscission zone extracts following 24h basipetal transport of IAA-2-^{14}C in the abscission zone explants

The chromatograms were developed in isopropanol/ammonia/water. The right hand chromatogram is IAA-2-^{14}C marker and the IAA has an R_f value of 0.58. The third spot is an extract of the cortical tissue of the abscission zone 'spiked' with IAA-2-^{14}C, and the R_f value of the IAA has been reduced to 0.50. Some IAA can be detected in the vascular tissue extract (second chromatogram) but not in the cortical tissue extract (first chromatogram).

III.6

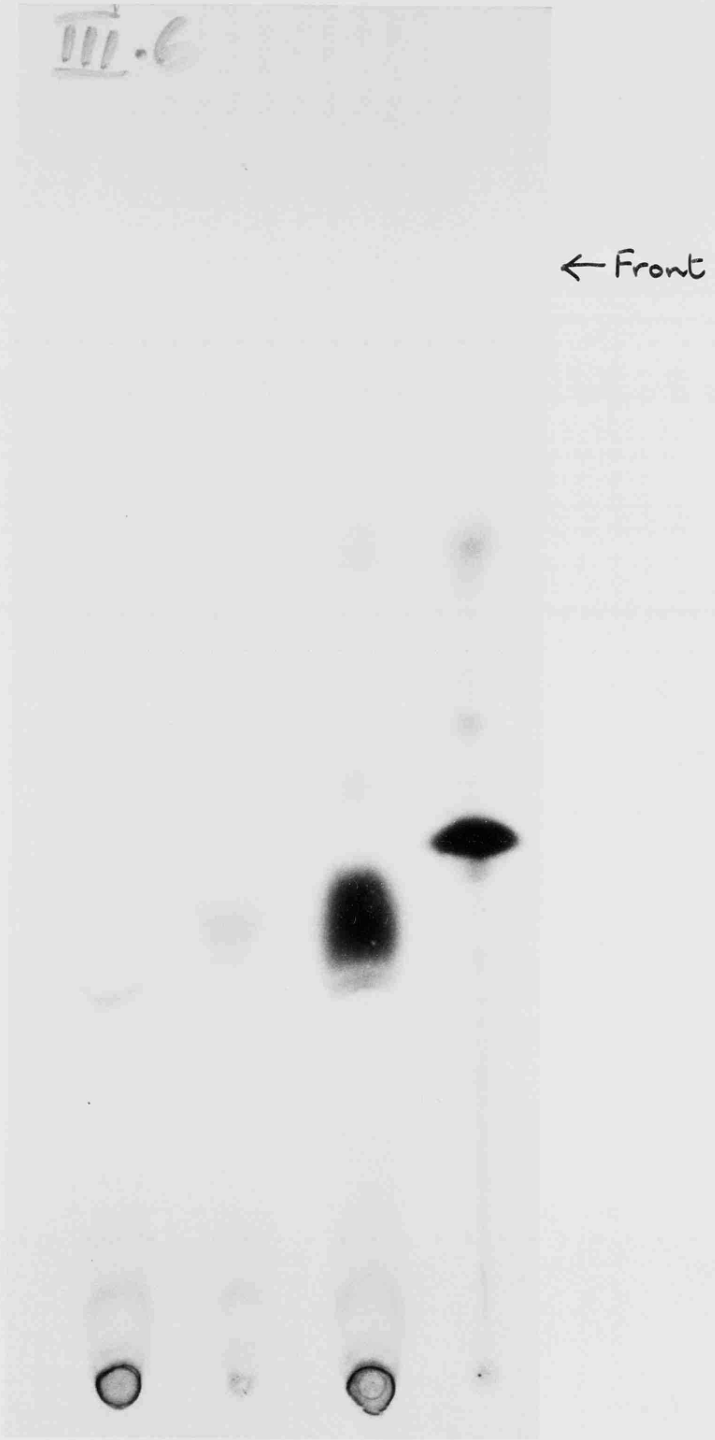
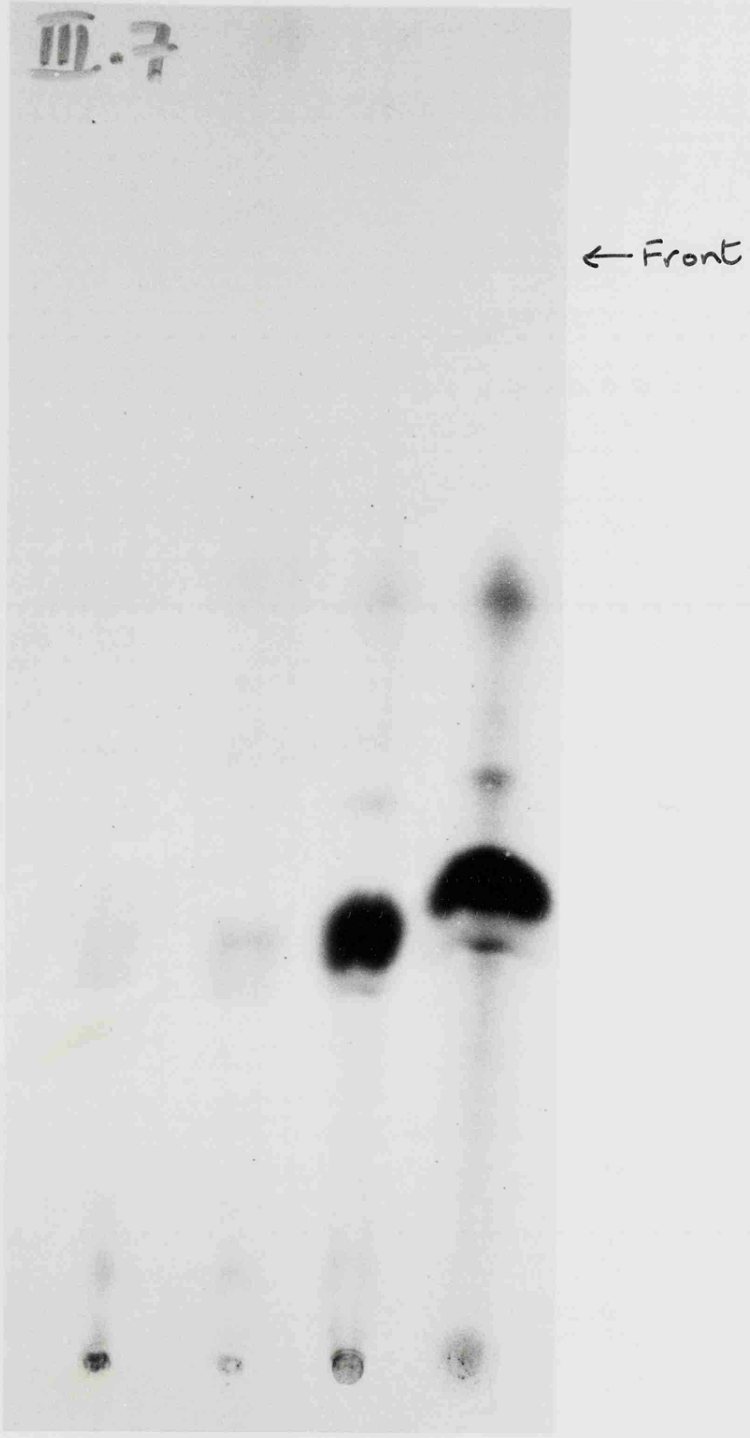


Plate III.7 Radiochromatograms of Coleus petiole extracts following 24 h basipetal transport of IAA-2-^{14}C in abscission zone explants

The chromatograms were developed in isopropanol/ammonia/water. The right hand chromatogram is an IAA-2-^{14}C marker. The IAA runs at an R_f value of 0.52. The third chromatogram is an extract of the cortical tissue 'spiked' with IAA-2-^{14}C and the R_f value of the IAA is reduced to 0.50. The first and second chromatograms are the cortical and vascular tissue extracts from the petiole and both appear to contain IAA.

III.7



contained less unchanged IAA than the other regions. IAAsp was again indicated in all extracts.

In another attempt to reproduce Halliday's results the experiment was repeated again and allowed to run for 48 h. His experiments had been of 43 h duration and the possibility existed that 24 h was not a long enough period to produce the result. Twenty four hours should certainly have been long enough for the transport system to carry large quantities of IAA into the region, but it was possible that the ageing of the explant was involved and that a freshly excised explant might not have the capacity to accumulate IAA in its abscission zone. However, the results (see Plates III.8 - III.11) were the same as those previously recorded. Slight differences were noticed in the minor constituents of the petiole vascular and non-vascular extracts, but the concentration of IAA appeared to be similar in all extracts.

These extraction and chromatography experiments did not confirm Halliday's findings, but as visual inspection of chromatograms cannot be regarded as conclusive evidence, it was decided to perform oxidation experiments to see if the distribution of activity he described could be confirmed. Twenty explants were set up as described, but the petiole stumps were only 7 mm long. Donors, each containing 10 μl of IAA-2-^{14}C solution, were applied to the cut surface of the petiole stumps. The transport time was 28 h and at the end of that time the donors were removed and the explants subdivided. The petiole was separated from the stem as close to the stem as possible to ensure inclusion of the abscission zone in the petiole material. The stem tissue was divided into three subsections, the node, the internode ablve and the internode below. The petiole stump was divided into seven sections (1-7, 1 being the closest to the donor). All components were pooled and oxidised and the filter paper which had been in contact with the explants was also counted. The results are recorded in Table III.3 and the log profile can be seen in Fig. III.2.A In the figure the stem and the filter paper circles are considered as the 'receiving system'.

Plate III. 8 Radiochromatograms of Coleus abscission zone extracts following 48 h basipetal transport of IAA-2-^{14}C in abscission zone explants

The chromatograms were developed in isopropanol/ammonia/water. The right hand chromatogram is an IAA-2-^{14}C marker and the IAA has an R_f value of 0.59. The third chromatogram is an extract of the cortical tissue of the abscission zone 'spiked' with IAA-2-^{14}C and the R_f value of the IAA is reduced to 0.55. The first and second chromatograms are the cortical and vascular tissue extracts of the abscission zone and both appear to contain small amounts of IAA.

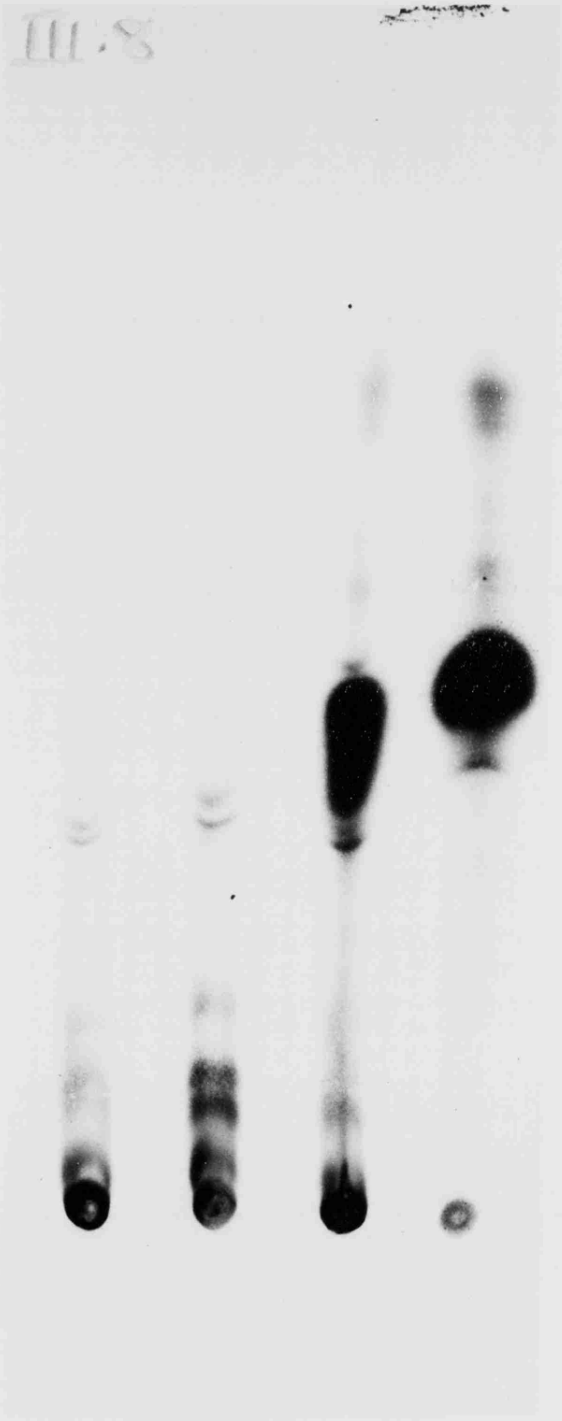

III.8
← Front

Plate III.9 Radiochromatograms of Coleus abscission zone extracts following 48 h basipetal transport of IAA-2-^{14}C in abscission zone explants

The chromatograms were developed inchloroform/methanol/acetic acid. The right hand chromatogram is cortical tissue extract 'spiked' with IAA-2-^{14}C. The IAA has an R_f value of 0.91. The first and second chromatograms are cortical and vascular tissue extracts from the abscission zone. Both appear to contain IAA.

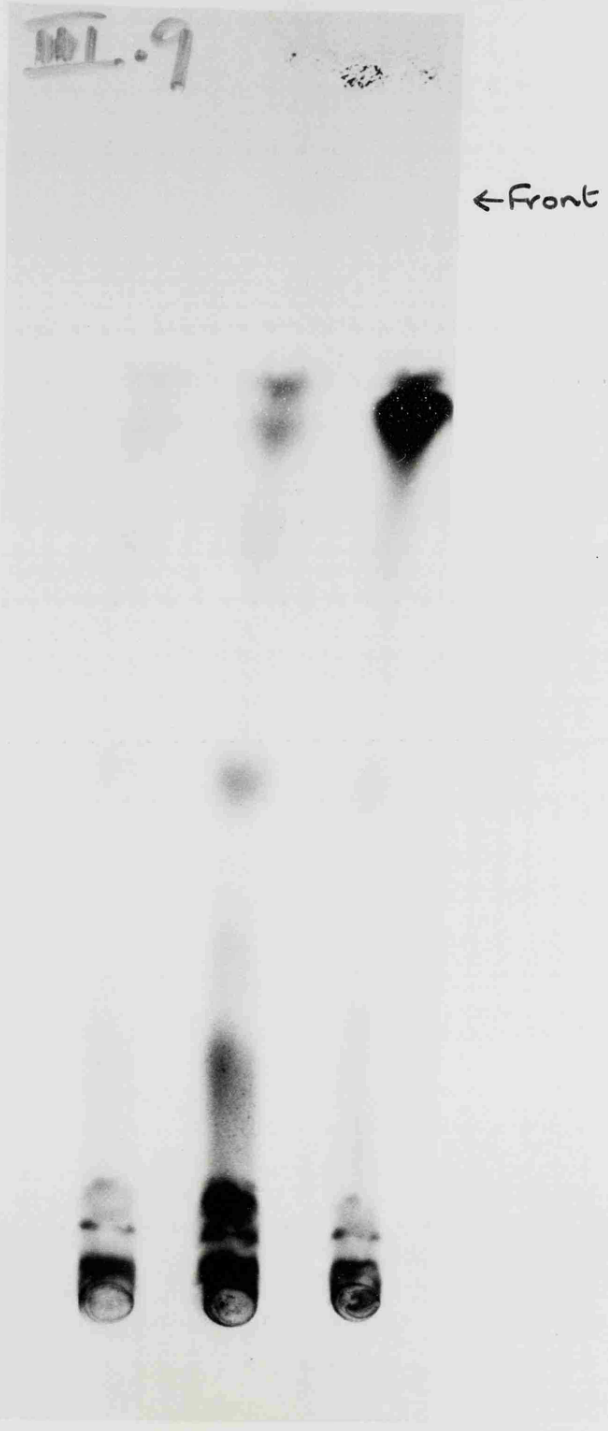

IDL.9
←Front

Plate III.10 Radiochromatograms of Coleus abscission zone extracts following 48 h basipetal transport of IAA-2-^{14}C in abscission zone explants

The chromatograms were developed in chloroform/ethyl acetate/ formic acid. The right hand chromatogram is the cortical tissue extract 'spiked' with IAA-2-^{14}C and the IAA has an R_f value of 0.67. The first and second chromatograms are the cortical and vascular tissue extracts from the abscission zone and neither appears to contain IAA.

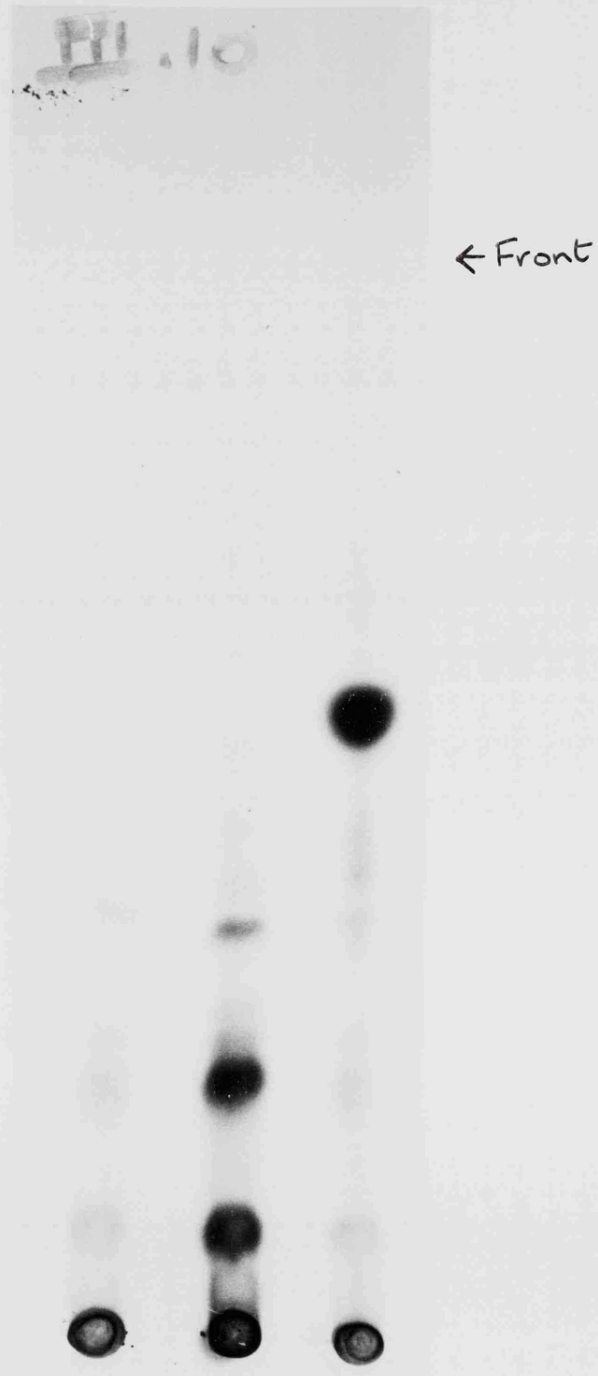

← Front

Plate III.11 Radiochromatograms of Coleus petiole extracts following following 48 h basipetal transport of IAA-2-^{14}C in abscission zone explants

The chromatograms were developed in chrloroform/ethyl acetate/formic acid. The right hand chromatogram is the cortical tissue extract 'spiked' with IAA-2-^{14}C and the IAA has an R_f value of 0.84. The first and second chromatograms are the cortical and vascular tissue extracts of the petiole and neither appears to contain IAA.

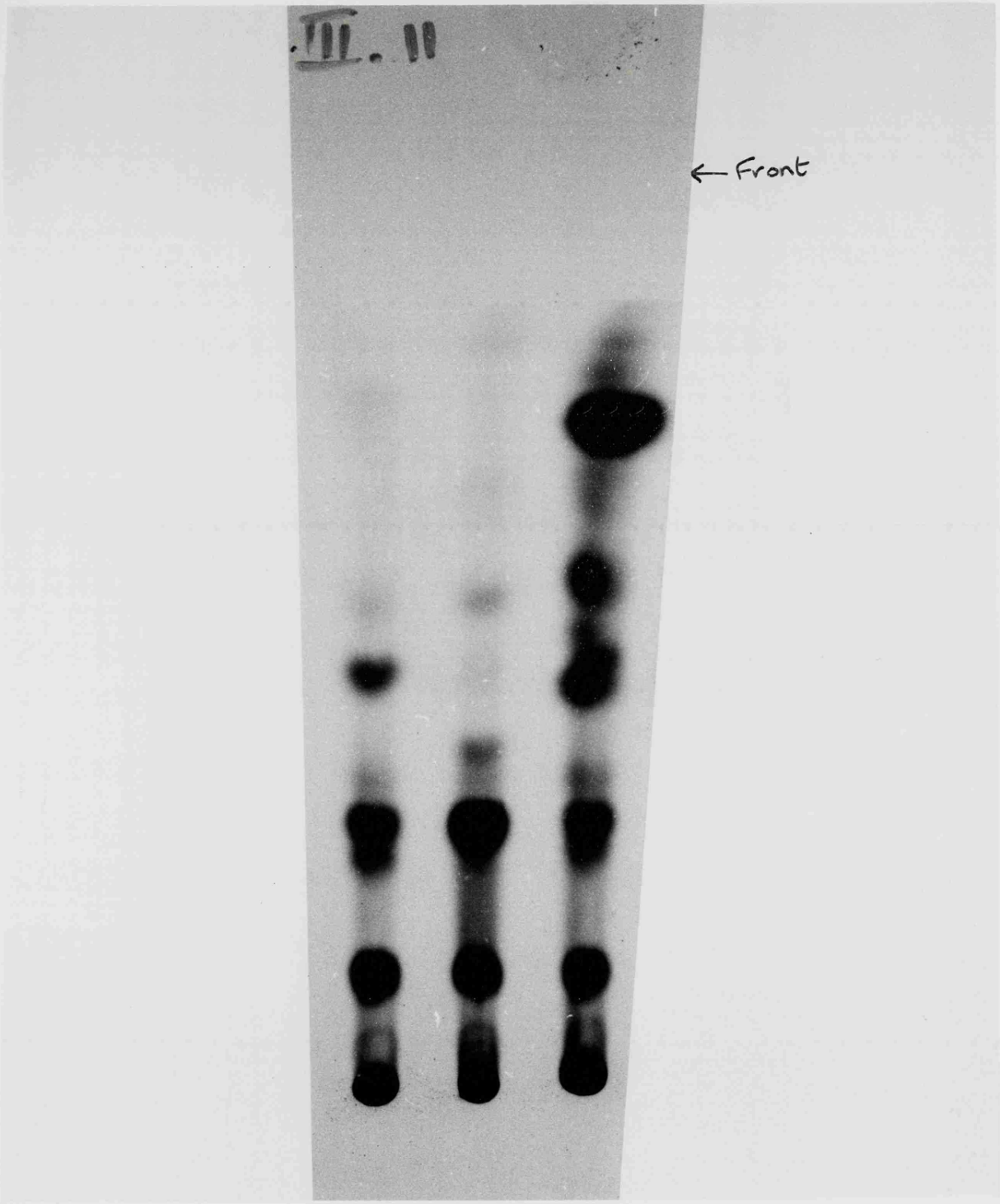

III. II
← Front

Fig. III. 2 Log profiles of the distribution of radioactivity in petiole segments which include the abscission zones after basipetal IAA-2-^{14}C transport

A shows the log profile of intact segments. It forms a smooth curve with no accumulation of radioactivity in the abscission zone (section 6). There is no basal accumulation because the petiole stump remained attached to the stem during the transport period.

B shows the log profiles of the vascular and non-vascular components of the petiole segments. There is more radioactive material in the non-vascular tissue than in the vascular tissue. Both log profiles are smooth curves with no discontinuity corresponding to the abscission zone.

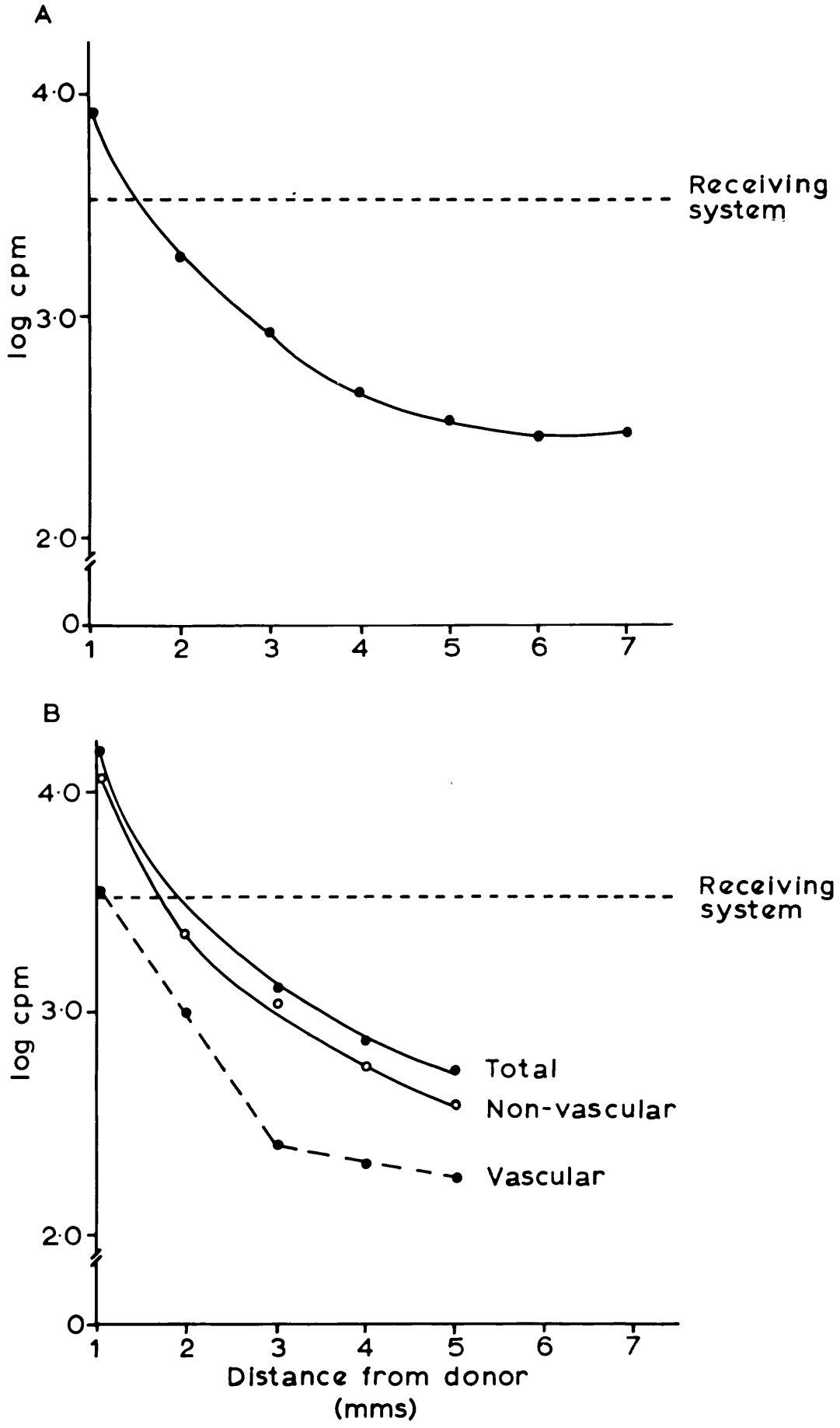

A
4·0
3·0
2·0
0
log cpm
Receiving system
1
2
3
4
5
6
7
B
4·0
3·0
2·0
0
log cpm
Receiving system
Total
Non-vascular
Vascular
1
2
3
4
5
6
7
Distance from donor (mms)

Table III.3 The distribution of radioactivity in the petiole and stem of an abscission zone explant after 28 h IAA-2-^{14}C treatment.

	Component	Count
petiole	Donor	37,967
	1	8,368
	2	1,815
	3	832
	4	451
	5	333
	6	283
	7	297
	Node	598
	Internode below	890
	Internode above	205
	Filter paper	1,685
	Total	53,724

It can be seen from Table III.3 and Fig. III.2 that there is no accumulation of activity in the abscission zone of the petiole. This would correspond to section 6 on the log profile, which demonstrates a smooth but non-linear decline. Polarity of transport in the stem was again demonstrated by the levels of activity in the internodes above and below the node. More than four times as much radioactive material moved basipetally as acropetally.

It seemed possible that Halliday's suggestion that the accumulation could be masked by the high levels of activity in the vascular tissue of the petiole could be correct, so another experiment was performed to investigate this. The transport period was again 28 h. The explants were set up as described, but the petiole stumps were further reduced to 5 mm in length, since long stumps were not necessary in these experiments. The stump was again divided into 1 mm sections , but this time the vascular tissue was carefully removed from each disc and vascular and non-vascular tissues were pooled, oxidised and counted separately. The results are shown in Table III.4 and the log profile is shown in Fig. III.2 B.

Table III.4 Activity distribution in the vascular and non-vascular components of the petiole stump in an abscission zone explant after 28 h IAA-2-^{14}C treatment

Component	Count	
	vascular	non-vascular
1	3,504	11,245
2	968	2,290
3	239	1,075
4	203	548
5	178	370
Stem (total)	3,287	

The figures show that even in this experimental arrangement no accumulation of radioactivity was found in the abscission zone. The curves for vascular and non-vascular tissue follow the same shape as the curve for whole sections. Moreover, changes in radioactivity in the cortical tissue were unlikely to be masked by activity in the vascular tissue in the previous experiments, because more radioactivity can be recovered from the non-vascular than the vascular tissues. Radioactive material is, nevertheless, concentrated in the vascular tissue because the cross-sectional area of the vascular tissue is very small compared with that of the non-vascular tissue. It is not possible to state from these results whether the radioactive material in the two regions is mobile or immobile, nor with which compounds the ^{14}C is associated. The relationship between the radioactivity in vascular and non-vascular tissue appeared to be fairly constant in all sections. Percentage values were therefore caculated and are shown in Table III. 5.

Table III.5 Percentage of radioactive material found outside the vascular tissue in individual sections of petiole stumps from abscission zone explants.

Section	% activity
1	76
2	70
3	82
4	73
5	68

It can be seen that there is little variation in the calculated percentage of radioactive material outside the vascular tissue (68-82%) and the value in

section 4 which contains the abscission zone is very close to the mean value. It is difficult to say why Halliday's results cannot be confirmed, since his micro-autoradiographs showed a distinct darkening in the abscission zone. However, a technique as sensitive as oxidation in the Oxymat would be expected to detect an accumulation such as he suggested. The results are in broad agreement with those of Jacobs et al,(1966) and Jacobs (1968), who report no major discontinuity of ^{14}C distribution at the abscission zone of Phaseolus petioles. Robinson et al (1968) reported that there is no barrier to transport distal to the abscission layer in petioles on cotton explants.

Small Coleus Cuttings

Most of the experimental work reported in this thesis was performed on excised plant segments, but the distribution of auxin in decapitated cuttings was also investigated. Coleus cuttings were allowed to root as described in 'Materials' and planted out in moist vermiculite in plastic pots. The cuttings were decapitated to 60 mm in length, and one pair of leaves was left on the cuttings. These leaves were about half expanded. Adventitious roots grew from the basal 10 mm or so of the cutting and were about 1 mm in diameter at their origins and between 2 and 10 mm long. An agar donor block was applied to the cut surface of each cutting. Each donor block contained 5μl of IAA-2-^{14}C and a further two applications of 5μl of IAA-2-^{14}C solution were made during the 48 h transport period. After the transport period the roots were carefully shaved off and pooled in combustion vessels. The leaves were removed and discarded and the stems were cut into 30 sections, each 2 mm thick (1-30). The results of the experiment are recorded in Table HI.6 and the log profile is shown in Fig. III.3.

Fig. III.3 Log profile of the distribution of radioactivity in a decapitated Coleus cutting after basipetal IAA-2-^{14}C transport

The log profile has a similar shape to those seen for internode segments. There is an apical decline and a basal accumulation of activity. The level of activity found in the roots from one cutting is quite high. It would appear that there is unrestricted flow of auxin into the adventitious roots.

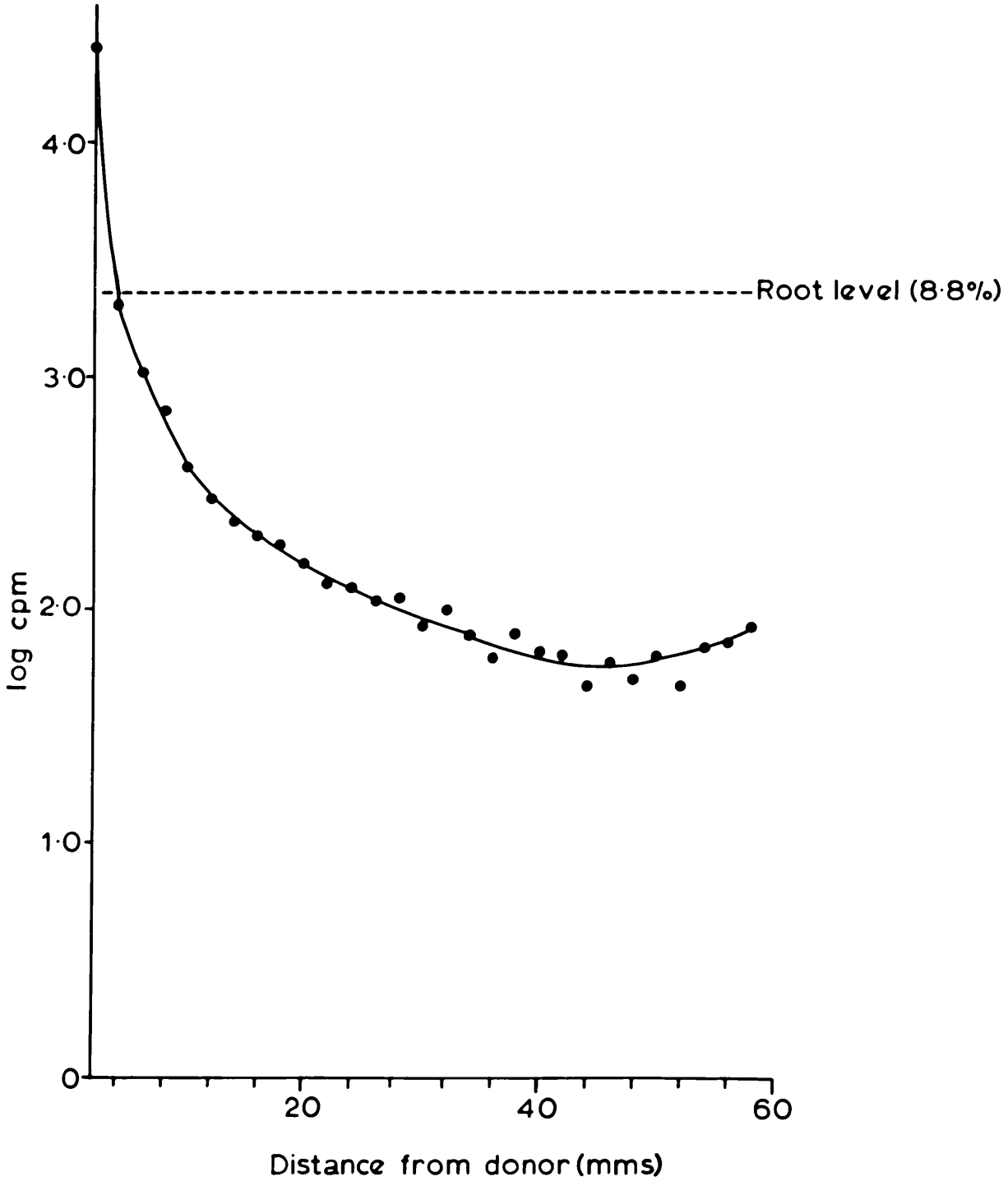

4·0
3·0
2·0
1·0
0
log cpm
Root level (8·8%)
20
40
60
Distance from donor (mms)

Table III. 6 Activity distribution down the stem of a decapitated cutting following 48 h basipetal IAA-2-^{14}C treatment.

Component	Count
1	16,150
2	2,004
3	1,040
4	711
5	410
6	303
7	245
8	214
9	196
10	161
11	132
12	126
13	113
14	115
15	85
16	100
17	78
18	61
19	78
20	66
21	64
22	47
23	60
24	52
25	65
26	49
27	68
29	73
30	83
Roots	2,310

The distribution over the stem resembles that seen in short segments, except that the basal accumulation is not very marked. The probable reason for this is that a great deal of radioactive material passed into the roots. The level of activity in the roots growing on a cutting is quite high, higher than that in section 2 of the stem. The amount of root tissue present on a cutting is small compared to the amount of stem tissue and diameter of the roots is only very small, so the level of activity recovered from the roots represents a considerable flow of auxin into the roots. There cannot be any barrier to transport between the stem and the adventitious roots of a Coleus cutting. The attempts reported in Chapter I to extract radioactive material from roots of small cuttings were not very successful but the presence of IAAsp was indicated.

Movement of auxin from shoot to roots of aspen cuttings has been reported by Eliasson (1972). Phillips (1964a and b) reported movement of auxin from shoot to root of mature Helianthus plants and Morris et al (1969) found that auxin moved from shoot to root of pea seedlings.

Transition Zones in Helianthus seedlings

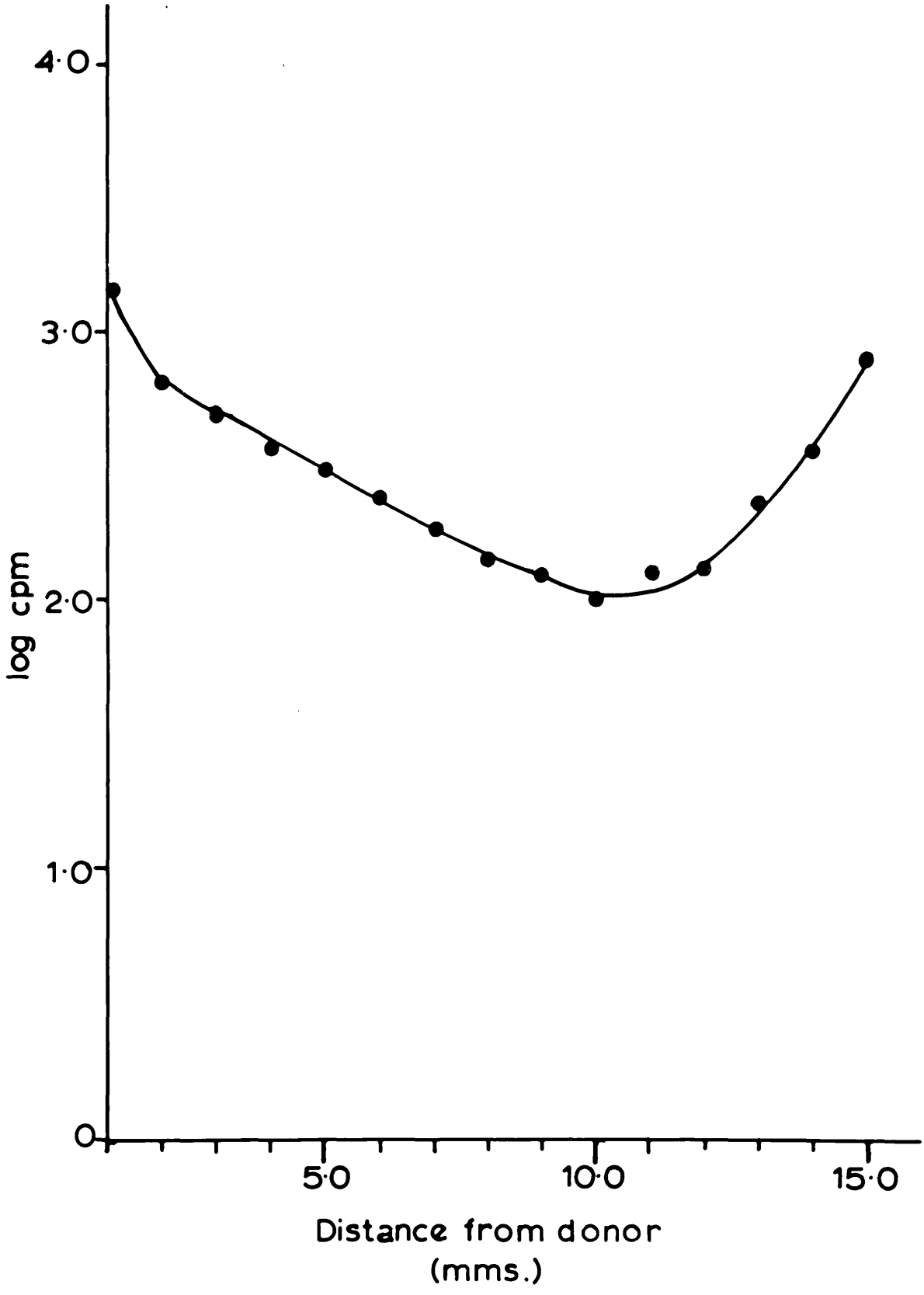
Auxin transport in young seedlings of Helianthus annus was also investigated. Anatomical investigations of seedlings with one pair of unfolded leaves showed a discontinuity of vascular tissue between hypocotyl and epicotyl. The vascular strands from the hypocotyl run into the cotyledons while the strands from the epicotyl run medially to the hypocotyl strands and appear to end below the junction of the cotyledons and stem. This means that there is a longitudinal overlap of the two sets of vascular strands but no apparent physical continuity. It is possible that there is a continuity of phloem but this is not readily discernible. It was decided to find out if the apparent discontinuity impeded the flow of auxin in the young seedling. Segments 15 mm long were, therefore, cut from such seedlings so that the apical cut surface occurred 3 mm above the V-shaped notch formed by the insertion of the cotyledons. Agar donor blocks containing 5 μl of IAA-2-^{14}C solution were applied to the apical cut surface of the segments which were supported in perspex bridges on receiver blocks. The transport period was 20 h after which time the donors were removed, and 15 sections cut, pooled and oxidised in the usual way. The results are shown in Table III.7 and the log profile is shown in Fig. III.4.

Table III.7 Activity distribution in the transition zone between the epicotyl and hypocotyl folowing 20 h apical IAA-2-^{14}C treatment

Component	Count
Donor	14,085
A	1,414
B	648
C	487
D	370
E	312
F	239
G	185
H	144
I	126
J	103
K	127
L	133
M	234
N	366
O	817
Receiver	783
Total	20,574

Fig. III.4 Log profile of the distribution of radioactivity in a segment from the stem/hypocotyl transition zone of a Helianthus seedling after basipetal transport of IAA-2-^{14}C

The log profile shows an apical decline and a basal accumulation similar to those seen in log profiles of internode segments. There is no indication of any discontinuity of flow of auxin in the transition zone.


4·0
3·0
2·0
1·0
0
log cpm
5·0
10·0
15·0
Distance from donor
(mms.)

It can be seen from Fig. III.4 that the epicotyl-hypocotyl transition zone does not cause any discontinuity of auxin flow. The log profile is very similar to that established in other plant segments investigated, although uptake is low compared with that in mature Coleus internode segments and no irregularity is obvious. The basal accumulation occurs as is usual with active transport and the count in the receiver is high which suggests that flow into the receiver has continued during the long period. The apparent discontinuity in the vascular tissue does not appear to inhibit the movement of auxin. Possibly vascular connections existed which were not readily visible as suggested earlier or possibly other tissues were capable of transporting the auxin actively. Certainly there was sufficiently rapid flux to produce a marked accumulation in the segment.

The shoot-root transition zone from the same seedlings was also investigated. No physical discontinuity of vascular tissue occured here but the diameter of the root was considerably smaller than that of the hypocotyl and the vascular strands converged. The hypocotyl of the seedling was therefore cut at a point 1 cm above what was judged (by pigmentation changes) to be the root-shoot transition region. The roots were replanted in vermiculite and an agar block containing 5 μl of IAA-2-^{14}C solution was applied to each cut surface. The transport period was 23 h after which time the lateral roots were removed and pooled and the main axis of the seedling was cut into 25 slices each 1 mm thick. The slices and lateral roots were pooled, oxidised and counted. The terminal portions of each root, which were too thin to section and which, in most cases, were about 1.5 cm long, were also pooled and counted. The results are shown in Table III.8 and the log profile is shown in Fig. III.5.

Fig. III. 5 Log profile of the distribution of radioactivity in a segment including the hypocotyl/root transition zone of a Helianthus seedling after basipetal transport of IAA-2-^{14}C

The log profile shows an apical decline and a slight basal accumulation. From the histograms shown alongside the curve it can be seen that quite high levels of radioactivity can be recovered from the terminal portion of the root and from the lateral roots in spite of the reduced diameter of these components.

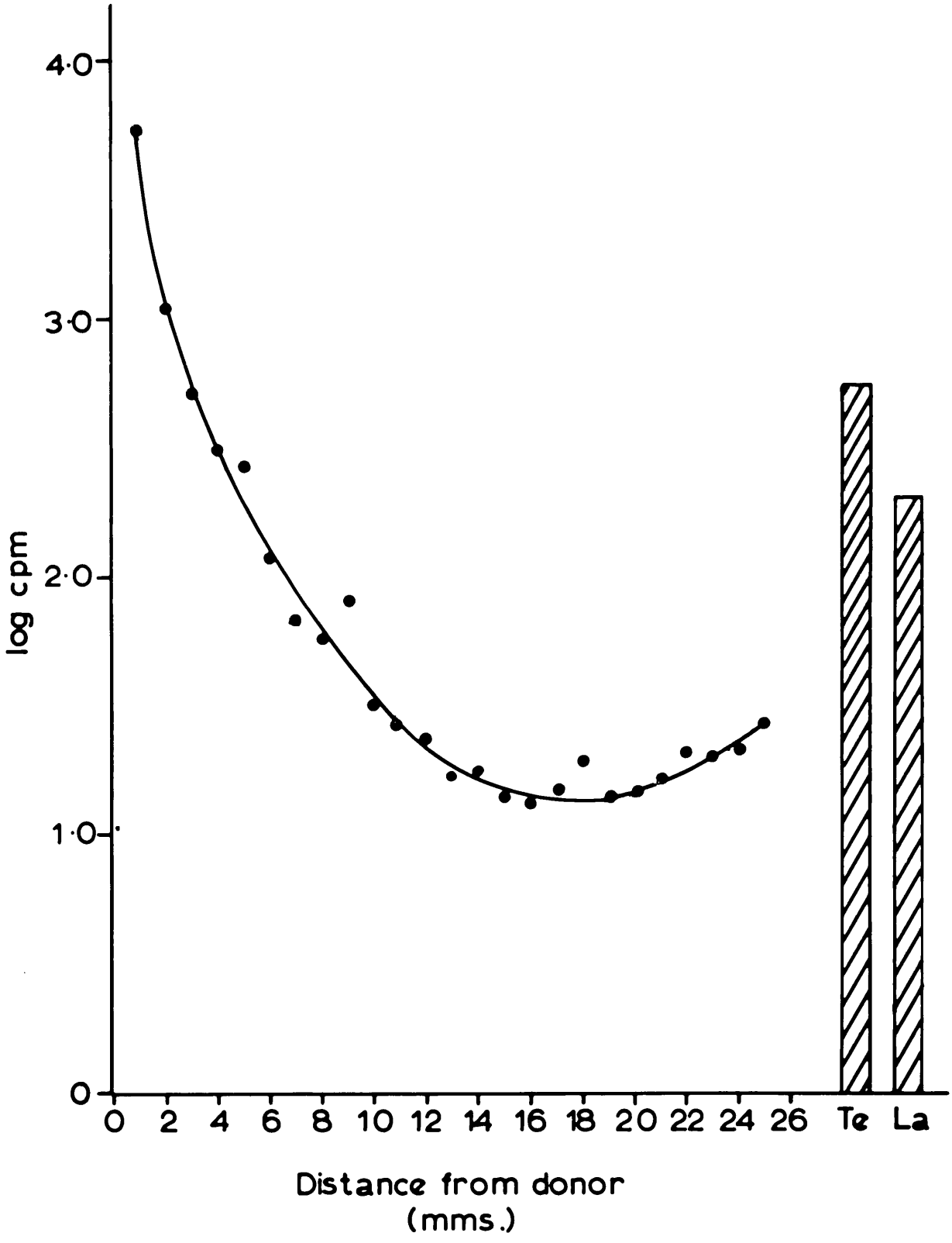

4·0
3·0
2·0
1·0
0
log cpm
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Te
La
Distance from donor
(mms.)

Table III. 8 A_c tivity distribution in the transition zone between the hypocotyl and root following 23 h basipetal transport of IAA-2-^{14}C

Component	Count	
Donor	9,224	
A1	5,339	
B	1,107	
C	514	
D	313	
E	270	
F	116	
G	69	
H	56	
I	79	
J	31	Estimated transition point
K	26	
L	23	
M	17	
N	17	
O	14	
P	13	
Q	14	
R	19	
S	14	
T	15	
U	16	
V	20	
W	19	
X	21	
Y	27	
Terminal root	537	
Lateral roots	200	
Total		

Again no discontinuity of flow was indicated in the transition region. The change in pigmentation which had been taken as a sign of the shoot-root junction occurred at about section 10 of the segment, and no irregularity of the profile can be seen in this region. The log profile is of the form usually seen for basipetal transport. There is a slight basal accumulation which is not very marked. If the terminal portion of the roots if taken into account, however, it can be seen that much radioactivity can be recovered from the root apex and levels in the individual 1 mm sections of this terminal portion would be much higher than those in the sections measured and recorded. This is the first time the basal accumulation has been seen to occur in the absence of a cut surface (since the Coleus cuttings had a cut surface at the base of the stem). A basal accumulation

apparently occurs whenever active basipetal transport is impeded by some physical obstruction. It can also be seen from these results that quite large amounts of radioactive material flow from the main root into the lateral roots.

Discussion

The findings reported in this chapter are not closely related to each other except in so far as they are concerned with auxin transport into regions of the plant where auxin is thought to exert specific influences.

The necessity for the control of auxin in root initiation is demonstrated, as is the ability of auxin to move acropetally from the main auxin stream into lateral buds. It is also shown that auxin can move freely through abscission zones and apparent vascular tissue discontinuities. The flow of auxin from shoots to roots, including adventitious roots, has also been shown to occur freely.

SUMMARY

The results arising from the experiments in the preceding three chapters can be briefly summarised under six headings.

1. IAAsp is the major metabolite formed from IAA during its transport in Coleus internode tissue. Other metabolites were indicated intermittently but they do not seem to be as important as IAAsp.

2. IAAsp is probably not formed in Helianthus internode tissue and 2,4-D does not seem to break down during transport in Coleus.

3. If radioactive IAA is applied apically to Coleus or Helianthus internode segments it is freely transported but not so freely exported and a basal accumulation of radioactive material occurs.

4. The accumulation is not found as a result of acropetal transport of IAA, nor as a result of acropetal or basipetal transport of 2,4-D.

5. Most of the radioactive materials found in an excised plant segment after a period of transport of IAA-^{14}C is immobile and cannot be 'chased' out of the segment. This is true even when it can be shown that most of the radioactive material in the segment chromatrographs as unchanged IAA. It is, therefore, proposed that immobilisation and conjugation of IAA are two processes.

6. IAA transport can continue across anatomical barriers.

APPENDIX

The Principles of the Intertechnique Oxymat

The Oxymat is a commercial version of the Peterson apparatus for tube furnace combustion of samples (Peterson et al., 1969). It oxidises ^{3}H and ^{14}C labelled samples automatically, delivering them dissolved in their respective scintillators ready for counting.

The sample, contained in a combustible polycarbonate capsule (Lexan R), is introduced into a vertical quartz furnace by the opening of a solenoid-operated poppet valve. The sample falls on a bed of quartz chips at the top of the furnace. The furnace temperature is 700°C and the sample is burned in a stream of pure oxygen. The products of combustion flow down into a two-section catalyst bed, of which the upper part comprises copper oxide needles and the lower part is a copper-manganese mixed oxide catalyst (Type SMR 7 35, 35, grade 908, supplied by the W.R. Grace Co.). The tube also contains quartz sand and quartz wool.

On leaving the combustion tube via a heated transfer line the final products of combustion ($^{14}CO_2$ and HTO) meet a stream of liquid scintillator and pass through an absorber where HTO condenses and mixes with the scintillator. The $^{14}CO_2$ is not absorbed at this stage but is conducted to another trap where it is absorbed by a second scintillator. Both absorbers operate on the 'wiped film' principle in which a rotor operating in a precision bore tube barely touches the sides and creates a thin film of liquid scintillator. The large surface of the scintillator so formed and the turbulence of the incoming vapour stream insures efficient trapping. A purge gas, usually nitrogen, deoxygenates the scintillator before it is dispensed into counting vials which are held in position at the outlets under pressure seals. This reduces quenching and also blows any dissolved $^{14}CO_2$ out of the HTO vial and back into the system, reducing contamination. Additional scintillator is pumped into the vials to

insure complete trapping. All excess oxygen passes to the outside air through a line running from the upper end of the CO_2 absorber.

The whole process is operated automatically by 'push button' controls and either one or both trapping systems can be selected for use.

The composition of the scintillators is as follows:

For ^{3}H trapping:

Dioxane	700 ml/l
Toluene	300 ml/l
Naphthalene	20 g/l
2-(4' tert. Butylphenyl)-5-(4''-biphenyl)-1,3,4-oxadiazole (Butyl PBD)	7 g/l

For $^{14}CO_2$ trapping:

Phenylethylamine (trapping agent)	330 ml/l
Absolute methanol	220 ml/l
Toluene	400 ml/l
Distilled water	50 ml/l
Butyl PBD	7 g/l

The system is washed out after use with a solution containing:

Methanol	660 ml/l
Toluene	340 ml/l

Operating specifications are as follows:

Recovery	Better than 95%
Memory (activity remaining in system and appearing in subsequent samples)	Less than 0.5%
Spillovers:	
^{14}C into ^{3}H	Less than 1.0%
^{3}H into ^{14}C	Less than 0.5%

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ABSTRACT

The products formed during transport in excised plant segments of radioactively labelled indolyl-3-acetic acid and 2,4-dichlorophenoxyacetic acid have been investigated. The techniques employed were extraction of the tissue followed by radiochromatography. It was found that indolyl-3-acetic acid is readily converted to indolyl-3-3 acetyl asparate in Coleus tissue but not in Helianthus tissue. 2,4-dichlorophenoxyacetic acid appears to undergo little change.

The distribution of radioactive material which results from the transport of labelled auxin in plant segments and small plants was also investigated by means of oxidation and subsequent scintillation counting. It was found that the system has a definite capacity and that much of the transported auxin is immobilised but not necessarily conjugated and that amounts of auxin reaching receiving systems is not a reliable basis for estimating auxin which is transported within tissue.

Auxin transport into root primordia, lateral buds, abscission zones and through developmental transition zones is also briefly considered and it is reported that no barrier to the transport is encountered in these regions.